THE TBE BOOK

SEVENTH EDITION

Prof. Dr. Gerhard Dobler

Dr. Wilhelm Erber

Dr. Michael Bröker

Dr. Lidia Chitimia-Dobler

Prof. Dr. Heinz-Josef Schmitt

GLOBAL HEALTH PRESS

Published by
Global Health Press Pte Ltd
50 Kaki Bukit Place
#04-01 Jean Yip Building
Singapore 415926

https://tbenews.com/tbe/

The TBE Book (7th Edition)

Publication of this book was funded by Pfizer Inc.

ISSN: 2661-3980

Foreword to the 7th Edition of THE TBE BOOK: A Comprehensive Guide to Tick-Borne Encephalitis

Dear Readers!

It is with great pleasure that we present to you the 7th edition of THE TBE BOOK, a comprehensive guide to Tick-Borne Encephalitis (TBE). Over the past two years, this book has reached an astonishing milestone of over 1 million readers worldwide, and our TBE News including newsletter accompanying The TBE BOOK in April 2024 reached more than 20,000 readers in the United States alone – although not a single autochthonous case of TBE has ever been reported from that country. We are immensely encouraged by the overwhelming response and continue our commitment to providing the most up-to-date information on TBE with this latest edition.

Embracing the E-CDC Definition

In this edition, we have fully embraced the European Centre for Disease Prevention and Control (ECDC) definition of “arbovirus disease risk”, where regions are classified as "predisposed" [climate and territory would allow TBE-virus (TBEV) circulation], "imperiled" (TBEV detected, but no case in a human), "affected" (single sporadic autochthonous TBE cases reported), or "endemic" (annual documentation of several human TBE cases). This appears to us to be the currently best scientifically sound approach to document the risk for TBEV-infections, as to date testing for the disease is largely incomplete, even in endemic countries and even for patients with symptoms of encephalitis during the active tick season.

Major Updates and New Chapters

The 7th edition of THE TBE BOOK features significant updates to the main chapters, including:

Historical Perspective: We delve into the roots of TBE, exploring the discovery of the TBEV in the Soviet Union in the early 1900s and the social and political circumstances that precipitated this discovery. Additionally, we provide a general summary of the TBE-associated work of the six main scientists who unraveled the mysteries surrounding TBE in Europe, including the recently “rediscovered” ground-breaking epidemiological work by Dr. Hans Schneider, elucidating TBE infections by types of exposures.

Microbial Species Transmitted by Ticks: We have added a comprehensive chapter on the ever-increasing number of pathogens transmitted by ticks, as they are relevant for differential diagnostic considerations. This chapter will assist physicians in their efforts to make accurate and timely diagnoses based on clinical findings and microbiological confirmation.

TBE in Children: We have included more comprehensive data on TBE in children, with a focus on long-term adverse outcomes. Different from the current general belief, TBE is NOT a mild and neglectable disease in the young but frequently results in long-term cognitive and psychological impairments – even if the initial disease is just a “mild encephalitis”.

Epidemiology and Public Health Aspects

The epidemiology section has been updated to reflect the increase in TBE cases in Northern and Central Europe, as well as the spreading (or just the “recent detection“) of the virus to Africa (Tunisia). We also discuss the concept of “risk areas" – predisposed, imperiled, affected, and endemic – as proposed by the ECDC, and how "incidence data" may be misleading in judging the risk of contracting the disease. Additionally, we explore the potential reasons behind the increase in reported cases, despite increasing vaccine uptake, and whether this is due to increased awareness and testing, increased exposure, or other factors.

Furthermore, we provide detailed information on the public health aspects of TBE, emphasizing that it has been a vaccine-preventable disease for more than 50 years. Recent studies have documented high and long-lasting vaccine effectiveness, leading countries like Switzerland and Finland to recommend a simplified (2+1) vaccination schedule with extended 10-year boosters for the two vaccines licensed in Europe. Information on the Russian and Chinese TBE vaccines has also been updated.

Underdiagnosis and the Way Forward

Despite the availability of vaccines, TBE – even severe cases – remains hugely underdiagnosed in Europe and Asia, and we are far from systematic testing for this disease. This 7th edition of THE TBE BOOK aims at raising awareness and providing valuable insights to combat these challenges by further research.

We hope that this comprehensive guide will prove valuable to healthcare professionals, public health officers, travel medicine colleagues, researchers, travelers and anyone interested in understanding, diagnosing and preventing TBE. Join us on this journey as we continue to unravel the mysteries surrounding this important disease.

We thank all authors for their valuable time, work and dedication; we thank all members of the publishing team with Global Health Press in Singapore for their hard and focused work; the language Editor for finding and correcting all the big and small errors in each manuscript and finally we thank the publisher for her commitment to this work.

Munich, Nierstein, Marburg (Germany), Vienna (Austria), Singapore

June, 2024
The Editors

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List of contributing authors

(in alphabetical order)

Prof. Dr. Judith H. Aberle,
Center for Virology, Medical University of Vienna, 1090 Vienna, Austria

Prof. Dr. Stephan W. Aberle,
Center for Virology, Medical University of Vienna, 1090 Vienna, Austria

Dr. Rahel Ackermann-Gäumann,
Microbiologie, ADMED Analyses et Diagnostics Médicaux, La Chaux-de-Fonds, Switzerland.
Swiss National Reference Center for Tick-transmitted Diseases, La Chaux-de-Fonds, Switzerland.

Dr. Heidi Åhman,
Senior Manager Medical Lead Finland & Baltics, Pfizer Vaccines, Helsinki, Finland

Dr. Kristian Alfsnes,
Norwegian Institute of Public Health

Prof. Dr. Åshild Andreassen,
Norwegian Institute of Public Health, Division for Infection Control and Environmental Health, Oslo, Norway

Dr. Uyanga Baasandagva,
Epidemiologist, National Center for Zoonotic Diseases, Ulaanbaatar, Mongolia

Prof. Dr. Burmaajav Badrakh,
Scientific Secretary of the Mongolian Academy of Medical Sciences and Head of Public Health Department of "Ach" Medical University, Ulaanbaatar, Mongolia

Prof. Dr. Greta Bălan,
MD, PhD, Associate Professor, Nicolae Testemiţanu State University of Medicine and Pharmacy of the Republic of Moldova, Microbiology and Immunology Discipline

Dr. Pavle Banović,
Assist. Dr. sci. med. Pavle Banović, Department of Microbiology with Parasitology and Immunology, Faculty of Medicine in Novi Sad, University of Novi Sad, 21000 Novi Sad, Serbia
Diagnostics and Laboratory Research Task Force, Balkan Association for Vector-Borne Diseases, 21000 Novi Sad, Serbia and Pasteur Institute Novi Sad, 21000 Novi Sad, Serbia

Prof. Dr. Ljubo Barbić,
Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine University of Zagreb, 10000 Zagreb, Croatia

Dr. Marshall E. Bloom,
National Institutes of Health, Laboratory of Virology, Rocky Mountain Laboratories, Hamilton, MT, USA

Dr. Maja Bogdanić,
National Reference Laboratory for Arboviruses, Reference Center for Diagnosis and Surveillance of Viral Zoonoses of the Croatian Ministry of Health, Department of Virology, Croatian Institute of Public Health, 10000 Zagreb, Croatia

Dr. Petra Bogovic,
Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

Dr. Michael Bröker,
Editor, Global Health Press, Singapore / Marburg, Germany

Dr. Olga Burduniuc,
MD, PhD, Head of Department of the Laboratory Diagnosis in Public Health, National Agency for Public Health

Dr. Lidia Chitimia-Dobler,
Bundeswehr Institute of Microbiology, Munich, Germany

Dr. Khrystyna Hrynkevych,
University Hospital Essen

Dr. Anu Jääskeläinen,
Department of Virology, Medicum, University of Helsinki, Helsinki, Finland
Helsinki University Hospital, Laboratory Services (HUSLAB), Department of Clinical Microbiology, Helsinki, Finland

Asst. Prof. Bernard Kaić,
Reference Center for Epidemiology of the Croatian Ministry of Health, Department of Communicable Disease Epidemiology, Croatian Institute of Public Health, 10000 Zagreb, Croatia

Dr. Jana Kerlik,
Department of Epidemiology, Regional Public Health Authority in Banská Bystrica, Slovakia

Dr. Burmaa Khoroljav,
Department of Epidemiology, National Center for Zoonotic Disease, Ulaanbaatar, Mongolia

Prof. Iryna Kolesnikova,
Professor of Epidemiology, Bogomolets National Medical University

Prof. Dr. Herwig Kollaritsch,
Institute for Specific Prophylaxis and Tropical Medicine, Medical University Vienna, Austria

Dr. Natalia Kolomiets,
Institute of Advanced Training and Retraining of Healthcare Personnel of the Belarusian State Medical University, Minsk, Belarus

Dr. Kuulo Kutsar,
Health Board, Tallinn, Estonia

Dr. András Lakos,
Centre for Tick-borne Diseases, Outpatient Service, Budapest, Hungary

Dr. Phung Lang,
Epidemiology, Biostatistics and Prevention Institute (formally Institute of Social and Preventive Medicine), University Zurich

Dr. Mirko Lange,
Institut für Mikrobiologie der Bundeswehr

Dr. Tinne Lernout,
Epidemiology of Infectious Diseases, Epidemiology and Public Health Directorate, Sciensano, Brussels, Belgium

Priv.-Doz.Dr Michael Leschnik,
Clinical Unit of Internal Medicine Small Animals, Department and Clinic of Companion Animals and Horses, University of Veterinary Medicine Vienna, Austria

Prof. Dr. Åke Lundkvist,
Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden

Urusha Maharjan
Medical Laboratory Technologist Norwegian Institute of Public Health

Dr. Anna Mazanik,
Max Weber Network Eastern Europe, Max Weber Foundation, Bonn, Germany

MScPH Eszter Mezei,
Department of Epidemiological and Vaccination Surveillance, National Center for Public Health and Pharmacy, Budapest, Hungary

Prof. Dr. Auksė Mickienė,
Department of Infectious Diseases, Medical Academy of the Lithuanian University of Health Sciences, Kaunas, Lithuania

Dr. Anna Nagy,
National Reference Laboratory for Viral Zoonoses, National Center for Public Health and Pharmacy, Budapest, Hungary

Dr. Uranshagai Narankhuu,
Department of Epidemiology, National Center for Zoonotic Disease, Ulaanbaatar, Mongolia

Dr. Tsogbadrakh Nyamdorj,
Director, National Center for Zoonotic Diseases, Ulaanbaatar, Mongolia

Assoc. Prof. Dr. Anna K. Överby,
Department of Clinical Microbiology, Virology, Umeå University, Sweden
The Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå, Sweden

Prof. Dr. Anna Teresa Palamara,
Istituto Superiore di Sanità, 00161 Rome, Italy

Dr. Katarzyna Pancer,
Department of Virology, National Institute of Public Health-NIH (NIPH-NIH), Warsaw, Poland

Prof. Dr. Petr Pazdiora,
Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

Dr. Eva Maria Pöllabauer,
Institute of Specific Prophylaxis and Tropical Medicine, Medical University Vienna, Austria

Maja Praprotnik,
Nacional Institute of Public Health, Slovenija

Prof. Dr. Martin Pfeffer,
Institute of Animal Hygiene and Veterinary Public Health, Center for Veterinary Public Health, Faculty of Veterinary Medicine, University of Leipzig, Germany

Simon Raffl,
Center for Virology, Medical University of Vienna, 1090 Vienna, Austria

Bsc. Johannes Hermanus Jozef Reimerink,
National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

Dr. Chantal B.E.M. Reusken,
National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

Dr. Flavia Riccardo,
Department of Infectious Diseases, Istituto Superiore di Sanità, Roma, Italy

Prof. Dr. Daniel Růžek,
Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
Faculty of Science, Masaryk University, Brno, Czech Republic
Veterinary Research Institute, Brno, Czech Republic

Rikard Rykkvin,
Norwegian Institute of Public Health Senior Medical Officer

Prof. Dr. Vladimir Savić,
Poultry Center, Croatian Veterinary Institute, 10000 Zagreb, Croatia

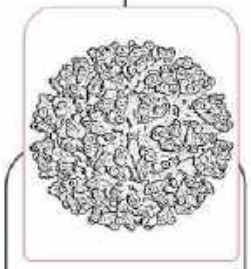
Prof. Dr. Heinz-Josef Schmitt,
Editor-in-Chief, Global Health Press, Singapore
Strategic and Scientific Advisor, “Vaccelerate” European Clinical Trial Consortium, Cologne University, Germany

Dr. Hannah M. Schmuck,
Institute of Animal Hygiene and Veterinary Public Health, Center for Veterinary Public Health, Faculty of Veterinary Medicine,

TBE milestones

20th Century

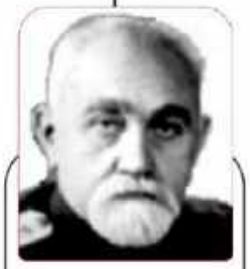
100 million years old: tick[1]



10,000 years old: TBE virus[2]

1931
Hans Schneider
First description of the clinical aspects of TBE[3]

1937
Lev Alexandrovich Zil'ber
First scientist in the Western World to isolate the TBEV[4]



1938
Evgeny Pavlovsky
First scientist to document the way of transmission of the TBEV[5]

1976
Christian Kunze
Pioneer of the first modern cell-culture based TBE vaccine[6]

Note:

1. Tick in Burmese amber, about 100 million years old. Credit: Dr. Lidia Chitimia-Dobler, Bundeswehr Institute of Microbiology, Munich, Germany
2. TBEV under an electron microscope. Credit: PD Dr. Sandra Essbauer, Bundeswehr Institute of Microbiology, Munich, Germany
3. Cover of the book The epidemic acute *"meningitis serosa"*, written by Hans Schneider and published by Maudric Verlag, Vienna, 1932
4. Lev Zilber. Photo from the book Lev *Alexandrovich Zliber* written by his sons L.L. Kisselev and E.S. Levina, published in 2005 by the publishing house *Science* in the series "Scientific biographies"
5. Evgeny Pavlovsky. Credit: Laboratory of Parasitology, Zoological Institute RAS
6. Christian Kunz. Credit: Pric.-Doz. Dr. Gerhard Dobler, Bundeswehr Institute of Microbiology, Munich, Germany

List of abbreviations

ADE	Antibody mediated disease enhancement
AE	Adverse Event
CMV	Cytomegalovirus
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computerized Tomography
DENV	Dengue virus
ECDC	European Center for Disease Prevention and Control
EEG	Electro-Encephalography
EMA	European Medicines Agency
FDA	(usually: The American) Food and Drug Administration
GMT	Geometric Mean Titer
HI	Hemagglutinin Inhibition
IFA	Immuno Fluorescence Assay
JEV	Japanese Encephalitis Virus
KFD	Kyasanur Forest Disease
NIP	National Immunization Program
NT	Neutralization Test
OHFV	Omsk Haemorrhagic Fever Virus
POWV	Powassan Virus
TBEV	Tick-Borne Encephalitis Virus
TBEV-EU	Tick-Borne Encephalitis Virus, European subtype
TBEV-FE	Tick-Borne Encephalitis Virus, Far-Eastern subtype
TBEV-SIB	Tick-Borne Encephalitis Virus, Siberian subtype
TBEV-HIM	Tick-Borne Encephalitis Virus, Himalaya subtype
TBEV-BKL	Tick-Borne Encephalitis Virus, Baikalian subtype
WHO	World Health Organization
WNV	West Nile Virus
YFV	Yellow Fever Virus

Chapter 1

Tick-borne human diseases around the globe

Tatjana Vilibić-Čavlek, Maja Bogdanić, Vladimir Savić, Ljubo Barbić, Vladimir Stevanović and Bernard Kaić

Key points

- The number of tick-borne diseases is increasing due to the geographical expansion of their tick vectors, higher frequencies of infected ticks, increased awareness of infection, and improved diagnostics.
- Ticks are vectors of numerous viruses (arboviruses), bacteria, and parasites.
- Tick-borne encephalitis (TBE) and Lyme disease (LD) are the most common and most widely distributed tick-borne infections in Europe. TBE is also endemic in northern and eastern Asia, while highly endemic areas for LD include the northeastern and north-central United States.
- The epidemiology of tick-borne infections differs according to the geographic region and season of the year.
- Clinical manifestations of tick-borne diseases vary from asymptomatic infection or mild febrile disease to hemorrhagic fever and neuroinvasive diseases.
- Diagnosis of tick-borne infections includes direct (cultivation, PCR/RT-PCR) and indirect methods (serology).

Introduction

Tick-borne diseases (TBDs) are emerging due to the geographical expansion of their tick vectors and represent an important public health problem worldwide.[1] Ticks are vectors of a wide variety of viruses, bacteria, and parasites. Tick-borne viruses include a large group of arboviruses (mainly flaviviruses and bunyaviruses) with diverse genetic and pathogenic properties. Some arboviruses cause severe disease with a high case fatality rate in humans, while others may pose risks to public health, but their role in human diseases is still unclear or neglected.[2] Clinical symptoms of tick-borne viral infections in humans range from mild fever to neuroinvasive diseases or hemorrhagic fevers.[3] The medically most important tick-borne bacteria are *Borrelia burgdorferi* s.l. complex (Lyme disease; LD) and other *Borrelia* spp. (relapsing fever), spotted-fever *Rickettsia* spp., *Anaplasma phagocytophilum* (human granulocytic anaplasmosis; HGA), and *Ehrlichia chaffeensis* (human monocytic ehrlichiosis; HME). Babesiosis is the most common human tick-borne parasitic disease of increasing public health importance.[1]

Tick-borne flaviviruses are responsible for about 10,000 hospital admissions in Europe, Russia, China, and Japan each year. Between 10,000 and 15,000 cases of Crimean-Congo hemorrhagic fever (CCHF) are estimated to occur each year, mostly in bunyavirus endemic countries.[1,4] LD is the most common tick-borne bacterial infection, with approximately 85,000 annual cases in Europe and 300,000 cases in the USA.[1] According to epidemiological data, the number of HGA cases in the USA has increased significantly over time.[5] Over three decades, there has been a noticeable increase in the identification of rickettsioses, mainly due to the advances in molecular diagnostics that have facilitated the identification of both previously recognized and novel rickettsia species.[6] The number of *Babesia microti* infections has been on the rise in recent decades. More than 2,000 cases of babesiosis are documented in the USA each year, however, the actual number is probably much higher.[7] In addition, in the USA, babesiosis has been one of the main causes of transfusion-transmitted infections.[8]

This chapter focuses on the epidemiology and clinical characteristics of the most common medically important tick-borne viral, bacterial, and parasitic diseases.

Tick-borne viruses

Among tick-borne arboviruses, tick-borne encephalitis virus (TBEV) is the most important human pathogen. Other medically important viruses include hemorrhagic fever viruses: Crimean-Congo hemorrhagic fever virus (CCHFV), Omsk hemorrhagic fever virus (OHFV), Kyasanur forest disease virus (KFDV) and Alkhumra hemorrhagic fever virus (AHFV) as well as other neurotropic arboviruses such as Powassan virus (POWV) and Louping ill virus (LIV). There

Table 1: The most common tick-borne viruses of medical importance

Virus	Main vector(s)	Reservoir(s)	Clinical presentation in humans	Geographic distribution
TBEV	*I. ricinus*, *I. persulcatus*	Rodents	Meningitis, encephalitis, myelitis	Europe, Asia
CCHFV*	*Hyalomma* spp.	Rodents, livestock	Hemorrhagic fever	Asia, Arabian peninsula, Middle East, Africa, Europe
CTFV	*D. andersoni*	Rodents	Febrile disease	USA
POWV	*Ixodes* spp., *D. andersoni*	Skunks, rodents, raccoons, foxes	Febrile disease, meningitis	Canada, USA
KFDV	*H. spinigera*	Monkeys, rodents, birds	Hemorrhagic fever	Karnataka (India)
OHFV	*D. reticulatus*, *D. marginatus*	Rodents	Hemorrhagic fever	Russia (Omsk, Novosibirsk, Kurgan, Tjumen)
LIV	*I. ricinus*	Sheep	Meningitis	United Kingdom, Ireland
AHFV	*H. dromedarii*, *O. savignyi*	Livestock	Hemorrhagic fever	Saudi Arabia, Egypt
BHAV	*Haemaphysalis* spp.	Hedgehogs, squirrels, hares	Febrile disease, meningitis	Africa, Asia, Southern Europe
KEMV	*I. persulcatus*	Rodents	Febrile disease, meningitis, encephalitis	Asia (Siberia)
LIPV	*I. ricinus*	Rodents	Meningitis	Europe
TRBV	*I. ricinus*	Rodents	Meningitis	Europe

TBEV=tick-borne encephalitis virus, **CCHFV**=Crimean-Congo hemorrhagic fever virus, **CTFV**=Colorado tick fever virus, **POWV**=Powassan virus, **KFDV**=Kyasanur forest disease virus, **OHFV**=Omsk hemorrhagic fever virus, **LIV**=Louping ill virus; **AHFV**=Alkhumra hemorrhagic fever virus, **BHAV**=Bhanja bandavirus, **KEMV**=Kemerovo virus, **LIPV**=Lipovnik virus; **TRBV**=Tribec virus, *Interhuman transmission possible

are many other still neglected viruses such as Bhanja bandavirus (BHAV) and Kemerovo-related viruses. Severe fever with thrombocytopenia syndrome virus (SFTSV), Bourbon virus (BRBV), and Heartland virus (HRTV) are newly emerged tick-borne viruses (Table 1).[1]

Tick-borne encephalitis virus

TBEV (Orthoflavivirus encephalitidis virus, according to the latest ICTV classification) is the most widely distributed neurotropic arbovirus that belongs to the family *Flaviviridae*, genus *Orthoflavivirus*, tick-borne encephalitis serocomplex. Three main subtypes are European (TBEV-Eu), Far-East (TBEV-FE), and Siberian (TBEV-Sib). *Ixodes ricinus* is the main vector of the TBEV-Eu, while *Ixodes persulcatus* is a vector for TBEV-FE and TBEV-Sib.[9,10] TBE is endemic in a large area from Central Europe and Scandinavia to Japan. Over the past two decades, the TBE incidence has increased in endemic areas; however, sporadic cases were also detected outside of known endemic regions. In many "non-endemic" areas of Eurasia, there are no commercial tests available or testing is not performed, therefore the possible cases are not reported. Human infections usually occur after a tick bite but the number of food-borne infections (consumption of unpasteurized goat milk) is increasing. The TBE-Eu is usually a biphasic disease. The first phase corresponds with viremia, while in the second phase symptoms of the central nervous system (CNS) occur (meningitis, encephalitis, myelitis). It is generally considered that TBEV-FE causes the most severe form of TBE and usually has a monophasic course. The case-fatality rate is 0.5-2% for the TBEV-Eu and 20% for the TBEV-FE.[11] The TBE diagnosis is based on the detection of the intrathecal production of specific IgM antibodies or TBEV RNA.[12]

Crimean-Congo hemorrhagic fever virus

CCHFV is a bunyavirus of the family *Nairoviridae*, genus *Orthonairovirus*. CCHFV strains are classified into seven genotypes (I- VII). Ixodid ticks from the genus *Hyalomma* are the main vectors of CCHFV. Different wild and domestic animals, such as cattle, goats, sheep, and hares represent

the virus reservoirs in nature.[13] Humans become infected by a tick bite or exposure to body fluids from viremic animals or humans.[2] People who have close contact with livestock (shepherds, farmers, butchers, slaughterhouse workers, and veterinarians) and those involved in outdoor activities (soldiers, farmers, forest workers, and hikers) are at high risk of exposure as well as healthcare personnel and close family members involved in patient care. CCHFV is widely distributed throughout Africa, the Middle East, Southeast Asia, and southern and eastern Europe. In humans, CCHF infections range from asymptomatic and mild infections (the majority of CCHFV cases) to severe and occasionally fatal hemorrhagic fever. In some regions, case fatality rates can be higher than 30%.[14] RT-PCR and serology (IgM antibodies or a fourfold increase of IgG antibodies) are used for the diagnosis of CCHFV.[4]

Colorado tick fever virus

Colorado tick fever virus (CTFV) is a neglected virus that belongs to the family *Spinareoviridae*, genus *Coltivirus*. Transmission to humans occurs through a bite of the adult Rocky Mountain wood tick, *Dermacentor andersoni*. Both adults and nymphs are permanently infected, providing an overwintering mechanism for the virus.[15] Because *D. andersoni* shows a broad host feeding preference, different vertebrate hosts have been identified as competent reservoirs for CTFV. The golden-mantled ground squirrel (*Callospermophilus lateralis*) is considered the most prominent natural reservoir of CTFV, while the other reservoirs include chipmunks, mice, rats, and hares. The CTFV is distributed in the western United States and southwestern Canada which correlates with the distribution of its tick vector. Human CTFV infections usually occur in the mid-summer when people are working or recreating in tick habitats. Infection in humans generally presents as a self-limiting febrile disease. Early diagnosis is primarily achieved using an RT-PCR or a 4-fold rise in IgG serology.[16]

Powassan virus

POWV is a tick-borne arbovirus of the family *Flaviviridae*, genus *Orthoflavivirus*. Two distinct genotypes are POWV lineage 1 and 2 (POWV-1 and POWV-1). Most human cases of POWV have been reported in the Great Lakes and Northeast regions of the USA and eastern Canada. In North America, the virus has been detected in four *Ixodes* species and *Dermacentor andersoni* ticks. The two enzootic cycles of POWV-1 include *Ixodes cookei* and groundhogs or mustelids, and *Ixodes marxi* and squirrels. POWV-2 is maintained in one enzootic cycle, primarily between *Ixodes scapularis* and the white-footed mouse.[17] Unlike some other tick-borne pathogens, such as borrelia and babesia, which require tick attachment for 48 and 24 hours for transmission, POWV transmission can occur 15 to 50 minutes after ticks attach. In humans, POWV causes sporadic but severe encephalitis; however, the disease severity can vary significantly. Case fatality rates are ~20% in adults and ~7% in children. Long-term neurological complications are frequently observed in adults.[18] The cerebrospinal fluid (CSF) serology is still the gold standard for confirmation of POWV neuroinvasive disease.[19]

Kyasanur forest disease virus

KFDV is a tick-borne arbovirus that belongs to the family *Flaviviridae*, genus Orthoflavivirus. After the first identification of KFDV in 1957 in monkeys from the Kyasanur Forest of Karnataka, India, 400-500 human cases have been reported annually. *Haemaphysalis spinigera* is the main vector of KFDV. Although the virus has been isolated from rodents, ground-dwelling birds, porcupines, cattle, and bats, only primates appear to develop the disease. Humans become infected by the bite of infected ticks or by handling of infecting mammals and birds.[20] In humans, KFDV causes hemorrhagic fever with a case fatality rate of 3-5%. Some patients (10-20%) develop a secondary phase of fever relapse with meningoencephalitis. Diagnosis is usually confirmed by RT-PCR in a blood sample. Humans usually show high-level viremia (about 10^6 pfu/mL) around day 3 after the onset of symptoms that persist for up to two weeks. The ELISA can be used for the detection of IgM and IgG antibodies.[21] A formalin-inactivated whole KFDV vaccine produced in chick embryo fibroblasts is available.[22]

Omsk hemorrhagic fever virus

OHFV is an arbovirus closely related to TBEV (family *Flaviviridae*, genus *Orthoflavivirus*). Humans become infected through tick bites or contact with the blood, feces, or urine of infected rodents, mainly muskrats (*Ondatra zibethicus*).[23] The disease is prevalent in four regions of western Siberia in Russia (Kurgan, Tyumen, Omsk, and Novosibirsk). The Ixodidae ticks *Dermacentor reticulatus* and *Dermacentor marginatus* are the main hosts for OHFV in the forests and steppes of Siberia. Very recently, the OHFV RNA has been detected in the CSF of two patients from Almaty, Kazakhstan. In addition, the virus was detected in ticks in the Akmola region in Kazakhstan. The disease occurs mainly in muskrat trappers (60%). Hunters are at risk of infection when skinning infected animals. Omsk hemorrhagic fever (OHF) is a self-limiting acute disease in most cases, although a small proportion progresses to hemorrhagic disease. The fatality of OHF is low (0.5-3%). Diagnosis of OHF is based on RT-PCR, OHFV-NS1 antigen detection, and serology.[24] Data suggest that the TBE vaccination provides a high degree of protection against OHF.[25]

Louping ill virus

Louping ill virus (LIV) is a tick-borne arbovirus closely related to TBEV, and belongs to the *Flaviviridae* family, genus *Orthoflavivirus*. Although LIV has previously been found exclusively on the British Islands, it has recently been discovered in Norway and on the Danish island of Bornholm

in the Baltic Sea. *Ixodes ricinus* is the only known tick vector for LIV while sheep, mountain hares, and red grouse are the most important hosts.[26] Human infections caused by LIV are rare and occur after a tick bite or occupational exposure to infected sheep tissues. Risk groups include professionally exposed individuals who have contact with sheep or other potentially infected animals, such as abattoir workers, butchers, and veterinarians. LIV infections in humans are mostly asymptomatic or present as a flu-like disease, while mild meningoencephalitis is rare.[27]

Alkhumra hemorrhagic fever virus

AHFV is a tick-borne virus of the family *Flaviviridae*, genus *Orthoflavivirus*. The virus was first isolated in 1995 from a 32-year-old male butcher from Alkhumra district (Jeddah, Saudi Arabia), who died of hemorrhagic fever. Since then, AHFV cases have been reported among residents of Saudi Arabia and tourists in Egypt and Djibouti. The AHFV epidemiology is not fully understood. Epidemiological studies have shown that AHFV cases were linked to direct or indirect contact with infected blood/organs of slaughtered livestock and ingestion of infected raw milk. The transmission through a tick bite has also been reported in the literature. The hard tick *Hyalomma dromedarii* and the soft tick *Ornithodoros savignyi* are potential vectors of AHFV.[28] Clinical symptoms in humans range from subclinical or mild to severe and rapidly fatal infection.[29] Acute febrile flu-like illness, hepatitis, and hemorrhagic manifestations are the main clinical features of AHFV infection. Mortality in hospitalized patients may reach 30%. RT-PCR or serology can confirm the diagnosis.[28]

Kemerovo related viruses

The Kemerovo serogroup (family *Reoviridae*, genus *Orbivirus*) contains more than 50 tick-borne viruses of which only Kemerovo virus (KEMV), Lipovnik virus (LIPV), and Tribeč virus (TRBV) have been associated with human diseases. An illness caused by the KEMV virus was first described in the taiga landscape in the Kemerovo region in Western Siberia in 1962, where the virus was isolated from ticks and the CSF of patients with meningitis and meningoencephalitis after a tick bite. In a natural cycle, rodents are reservoirs and *I. persulcatus* tick is a vector of KEMV. In humans, KEMV causes febrile disease and occasionally meningitis.[30,31] LIPV was isolated from *I. ricinus* ticks collected in 1963 in Lipovnik village, Slovakia. Meningoencephalitis and polyradiculitis have been linked to LIPV in the Czech Republic. TRBV was isolated in 1963 from *I. ricinus* ticks and the blood of small rodents in the Tribeč mountains, Slovakia.[32] A TRBV was detected from Siberia to central Europe by virus isolation from ticks and antibodies detected in animals. In humans, TRBV-specific antibodies were detected in patients with febrile disease and meningitis.[30,33,34]

Bhanja bandavirus

BHAV is a neglected tick-borne bunyavirus of the family *Phenuiviridae*, genus *Bandavirus*. The virus was isolated in 1954 from the *Haemaphysalis intermedia* tick collected from goats in Bhanjanagar, India, while the first human case of BHAV infection was reported in 1974. BHAV is widely distributed in central Europe, the Mediterranean basin, the Middle East to India, and in Sub-Saharan Africa, however, human clinical infections are rare. The natural reservoirs of BHAV are sheep, goats, hares, hedgehogs, and squirrels, while *Haemaphysalis* ticks are the main vectors in Europe.[11] Only a few human cases of neuroinvasive diseases caused by BHAV have been reported.[35,36] RT-PCR and serology are used for the diagnosis of BHAV infection.[11]

Dabie bandavirus (Severe fever with thrombocytopenia syndrome virus)

SFTSV is one of the emerging pathogenic tick-borne viruses reported in patients with severe fever, thrombocytopenia, and leukocytopenia and an initial fatality rate of up to 30%.[37] SFTSV was first discovered in China (2009) and later in South Korea and Japan. Some patients reported a history of tick bites, and the virus was detected primarily in *Haemaphysalis longicornis* ticks originating from regions where the patients lived.[38] Several studies indicated that infected patients can spread the virus to family members or healthcare workers, primarily through contact with contaminated blood or body fluids.[39] Hemorrhagic fever with thrombocytopenia, leukocytopenia, and increased liver enzymes are the main clinical and laboratory findings in patients with severe SFTSV infection. Fatalities mainly occur in patients over 50, with mortality rates ranging from 10 to 19%. RT-PCR is the gold standard diagnostic method for the detection of SFTSV.[40]

Bourbon virus

Bourbon virus (BRBV) is a recently discovered tick-borne virus of the genus *Togotovirus*, family *Orthomyxoviridae* that was first identified in a fatal human case in Bourbon County, Kansas, USA in 2014. The virus has been associated with several cases of severe acute febrile illness in patients in the Midwest US, but since 2020, the BRBV has been reported in North Carolina, Virginia, New Jersey, and New York State. *Amblyomma americanum* is considered to be the primary vector of BRBV, while the mammalian reservoir has not been identified yet. However, serological testing has identified white-tailed deer and raccoons as potential sentinels to track the spread of BRBV. Clinical symptoms of BRBV infection include fever, weakness, fatigue, myalgia, arthralgia, and nausea that occur 2-7 days after a tick bite. Shock, organ failure, cardiac dysregulation, pleural effusions, and acute bone marrow suppression were linked to fatal cases. RT-PCR is used to diagnose the BRBV.[41-43]

Table 2: Epidemiological and clinical characteristics of the most common tick-borne bacteria

Bacteria	Main vector(s)	Clinical presentation in humans	Geographic distribution
B. burgdorferi s.l.	*I. ricinus*	Erythema migrans, meningitis	North America, Europe, Asia
B. miyamotoi	*I. ricinus*	Febrile disease	North America, Europe, Asia
B. duttoni, B. hispanica, B. persica	*Ornithodoros* spp.	Relapsing fever	North America, Europe, Asia
A. phagocytophilum	*I. ricinus*	Human granulocytic anaplasmosis	USA, Europe, Southeast Asia
E. chaffeensis	*A. americanum, I. ricinus*	Human monocytic ehrlichiosis	USA, Europe
R. conorii (subsp. *conorii, indica, israelensis, caspia*)	*R. sanguineus*	MSF, Indian tick typhus, Israeli spotted fever, Astrakhan fever	Europe, Africa, India, Asia, Middle East
R. rickettsii	*A. americanum*	Rocky Mountain spotted fever	North America
R. africae	*Amblyoma* spp.	African tick bite fever	Africa
R. aeschlimannii	*Amblyomma, Dermacentor*	Similar to MSF	Europe, Africa, Asia
R. heilongjiangensis	*Dermacentor, Haemaphysalis*	Far-eastern spotted fever	China, Japan
R. australis	*Ixodes* spp.	Queensland tick typhus	Australia, Torres Strait Islands
R. helvetica	*D. reticulatus*	Fever, headache, rash	Europe, Asia
R. honei	*Bothriocroton hydrosauri*	Flinders Island spotted fever	Flinders Island, Australia
R. japonica	*D. taiwanensis*	Japanese or Oriental spotted fever	Japan, South Korea, Thailand
R. massiliae	*A. sylvaticum*	Similar to MSF	Sicily, France
R. monacensis	*A. dissimile*	Fever, rash	Europe
R. philipii	*D. occidentalis*	Pacific Coast tick fever	California, Pacific Coast
R. sibirica (subsp. *sibirica, mongolitimonae*)	*D. nuttalli, D. marginatus*	Siberian tick typhus, lymphangitis-associated rickettsiosis	Russia, Mongolia
R. slovaca	*D. marginatus*	TIBOLA, DEBONEL	Europe, Asia
R. raoultii	*A. testudinarium, Dermacentor* spp.	TIBOLA, DEBONEL	Europe, Asia
R. tamurae	*A. testudinarium*	Local skin inflammation	Japan

TIBOLA= tick-borne lymphadenitis, **DEBONEL**= dermacentor-borne necrosis erythema lymphadenopathy

Heartland virus

Heartland virus (HRTV) is an emerging bunyavirus first discovered in the USA in 2009. Originally classified in the genus *Phlebovirus*, family *Phenuiviridae*, the virus is now reclassified in the *Bandavirus* genus alongside BHAV and SFTSV. HRTV infections are reported mainly east of the Mississippi River, mostly in the summer months. The Lone Star tick, *Amblyomma americanum* is considered the primary vector of HRTV zoonotic transmission. It is also possible that *Amblyomma* or *Haemaphysalis* tick species are the sole reservoirs of HRTV. Numerous possible amplification hosts, including raccoons, white-tailed deer, coyotes, domestic dogs, and opossums, have been identified based on serosurveillance studies. However, clinical infections have been reported only in humans.[44] Clinical symptoms of HRTV infection include fever, headache, fatigue, myalgia, nausea, and diarrhea with

leucopenia and thrombocytopenia. RT-PCR is most commonly used for the diagnosis of HRTV. The plaque reduction neutralization test (PRNT) is used for screening both human and animal serum samples in serosurveillance studies.[45]

Tick-borne bacteria

Borrelia burgdorferi s.l., a causative agent of LB, is the most frequently detected tick-borne bacteria with a worldwide distribution.[46] Cases of HGA have been identified in the upper Midwest and the Northeast USA, Northern Europe, and Southeast Asia.[47] The majority of HME cases in the USA are caused by *E. chaffeensis*.[48] Spotted-fever group (SFG) rickettsia are a neglected group of bacteria of the genus *Rickettsia*, family *Rickettsiaceae* that includes numerous emerging infectious diseases with a worldwide distribution.[49] The main tick-borne bacteria are presented in Table 2.

Borrelia spp.

The three main species of *Borrelia burgdorferi* sensu lato (s.l.) complex associated with human LD are *B. burgdorferi* sensu stricto (s.s.), *Borrelia afzelii* and *Borrelia garinii*. *Ixodes ricinus* is the main tick vector in Europe. *Ixodes persulcatus* and *Ixodes hexagonus* are also proven vectors of *B. burgdorferi* s.l. Rodents are the principal reservoir hosts of borrelia. Clinical manifestations of LD may be localized (*erythema migrans*) or disseminated (arthritis, carditis, neuroborreliosis).[50] Serology tests (ELISA, IFA, immunoblot) for the detection of borrelia antibodies in the blood or CSF are most commonly used for the diagnosis of LD. Therapy of LD depends on the patient's age and the stage of the disease. Doxycycline is recommended for patients older than 8 years with localized disease. Patients under the age of 8 should receive amoxicillin or cefuroxime. Parenteral therapy may be required for more severe manifestations such as arthritis, carditis, meningitis, or encephalitis.[51]

Relapsing fever (RF) is another tick-borne borreliosis distributed in the Northern Hemisphere, Africa, and Central America. *Borrelia duttoni*, *B. hispanica*, and *B. persica* are the main tick-borne borreliae transmitted by soft-bodied or argasid ticks. Small rodents and other mammals, including bats serve as a reservoir for tick-borne *Borrelia* species.[52] Clinical symptoms of RF typically include a high fever for a few days followed by a period of well-being and another relapse. Without antibiotic therapy, relapses can occur several times.[53] The diagnosis of RF can be confirmed by direct microscopic detection of borrelia in Giemsa-stained blood films, serologic analysis, or PCR. RF is treated with doxycycline. Penicillin or erythromycin are preferred in pregnant women and children under 8 years of age.[52]

Borrelia miyamotoi is a new tick-borne *Borrelia* species discovered in Japan in 1995. The pathogenicity was suggested in 2011 in Russia when 51 patients with suspected tick bites developed a nonspecific febrile illness and *B. miyamotoi* was confirmed by PCR or specific antibodies. Immunocompetent individuals present with a mild flu-like disease, but the disease may be more severe in immunocompromised patients. PCR that detects *B. miyamotoi* DNA in blood or CSF and serologic assays are used for disease confirmation.[54] *Borrelia miyamotoi* infections are treated with doxycycline. Amoxicillin and ceftriaxone have also been successfully used for the treatment of *B. miyamotoi*.[55]

Anaplasma phagocytophilum

A. phagocytophilum, an obligate intracellular bacteria is the most important species within the *Anaplasma* genus that causes HGA. The *Ixodes ricinus* tick is the main vector of HGA in Europe, while *I. scapularis* and *I. pacificus* are vectors in the USA.[56] Whereas some patients with HGA remain asymptomatic, others develop a nonspecific febrile disease, and only a small proportion develop severe disease. The most common symptoms of HGA include fever, headache, malaise, myalgia, and arthralgia. The mortality rate is about 0.6%. Whole-blood PCR is the most sensitive method to diagnose HGA. A Giemsa-stained peripheral blood smear may reveal morulae within the polymorphonuclear leukocytes. IFA can be used for the detection of specific IgM and/or IgG antibodies.[5] Doxycycline is the recommended first-line therapy for HGA.[47]

Ehrlichia spp.

The genus *Ehrlichia* includes several tick-borne obligate intracellular bacteria that infect humans and other mammals. The most important species are *Ehrlichia chaffeesis*, which causes HME, and *Ehrlichia ewingii*, which causes *Ehrlichia ewingii* ehrlichiosis. The Lone Star tick (*A. americanum*) is the most common vector in the USA,[48] while *I. ricinus* is a vector in Europe.[57] Ehrlichia infections are reported most often in the elderly. Since children frequently develop milder or subclinical infections, the disease is probably underreported in this population group. Patients with ehrlichiosis typically present with a flu-like febrile disease. CNS involvement including meningitis and meningoencephalitis occurs in up to 20% of patients.[48] The overall case fatality rate is 1%. Diagnosis of ehrlichiosis is usually confirmed using PCR or serology. Tetracyclines are highly efficacious for the therapy of ehrlichiosis.[58]

Rickettsia spp.

Tick-borne rickettsioses are caused by obligate intracellular bacteria belonging to the spotted fever group (SFG) of the *Rickettsia* genus. The most widely distributed SFG rickettsia

include *Rickettsia rickettsii* (Rocky Mountain spotted fever; RMSF), R. conorii (Mediterranean spotted fever; MSF), *R. africae* (African tick bite fever), *R. helvetica*, *R. aeschlimannii*, *R. slovaca* (tick-borne lymphadenitis; TIBOLA Dermacentor-borne necrosis erythema lymphadenopathy; DEBONEL), and *R. raoultii*.[6,59] In addition to pathogenic rickettsia species, there are many potentially pathogenic "candidates" for new species. Most SFG rickettsiae are transmitted by ixodid tick bites during blood feeding. The distribution of SFG rickettsioses varies geographically and correlates with the distribution of tick vectors.[6] Localized rickettsial infections appear as an eschar (also known as a "tache noir") at the site of tick inoculation. However, disseminated infection can cause severe vasculitis and endothelial damage, which can manifest as cutaneous necrosis and digital gangrene, pneumonitis, meningoencephalitis, and multiorgan failure.[60] Serology (IFA) is most commonly used for the diagnosis of rickettsioses. PCR enables species-specific identification.[61] Doxycycline is the therapy of choice for SFG rickettsial diseases.[62]

Tick-borne parasites

Babesia microti, *B. divergens*, *B. duncani* and *B. venatorum* are the main zoonotic babesia species that can cause human diseases. *Babesia microti* is the most reported species in North America, while *B. divergens* is the most common cause of human babesiosis in Europe. The tick vectors of babesia include *I. scapularis* (North America), *I. ricinus* (Europe), and *I. persulcatus* (Asia). Babesiosis is typically asymptomatic and self-limiting in healthy individuals. However, in elderly, splenectomised, and other immunocompromised individuals the disease may be severe with hemolytic anemia, splenomegaly, hepatomegaly, and renal failure, sometimes with fatal outcomes.[63] Peripheral thick and thin blood smear examination has been the standard method for diagnosing human babesiosis. Serological tests (EIA, IFA, IB) have been used to support or confirm the diagnosis of babesiosis in endemic regions. PCR targeting the *Babesia* spp. is 18S rRNA can also be used.[64] The current therapy for human babesiosis includes combinations of atovaquone and azithromycin or clindamycin and quinine.[65]

Concluding remarks

The number of TBDs is increasing, and this trend is expected to continue. Based on information from animal experiments, a large number of potential tick-borne pathogens have already been proposed. It was also noted that the clinical spectrum of TBDs is becoming more diverse, including underrecognized manifestations of previous well-known pathogens. To effectively develop strategies to mitigate the increasing incidence of TBDs, a deeper understanding of the ecological and biological factors driving the expansion of tick vectors and reservoir host distributions, as well as the microbiological dynamics within ticks that modulate pathogen emergence, is required.[66]

Contact: tatjana.vilibic-cavlek@hzjz.hr

Citation:
Vilibić-Čavlek T, Bogdanić M, Savić V, Barbić L, Stevanović V, Kaić B. Tick-borne human diseases around the globe. Chapter 1. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_1-7

References

1. Rochlin I, Toledo A. Emerging tick-borne pathogens of public health importance: a mini-review. *J Med Microbiol.* 2020;69 (6):781-791. doi: 10.1099/jmm.0.001206.
2. Shi J, Hu Z, Deng F, Shen S. Tick-Borne Viruses. *Virol Sin*. 2018;33:21-43. doi: 10.1007/s12250-018-0019-0.
3. Shah T, Li Q, Wang B, Baloch Z, Xia X. Geographical distribution and pathogenesis of ticks and tick-borne viral diseases. *Front Microbiol*. 2023;14:1185829. doi: 10.3389/ fmicb.2023.1185829.
4. Raabe VN. Diagnostic Testing for Crimean-Congo Hemorrhagic Fever. *J Clin Microbiol*. 2020;58(4):e01580-19. doi: 10.1128/ JCM.01580-19.
5. Dumic I, Jevtic D, Veselinovic M, et al. Human Granulocytic Anaplasmosis-A Systematic Review of Published Cases. *Microorganisms*. 2022;10(7):1433. doi: 10.3390/ microorganisms10071433.
6. Piotrowski M, Rymaszewska A. Expansion of Tick-Borne Rickettsioses in the World. *Microorganisms*. 2020;8(12):1906. doi: 10.3390/microorganisms8121906.
7. Kumar A, O'Bryan J, Krause PJ. The Global Emergence of Human Babesiosis. *Pathogens*. 2021;10(11):1447. doi: 10.3390/pathogens10111447.
8. Bloch EM, Krause PJ, Tonnetti L. Preventing Transfusion-Transmitted Babesiosis. *Pathogens*. 2021;10:1176. doi: 10.3390/pathogens10091176.
9. Kwasnik M, Rola J, Rozek W. Tick-Borne Encephalitis-Review of the Current Status. *J Clin Med.* 2023;12(20):6603. doi: 10.3390/jcm12206603.
10. Pustijanac E, Buršić M, Talapko J, Škrlec I, Meštrović T, Lišnjić D. Tick-Borne Encephalitis Virus: A Comprehensive Review of Transmission, Pathogenesis, Epidemiology, Clinical Manifestations, Diagnosis, and Prevention. *Microorganisms*. 2023;11(7):1634. doi: 10.3390/microorganisms11071634.
11. Vilibić-Čavlek T, Savić V, Židovec-Lepej S, Bogdanić M, Stevanović V, Barbić Lj. Emerging and neglected viral zoonoses in Europe. In: Rodriguez-Morales AJ, ed. Current Topics in Zoonoses. IntechOpen: London, United Kingdom, 2023; doi: 10.5772/intechopen.112779

12. Phipps LP, Johnson N. Tick-borne encephalitis virus. *J Med Microbiol*. 2022;71(5). doi: 10.1099/jmm.0.001492.

13. Centers for Disease Control and Prevention (CDC). Crimean-Congo Hemorrhagic Fever. Available from: https://www.cdc.gov/vhf/crimean-congo/transmission/index.html

14. Hawman DW, Feldmann H. Crimean-Congo haemorrhagic fever virus. *Nat Rev Microbiol*. 2023;21(7):463-477. doi: 10.1038/s41579-023-00871-9.

15. Centers for Disease Control and Prevention (CDC). Colorado tick fever. Available from: https://www.cdc.gov/coloradotickfever/index.html

16. Harris EK, Foy BD, Ebel GD. Colorado tick fever virus: a review of historical literature and research emphasis for a modern era. *J Med Entomol*. 2023;60(6):1214-1220. doi: 10.1093/jme/tjad094.

17. Corrin T, Greig J, Harding S, Young I, Mascarenhas M, Waddell LA. Powassan virus, a scoping review of the global evidence. *Zoonoses Public Health.* 2018;65(6):595-624. doi: 10.1111/zph.12485.

18. Kakoullis L, Vaz VR, Kaur D, et al. Powassan Virus Infections: A Systematic Review of Published Cases. *Trop Med Infect Dis*. 2023; 8(12):508. doi: 10.3390/tropicalmed8120508.

19. Kapoor AK, Zash R. Powassan Virus. [Updated 2023 Mar 27] [Accessed 2024 Feb 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK570599/

20. Bhatia B, Feldmann H, Marzi A. Kyasanur Forest Disease and Alkhurma Hemorrhagic Fever Virus-Two Neglected Zoonotic Pathogens. *Microorganisms*. 2020;8(9):1406. doi: 10.3390/microorganisms8091406.

21. Chunduru K, Saravu K. Kyasanur forest disease: A review on the emerging infectious disease. *J Clin Infect Dis Soc*. 2023;1:5-11.

22. Srikanth UGK, Marinaik CB, Gomes AR, et al. Evaluation of Safety and Potency of Kyasanur Forest Disease (KFD) Vaccine Inactivated with Different Concentrations of Formalin and Comparative Evaluation of In Vitro and In Vivo Methods of Virus Titration in KFD Vaccine. *Biomedicines*. 2023;11(7):1871. doi: 10.3390/biomedicines11071871.

23. Kovalev SY, Mazurina EA. Omsk hemorrhagic fever virus is a tick-borne encephalitis virus adapted to muskrat through host-jumping. *J Med Virol*. 2022;94(6):2510-2518. doi: 10.1002/jmv.27581.

24. Wagner E, Shin A, Tukhanova N, et al. First Indications of Omsk Haemorrhagic Fever Virus beyond Russia. *Viruses.* 2022;14(4):754. doi: 10.3390/v14040754.

25. Chidumayo NN, Yoshii K, Kariwa H. Evaluation of the European tick-borne encephalitis vaccine against Omsk hemorrhagic fever virus. *Microbiol Immunol*. 2014;58(2):112-118. doi: 10.1111/1348-0421.12122.

26. Ytrehus B, Rocchi M, Brandsegg H, et al. Louping-ill virus serosurvey of willow ptarmigan (Lagopus lagopus lagopus) in Norway. *J Wildl Dis.* 2021;57(2):282-291. doi: 10.7589/JWD-D-20-00068.

27. Jeffries CL, Mansfield KL, Phipps LP, Wakeley PR, Mearns R, Schock A, Bell S, Breed AC, Fooks AR, Johnson N. Louping ill virus: an endemic tick-borne disease of Great Britain. *J Gen Virol*. 2014;95(Pt 5):1005-1014. doi: 10.1099/vir.0.062356-0.

28. Abdulhaq AA, Hershan AA, Karunamoorthi K, Al-Mekhlafi HM. Human Alkhumra hemorrhagic Fever: Emergence, history and epidemiological and clinical profiles. *Saudi J Biol Sci.* 2022;29(3):1900-1910. doi: 10.1016/j.sjbs.2021.10.031.

29. Madani TA, Abuelzein EME. Alkhumra hemorrhagic fever virus infection. *Arch Virol*. 2021;166(9):2357-2367. doi: 10.1007/s00705-021-05083-1.

30. Peňazziová K, Korytár Ľ, Cingeľová Maruščáková I, et al. Serologic Investigation on Tick-Borne Encephalitis Virus, Kemerovo Virus and Tribeč Virus Infections in Wild Birds. *Microorganisms*. 2022;10(12):2397. doi: 10.3390/microorganisms10122397.

31. Migné CV, Braga de Seixas H, Heckmann A, et al. Evaluation of Vector Competence of Ixodes Ticks for Kemerovo Virus. *Viruses*. 2022;14(5):1102. doi: 10.3390/v14051102.

32. Belhouchet M, Mohd Jaafar F, Tesh R, et al. Complete sequence of Great Island virus and comparison with the T2 and outer-capsid proteins of Kemerovo, Lipovnik and Tribec viruses (genus Orbivirus, family Reoviridae). *J Gen Virol.* 2010;91(Pt 12):2985-2993. doi: 10.1099/vir.0.024760-0.

33. Dilcher M, Hasib L, Lechner M, et al. Genetic characterization of Tribeč virus and Kemerovo virus, two tick-transmitted human-pathogenic Orbiviruses. *Virology*. 2012; 423(1):68-76. doi: 10.1016/j.virol.2011.11.020.

34. Hubálek Z. History of Arbovirus Research in the Czech Republic. *Viruses*. 2021; 13(11):2334. doi: 10.3390/v13112334.

35. Calisher CH, Goodpasture HC. Human infection with Bhanja virus. *Am J Trop Med Hyg*. 1975;24(6 Pt 1):1040-1042. doi: 10.4269/ajtmh.1975.24.1040.

36. Vilibic-Cavlek T, Stevanovic V, Krcmar S, et al. Detection of Bhanja Bandavirus in Patients with Neuroinvasive Disease of Unknown Etiology in Croatia. *Microorganisms*. 2023;11(9):2155. doi: 10.3390/microorganisms11092155.

37. Li J, Li S, Yang L, Cao P, Lu J. Severe fever with thrombocytopenia syndrome virus: a highly lethal bunyavirus. *Crit Rev Microbiol*. 2021;47(1):112-125. doi: 10.1080/1040841X.2020.1847037.

38. Luo LM, Zhao L, Wen HL, et al. Haemaphysalis longicornis Ticks as Reservoir and Vector of Severe Fever with Thrombocytopenia Syndrome Virus in China. *Emerg Infect Dis*. 2015;21(10):1770-1776. doi: 10.3201/eid2110.150126.

39. Bae S, Chang HH, Kim SW, et al. Nosocomial outbreak of severe fever with thrombocytopenia syndrome among healthcare workers in a single hospital in Daegu, Korea. *Int J Infect Dis*. 2022;119:95-101. doi: 10.1016/j.ijid.2022.03.048.

40. Kim EH, Park SJ. Emerging Tick-Borne Dabie Bandavirus: Virology, Epidemiology, and Prevention. *Microorganisms*. 2023;11(9):2309. doi: 10.3390/microorganisms11092309.

41. Lange RE, Dupuis AP 2nd, Ciota AT. Diversification of Bourbon Virus in New York State. *Microorganisms*. 2023; 11(6):1590. doi: 10.3390/microorganisms11061590.

42. Roe MK, Huffman ER, Batista YS, et al. Comprehensive Review of Emergence and Virology of Tick-borne Bourbon Virus in the United States. *Emerg Infect Dis.* 2023;29(1):1-7. doi: 10.3201/eid2901.212295.

43. Bendl E, Fuchs J, Kochs G. Bourbon virus, a newly discovered zoonotic thogotovirus. *J Gen Virol*. 2023;104(8). doi: 10.1099/jgv.0.001887.

44. Mantlo EK, Haley NJ. Heartland Virus: An Evolving Story of an Emerging Zoonotic and Vector-Borne Disease. *Zoonotic Dis*. 2023;3:188-202. doi: 10.3390/zoonoticdis3030016

45. Dembek ZF, Mothershead JL, Cirimotich CM, Wu A. Heartland Virus Disease—An Underreported Emerging Infection. *Microorganisms*. 2024;12(2):286. doi: 10.3390/microorganisms12020286.

46. Marques AR, Strle F, Wormser GP. Comparison of Lyme disease in the United States and Europe. *Emerg Infect Dis.* 2021; 27(8):2017-2023. doi: 10.3201/eid2708.204763.

47. Guzman N, Yarrarapu SNS, Beidas SO. Anaplasma phagocytophilum. [Updated 2023 Aug 8] [Accessed 2024 Mar 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513341/

48. Snowden J, Bartman M, Kong EL, et al. Ehrlichiosis. [Updated 2022 Sep 12] [Accessed 2024 Mar 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441966/

49. Robinson MT, Satjanadumrong J, Hughes T, Stenos J, Blacksell SD. Diagnosis of spotted fever group Rickettsia infections: the Asian perspective. *Epidemiol Infect*. 2019; 147:e286. doi: 10.1017/S0950268819001390.

50. Steinbrink A, Brugger K, Margos G, Kraiczy P, Klimpel S. The evolving story of Borrelia burgdorferi sensu lato transmission in Europe. *Parasitol Res.* 2022; 121(3):781-803. doi: 10.1007/s00436-022-07445-3.

51. Skar GL, Simonsen KA. Lyme Disease. [Updated 2024 Feb 4] [Accessed 2024 Jan 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK431066/

52. Snowden J, Yarrarapu SNS, Oliver TI. Relapsing Fever. [Updated 2023 Jul 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441913/

53. Centers for Disease Control and Prevention (CDC). Tick- and Louse-Borne Relapsing Fever. Available from: https://www.cdc.gov/relapsing-fever/symptoms/index.html

54. Cleveland DW, Anderson CC, Brissette CA. Borrelia miyamotoi: A Comprehensive Review. *Pathogens.* 2023;12(2):267. doi: 10.3390/pathogens12020267.

55. Centers for Disease Control and Prevention (CDC). Borrelia miyamotoi. Available from: https://www.cdc.gov/relapsing-fever/miyamotoi/index.html

56. Matei IA, Estrada-Peña A, Cutler SJ, et al. A review on the eco-epidemiology and clinical management of human granulocytic anaplasmosis and its agent in Europe. *Parasit Vectors*. 2019;12(1):599. doi: 10.1186/s13071-019-3852-6.

57. Stanilov I, Blazhev A, Miteva L. Anaplasma and Ehrlichia Species in Ixodidae Ticks Collected from Two Regions of Bulgaria. *Microorganisms.* 2023;11(3):594. doi: 10.3390/microorganisms11030594.

58. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. *Clin Infect Dis*. 2007;45 Suppl 1:S45-51. doi: 10.1086/518146.

59. Parola P, Paddock CD, Socolovschi C, et al. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev*. 2013;26(4):657-702. doi: 10.1128/CMR.00032-13.

60. Abdad MY, Abou Abdallah R, Fournier P-E, Stenos J, Vasoo S. A concise review of the epidemiology and diagnostics of rickettsioses: Rickettsia and Orientia spp. *J Clin Microbiol*. 2018;56:e01728-17. doi: 10.1128/JCM.01728-17.

61. Stewart AG, Stewart AGA. An Update on the Laboratory Diagnosis of Rickettsia spp. Infection. *Pathogens*. 2021;10(10):1319. doi: 10.3390/pathogens10101319.

62. Binder AM, Armstrong PA. Patient characteristics, treatment patterns, and outcomes of Rickettsial diseases among a commercially insured population in the United States, 2005-2017. *Sci Rep*. 2021;11(1):18382. doi: 10.1038/s41598-021-96463-9.

63. Young KM, Corrin T, Wilhelm B, Uhland C, Greig J, Mascarenhas M, Waddell LA. Zoonotic Babesia: A scoping review of the global evidence. *PLoS One*. 2019; 14(12):e0226781. doi: 10.1371/journal.pone.0226781.

64. Parija SC, Kp D, Venugopal H. Diagnosis and management of human babesiosis. *Trop Parasitol.* 2015;5(2):88-93. doi: 10.4103/2229-5070.162489.

65. Renard I, Ben Mamoun C. Treatment of Human Babesiosis: Then and Now. *Pathogens*. 2021;10(9):1120. doi: 10.3390/pathogens10091120.

66. Paddock CD, Lane RS, Staples JE, et al. Changing paradigms for tick-borne diseases in the Americas. In: Forum on Microbial Threats; Board on Global Health; Health and Medicine Division; National Academies of Sciences, Engineering, and Medicine. Global Health Impacts of Vector-Borne Diseases: Workshop Summary. Washington (DC): National Academies Press (US); 2016 Sep 21. A8. Available from: https://www.ncbi.nlm.nih.gov/books/NBK390439/

Tick-borne-flavivirus serocomplex: Phylography and bio-geography

Daniel Lang; Teemu Smura; Gerhard Dobler; Olli Vapalahti

COMING SOON

Chapter 3a

Early TBE research in the Soviet Union: revisiting the narrative

Anna Mazanik

Key points

- The TBE virus was first isolated in 1937 by the team of Lev Zilber during their expedition to the Soviet Far East (today the Khabarovsk and Primorie regions of Russia). The same expedition also established the connection between the disease and the tick vector.
- After the isolation of the virus, several studies established numerous older cases of TBE in the Soviet Far East, Siberia, and the Urals dating back to the early 1900s. The first retrospectively diagnosed case was identified by Mikhail Chumakov in Tatarstan and dates back to 1895.
- A separate line of Soviet research studied Kozhevnikov epilepsy (epilepsia partialis continua), one of the many possible symptoms of TBE and/or TBE sequelae. In 1922 Vladimir Omorokov examined 27 cases of Kozhevnikov epilepsy from Western Siberia and suggested that the infectious agent was linked to the forest and its insects.
- Although TBE was present in many parts of Russia at the turn of the twentieth century, it became much more visible in the Soviet Far East in the 1930s due to the mass deportations and forced labor in the region, which resulted in higher exposure and severity of disease.
- In 1938-39, Soviet virologists Nadezhda Kagan and Elizaveta Levkovich developed the first vaccine against TBE, which was then tested on the unfree population in the Khabarovsk region.
- Due to the extreme conditions in which that population lived, including severe malnutrition and exploitation, the early Soviet epidemiological data on TBE needs to be used and interpreted with caution.

The history of the discovery of TBE in the Soviet Far East and the isolation of the virus is well known in the scientific literature. It has been a subject of a number of publications, both in Russian and in English[1-6] including also the earlier editions of the TBE Book.

In the 1930s, an outbreak of a severe paralytic disease was recorded in the southern parts of the Soviet Far East. In 1937, the People's Commissariat of Public Health, the Soviet equivalent of a public health ministry, organized a scientific expedition, led by Jewish virologist Lev Zilber (Silber), to investigate the reports of the unknown disease in the region of Khabarovsk. Zilber's expedition established the viral etiology of disease, which soon became known in Russian as "tick-borne encephalitis" (kleshchevoi entsefalit) and in English as "Russian spring-summer encephalitis"; the expedition isolated the causative virus from the patients and the ticks using mouse brain, thus identifying ixodid ticks as its vectors. The subsequent expedition in 1938-1939 described the circulation of the virus, vector species and reservoir hosts. Largely on the basis of that research, parasitologist Evgeny Pavlovsky developed his famous natural nidality theory of transmissible disease, which applied the ecological niche approach to the study of zoonoses and soon became the key to studies of the environmental circulation of arthropod-borne viruses.

That early Soviet research on TBE in the 1930s and 1940s has been crucial for the understanding of TBE, its etiology, clinical picture, and epidemiology until the present day, both in Russia and internationally. However, some of this early research has in fact been misrepresented in the scientific literature and obscured by Soviet censorship. In the current chapter, based on the analysis of previously unstudied historical documents, I would like not only to retell the key steps of that familiar story, but to discuss how those early expeditions fit into the broader Soviet scientific, environmental, and socio-political context and what it means for the interpretation of Soviet TBE research and the history of TBE.

Considering the wide spread of TBE across Eurasia and Russia, it is remarkable that TBE – supposedly - captured scholarly attention only in the 1930s. The first subchapter here analyzes the history of TBE "before the TBE virus", that is before 1937, and puts together scientific records on the localization and understanding of this disease before it received its name and before its etiology became known. The second subchapter asks why, then, this disease became

particularly visible in the 1930s and why specifically in the Soviet Far East. Looking at the social, environmental, and political developments in the region, it shows the "emergence" of this disease was inseparable from the geopolitical agendas and the Stalinist colonization of the Far Eastern peripheries through involuntary resettlement and forced labor. Finally, the last subchapter looks at how this influenced early Soviet studies of TBE and the interpretation of their findings.

TBE "before the TBE virus"

The story of TBE in the Soviet Union typically begins in the early 1930s. Since 1932 physicians in the Soviet Far East observed clusters of cases of a severe infection with a high case-fatality rate. Depending on the symptoms, it was described as poliomyelitis, meningitis, or "toxic influenza". In 1935 Vladivostok-based navy neurologist A.G. Panov recognized this disease as infectious encephalitis and noted its distinct spring-summer seasonality.[7] This opened the way for a suggestion that the disease might in fact be a form of Japanese encephalitis, for which the causative agent had been identified in Japan shortly before that—the misconception that spread beyond scientific circles and, as I will show later in the chapter, played a tragic role in the careers of early TBE researchers. In 1936 the Khabarovsk regional department of public health created a special medical unit of local neurologists and physicians led by Israel Finkel to carry out the studies of this disease, but its exact etiology remained unknown. Finkel also authored the first publication on "Far Eastern encephalitis" in a local medical journal. There were some attempts to isolate viruses from the brain of those succumbed to the disease, but the strain was quickly lost, and the causative link could not be proven.[3,8,9] Although these early studies in themselves contained no major scientific breakthroughs, they helped accumulate important epidemiological and clinical evidence to suggest that the disease was likely viral and vector-borne. This evidence provided a starting point for Zilber's scientific mission in 1937 and contributed to its quick success.

The observed disease clusters of the 1930s were, however, not the earliest cases of probable TBE. Already the first expeditions tried to find earlier cases through checking the hospital records and patients' histories and examining the local population in search of the long-term symptoms of the past disease. N. Dankovskii and A. Drobyshevskaia identified two local cases of TBE from the early 1920s with residual paresis of the extremities that was still visible seventeen years later. Serum of the survivors protected mice from a challenge with TBEV-preparations from mouse brain.[10,11] Panov mentions reports of local physicians suggesting that cases of a disease similar to TBE had been observed in 1920 among the partisans hiding in the Far Eastern taiga during the Russian Civil War.[7]

The earliest retrospectively identified cases of TBE in the Far East were later reported by Aleksei Shapoval, a local neurologist who had been involved in the Khabarovsk medical group on TBE in 1936, in Zilber's expedition, and in many subsequent investigations of this disease in the region. He described several patients from the regions of Khabarovsk and Primorie with residual symptoms of possible TBE, which had started after a severe febrile illness during the summer months, one from 1909, examined in 1937, another one from 1917, examined in 1941, and the third one from 1911, examined in 1949. Additionally, Shapoval also mentioned a possible cluster of TBE in 1904 in a forestry near Nikolaievsk-on-Amur with 17 cases and 3 deaths with symptoms of fever, headaches, vomiting, blurred consciousness, and paralysis. One of the survivors of this outbreak was examined in 1939 by S. Vaflin (so not by Shapoval himself) and was found to have paresis of the upper extremities. If we accept this indirect evidence, this 1904 outbreak can be considered to be the earliest known historical cluster of possible TBE cases in the Russian Far East – and also the biggest before the Soviet period.[12]

Importantly, the Far East was not the only location of the early TBE reports in Russia. Cases of a very similar disease had already caught the attention of physicians in other parts of the country, in particular, in the Urals and Western Siberia, but had been described under different names, for example, as atypical poliomyelitis.[6,13] A.A. Pecherkin (Perm), M.G. Polykovskii (Sverdlovsk / Yekaterinburg) and N.V. Shubin (Tomsk) had sent reports about this disease to the All-Union Institute of Experimental Medicine, but it was not until early 1939 that they, together with the serum samples from recovered patients, were tested by Moscow virologists and the link to TBE was confirmed by using the serum of survivors in a TBEV-mouse-challenge test. As a result, a special expedition was sent to the regions of Sverdlovsk and Perm to investigate the presence of TBE there. This expedition was led by Mikhail Chumakov, a talented virologist who had survived and had been left permanently disabled by a TBE infection he had contracted during Zilber's expedition in 1937 by conducting the autopsy on a patient who had died from TBE. Through retrospective diagnosis, confirmed by serological studies, Chumakov and Zeitlenok managed to identify several possible past TBE cases in the Urals, the earliest of which went back to 1914.[14] Ten years later, however, Mikhail Chumakov managed retrospectively to identify an even earlier case. In 1949, Chumakov, by then a very established virologist, was sent to investigate a TBE outbreak in the Tatar ASSR (today the Republic of Tatarstan in Russia). There he found a 72-year old man from the village of Urgancha (about 200 km east of Kazan) with post-encephalitis symptoms, who had fallen ill in May 1895, diagnosed by Chumakov as TBE. He emphasized the "historical importance" of this case and described it in his report to the Russian (RSFSR) Ministry of Public Health, preserved in the ministry's archival fonds.[15]

This may be the earliest historical (retrospectively) clinically diagnosed case of tick-borne encephalitis.

Of separate importance for reconstructing the history of TBE in Russia is the question of the relation between TBE and Kozhevnikov epilepsy. Kozhevnikov epilepsy (epilepsiea partialis continua), first described by Russian neurologist Aleksei Kozhevnikov in 1894, is a syndrome with many possible causes.[16] One of these causes is TBEV infection, and this causality is common in the Eastern parts of Russia.[17] In 1922 L.I. Omorokov, a professor from Tomsk in Western Siberia, published a study of 27 cases of Kozhevnikov epilepsy observed over three years. Based on his cases, Omorokov described Kozhevnikov epilepsy as a syndrome of encephalitis, caused by an infectious agent. Even more importantly, he suggested the link between this disease and the taiga and its insects:

"What is striking is the fact that all the sick are peasants, manual workers, living mostly in the taiga, who were born in the Tomsk, Tobolsk, Altai and Yenisei gubernia. This fact in our opinion can shed some light on the etiology of this suffering that is so rare in Europe and in European Russia and is so frequent in Siberia […] In our large material there has not been a single case from the intellectual classes. Therefore we need to recognize that Kozhevnikov epilepsy is tightly linked to the peasant population of the Siberian taiga. Perhaps the climatic conditions, the harshness of the climate, the difficult conditions of living in the taiga as well as the abundance of insects, that is mosquitos and flies, is one of the preconditions of the appearance of this form of encephalitis." [18]

In 1939 it was recognized that the cases studied by Omorokov had possibly been cases of TBE based on clinical descriptions and the epidemiological situation. Omorokov's 1922 article can be considered the first description of that specific manifestation of TBE and the first suggestion of its link to the forest and the possibility of the vector-borne etiology.[19] In some of the cases observed by Omorokov the onset of the symptoms started long before the examination, with the earliest case from 16 years before, that is from the 1900s, and at least six cases dating back to the 1910s. The majority of the cases, however, were very recent or new, from 1917-1922, the period of the Russian revolution and Civil War, a time of extreme hardship, violence, displacement and severe food scarcity as well as radical food expropriations from the peasants by the new Soviet authorities. It is possible that this time of crisis contributed to a certain emergence of TBE in the region, as the local population intensified their contact with the forest (as a place to hide or to search for food) while malnutrition could have increased the severity of disease.

What all this evidence suggests is that there clearly had been sporadic cases of TBE in the Far East, the Urals, Western Siberia and Tatarstan going back to the 1890s-1920s. Although those cases were rare, they were often severe and noticeable enough to attract the attention of local physicians and scientists who presented their materials in published papers and reports to their superiors, even if they described this disease as Kozhevnikov epilepsy or atypical poliomyelitis, but these reports did not result in further investigation. What, then, made the outbreak in the Soviet Far East in the 1930s so distinct to ensure that a special expedition with considerable resources, equipment, and experts from the top research institutions in Moscow and Leningrad went there?

The emergence of TBE in the Soviet Far East: Environmental, social, and political factors

Early Soviet research on TBE often described Far Eastern taiga as virgin, as a kind of "untouched" nature, *tabula rasa* unaffected by humans, which was to be transformed, cultured and "healthified" under socialism. Such bias was quite typical of many modern European scientists in colonial spaces, who often failed to grasp the complexities of human -environment interaction in local and indigenous communities but was exacerbated by the Soviet tendency to downplay pre-revolutionary developments. -In fact, the region that became the space of the early TBE research experienced dramatic socio-environmental transformation in the late imperial period. It was annexed by the Russian Empire in 1858-1860 and at that moment was sparsely populated by Indigenous hunter-gatherer communities. In the following half a century it experienced dramatic population growth because of the arrival of Russian, Ukrainian, and Korean settlers, re-emergence of settled agriculture (that had ceased to exist for several hundred years following the destruction of medieval settlements by the Mongol invasion), deforestation (due to clearing, the construction of infrastructure, wood-logging, erosion, and mass forest fires) as well as widely reported overhunting of predators. Throughout the 1920s and 1930s these processes were supplemented by the early conservation policies, the creation of nature reserves and the establishment of deer farms to harvest deer antlers (which were considered a precious export commodity because of their value in Chinese medicine).[20] These processes are not only of historical but also of biological importance as they could have affected animal migration, the population of vectors and their hosts and the circulation of the virus.

Clearly, imperial colonizers - peasants, Cossacks, scientists, explorers, forestry workers - had to come into frequent contact with the taiga. Furthermore, late imperial accounts often mention the abundance of ticks in Far Eastern forests and report frequent tick bites, usually multiple at the same time.[21,22] So there had clearly been humans in the Far Eastern taiga before the 1930s and they had frequently been bitten by ticks, yet there seems to have been only sporadic cases of TBE. Later studies also specifically looked

for the cases compatible with a TBEV infection among the Indigenous Orochen and Udeghe peoples in the region, whose life had been directly tied to the forest, but failed to identify more than a couple of cases.[12]

The situation changed radically in the Stalinist period when suddenly TBE cases in the region started to appear in dozens. Not only did the colonization and industrial development of the region intensify, but it relied strongly on involuntary and semi-voluntary resettlement and forced labor. By the end of the 1930s, about 20% of the population of the entire Far Eastern region were unfree, and it was these groups that were used in the heavy labor in wood-logging, mines, and infrastructure construction.[23,24] Due to its remote location and the shortcomings of Soviet central planning and distribution system, throughout the 1930s this region remained constantly undersupplied. The scarcity of food and clothes was known even to the privileged groups, such as the military and the free administrative personnel,[25] but the conditions of prisoners and deported special settlers in the GULAG (Glavnoie upravleniie ispravitel'no-trudovykh lagerei) system of camps and special settlements were simply horrendous, characterized by extreme undernutrition, vitamin deficiencies, lack of basic supplies, exhaustive labor and constant stress connected to the arrest, deportations, and imprisonment. There exists considerable scholarship on the influence of social factors on the TBE morbidity as well as on the relations between malnutrition and viral infections, that show that malnutrition, low calorie intake and vitamin deficiencies weaken the immune system and increase the risk of severe course and complications and death.[26-29] All of these factors were present in the GULAG camps and settlements and to some extent also influenced other forms of organized labor (military units, Komsomol brigades, worker parties), which also depended on the very poor centralized supply system. Furthermore, many of the newcomers of the Stalinist period came from the steppe regions and had likely not been previously exposed to the TBEV. The morbidity among newcomers was much higher than that of earlier (for example, Korean) settlers in the same location.[12]

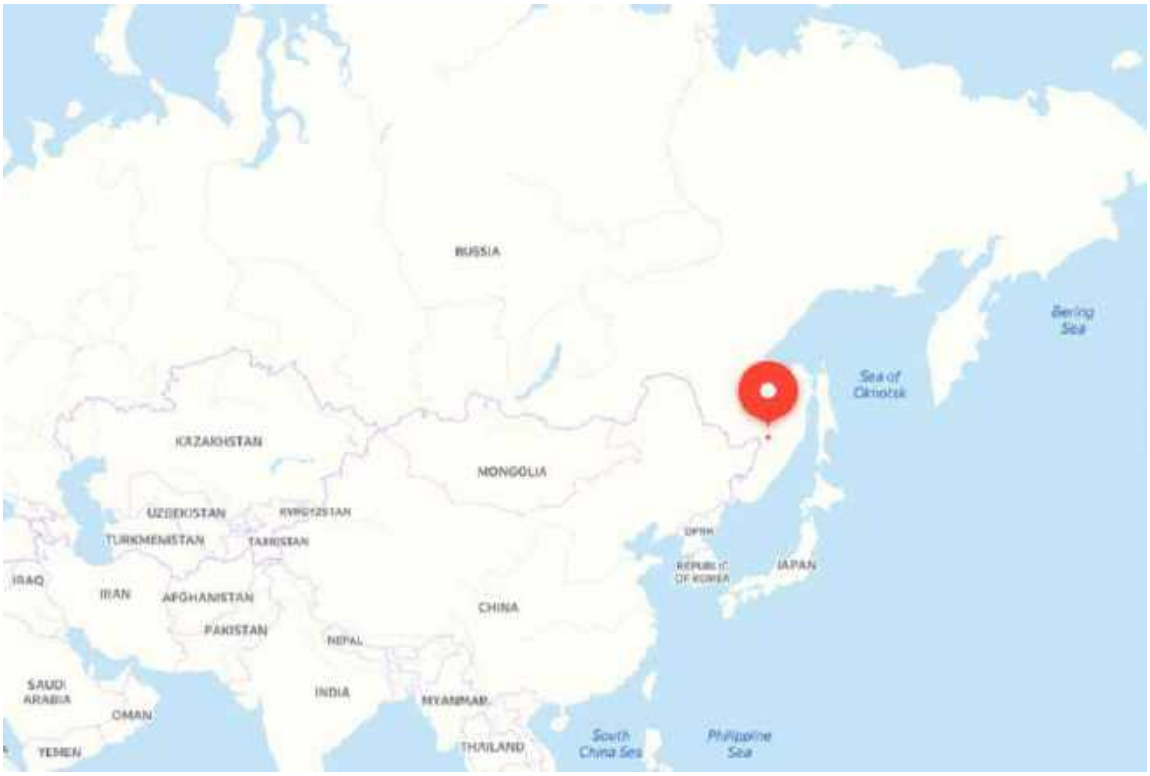
Another important factor in the apparent emergence of TBE in the Far East, or rather its perception, was geopolitical. At that time this remote Russian periphery was gaining strategic importance following the occupation of Manchuria by Japan in 1931. The repeated border clashes and the fear of a Japanese attack forced Soviet leadership to station considerable military and industrial forces along the border with Manchuria. It was the Red Army that requested the special expedition to study encephalitis in the Far East.[3] Apart from the general concern about the potential spread of disease among the military personnel, the possible connection of the new disease to Japanese encephalitis led to a fear that the outbreaks could have been a result of the Japanese attack. This view was shared by the highest ranks of the Soviet military and was in fact not as bizarre as it might sound today, considering the existence of the strong bioweapon program in Japan at the time. It was therefore the military concerns that ensured that the disease outbreaks in the Far East would not go unnoticed as those in the Urals and Western Siberia but that a special expert mission from the center, located 7,000 km away, would be sent there and would eventually succeed in identifying the virus.

The implications for early Soviet TBE research

When commissioned with the tasks to lead an expedition to the Far East, Zilber managed to bring together an interdisciplinary team of virologists, entomologists, epidemiologists and clinicians. Importantly, about half of the members of Zilber's expedition were women, including both deputy heads, virologists Elizaveta Levkovich and Alexandra Sheboldaeva. The mission arrived at Khabarovsk in mid-May 1937 and was divided into two units. The southern unit was located in Vladivostok at the local microbiological laboratory and the northern in the village of Obor. It is worth having a closer look at it, as much of the early research was shaped by disease ecologies of this specific location.

Obor (Figure 1) is located on the banks of the river with the same name southeast of Khabarovsk (ca. 100 km away by road today). The development of this area started at the turn of the 1930s with the construction of the Obor railway and the Obor forestry industrial complex. Its population had a very distinct composition, as it consisted primarily of deported special settlers, distributed across several camps belonging to the forestry. The first large cohort of special settlers—7,400 persons deported from the south of European Russia and the Volga region--was brought there in 1931.10,30 In addition to the deportees, the Obor forestry complex also used the labor of prisoners--the GULAG report of 1933 sets the quota of 800 prisoners to be sent to the area.[31] The conditions in the Obor forestry were typical for the GULAG structures with their extreme undernutrition, exploitation and abuse, and in the first half of the 1930s perhaps even worse than average in that outstandingly brutal and inhumane system. For example, a 1932 security service report states that "the food situation was particularly acute in the Obor and Tygda districts of special settlements where the shortages of supplies resulted in a true famine."[32] Food scarcity remained severe in the following years. Undernutrition must have significantly affected the interaction of human bodies with the virus and could have disadvantaged the new migrants to the Far East vis-à-vis the colonizers of the late imperial period, contributing to the rise in the numbers and severity of symptomatic TBE infections. There were other factors that undermined the health status of the residents of Obor and could have influenced the ways their bodies responded to the virus when infected—exploitative physical labor in wood-logging with low mechanization, hypothermia

Figure 1: The map of the Russian Far East with the village of Obor



because of the constant work outside in a wet, swampy area, lack of warm clothes and footwear and inadequate housing, various comorbidities that were common in the conditions of overcrowding, lack of sanitation and very poor healthcare, extreme stress connected to the traumatic experiences of deportation, arrest, family separation and adaptation to the camp environment, as well as direct torture and abuse.

In addition, the residents of Obor had a significantly increased exposure risk. They spent long working hours in the taiga thickets because of the nature of their labor with minimal precautions of occupational health. Furthermore, in the situation of dramatic undersupply of food, the forest was not only a place of their hard labor but also their main ally in the fight against starvation and scurvy. The camp administration encouraged foraging as the berries, mushrooms, and herbs could compensate for the lack of provisions evident from official reports.[33] The other dimension was the lack of any protection against exposure to tick-bites, and this too could have distinguished settlers of the Stalinist times from the earlier colonizers and the Indigenous people who lived in the area. Even today the key protection against tick-borne disease, apart from vaccination, is adequate clothing and footwear, and regular inspection of the body to remove ticks before they bite. All of these were unavailable to the special settlers and prisoners in Obor. First of all, the wear and tear of cloths was intense in the thickets of the Far Eastern taiga. New--or any other--clothes were, however, virtually impossible to procure. The lack of clothes and footwear was a constant refrain of the official reports of the time, which affected not only prisoners and special settlers but also peasants, soldiers, and the camp administration. Furthermore, the inspection of the body and the early detection of ticks was also extremely complicated among the exhausted workers living in the poorly heated and lit overcrowded barracks.

Medical research in the GULAG has recently come to the attention of historians, who revealed the "conspicuous silence" of Soviet scientists, many of whom were also prisoners, about the social context of their research subjects, when any references to camps, starvation and ruthless exploitation were avoided.[34-36] Clearly, these conditions could not have evaded either the local medical researchers in the Far East, or the members of the Zilber's expedition but due to political reasons they could only hint at the social status of the Obor residents in their early publications, for example, by referring to the local population as a "contingent" that "was brought" rather than "came" to the area and describing their working and living conditions as "difficult" or "unsatisfactory". The TBE morbidity and fatalities that they recorded in the Obor forestry were remarkable, with 60-80 symptomatic cases per season and 15-20 deaths (see Table 1). There is no exact data on the severity of disease and the complications, but the expedition's epidemiological study mentions that out of 8 confirmed cases of TBE in 1933, 6 survivors remained severely disabled which suggests that post-infection disability was very frequent.[37]

Table 1: TBE cases and fatality rates in the Obor forestry industrial complex.[37]

Year	Confirmed cases		Confirmed plus possible and suspicious	
	Cases	Deaths	Cases	Deaths
1931	0	0	2	0
1932	0	0	6	4
1933	8	1 (13%)	13	4 (13%)
1934	9	1 (11%)	20	10 (30%)
1935	57	16 (28%)	72	17 (24%)
1936	63	15 (24%)	84	20 (24%)
1937	62	15 (24%)	62	15 (24%)

"Confirmed cases": neurological residual symptoms after infectious encephalitis; "possible cases": infectious encephalitis without a neurological examination or no residual symptoms observed; "suspicious cases": death at a young age with a diagnosis labeled as "meningitis", "paralysis", "paresis" or "intracerebral hemorrhage".

Zilber's expedition lasted for three months, and in this short period it identified a new distinct form of viral encephalitis and isolated 29 strains of the causing virus, described the tick vector, the epidemiology and pathophysiology of disease and its clinical manifestation and showed some efficiency of serotherapy against it. Although Zilber's success is usually told as an exclusively Soviet story, it of course did not happen in isolation from the international science. Zilber and his colleagues read and widely cited foreign research on encephalitis, particularly American and Japanese. Even more importantly, there was also a transborder exchange of viral strains. Already during the expedition, in summer 1937 Zilber asked for and received a Kalinin strain of the Japanese encephalitis virus from Japan, through the Soviet Embassy in Tokyo--quite remarkable given the political and military tensions between the two countries. The strain of the St. Louis encephalitis virus was received from L.T. Webster in New York. These strains were immediately used in the expedition research and helped confirm the distinctiveness of the TBE virus.[3,10]

Such international cooperation had tragic consequences for Zilber and some of his colleagues. At the height of the Stalinist purges it seemed to be more fitting for the Soviet security authorities and military leadership to view the disease outbreaks in the Far East as cases of Japanese encephalitis and therefore not as a result of their mismanagement but as an act of sabotage and Japanese attack. Upon his return to Moscow, Zilber was arrested on the accusation of being a Japanese spy and intentionally spreading Japanese encephalitis among the Soviet population. Two of his female colleagues--Alexandra Sheboldaeva and Tamara Safronova--were arrested because of their connection to Zilber. Israel Finkel was also arrested and most likely perished in prison.[3] Zilber was soon released and managed to take part in the all-union conference of microbiologists in January 1939 and since then the distinctiveness of tick-borne encephalitis was recognized in Soviet publications. However, in 1940 he was arrested again and released only in 1944, following the intervention of several prominent virologists and his former partner, Soviet penicillin researcher Zinaida Ermolieva.[38] The research on the virus and the expeditions to the Far East continued without Zilber, but it is clear that his arrest must have made Soviet scientists even more cautious.

Given the high case fatality and disability rates, including among the Soviet scientists themselves, the prevention of disease immediately became a priority of research. Work on the vaccine started in 1938 and was led by two female virologists, both affiliated with Moscow's All-Union Institute of Experimental Medicine: Nadezhda Kagan in Moscow and Elizaveta Levkovich, who had been a deputy head of Zilber's expedition in the field in the Khabarovsk region. The laboratory where the research was conducted was also staffed with female personnel. In the autumn of 1938 Kagan contracted TBEV after exposure in the laboratory and died, and Levkovich took over her work. Two months later, a laboratory technician Natalia Utkina also died after contracting TBE. Women's bodies were also the first to try the new vaccine, based on the Sofyin strain, when Levkovich and her assistant Galina Zorina-Nikolaieva tested the vaccine on each other in 1939.[3,39,40]

To check the efficiency of the vaccine, the 1939 expedition

conducted trials, designed as a kind of unblinded cluster-randomized trials, on the population of the endemic area in Obor. The 1941 publication of the results speaks of 925 vaccinated subjects and a control group of 1,185 unvaccinated subjects that were distributed across four locations within the Obor forestry-industrial complex and had a comparable age, gender and occupational composition. This account does not mention the legal status of the participants but says that both groups were offered "sanitary explanation" about the trials although it is unclear what exactly that explanation implied. It was not until 2001 that the memoir of the neurologist Aleksei Shapoval, involved in those trials, revealed the circumstances in which they were conducted. Shapoval speaks of 1,987 vaccinated subjects and explicitly states that they were inmates of a forced labor camp while another camp with 2,387 prisoners in the same area was used as a control group. Such composition of participants would suggest that the involvement in the trial was not voluntary. Luckily for those vaccinated, both accounts agree that the vaccine seemed to be successful and offered some protection against the disease (the official publication reported only 2 mild TBE cases among the vaccinated compared to 27 cases and 7 deaths among the control group; Shapoval recalls 9 mild TBE cases among the vaccinated compared to 37 TBE cases and 12 deaths in the control group).[39,40,41] The case-fatality rate of TBE observed in the early trials (27-32% in the unvaccinated group) was dramatic. These most likely involuntary vaccine trials on the unfree population did not contradict the scientists' compassion and probably sincere desire to protect that population from a potentially deadly disease --after the arrest of Zilber and his colleagues, the expedition members knew very well that they could easily end up in a similar camp themselves. Yet, again, the concealment of the camp context had not only ethical but also empirical implications. The health status and post-infection survival chances of forced laborers or settlers had been severely compromised by very poor nutrition, exhaustive work, the lack of adequate healthcare, and extreme stress connected to deportation and imprisonment. However, Soviet scientists did not reflect on how those factors could have influenced the striking TBE mortality and morbidity they observed and in their publications attributed them exclusively to the properties of the virus, reinforcing the image of tick-borne encephalitis, especially in its Far Eastern manifestation, as highly lethal.

Of all the Soviet scientists involved in the early TBE research, Aleksei Shapoval deserves credit for consistently trying, if not to reveal, then to hint at the social conditions in which TBE emerged to the extent Soviet censorship allowed. Lev Goldfarb, who later worked with Shapoval, mentioned that Shapoval had been deeply affected by Zilber's arrest,[42] perhaps this was one of the reasons why he did not let this important aspect fall into oblivion. In 1947, Shapoval coordinated the treatment of the TBE patients in the Khabarovsk region and it becomes clear from his report to the Public Health Ministry that most of the patients were Japanese prisoners-of-war.[43] The forced labor of prisoners-of-war was widely used in the Soviet Far East, and their conditions were comparable to those of other prisoners and special settlers, with undernutrition as a crucial factor affecting their health status and mortality. In Primorie, another Far Eastern region, the situation was very similar – in 1948 the majority of 240 recorded TBE cases occurred among the Japanese prisoners-of-war (the method of TBEV diagnosis is not specified in the source).[44] These were unpublished internal reports, but after Stalin's death with the certain liberalization of the Soviet regime some of this information made it into scientific publications.

In 1961, Shapoval published a monograph entitled "Tick-borne Encephalitis", in which he questioned the assumption that the changes in the TBE morbidity in the Far East were connected exclusively to the frequency of the contacts with the forest and argued, although with careful phrasing, that the severity of disease depended on the living conditions of the human population. Comparing TBE outcomes across several locations in the Khabarovsk region in 1947, he showed that in settlements with good living conditions and decent food supply the lethality was 8%, in Obor, where the situation had somewhat stabilized by the late 1940s, it was 20%, while in the Amgun unit, where there were "problems with food supply" (probably a euphemism for extreme undernutrition) and where "workers had to build housing for themselves" away from any settlements, the disease was particularly severe and the case fatality was as high as 56%.[12] What Shapoval described here was most likely the GULAG Amgun labor camp which used the forced labor of Soviet prisoners and Japanese prisoners-of-war. He also specifically mentioned that the disease was particularly severe among the workers with hunger dystrophy. Admitting that in the socialist state there had been workers with hunger dystrophy was in itself very daring, and this was the kind of diagnosis that for his contemporaries must have signaled that he was describing the workers in the camps. It took, however, forty more years until Shapoval was able to speak about it openly in his memoirs.

Conclusion

The year 1937 and the work of the early Soviet Far Eastern expeditions should always have a very special place in the history of TBE. This is when the virus was first isolated in the mouse brain and the disease etiology was understood and described. It also has to be emphasized that scientists, many of whom were women, worked on this disease at a significant risk for themselves, both medical and political, and although this research propelled the career of some, others had to pay a very high price with their life, health or freedom.

Yet, it is clear that not only the biological but also the documented social history of TBE is longer and broader. Cases of this disease have been recorded in Russian/Soviet territory at least since the 1890s and they occurred both in the Asian and the European parts of the country. In the 1920s and 1930s TBE started capturing the attention of scientists and physicians in various locations, not only in the Far East, but did this under different names such as atypical poliomyelitis or Kozhevnikov epilepsy. This increased attention was linked to the transnational developments in medical sciences and general interest in neuroinfections following the epidemic of the Encephalitis lethargica (Economo encephalitis)—a mysterious infectious brain disease that swept the world in the 1910s and 1920s. It is possible, however, that there was some real increase in the number and severity of TBE cases across the Soviet Union in this time due to the changing patterns of human interaction with the environment and the virus, connected to the hardship and food scarcity during the times of the Civil War, military communism, and collectivization.

The well-known emergence of TBE in the Far East in the 1930s, that eventually led to the isolation of the virus, happened in very special circumstances of Stalinist colonization of the region. That socio-political context dramatically affected the composition, the health status and exposure of the population that lived in or was brought to the region, often by force, and must have influenced the TBE epidemiology, including the severity, clinical manifestations, and lethality of disease. These social circumstances, including extreme undernutrition and exploitative forced labor in the forest without any protection, were a long-lasting reality that continued to affect local disease epidemiology at least until the turn of the 1950s, if not later.

It is important to acknowledge this social context when reconstructing the history of tick-borne encephalitis. One aspect here is ethical, that is the need to, at least in this form, commemorate the many people in the inhumane circumstances who were exposed to this infection and deprived of all the means to resist it. But there can also be empirical implications for scientific research. Since the living conditions of the European and Russian population today--and in fact of the late-Soviet population as well--were and have been, thankfully, very different from that of the Stalinist period, early Soviet epidemiological studies have to be interpreted and used for comparison with caution. These differences in the social context, health status and exposure need to be considered in the long-term and cross-regional TBE epidemiology, especially its Far Eastern variant, as well as the historical evaluation of preventive strategies. At the same time, this new interpretation of early Soviet research could provide important historical precedents for the studies on the role of the social factors in the TBE emergence in the 1990s and could inform future investigations.[27,28,45]

Acknowledgements:

The author would like to thank Anastasia Fedotova for her help in getting access to the 1939 issue of the journal *Arkhiv biologicheskikh nauk.*

Contact: anna.mazanik@mws-osteuropa.org

Citation: Mazanik A. Early TBE Research in the Soviet Union: revisiting the narrative. Chapter 3a. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi: 10.33442/26613980_3a-7

References

1. Kisselev LL, Abelev GI, Kisseljov F. Lev Zilber: The Personality and the Scientist. *Adv Cancer Res*. 1992;59:1-40. doi:10.1016/s0065-230x(08)60301-2
2. Kiselev LL, Levina ES. Lev Aleksandrovich Zil'ber, 1894-1966: zhizn' v nauke [Lev Aleksandrovich Zilber, 1894-1966: Life in science]. Moscow: Nauka; 2005.
3. Levina ES. Pervyie virusologi i meditsinskaia virusologiia v SSSR 1930-kh godov [The first virologists and medical virology in the USSR of the 1930s]. *Istoriko-biologicheskie issledovaniia*. 2010;2(1):10-50.
4. Pogodina VV, Karan LS, Levina LS, Kolyasnikova NM, Gerasimov SG, Malenko GV. 75-letie otkrytiya virusa kleshchevogo entsefalita. Sravnenie rannykh (1937-1945) i sovremennykh shtammov [75th anniversary of the discovery of tick-borne encephalitis virus. Comparison of early (1937-1945) and modern strains]. *Voprosy virusologii*. 2012;(S1):66-75.
5. Zlobin VI, Pogodina VV, Kahl O. A brief history of the discovery of tick-borne encephalitis virus in the late 1930s (based on reminiscences of members of the expeditions, their colleagues, and relatives). *Ticks and Tick-borne Diseases.* 2017; 8 (6):813-820; https://doi.org/10.1016/j.ttbdis.2017.05.001.
6. Dobler G, Gniel D. A History of Tick-Borne Encephalitis and Its Virus. In: Vasilakis N, Kramer LD, eds. History of Arbovirology: Memories from the Field. *Springer*; 2023. https://doi.org/10.1007/978-3-031-22003-6_21
7. Panov AG. Sezonnye letnie entsefality [Seasonal summer encephalitis]. Vladivostok; 1940.
8. Finkel IZ. K voprosu ob osobennostyakh techeniya entsefalita [About the particularities of the course of encephalitis]. *Dal'nevostochnyĭ meditsinskiĭ zhurnal*. 1936;(3):30-39.
9. Walker L. Public health in the Soviet periphery: The case of tick-borne encephalitis (TBE). NCEEER Working Paper. 2011.
10. Zilber LA. Vesennii (vesenne-letnii) endemicheskii kleshchevoi entsefalit [Spring (spring-summer) endemic tick-borne encephalitis]. *Arkhiv biologicheskikh nauk*. 1939;56(2):9-37.
11. Dankovskii NL. Epidemiologicheskie osobennosti vesenne-letnego (taezhnogo) entsefalita [Epidemiological characteristics of the spring-summer (taiga) encephalitis]. *Arkhiv biologicheskikh nauk*. 1939;56(2):176-184.

12. Shapoval AN. Kleshchevoy entsefalit [Tick-borne encephalitis]. Leningrad: Medgiz; 1961.

13. Stenogram of the scientific conference of the All-Union Institute of Experimental Medicine and the Society of Neurologists and Psychiatrists on the problem of encephalitis, 22 December 1938. State Archive of the Russian Federation, 6742:1:104:82.

14. Chumakov MP and Zeitlenok NA. Kleshchevoi vesenne-letnii entsefalit na Urale I v Priuralie (Tick-borne spring-summer encephalitis in the Ural region). *Arkhiv biologicheskikh nauk*. 1939;56(2):112-120.

15. Report of Prof. M.P. Chumakov and laboratory assistant L.N. Shmelkova about the RSFSR Ministry of Public Health expedition to the Tatar ASSR in July 1949. State Archive of the Russian Federation, 482:49:334:72.

16. Kozhevnikov A Ia. Osobyi vid kortikalnoi epilepsii [A special type of cortical epilepsy]. *Meditsinskoe obozrenie.* 1894; 12 (14):97-118.

17. Mukhin KY, Mameniškienė R, Mironov MB, Kvaskova NE, Bobylova MY, Petrukhin AS, Wolf P. Epilepsia partialis continua in tick-borne Russian spring-summer encephalitis. *Acta Neurol Scand*. 2012;125(5):345-352.

18. Omorokov LI. Epilepsia partialis continua Kozhevnikova. *Sibirskii meditsinskii zhurnal*. 1922;1-2:8-18.

19. Propper-Grashchenkov NI. Klinicheskaia i gistopatologicheskaia kharakteristika sezonnykh virusnykh entsefalitov i kozhevnikovskaia epilepsiia, kak raznovidnost' ikh [Clinical and histopathological characteristic of seasonal viral encephalitis and Kozhevnikov epilepsy as their variant]. In: Vsesoiuznoie obshchestvo nevropatologov i psikhiatrov. Rasshirennyi plenum pravleniia. Tezisy dokladov. Moscow: s.n.; 1941.

20. Sokolsky M. Taming Tiger Country: Colonization and the Environment in the Russian Far East, 1860-1940. PhD dissertation, Ohio State University; 2016.

21. Przhevalskii N. Puteshestvie v Ussuriiskom kraie [A travel in the Ussuri land]. Moscow: Gosudarstvennoie sotsialno-ekonomicheskoie izdatel'stvo; 1937 (1870).

22. Arseniev VK. Po Ussuriiskomu kraiu (Dersu Uzala) [In the Ussuri Land (Dersu Uzala)]. Vladivostok: Ekho; 1921:71.

23. Chernolutskaia EN. Prinuditel'nye migratsii na sovetskom Dal'nem Vostoke v 1920–1950-e gg. [Forced migration in the Soviet Far East in the 1920s-1950s]. Vladivostok: Dal'nauka; 2011.

24. Kuzmina MA. Ispol'zovanie prinuditel'nogo truda zakliuchennykh na "velikikh stalinskikh stroikakh" v Nizhnem Priamur'e: 1929-1955 gg. [The use of forced labor of prisoners at the great Stalin's construction projects on the lower Amur, 1929-1955]. Dissertation in History. Komsomolsk-on-Amur; 2004.

25. Dudar LA. Kommercheskaia i obraztsovo-pokazatel'naia torgovlia na sovetskom Dal'nem Vostoke v 1930-1940-e gg. [Commercial and exemplary trade in the Soviet Far East in the 1930s and 1940s]. In: Chernolutskaia EN ed. Sovetskii Dalnii Vostok v stalinkuiu i post-stalinskuiu epokhu. Vladivostok: IHAE FEB RAS, 2014:46-51.

26. Randolph S. Tick-borne encephalitis incidence in Central and Eastern Europe: consequences of political transition. *Microbes Infect*. 2008;10(3):209-216.

27. Sumilo D, et al. Socio-economic factors in the differential upsurge of tick-borne encephalitis in Central and Eastern Europe. *Rev Med Virol*. 2008;18(2):81-95.

28. Godfrey E, Randolph S. Economic downturn results in tick-borne disease upsurge. *Parasit Vectors*. 2011;4(1):35.

29. Singh P, Bhatt GC, Singh V, Kushwaha KP, Mittal M, Mehta A, Sharma B, Pakhare AP, Kumar A. Influence of malnutrition on adverse outcome in children with confirmed or probable viral encephalitis: a prospective observational study. BioMed Res Int. 2015;2015:407473. doi:10.1155/2015/407473

30. Kochegarova ED. Starymi metodami: Osobennosti formirovaniia trudovykh resursov v Dalnevostochnoi zolotopromyshlennosti v 20-30-e gg XX v. [With the old methods: The formation of the labor resources in the Far Eastern gold industry in the 1920s and 1930s]. Rossiia I ATR 2002, 4:11-20, 17.

31. Berman MD, Berenzon LI, Kogan LI. to Deputy Chairmen of the OGPU G.G. Yagoda and G.E. Prokofiev on the improvement of the use of prisoners in camps for production. May 17, 1933. Central Archive of the Federal Security Service of the Russian Federation. 2:11:546:20-25, accessed online https://docs.historyrussia.org/ru/nodes/48419#mode/inspect/page/3/zoom/4

32. Special report of the Special Department of the OGPU on the political attitudes and counter-revolutionary activities of special settlers, as of October 25, 1932. November 1, 1932. Central Archive of the Federal Security Service of the Russian Federation, 2:10:514:208-228, accessed online http://docs.historyrussia.org/ru/nodes/88496#mode/inspect/page/8/zoom/4

33. GULAG Directive No. 674514 to the chief sanitary Officers of corrective labor camps on strengthening the fight against scurvy in the camps. November 2, 1933. State Archive of the Russian Federation, P-9414:1:2741:54-55, accessed online http://docs.historyrussia.org/ru/nodes/50153#mode/inspect/page/3/zoom/4

34. Alexopoulos G. Medical research in Stalin's Gulag. *Bull Hist Med*. 2016;90(3):363-393.

35. Healey D. Lives in the balance: Weak and disabled prisoners and the biopolitics of the Gulag. Kritika: Explorations in Russian and Eurasian History. 2015;16(3):527-556.

36. Nakhapetov BA. Ocherki istorii sanitarnoi sluzhby GULAGa. Moscow: ROSSPEN; 2009.

37. Olshevskaya VL. Vesenne-letnii entsefalit v lespromkhoze (LPKh) [Spring-summer encephalitis in an industrial forestry complex]. *Arkhiv biologicheskikh nauk.* 1939;56(2):164-175.

38. Letter from leading medical researchers to I.V. Stalin requesting the release of Professor L.A. Zilber. March 1944. Archive of the President of the Russian Federation,

3:58:255:187-191, accessed online https://docs.historyrussia.org/ru/nodes/330508

39. Pogodina VV, ed. Vospominaniia o Elizavete Nikolaievne Levkovich [Memoirs about Elizaveta Nikolaievna Levkovich]. Moscow: Institut poliomielita i virusnykh entsefalitov im. Chumakova; 2001.

40. Mazanik A. Arbovirology and Cold War collaborations: a transnational history of the tick-borne encephalitis vaccine, 1930-1980. *J Hist Med Allied Sci.* 2023. doi:10.1093/jhmas/jrad054

41. Smorodintseff AA, Kagan NW, Levkovitsch EN, et al. Experimenteller und epidemiologischer Beitrag zur aktiven Immunisierung gegen die Frühling-Sommer-Zeckenencephalitis. Archiv f Virusforsch. 1941;2:1–25. doi:10.1007/BF01240711

42. Lev Goldfarb, personal communication, 09.05.2023.

43. Shapoval AN. Report on Tick-Borne Encephalitis in the Khabarovsk Region, 1947. State Archive of the Russian Federation, A482:47:7997:10.

44. Kravchenko A, Soloviev V. Secret note to the USSR Public Health Minister E. Smirnov on the liquidation of tick-borne encephalitis. 1949. State Archive of the Russian Federation, 8009 (secret part):32:668:9-13.

45. Zavadska D, Odzelevica Z, Karelis G, Liepina L, Litauniece ZA, Bormane A, et al. Tick-borne encephalitis: A 43-year summary of epidemiological and clinical data from Latvia (1973 to 2016). *PLoS ONE.* 2018. 13(11): e0204844. https://doi.org/10.1371/journal.pone.0204844

Chapter 3b

Short history of TBE research and the scientists behind it

Mirko Lange, Lidia Chitimia-Dobler, Gerhard Dobler

Key Points

- Tick borne encephalitis came to the attention of human medicine in the 1920s and 1930s due to economic and political changes in Far Eastern Russia and Central Europe.
- Russian scientist identified the TBEV in Far Eastern Russia in the 1930s.
- Czechoslovak scientists in the late 1940s were the first to detect TBEV in Central Europe.
- In the 1960s and 1970s the transmission cycle of TBEV was elucidated mainly by Czechoslovakian and Austrian scientists.
- First trials to protect exposed humans by vaccination started shortly after the discovery of TBEV in Far Eastern Russia.
- In Austria and Germany currently used, cell culture based TBEV vaccines were developed in the 1970s and 1980s.

Tick-borne encephalitis (TBEV) is the most important tick-borne viral disease in humans and has increasingly shown its importance also in veterinary medicine. Although TBE virus (TBEV) probably evolved several thousand years ago, it was only due to political and economical changes in the 1920s that it came into the focus of human medicine in two independent locations on the European-Asian landmass. The history of TBE therefore is also a history of studying and understanding the connection of ecology of naturally occurring microorganisms and their interplay with vectors and hosts and their connection to the epidemiology of human and animal disease and underscores the importance of understanding these interrelationships for a better understanding and prevention and control of vector-borne zoonoses.

Introduction

Tick-borne encephalitis (TBE) is one of the most important arthropod-borne viral infections in Europe and Asia. Ecologically, TBE virus (TBEV) is an arbovirus. Taxonomically, it belongs to the Flavivirus genus, together with other medically relevant arboviruses such as dengue and yellow fever viruses.[1] TBEV is endemic in Europe and Asia and circulates between its principal vectors, hard ticks (Ixodidae; mainly of the genus Ixodes, and small mammals (reservoir hosts). Human infection most commonly occurs through the skin via the bite of a tick. Several thousands of people are affected by TBE every year. In the literature, the first cases were assumed to be mentioned in church records from the Åland Islands (Finland) in the 18th century.[2] This was long before two scientists, Smith and Kilbourne, discovered that ticks are vectors of pathogens.[3] In the 20th century, a disease, which was referred to as "taiga encephalitis" or "biphasic encephalitis", was described in soldiers, railway workers and woodcutters in the eastern parts of the former Soviet Union (USSR; see chapter 3a).

In 1931, Schneider wrote the first detailed medical description of what is today known as TBE.[4] In a monograph that was published in 1932, he described more than sixty cases of "epidemic acute meningitis serosa". Forest workers were mainly affected. As a result, TBE ultimately became the first disease that was recognised as an occupational disease in Austria, where it was known as the resin workers' disease or Schneider's disease. The first detailed description of the clinical picture of "summer encephalitis" in the Russian Far East was published by Panov in 1938.[5]

This chapter provides a brief historical overview of TBE in Europe and Asia and of the most important developments in TBE and TBEV research.

The discovery of TBEV in Europe and Asia

Based on molecular biological data, it can be assumed that western Siberia (Russia) is the area of origin of the TBEV. These scientific data also indicate an origin of this virus approximately 3100 [1800–4900] years ago.[6] Whereas the eastern TBEV groups spread from western Siberia through Asia eastward, the western TBEV groups dispersed westward and may have arrived in (central) Europe approximately 2000 years ago.

In Europe, the first medical description of four cases of what today is referred to as TBE was provided in 1931 by

Figure 1: Old man with child at the resin harvest (Pecher) in the 19th century (Source: Postcard)

Hans Schneider, a physician in Lower Austria. Hans Schneider (born under the name Johann Schneider) studied medicine at the medical faculty of the University of Vienna from 1911 to 1918. During his course of study, he received two scholarships in 1912. The first scholarship was granted by the Imperial-Royal Landwehr Command in Vienna and the second by the Theobald Uffenheimer Scholarship Foundation. After having passed his final oral examination, Schneider was awarded a degree in medicine on 31 January 1919. During World War I, he joined the Imperial and Royal Army and served time with the elite Hoch- und Deutschmeister Regiment. He was awarded the Silver Medal for Bravery 1st Class (1916), the Karl Troop Cross (1916), and the Austrian Red Cross Silver Medal for his work in internal medicine (1917). From 1919 to 1924, Schneider worked as a resident at the Vienna-Lainz hospital. During this time, he familiarised himself with the most modern diagnostic procedures available at the time. This applies in particular to the early stages of clinical microbiological and serological diagnostic techniques. This was when he published his first scientific papers. In 1925, when there were severe influenza and typhoid epidemics, he was appointed as a specialist in infectious diseases to a hospital in Neunkirchen, the capital of a district in Lower Austria. In 1926, Schneider became the head of the medical department and in particular of the infectious diseases ward that was being set up. As a chief physician in internal medicine, Schneider increasingly focused on diseases of the at that time increasingly important petrochemical industry and documented all cases of workers in this industry meticulously. In 1927, he observed an epidemic increase of a usually benign form of “acute serous meningitis” and detected an association between this disease and resin tapping, which was the basis of petrochemical industry in the area south of Vienna at that time. He found that a special type of meningitis often occurred in resin tappers who harvested resin from black pines.

In his experiments, Schneider even sent clinical materials of patients (cerebrospinal fluid) to the Vienna University to infect monkeys, which, however, in contrast to poliomyelitis, which was causing similar symptoms, did not cause disease in the animals. Schneider was thus able to clearly differentiate this disease from poliomyelitis, which too was widespread at the time. Within only three years, he documented more than sixty cases of this specific disease and provided first evidence of the possibility of milk-borne transmission at that time. In 1931, Schneider reported on four cases in the *Wiener Klinische Wochenschrift* and thus provided the first detailed clinical description of the disease worldwide. In 1932, he published a comprehensive monograph on “Epidemic acute meningitis serosa”, in which he described a total of 66 cases from the years 1927 to 1931 and presented his studies on the differentiation of this disease from other infectious diseases (poliomyelitis, typhoid fever). Owing to Schneider’s propaedeutic skills, this previously unknown disease was recognised as a new infectious disease that was associated with resin tapping. TBE was the first disease that was recognised as an occupational disease in Austria (where it was known as the resin workers’ disease). It was not until Schneider’s death in 1954 that this new entity became known, especially in German-speaking areas, as Schneider’s disease, named after the person who first described it.[7]

Figure 2: Dr. Johann (Hans) Schneider (1891 – 1954).

(Source: Niederösterreichische Ärztechronik - Geschichte der Medizin und der Mediziner Österreichs, Wien: Verlag Oskar Möbius GmbH 1990; S. 695 – 696: Nr. 20.)

Figure 3: Diffuse biotope of the ticks *Ixodes persulcatus*, *Haemaphysalis japonica* and *H. concinna* in the taiga.

(Source: Natural nidality of transmissible diseases with special reference to the landscape epidemiology of zooanthroponoses.] Moscow, Leningrad: Nauka (in Russian), 1966)

In the Soviet Far East taiga, a severe and usually fatal neurological disease was observed in 1934 and 1935 among Red Army soldiers who were stationed in this area and among the local population (see also chapter 3a). First attempts to identify the causative agent were unsuccessful and the etiology of the disease was unknown. In 1937, an expedition team led by Professor Lev Aleksandrovich Zilber (head of the first medical virology laboratory in the USSR) and Professor Evgenyi Nikanorovich Pavlovsky was sent to the taiga region on behalf of the Soviet health ministry in order to investigate the cause of the disease. Pavlovsky did not personally participate, but four scientists from his institute took part in this first expedition. The team of specialists (scientists and technical assistants) was divided into two groups. The first group investigated the Khabarovsk territory in the north and the second group the Primorsky territory in the south. In spite of extremely difficult conditions in remote areas (absence of infrastructure), the teams found that many local people showed neurological symptoms. Of 64 patients who were treated in a hospital, 12 died. The virus was isolated from 29 patient samples.[8-11]

In order to prevent infection, the teams informed the local population about the potential hazards associated with ticks. As a result, the number of new cases was significantly reduced within a short period of time. At the end of their mission, Zilber and his expedition team were able to provide convincing results suggesting that they had identified the causative virus and its vector (*Ixodes persulcatus*).

Some team members became infected with TBEV during the mission and showed typical disease symptoms. Since the virus is highly contagious and the conditions were challenging, it was almost a miracle that none of the affected team members died from the disease. Dr Chumakov, for example, became infected with the virus after having cut his finger during an autopsy. After a short while, he developed first symptoms such as paralysis of his right arm and loss of hearing. Later in his scientific career, Chumakov became a highly esteemed virologist and discovered TBEV foci at a great distance from the Far East in

the Ural and Trans-Ural regions.[12]

On the whole, the first expedition under the direction of Lev A. Zilber was a success and a major scientific achievement. During this mission, the team successfully isolated the virus several times, worked out the epidemiology of the disease, and took measures to prevent further infections. Owing to this success, it is no surprise that other expedition teams led by Evgenyi N. Pavlovsky were sent to the Far East in order to gather more information on the disease and especially on the virus.[13]

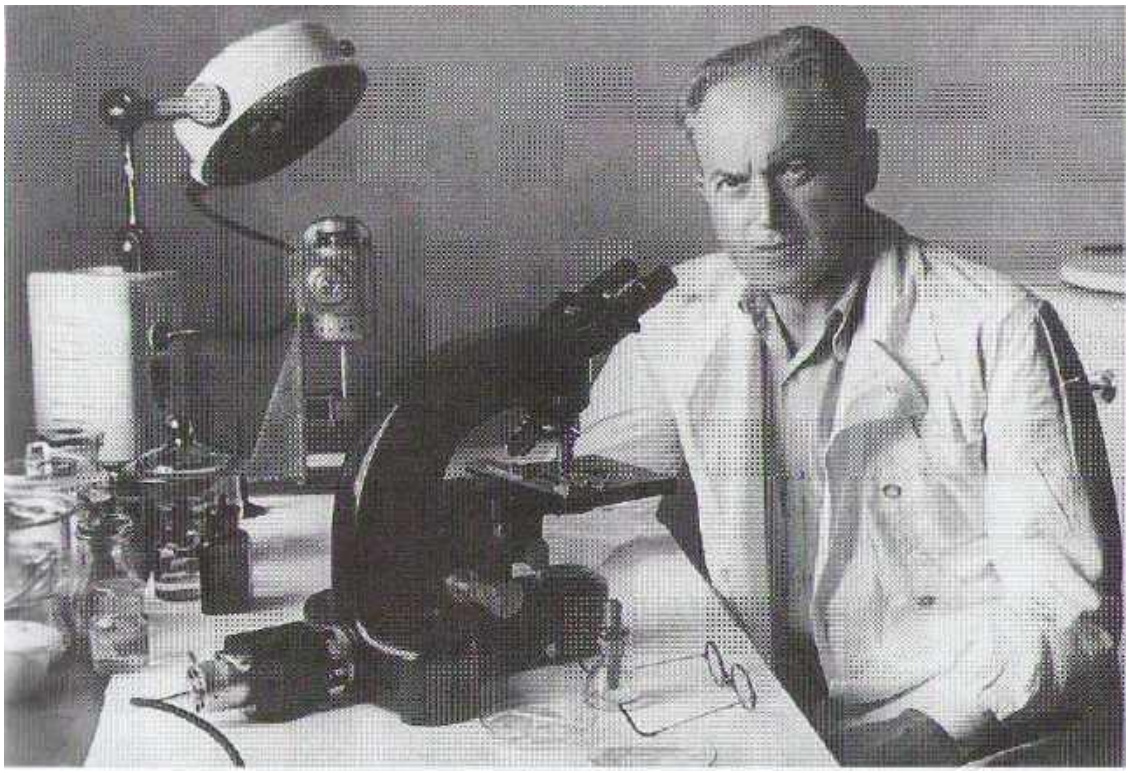
In Europe, the first isolation of TBEV was achieved in Belarus in 1939 from *Ixodes ricinus* ticks.[14] In the People's Republic of China, the first cases were reported in 1943. The virus was isolated for the first time from brain samples from deceased patients in 1944 (review by Yoshii et al., 2017).[15] In the early 1940s, US scientists at the Rockefeller Institute for Medical Research detected cross-reactivity between hyperimmune sera of Louping ill virus and Russian spring-summer encephalitis virus.

As early as in 1948, the second virus isolation (Hanzalova strain) was achieved in Europe in the present-day Czech Republic (near Prague).[16] The early 1950s played a special role in the history of TBE. In 1952, only a few years after the first virus isolation, a strain that was named "KEM I" was isolated in Hungary. Virus isolations were also achieved in Slovenia in 1953 and in Poland in 1954. In 1954, the first cases of TBE were reported on the island of Bornholm (Denmark). In the same year, TBEV was detected for the first time in Sweden. In Austria, the first TBEV isolates came from Styria in collaboration with Czechoslovak scientists in 1953. The Scharl strain, an isolate form the brain of a fatal human case, was isolated in Vienna, Austria in 1954. In 1958, the virus was detected for the first time in Slovakia. The Kumlinge strain was isolated in Finland in 1959.[17, 18] In divided Germany, the first virus isolation was achieved by scientists in the German Democratic Republic in the late 1950s.[19] In addition, the first case of TBE in Norway was reported as late as 1997.[20] In 2020, the virus and human cases were documented for the first time in the British Isles.[21] Two years later, TBEV was detected for the first time outside of Europe and Asia on the African continent (Tunisia).[22]

In the Federal Republic of Germany, the Zimmern TBEV strain was isolated for the first time in the region of Lower Franconia in 1970.[23,24] French scientists successfully isolated a TBEV strain in Alsace in 1970.[25] It was only in 2016 that the Netherlands reported the first autochthonous cases of TBE and the successful isolation of the Sallandse TBEV strain.[26]

Figure 4: Prof. Dr. Lev Alexandrovich Zilber (1894 – 1966)

(Source: "Lev Alexandrovich Zilber" written by his son L.L. Kisselev and E.S. Levina, The Publishing House "Science", Series "Scientific biographies")



The detection and natural transmission cycle of TBEV

The first expedition to the Russian Far East was led by Zilber in 1937 and provided first important information on the eco-epidemiology of TBEV within a few months. The causative agent was found to be a virus that was transmitted to a human host via the bite of an *Ixodes persulcatus* tick (*Ixodidae* family).

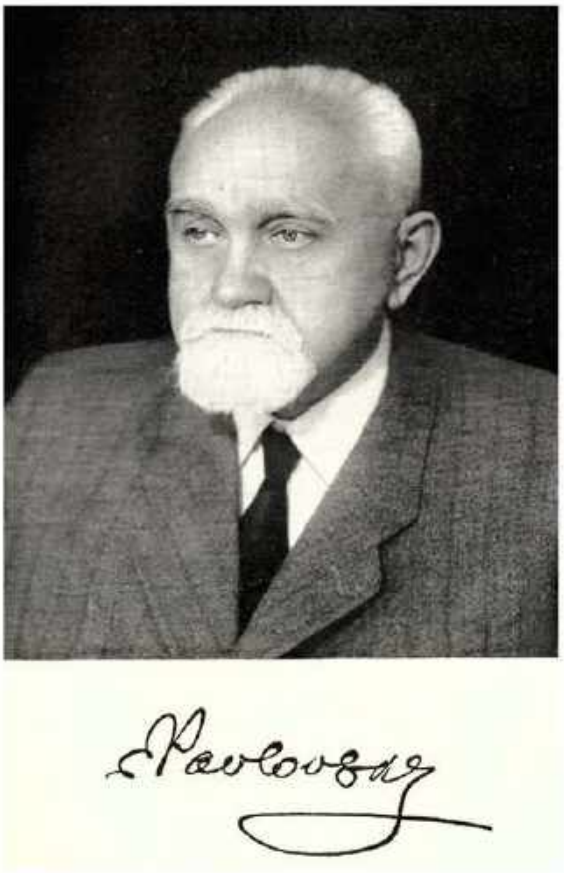
As mentioned before, the first expedition was followed by two further expeditions to the Russian Far East under the direction of Professor Evgenyi Nikanorovich Pavlovsky, who also was a general in the Red Army. The purpose of the second expedition (1938) was to investigate the spread of TBEV in the field and to identify the reservoir hosts of the virus. The results of the expedition were incorporated into Pavlovsky's widely acclaimed ideas about the ecology of zoonotic diseases (*Natural Nidality of Transmissible Diseases*).[27,28] TBEV is transmitted from a natural (transmission-competent) reservoir host to a vector (Ixodes ticks) through a blood meal. Infected vectors may transmit the virus to their accidental hosts (humans) during the next blood meal through the skin via a bite. These reservoir hosts are infected via the bite of an infected tick and transmit the virus to other ticks feeding on the host's blood. Long-term circulation of the virus depends on the presence of all necessary biotic factors (vectors, hosts) and an appropriate abiotic environment.

The scientists Chumakov and Naidenova[29] found that *Ixodes ricinus*, which is related to *Ixodes persulcatus*, is a vector that transmits a milder form of TBE in some European regions of the former USSR. This description was later confirmed by several European researchers (e.g. from Belarus and the former Czechoslovakia). In the former Czechoslovakia, Rampas and Gallia were the first outside of the USSR to isolate TBEV from field-collected ticks.[30-32]

From 1947 to 1951, a different route of transmission of TBE to humans was observed in the European part of the former Soviet Union.[33] TBEV was found to be transmitted through the ingestion of unpasteurised milk or milk products (e.g. cheese) from viraemic goats. One of the largest epidemics outside of the USSR occurred in the southeastern part of Slovakia (including the town of Rožňava) in 1951. More than 600 cases were documented.[34] Ten years later, cases resulting from alimentary transmission were reported in the former German Democratic Republic (e.g. in the town of Niesky).[35]

The 1970s and 1980s witnessed a substantial decrease in field work in many European countries. Since the ecology of TBEV had been well studied and understood by the scientific community, the focus of research attention shifted to molecular biological studies of TBEV and to Borrelia burgdorferi, a newly identified causative agent of Lyme

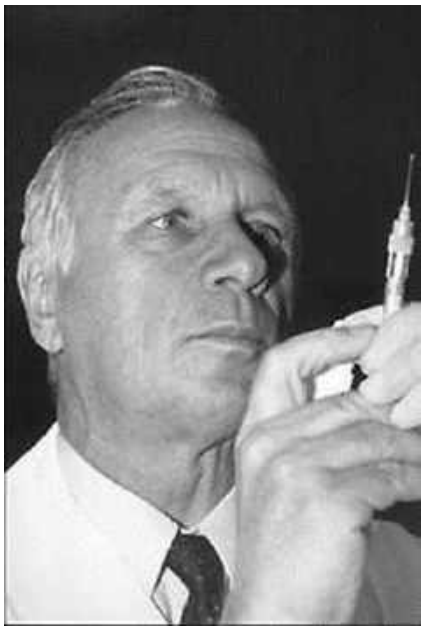
Figure 5: Prof. Dr. Evgenyi Nikanorovich Pavlovski.
(J. N. Pawlowski - Leben und Werk, Berlin: VEB Deutscher Verlag der Wissenschaften 1959)



disease. It is interesting to note that this coincided with the time when the first European vaccine became available in 1976[36] and it was believed that all problems associated with TBE had been solved. Today we know that this assumption was wrong.

Jones et al.[37] found that guinea pigs acquired Thogoto virus through Rhipicephalus appendiculatus ticks but did not develop detectable levels of virus in their blood. Alekseev and Chunikhin[38] as well as Labuda et al.[39] demonstrated the non-viraemic transmission of TBEV from small mammals to uninfected blood-feeding ticks. This was an important contribution to the understanding of the field ecology of the virus, and TBEV ecology once again became a focus of scientific attention. Milan Labuda et al.[40-42] found that (a) TBEV was transported in Langerhans cells of infected hosts, (b) non-viraemic transmission was also possible in immune hosts, and (c) this type of transmission occurred in small mammals but not in large mammals. This non-viraemic transmission now is more commonly referred to as infection by co-feeding.

Figure 6: Univ.-Prof. Dr. Christian Kunz (1990);
(Source: ©Michaela Seidler-Bruckberger))



The detection of different TBEV subtypes

On the basis of its general characteristics (physical and chemical properties, virion structure, arthropod carriers, and cross-reactivity), the *Flavivirus* genus was considered to belong to the *Togaviridae* family. This term was first used by Lwoff and Tournier in 1966.[43]

The *Togaviridae* family included the Alphavirus genus (formerly Group A arboviruses) and flaviviruses (formerly Group B arboviruses). Group B included dengue virus type 1 and other viruses.[44,45] Based on the plaque reduction neutralisation test (PRNT) and virus structure and viral replication, it was recognized that the former family of *Togaviridae* comprised two completely different groups of viruses. These finally were divided into two families, the genus Alphavirus in the family Togaviridae, and the newly created family Flaviviridae. This newly created genus, now called *Orthoflavivirus* in family Flaviviridae was further divided according to cross neutralization into seven subgroups.[46] One of these subgroups, the so-called "Tick-borne flavivirus group" contain the mammalian tick-borne flavivirus group (among others TBEV, Omsk haemorrhagic fever virus, Louping ill virus, Langat virus, Powassan virus, and Kyasanur Forest virus) and the Seabird tick-borne flavivirus group (among them Gadgets Gully virus, Saumarez, Reef virus, and Tyuleniy virus).

Although all these viruses have similarities, there are differences between them in their geographical distribution, associations with different vertebrates and ticks, and pathogenicity for humans. The *Flaviviridae* family comprises more than 70 species and includes ten sero-complexes.[48] TBEV belongs to the group of flaviviruses, which are mainly transmitted by ticks feeding on the blood of mammals. It has three subtypes: European, Far Eastern and Siberian.[49]

Two geographic and antigenic TBEV variants (eastern and western) have been known for more than 40 years.[1, 50-51] Clarke[52] divided 28 strains into two antigenic variants using the gel precipitation test with cross-absorbed sera and found that there were two types of antigens: eastern and western (European). Chumakov et al.[53] believed that there were differences between the Eastern and Western subtypes of TBEV and proposed a classification into the antigenic variants "persulcatus" and "ricinus" depending on viral ecology. Votyakov et al.[54,55] emphasised differences in antigenic profiles, geography, and clinical and pathological features in animals and humans.

Pletnev et al.[56,57] and Mandl et al.[58,59] decoded the whole genomes of Eastern (Sofjin) and European (Neudoerfl) subtype TBEV strains. This was the beginning of a new phase of the genetic classification of TBEV. Data showed significant genetic differences between the Western and Eastern variants in nucleotide substitutions (16.8–16.9%) and amino acid substitutions (6.9–7.2%). Also two Eastern strains were found to differ significantly in nucleotide (4.6%) and amino acid (1.8%) substitutions.

Rubin and Chumakov[60] were the first to publish these results for the Siberian subtype and, for example, described the isolation of a TBEV strain (Aina) from a child in the Irkutsk region (USSR). Pogodina et al.[61,62] reported the isolation of a group of strains from Ixodes persulcatus in eastern Siberia. These strains are serologically related to the Aina strain. Gritsun et al.[33,63] and Zlobin et al.[64-66] provided the first genotypic characterisation of what is today known as the Siberian subtype by sequencing the E gene and then the whole genome.

Sequencing a gene E fragment of eight and then 29 strains that were isolated in different geographical regions allowed the three major subtypes (Far Eastern, Western and Ural-Siberian) to be identified. Ecker[67] believed that there were three TBEV subtypes corresponding to the three major genotypes. Grard[68], however, provided a new interpretation of the genetic relationships between arthropod-borne viruses and proposed that TBEV be divided into four subtypes: (1) Louping ill virus (Spanish, British and Irish subtypes), (2) Western TBEV (European subtype), (3) Eastern TBEV (Far Eastern and Siberian subtypes), and (4) Turkish sheep encephalitis virus, including the Greek goat encephalitis virus subtype.

In addition to the three known and accepted subtypes, Russian researchers described two further strains that were denoted as 178/79 and 886/84. They proved that these two Russian strains were not closely related to the other three

subtypes. The latter strain with a number of isolated is now accepted as a fifth subtype of TBEV, named Baikalian subtype. The classification of the 178/79 strain is rather unclear as only on single isolate so far exists.[69] Further studies are required to assess whether these two new strains can be classified as further TBEV subtypes.

The development of TBEV vaccines

Pavlovsky was a pioneer in the development of a TBEV vaccine. A TBEV vaccine derived from mouse brain was for the first time administered to the local population in 1939 during the third expedition, which was led by E. N. Levkovich and N. L. Dankovsky. In 1940, mass vaccination was carried out for the first time in the Russian Far East (Khabarovsk) under the direction of Elizaveta Nikolaevna Levkovich.[70]

In order to address the increasing medical importance of TBE in Austria, Professor Christian Kunz decided to develop a vaccine against TBE. For this purpose, he cooperated with the British biological warfare research centre at Porton Down. This was possibly a result of many years of cooperation with the US armed forces. This cooperation was of utmost importance to Kunz since zonal ultracentrifugation, a purification method which was absolutely necessary for the production of vaccines, was available at this research facility. Kunz administered the first two TBE vaccines to himself and to his colleague Professor Hanns Hoffmann, a virologist. He carried out the first major vaccination campaigns and tested the vaccine on approximately 30,000 farmers and forest workers in Austria. He was personally liable for these activities. His private liability insurance covered 10 million schillings (approximately € 720,000).[71]

Kunz was unable to convince some of the major vaccine manufactures to become a partner in vaccine production. In 1976, the founders of IMMUNO AG, an Austrian pharmaceutical company, joined the project and started mass production of the vaccine (FSME-Immun®). Since then, 85% of the Austrian population have been vaccinated and the number of TBE cases in Austria has been reduced by approximately 90%. It should be noted that vaccination rates have been decreasing in Austria over the years as a result of a lack of acceptance in society. In 1991, another TBE vaccine (Encepur®) was approved in various European countries. This vaccine had been developed by the German pharmaceutical company Behringwerke and was then supplied by Chiron Corporation after the latter had acquired the vaccine businesses of Behringwerke.[71]

Professor Franz Xaver Heinz, director of the Institute of Virology in Vienna, and his team discovered a new mechanism of membrane function between virus and cellular membranes, a mechanism which is unique to flaviviruses. He was also the first to determine the three-

Figure 7: Franz Xaver Heinz

(Franz X. Heinz mit Loeffler-Frosch-Medaille ausgezeichnet; meduniwien.ac.at)

dimensional structure of the envelope protein E. Heinz and his research group thus enhanced the existing knowledge about fundamental mechanisms in virology. These scientific discoveries provided the molecular basis for many aspects of TBE immunology and pathogenesis.

TBE has become an important model for studying different cellular and virological mechanisms. In addition, this was also the time when the first infectious clone of TBEV was constructed allowing comprehensive studies on the genetics of TBEV.[72] Due to the nature of TBE as a zoonosis it will probably not be possible to extinguish this disease from our world. TBE will stay a permanent medical problem in Europe, Asia and probably will also expand its importance to Africa in the near future, as the TBEV was detected there, recently. In many of the endemic regions in Europe, there is an increasing trend of human TBE cases, even in highly vaccinated populations, like in Austria. The reason for this development has so far not understood, but may be related to the massive changes in global, regional and local ecological and environmental interactions due to human activities. Therefore, the prevention of TBE in humans and animals will remain a challenge although all instruments for control of human disease have been provided in the past by many brave, innovative and engaged researchers from different countries which faced the same problems although coming from political and economic suppositions.

Contact: Mirko1Lange@bundeswehr.org

Citation: Lange M, Chitimia-Dobler L, Dobler G. Short history of TBE research and the scientists behind it. Chapter 3b. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_3b-7

References

1. CASALS J. Viruses: the versatile parasites; the arthropod-borne group of animal viruses. *Trans N Y Acad Sci.* 1957;19 (3):219-235. doi:10.1111/j.2164-0947.1957.tb00526.x
2. Kunz C, Heinz FX. Tick-borne encephalitis. *Vaccine*. 2003;21 Suppl 1:S1-S2. doi:10.1016/s0264-410x(02)00810-1
3. Smith T, Kilbourne FL. Investigations into the nature, causations and prevention of Texas or southern cattle fever. *Bureau Anim Ind Bull*. 1893:322pp.
4. Schneider H. Über epidemische akute Meningitis serosa. *Wiener Klin Wochenschr*. 1931;44:350-2.
5. Panov AG. Klinika vesenne-letnikh entsefalitov. Nevropat I Psikhiat. 1938;7:18-32.
6. Heinze DM, Gould EA, Forrester NL. Revisiting the clinal concept of evolution and dispersal for the TB flaviviruses by using phylogenetic and biogeographic analyses. *J Virol*. 2012;86:8863-71. doi:10.1128/JVI.01013-12
7. Lange M, Chitimia-Dobler L, Dobler G. Dr. Hans (Johann) Schneider – Medical Officer and First Describer of Tick Borne Encephalitis. WMM 2021; 65(8): 294-301. doi 10.48701/opus4-51.
8. Pavlovskij EN., 1964. [Natural nidality of transmissible diseases with special reference to the landscape epidemiology of zooanthroponoses.] Moscow, Leningrad: Nauka, 211. (engl. transl. 1966, edited by N.D. Levine, University of Illinois Press, 257 pp.).
9. Pavlovskij EN., 1966. [Natural nidality of transmissible diseases in relation to landscape epidemiology of zooanthroponoses.] Moscow, Leningrad (engl. transl. 1966, edited by Y. Shirokov, Peace Publishers).
10. Pavlovsky YN. Human Diseases with Natural Foci. Moscow, UdSSR. Foreign Languages Publishing House. 1963.
11. Borchert A. J. N. Pawlowski Leben und Werk. Berlin. VEB Deutscher Verlag der Wissenschaften. 1959.
12. Chumakov MP, Seitlenok NA. TICK-BORNE HUMAN ENCEPHALITIS IN THE EUROPEAN PART OF USSR AND SIBERIA. *Science.* 1940;92(2386):263-264. doi:10.1126/science.92.2386.263
13. Zlobin VI, Pogodina VV, Kahl O. A brief history of the discovery of tick-borne encephalitis virus in the late 1930s (based on reminiscences of members of the expeditions, their colleagues, and relatives). *Ticks Tick Borne Dis*. 2017;8 (6):813-820. doi:10.1016/j.ttbdis.2017.05.001
14. Chumakov MP. [Tick-borne encephalitis in humans]. *PhD of Med Sci*. Moscow, 1944.
15. Yoshii K, Song JY, Park SB, Yang J, Schmitt HJ. Tick-borne encephalitis in Japan, Republic of Korea and China. *Emerg Microbes Infect*. 2017;6(9):e82. Published 2017 Sep 20. doi:10.1038/emi.2017.69
16. Gallia F, Rampas J, Hollender L. [Laboratory infection with encephalitis virus]. *Cas Lék Ces*. 1949;88:224-229.
17. Oker-Blom N, Kääriäinen L, Brummer-Korvenkontio M, Weckström P. Symp. *Czech. Acad. Sci.* 1962;3:423. (cited after Brummer-Korvenkontio et al., 1973)
18. Brummer-Korvenkontio M, Saikku P, Korhonen P, Oker-Blom N. Arboviruses in Finland. I. Isolation of tick-borne encephalitis (TBE) virus from arthropods, vertebrates, and patients. *Am J Trop Med Hyg*. 1973;22(3):382-389.
19. Sinnecker H. Zeckenencephalitis in Deutschland. *Zbl Bakt*., I. Abt Orig. 1960;180:12-18.
20. Skarpaas T, Ljøstad U, Sundøy A. First human cases of tickborne encephalitis, Norway. *Emerg Infect Dis.* 2004;10:2241-3. doi:10.3201/eid1012.040598
21. Holding M, Dowall SD, Medlock JM, et al. Tick-Borne Encephalitis Virus, United Kingdom. *Emerg Infect Dis*. 2020;26(1):90-96. doi:10.3201/eid2601.191085
22. Fares W, Dachraoui K, Cherni S, et al. Tick-borne encephalitis virus in Ixodes ricinus (Acari: Ixodidae) ticks, Tunisia. *Ticks Tick Borne Dis*. 2021;12(1):101606. doi:10.1016/j.ttbdis.2020.101606
23. Rehse-Küpper B, Danielová V, Klenk W, Abar B, Ackermann R. The isolation of Central European encephalitis (tick-borne encephalitis) virus from *Ixodes ricinus* (L.) ticks in southern-Germany. *Zentralbl Bakteriol Orig A*. 1978;242(2):148-155.
24. Müller W. Experimentelle Untersuchungen über das Vorkommen von Arboviren inUnterfranken. 2. Charakterisierung zweier isolierter Virusstämme [Experimental research on the existence of arboviruses in Lower Franconia-W. Germany. 2. Characterization of virus strains isolated from ticks]. *Zentralbl Bakteriol Orig*. 1970;14 (4):465-479.
25. Hannoun C, Chatelain J, Krams S, Guillon JC. Isolement, en Alsace, du virus de l'encéphalite à tiques (Arbovirus, groupe B) [Isolation, in Alsace, of the tick encephalitis virus (arbovirus, group B)]. *C R Acad Hebd Seances Acad Sci D.* 1971;272(5):766-768.
26. de Graaf JA, Reimerink JH, Voorn GP, et al. First human case of tick-borne encephalitis virus infection acquired in the Netherlands, July 2016 [published correction appears in Euro Surveill. 2016 Aug 25;21(34):]. *Euro Surveill*. 2016;21 (33):30318. doi:10.2807/1560-7917.ES.2016.21.33.30318
27. Pavlovsky EN. [On the natural focality of infectious and parasitic diseases]. *Vestn. Akad.* Nauk SSSR 1939;10:98–108.
28. Pavlovsky EN. Natural Nidality of Transmissible Diseases: With Special Reference to the Landscape Epidemiology of Zooanthroponoses. University of Illinois Press; 1966.
29. Chumakov MP, Naidenova GA. [The tick *Ixodes ricinus* as a vector of the tick-borne (spring-summer) encephalitis]. *Med Parazitol. Parazit Bolezni. (Moscow)* 1944;4:89-93.
30. Blaškovič D. The public health importance of tick-borne encephalitis in Europe. *Bull World Health Organ*. 1967;361967;36(Suppl 1):5-13.
31. Rampas J, Gallia F. The isolation of encephalitis virus from *Ixodes ricinus*. *Cas Lek Ces*. 1949;88:1179-1180.
32. Hubálek Z. History of Arbovirus Research in the Czech Republic. *Viruses*. 2021;13(11):2334. Published 2021 Nov 22. doi:10.3390/v13112334
33. Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. *Antiviral Res*. 2003;57(1-2):129-146. doi:10.1016/s0166-3542(02)00206-1
34. Ruzek D, Kaucka K. A brief tale of two pioneering moments: Europe's first discovery of Tick-Borne Encephalitis (TBE) virus beyond the Soviet Union and the largest alimentary

TBE outbreak in history. Ticks Tick Borne Dis. 2024;15 (3):102314. doi:10.1016/j.ttbdis.2024.102314
35. Helpert A, Sinnecker H. Ausgewählte Erhebungen zur Zeckenenzephalitis-Epidemie im Kreis Niesky, Bezirk Dresden, 1961 [Selective assessments on the tick-borne encephalitis epidemic in the County of Niesky Dresden, district 1961]. *Dtsch Gesundheitsw*. 1966;21(27):1277-1279.
36. Kunz C. Vaccination against TBE in Austria: the success story continues. *Int J Med Microbiol*. 2002;291 Suppl 33:56-57. doi:10.1016/s1438-4221(02)80011-x
37. Jones LD, Davies CR, Steele GM, Nuttall PA. A novel mode of arbovirus transmission involving a nonviremic host. *Science*. 1987;237(4816):775-777. doi:10.1126/science.3616608
38. Alekseev AN, Chunikhin SP. [The exchange of the tick-borne encephalitis virus between ixodid ticks feeding jointly on animals with a subthreshold level of viremia]. *Parazit Bolezni*. (Moscow). 1990:48-50
39. Labuda M, Jones LD, Williams T, Danielova V, Nuttall PA. Efficient transmission of tick-borne encephalitis virus between cofeeding ticks. *J Med Entomol*. 1993;30(1):295-299. doi:10.1093/jmedent/30.1.295
40. Labuda M, Austyn JM, Zuffova E, et al. Importance of localized skin infection in tick-borne encephalitis virus transmission. *Virology.* 1996;219(2):357-366. doi:10.1006/viro.1996.0261
41. Labuda M, Kozuch O, Zuffová E, Elecková E, Hails RS, Nuttall PA. Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology*. 1997;235(1):138-143. doi:10.1006/viro.1997.8622
42. Nuttall PA, Labuda M. Tick-borne encephalitis subgroup. In: *Ecological Dynamics of Tick-borne Zoonoses*. Eds Sonenshine DE, Mather TN: Oxford University Press; 1994;pp.351-391.
43. Lwoff A, Tournier P. The classification of viruses. *Annu Rev Microbiol*. 1966;20:45-74. doi:10.1146/annurev.mi.20.100166.000401
44. Fenner F, McAuslan BR, Mims CA. The Biology of Animal Viruses. New York – London: Academic Press; 1974.
45. Horzinek M. Togaviruses. *Ann Med Vet*. 1978;122:293-9.
46. De Madrid AT, Porterfield JS. The flaviviruses (group B arboviruses): a cross-neutralization study. *J Gen Virol*. 1974;23(1):91-96. doi:10.1099/0022-1317-23-1-91
47. Gaidamovich SY, Loginova NV. [Family Togaviridae]. In: *General and Particular Virology*, Moscow: Medicina. 1982;2:520pp
48. Westaway EG, Brinton MA, Gaidamovich SYa, et al. Flaviviridae. *Intervirology.* 1985;24(4):183-192. doi:10.1159/000149642
49. Thiel H-J, Collett MS, Gould EA, et al. Family Flaviviridae. In: Virus Taxonomy: Classification and Nomenclature. *Eighth Report of the International Committee on the Taxonomy of Viruses*. Eds Fauquet CM, et al. Amsterdam: Elsevier; 2005;979 -996
50. Chumakov MP. [Investigations on ultraviral encephalitides. VI. Transmission of tick-borne encephalitis virus to the progeny of ixodid ticks and the problem of natural reservoirs of this infection]. *Med Parazit (Moscow)*. 1944;6:38.
51. Clarke DH. Antigenic analysis of strains group B arthropod-borne viruses by antibody absorption. *J Exp Med*. 1960;1: 21 -32.
52. CLARKE DH. FURTHER STUDIES ON ANTIGENIC RELATIONSHIPS AMONG THE VIRUSES OF THE GROUP B TICK-BORNE COMPLEX. *Bull World Health Organ*. 1964;31 (1):45-56.
53. Chumakov MP, Rubin SG, Linev MB. [Three antigen types of tick -borne encephalitis virus, their dependence on arthropod vectors and geography]. *Problems of Medical Virology (Moscow*). 1975:371-5.
54. Votyakov VI, Protas II, Zhdanov VM. [*Western tick-borne encephalitis*]. Minsk: Belarus;1978;255pp.
55. Votyakov VI, Zlobin VI, Mishayeva NP. [*Tick-borne encephalitis of Eurasia: Ecology, Molecular epidemiology, Nosology and Evolution*]. Novosibirsk Nauka. 2002.
56. Pletnev AG, Yamshchikov VF, Blinov VM. Tick-borne encephalitis virus genome. The nucleotide sequence coding for virion structural proteins. *FEBS Lett*. 1986;200(2):317-321. doi:10.1016/0014-5793(86)81160-7
57. Pletnev AG, Yamshchikov VF, Blinov VM. Nucleotide sequence of the genome and complete amino acid sequence of the polyprotein of tick-borne encephalitis virus. *Virology*. 1990;174(1):250-263. doi:10.1016/0042-6822(90)90073-z
58. Mandl CW, Heinz FX, Kunz C. Sequence of the structural proteins of tick-borne encephalitis virus (western subtype) and comparative analysis with other flaviviruses. *Virology*. 1988;166(1):197-205. doi:10.1016/0042-6822(88)90161-4
59. Mandl CW, Heinz FX, Stöckl E, Kunz C. Genome sequence of tick-borne encephalitis virus (Western subtype) and comparative analysis of nonstructural proteins with other flaviviruses. *Virology*. 1989;173(1):291-301. doi:10.1016/0042-6822(89)90246-8
60. Rubin SG, Chumakov MP. New data on the antigenic types of tick-borne encephalitis (TBE) virus. *Zentralbl Bakteriol*. 1980;Suppl. 9:231-6.
61. Pogodina VV, Bochkova NG, Levina LS, et al. [Immunological and some etiology aspects of the Aina/1448 serotype of tick -borne encephalitis virus]. *Voprosy Virusol*. 1981;6:735-41.
62. Pogodina VV, Bochkova NG, Koreshkova GV. Properties of strains of tick-borne encephalitis virus, Aina/1448 serotype. *Voprosy Virusol*. 1981:741-5.
63. Gritsun TS, Frolova TV, Pogodina VV, Lashkevich VA, Venugopal K, Gould EA. Nucleotide and deduced amino acid sequence of the envelope gene of the Vasilchenko strain of TBE virus; comparison with other flaviviruses. *Virus Res*. 1993;27(2):201-209. doi:10.1016/0168-1702(93)90082-x
64. Zlobin VI, Mamayev LV, Dzhioev YP, Kozlova IV. [Genetic types of tick-borne encephalitis virus]. *J Infect Pathol* (Irkutsk). 1996;3:13-7.
65. Zlobin VI, Demina TV, Belikov SI, et al. [Genetic typing of tick -borne encephalitis virus based on an analysis of the levels of homology of a membrane protein gene fragment]. *Voprosy Virusol*. 2001;1:16-21.
66. Zlobin VI, Demina TV, Mamayev LV, et al. [Analysis of genetic variability of strains of tick-borne encephalitis virus by primary structure of a fragment of the membrane protein E gene]. *Voprosy Virusol*. 2001:13-6.
67. Ecker M, Allison SL, Meixner T, Heinz FX. Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia. *J Gen Virol.* 1999;80 (Pt 1):179-185. doi:10.1099/0022-1317-80-1-179
68. Grard G, Moureau G, Charrel RN, et al. Genetic characterization of tick-borne flaviviruses: new insights into evolution, pathogenetic determinants and taxonomy. *Virology.* 2007;361(1):80-92. doi:10.1016/j.virol.2006.09.015

69. Demina TV, Dzhioev YP, Verkhozina MM, et al. Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. *J Med Virol.* 2010;82(6):965-976. doi:10.1002/jmv.21765
70. Zlobin VI, Pogodina VV, Kahl O. A brief history of the discovery of tick-borne encephalitis virus in the late 1930s (based on reminiscences of members of the expeditions, their colleagues, and relatives). *Ticks Tick Borne Dis*. 2017;8(6):813-820. doi:10.1016/j.ttbdis.2017.05.001
71. Dobler, G. Die FSME – eine durch Zecken übertragene Zoonose mit wehrhistorischer und wehrmedizinischer Bedeutung. WMM. 2021;65(8): 302-307.
72. Dobler, G Gniel D. A History of Tick-Borne Encephalitis and Its Virus. In: Vasilakis N, Kramer L. D, eds. History of Arbovirology: Memories from the Field, Volume II: Virus Family and Regional Perspectives, Molecular Biology and Pathogenesis, pp. 453-467. Cham, Switzerland. *Springer Nature*. 2023

Chapter 4

TBE virology

Daniel Růžek, Kentaro Yoshii, Marshall E. Bloom and †Ernest A. Gould

Key points

- TBEV is the most medically important member of the tick-borne serocomplex group within the genus *Orthoflavivirus*, family *Flaviviridae*.
- Three antigenic subtypes of TBEV correspond to the 3 recognized genotypes: European (TBEV-EU), also known as Western, Far Eastern (TBEV-FE), and Siberian (TBEV-SIB).
  An additional 2 genotypes have been identified in the Irkutsk region of Russia, currently named
  TBE virus Baikalian subtype (TBEV-BKL) and TBE virus Himalayan subtype (Himalayan and "178-79" group; TBEV-HIM).
- TBEV virions are small enveloped spherical particles about 50 nm in diameter.
- The TBEV genome consists of a single-stranded positive sense RNA molecule.
- The genome encodes one open reading frame (ORF), which is flanked by untranslated (non-coding) regions (UTRs).
- The 5'-UTR end has a methylated nucleotide cap for canonical cellular translation. The 3'-UTR is not polyadenylated and is characterized by extensive length and sequence heterogeneity.
- The ORF encodes one large polyprotein, which is co- and post-translationally cleaved into 3 structural proteins (C, prM, and E) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).
- TBEV replicates in the cytoplasm of the host cell in close association with virus-induced intracellular membrane structures. Virus assembly occurs in the endoplasmic reticulum.
  The immature virions are transported to the Golgi complex, and mature virions pass through the host secretory pathway and are finally released from the host cell by fusion of the transport vesicle membrane with the plasma membrane.

Virus classification

Tick-borne encephalitis virus (TBEV) is the most medically important member of the tick-borne serocomplex group within the genus *Orthoflavivirus*, family *Flaviviridae* (from the Latin *flavus* – 'yellow', referring to the prototype virus, yellow fever virus).[1]

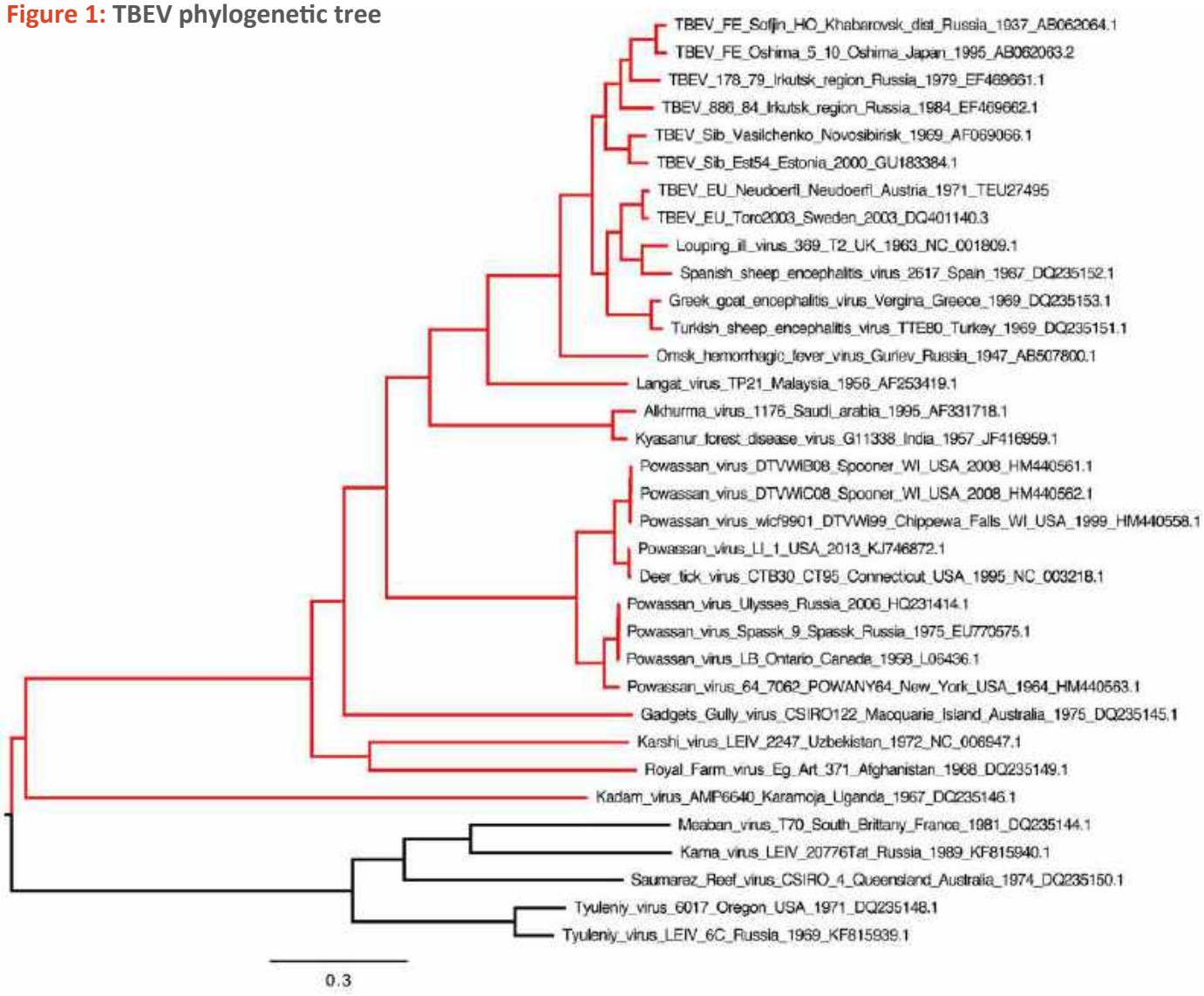
The genus *Orthoflavivirus* comprises over 70 virus species, many of which are important human pathogens.[2] Besides TBEV, these include mosquito-borne viruses such as dengue viruses, Japanese encephalitis virus, yellow fever virus, Zika virus, and many others. Virtually the entire human population lives where at least one flavivirus species is endemic.[2] Moreover, many orthoflaviviruses have recently expanded their endemic areas, being introduced to novel loci either on new continents (West Nile virus, Zika virus, etc.) or to areas with higher altitude or latitude (TBEV as an example).[3,4] For these reasons, flaviviruses pose an important threat to public and animal health. Moreover, they have high zoonotic potential because they can infect a broad range of hosts and vectors including domestic animals.

Most of the known flaviviruses are transmitted horizontally between hematophagous arthropods (ticks or mosquitoes) and their vertebrate hosts. They are therefore considered to be dual-host viruses. Depending on the recognized arthropod vector, they are divided into mosquito-borne or tick-borne viruses.

The term 'arbovirus' (an acronym from '**ar**thropod-**bo**rne **virus**') is non-taxonomic but is frequently used for viruses that cycle between vertebrates and arthropod vectors. However, not all orthoflaviviruses are arboviruses – some are vertebrate-specific (also called 'No known vector' and further divided into rodent-specific and bat-specific flaviviruses, with best-characterized representatives Rio Bravo and Modoc viruses)[5] while some are insect-specific.[6] These classifications reflect the adaptation of the viruses to particular invertebrate or vertebrate hosts, and modes of virus transmission in nature.

Tick-borne orthoflaviviruses (TBFVs) are further divided into mammalian and seabird TBFVs. While the seabird TBFV are non-pathogenic for humans, mammalian TBFV include several important human pathogens; in particular, TBEV, Kyasanur Forest disease virus (KFDV), Omsk hemorrhagic

Figure 1: TBEV phylogenetic tree



Phylogenetic tree illustrating the relationships between representative members of the TBEV complex (highlighted in red). Complete genome open reading frame sequences were retrieved from genbank and aligned using the gins option in mafft v7.266. The tree was constructed with RAxML v.8.2.9 using the GTR+G model of nucleotide evolution and 1,000 bootstrap replicates. The resulting tree was visualized and edited in Figtree v.1.4.1. All branches have maximum bootstrap support (not shown). The tree was midpoint rooted for visual purposes only. The lowest clade (black) contains members of the divergent seabird tick-associated virus complex (Meaban virus through Tyuleniy virus). We gratefully acknowledge the assistance of Dr John Pettersson (Zoonosis Science Center, Uppsala University, Sweden) who prepared and supplied the tree.

fever virus (OHFV), Powassan/Deer tick virus (POWV), and louping ill virus (LIV), which together with Langat virus (LGTV), for which there are no known cases of natural human disease, comprise a group known as the 'TBEV serocomplex' (Figure 1). All TBFVs are closely related antigenically and antibodies against one TBFV often cross-react with the other TBFVs, which should be taken into consideration when interpreting serological tests in areas where more than one TBFV co-circulates. The broadest cross-reactivity is seen in hemagglutination inhibition assays, whereas the highest specificity is seen in neutralization assays.[7]

Although all TBFVs are closely related genetically and antigenically, they cause diverse clinical manifestations in humans: OHFV and KFDV (including a subtype of this virus, Alkhurma hemorrhagic fever virus) induce hemorrhagic fever syndromes, while the others cause neurological disease. Importantly, the hemorrhagic fever associated TBFVs and encephalitogenic TBFVs do not form separate phylogenetic lineages and no specific determinants in the genomes of these viruses have been associated with particular disease manifestations.[8,9]

Three main antigenic subtypes of TBEV correspond to the 3 recognized genotypes: Western, also known as European (TBEV-EU; previously Central European encephalitis; prototype strain Neudoerfl), Far Eastern (TBEV-FE;

previously Russian spring-summer encephalitis; prototype strain Sofjin), and Siberian (TBEV-Sib; previously Western Siberian encephalitis; prototype strains Zausaev and Vasilchenko).[10] Two additional lineages; i.e., "178-79" and "886-84 group", named as Baikalian TBEV (TBEV-Bkl) respectively, have been identified in Eastern Siberia and proposed as TBEV subtypes.[11] The geographical distribution and clinical significance of these newly identified genotypes remains to be determined. However, some studies indicate that 0.6-6% of TBEV strains circulating in Eastern Siberia might belong to these new genotypes.[11] Another new potential TBEV subtype (Himalayan – TBEV-Him) was identified recently in wild rodents in Qinghai-Tibet Plateau in China.[12]

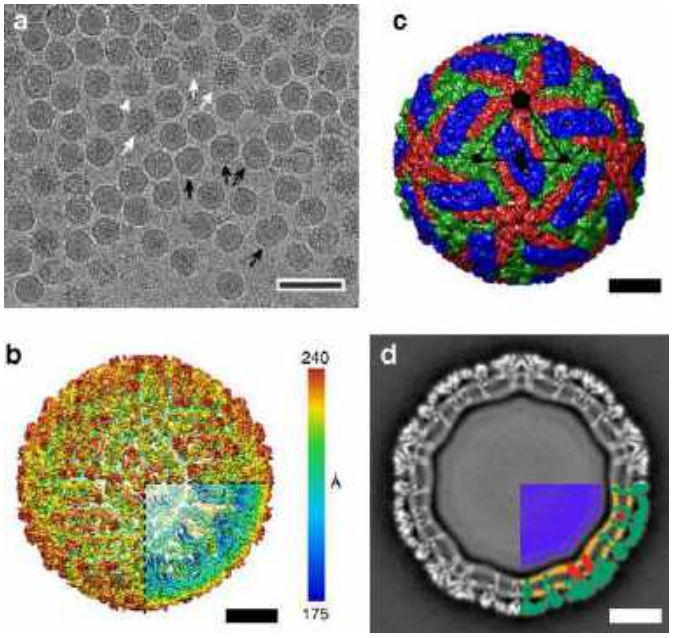
Comparison of the complete coding sequences of all recognized TBFV species led to a new taxonomic proposal, viz. the assignment of TBEV and LIV to a single species (TBEV) encompassing 4 viral types; i.e., Western TBEV (TBEV-EU); Eastern TBEV (TBEV-Sib and TBEV-FE); Turkish sheep TBEV, including Greek goat encephalitis virus subtype; and Louping ill TBEV, the latter having Spanish, British, and Irish subtypes.[13] This classification was supported by the fact that, based on antigenic properties, the European TBEV strains are more closely related to LIV than to TBEV-FE and TBEV-Sib strains.[14,15]

All TBFVs are thought to have shared a common ancestor, which diverged from mosquito-borne flaviviruses in Africa less than 5,000 years ago.[16-18] However, some studies suggest that this split might have occurred as long as 50,000 years ago.[19] The descendant TBFV species evolved and spread through Asia and then more recently westwards through Europe as they adapted to different host and tick species. [16-18] In comparison with mosquito-borne flaviviruses, TBFVs evolved nearly twice as slowly, primarily due to the long life-cycle of the Ixodes tick vector.[16,20,21] Overall, it was concluded that there is a direct correlation between genetic and geographic distance of individual TBFV species[16,22] and, furthermore, that the evolution and dispersal of these viruses is relatively slower than that of the mosquito-transmitted viruses. In addition, the evolution is not significantly influenced by migratory birds or international trade.[18]

Virion structure and morphology

Infectious TBEV virions are small spherical particles about 50 nm in diameter with no obvious distinct projections. The mature virions contain an electron-dense core approximately 30 nm in diameter which is surrounded by a lipid bilayer (Figure 2).[23,24] The nucleocapsid core consists of single-stranded positive-polarity genomic ribonucleic acid (RNA) molecule (11 kb) and the capsid protein C (12 kDa). The surface of the lipid membrane incorporates an envelope glycoprotein (E, 53K) and a membrane glycoprotein (M, 8K) (Figure 2).

Figure 2: TBEV particles



A. Cryo-EM micrograph of TBEV particles. The sample contained mature, immature (white arrows), half-mature (white arrowheads), and damaged (black arrows) particles. Scale-bar, 100 nm

B. B-factor sharpened electron-density map of TBEV virion, rainbow-colored according to distance from particle center. Scalebar, 10 nm.

C. Molecular surface of TBEV virion low-pass filtered to 7 Å. The three E-protein subunits within each icosahedral asymmetric unit are shown in red, green, and blue. Scalebar, 10 nm.

D. Central slice of TBEV electron density map perpendicular to the virus 5-fold axis. The virus membrane is deformed by the transmembrane helices of E-proteins and M-proteins. The lower right quadrant of the slice is color-coded as follows: nucleocapsid—blue; inner and outer membrane leaflets—orange; M-proteins—red; E-proteins—green. Scalebar, 10 nm.

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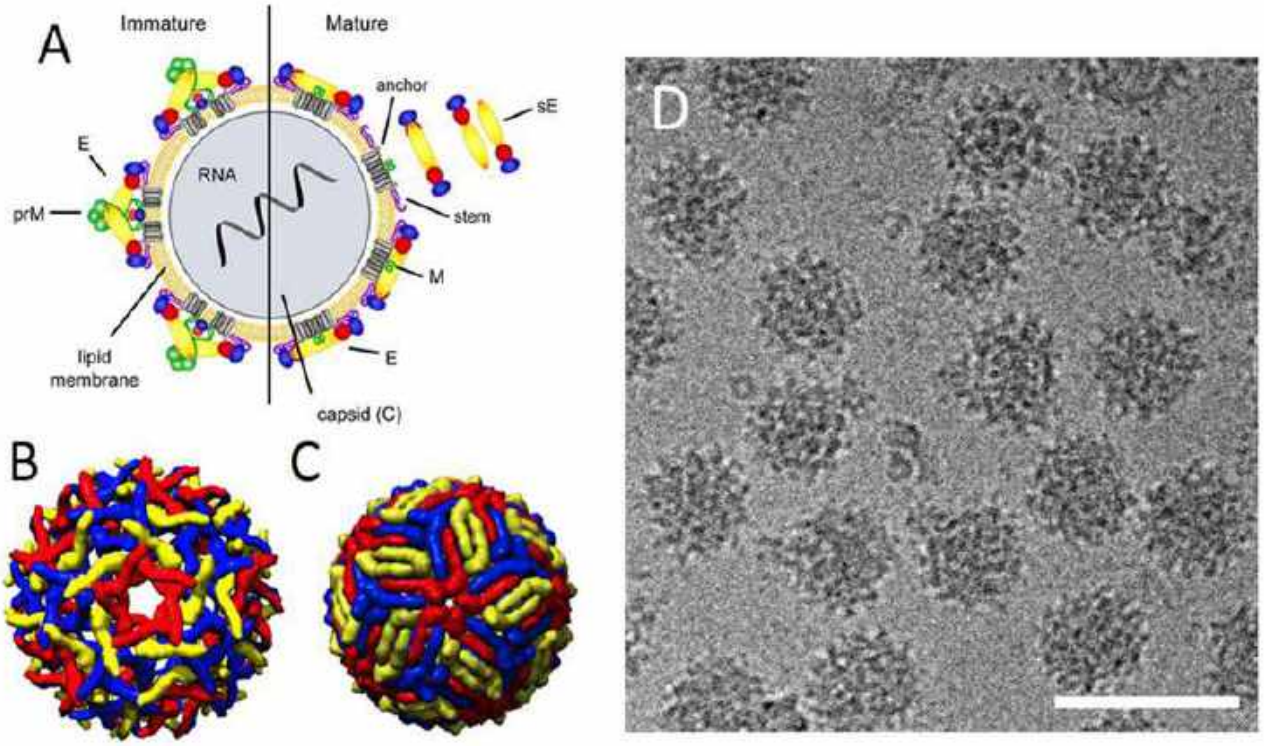
The glycosylated E protein is also a major antigenic determinant of the virus and induces immune responses in infected mammalian hosts. It also contains the sites for virus binding to receptors on the surface of susceptible host cells and subsequent pH-mediated fusion of the viral E protein with endosomal membranes during entry of viral RNA into the cell.

In the mature infectious virions, the M protein has been proteolytically cleaved from the precursor (pr)M protein. This post-translational process occurs during the maturation of nascent viral particles within the secretory pathway and immediately before release of the infectious virions from the infected cell. In immature non-infectious particles, prM and E proteins form hetero-dimers and

Figure 3



A. *Schematic model of a flavivirus particle. Left panel: immature virion, right panel: mature virion. The surface of immature particles consists of 60 spikes composed of trimers of prM-E heterodimers. Mature particles are formed after prM cleavage and contain 90 E homodimers. (From[25] (CC BY)).*

B. *Pseudoatomic cryo-EM reconstruction model of the immature flavivirus particle (PDB: 2OF6).*

C. *Pseudoatomic cryo-EM reconstruction model of the mature flavivirus particle (PDB: 3J0B).*

D. *Cryo-EM micrograph of immature TBEV particles (kindly provided by Tibor Füzik and Pavel Plevka, with permission). Scalebar, 100 nm.*

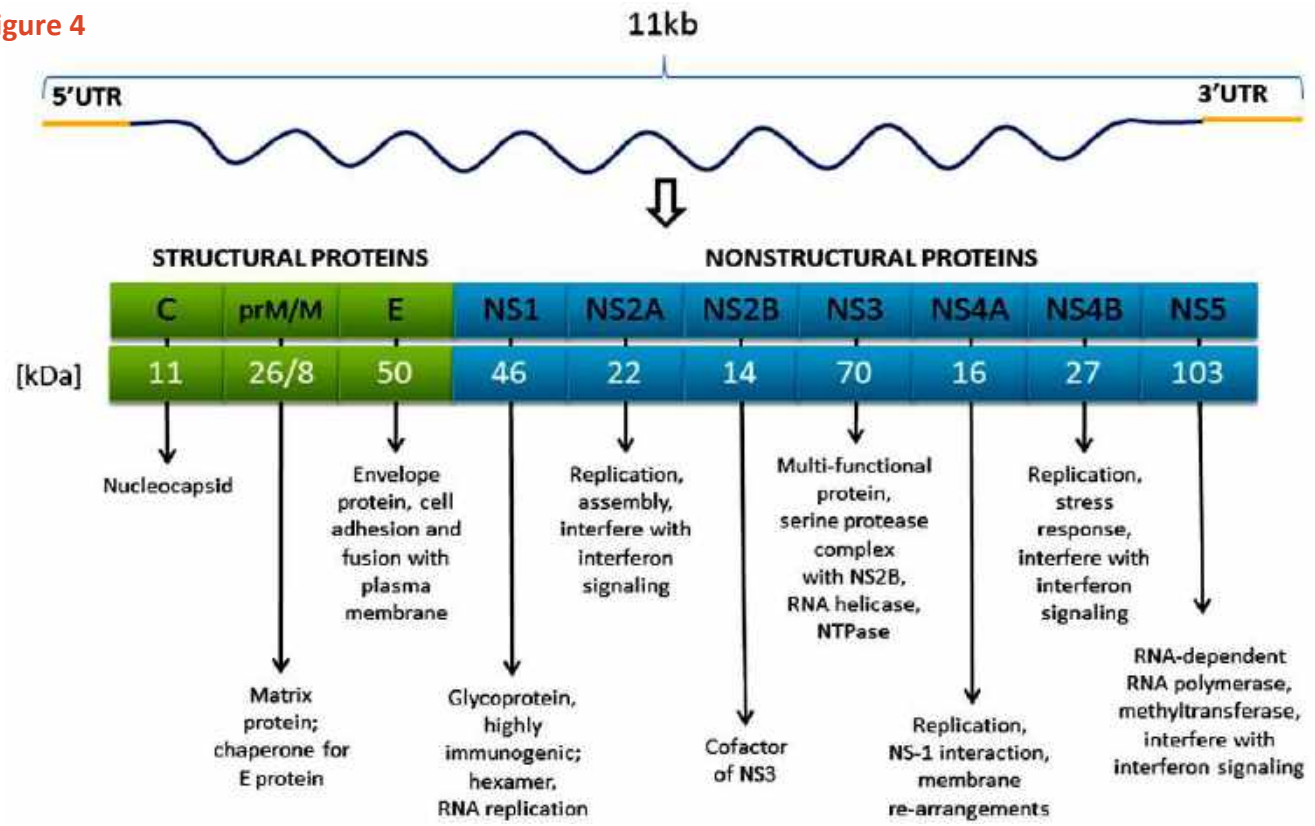
exist as trimers covering the virion surface. At this stage, the pr part of prM occludes the fusion domain of the E glycoprotein, preventing premature fusion with cell membranes within the secretory pathway (Figure 3).

In the trans-Golgi compartment, the pr is cleaved from prM by a cell furin-like protease; this is followed by the conformational change, rotation, and rearrangement of E proteins from 60 antiparallel trimers into 90 anti-parallel dimers, forming an unusual 'herring-bone' pattern with icosahedral symmetry and resulting in the viral particles being mature and fully infectious. However, the efficiency of prM cleavage varies for different flaviviruses; cleavage is therefore not always absolute. Thus, immature particles may also be released as a proportion of the infectious/non-infectious virus pool.[23]

The structure of purified mature TBEV particles has been determined at near atomic resolution of 3.3 (strain Kuutsalo-14) or 3.9 Å (strain Hypr) by reconstruction of cryo-electronmicroscopic images (Figure 2).[23,24] These studies revealed a relatively smooth outer surface of the particle, and E and M proteins organized in a similar manner to that in other flaviviruses. The surface of the TBEV virion is covered with small protrusions formed by glycans attached to the E-protein molecules.[23,24] Both E-proteins and M-proteins are anchored in the virion membrane, each by two trans-membrane helices. Viral envelope membrane is not spherical; instead the shape of the membrane closely follows the inner surface of the protein envelope and is deformed by insertions of the trans-membrane helices of E-proteins and M-proteins.[23]

Cryo-electronmicroscopic analysis was employed to explore the structure of three immature TBEV strains: Hypr, Neudoerfl, and Kuutsalo-14. The immature TBEV particle exhibited a diameter of 56 nm, with surface glycoproteins organized into characteristic spikes reminiscent of immature flaviviruses. The topology and domain assignment of prM in immature TBEV closely resembled that of the mosquito-borne Binjari virus, however was significantly different from other immature flavivirus models.[26] Recombinant sub-viral particles (RSPs) are of T-1 icosahedral symmetry formed by 30 E protein dimers. They have the same antigenic properties as wild-type virus. They can be used for vaccination purposes and represent an

Figure 4



Genome organization of TBEV and processing pathways of the polyprotein. A schematic representation of the TBEV genome with the 5' and 3' non-translated regions (NTRs) is shown in the top; the translation products are given below (adapted from[38], with permission).

established model system for flavivirus membrane fusion because they have fusion characteristics similar to those of infectious virions.[27]

Viral genome

The nucleocapsid is formed from a single viral RNA genome and multiple copies of the C protein. The RNA binding domains of the C protein molecules are located at their N- and C-termini and are separated by hydrophobic regions. The nucleocapsid is less ordered and as for other flaviviruses, no discernible symmetry was detected in cryoelectron microscopic reconstructions.[23] Instead, the C protein is arranged in a cage-like structure surrounding the viral genome. The icosahedral symmetry is, therefore, directed by surface proteins rather than by the nucleocapsid protein.

In addition to mature virions, smaller (approximately 14 nm in diameter) non-infectious particles are released from the infected cells. These particles lack nucleocapsid and consist of E and M proteins only; they are called sedimenting (70S) hemagglutinin (SHA).

Similar RSPs of a slightly larger size (approximately 30 nm in diameter) can be produced by cells expressing only prM and E proteins.[28]

The TBEV genome consists of a single-stranded positive sense RNA molecule, approximately 11 kilobases in length. The genome encodes 1 open reading frame (ORF) of over 10,000 bases, which is flanked by untranslated (non-coding) regions (UTRs). The ORF encodes 1 large polyprotein of approximately 3,400 amino acids, which is co- and post-translationally cleaved by viral and cellular proteases into 3 structural proteins (C, prM, and E) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5)[29] (Figure 4). A second short upstream ORF is present in the 5'-UTR of some TBEV strains. However, no protein encoded by this ORF has been found in TBEV-infected cells, indicating that it is neither expressed nor present at undetectable concentrations, suggesting that this additional ORF has either a minor or no biological role in the TBEV replication cycle.[30] A common feature of all flavivirus genomes is their high purine content and low GC and UA doublet frequencies, which may influence translation of the genome and/or reflect the requirement for flaviviruses to grow in different hosts and cell types; however, a specific role for this unique genomic characteristic remains unclear.[31] A replication enhancer element (REE) has been found within the capsid gene of TBEV. The REE folds as a long stable stem-loop (designated SL6), conserved among all TBFVs. Although SL6 REE is not essential for growth in tissue culture, it acts to up-regulate virus replication.[32]

In addition to coding for the polyprotein, the genome has

RNA structural motifs that play a crucial role in the viral life-cycle.[33] In particular, the untranslated regions form secondary stem-loop structures that probably serve as cis-acting elements for genome replication, translation, and/or packaging.[33-36] The 5'-UTR contains a type 1 cap (m7GpppAmG), followed by a conserved stem-loop structure. The 3'-UTR is not polyadenylated and is characterized by extensive length and sequence heterogeneity.[37] This region of the viral genome can be divided into 2 parts: a proximal (localized behind the 'stop' codon of the ORF) and a distal ('core', the 3' terminus itself). The distal part of this region (approximately 340 nt) is highly conserved, whilst the proximal part is a noticeably variable segment with common deletions and insertions.[34-36]

RNA structural models demonstrate that flavivirus genomes, including TBFVs, form dsRNA cyclization stems or 'panhandles' at their 5'- and 3'-termini. The 'panhandle' of the TBFV group (5'CYCL) is formed by a perfectly conserved continuous 21-nucleotide sequence located in the 5'-UTR. The 5'-UTR and 3'-UTR sequences directly involved in cyclization are located downstream from the 5' Y-shaped structure and the 3' long stable hairpin, respectively. The terminal 5'-UTR and 3'-UTR regions not involved in cyclization also show homology, suggesting they are evolutionary remnants of a long cyclization domain that probably emerged through duplication of 1 of the UTR termini.[39]

5'-untranslated region

The 5'-UTR is 132 nucleotides long in most TBEV strains and its secondary structure is highly conserved among different TBEV strains.[36] Common secondary structures in this region can also be found among different flaviviruses, although the sequence is diverse.[31] The function of these conserved secondary structures is probably related to translation of the genome and in the complementary RNA strand serves as a site for initiation of synthesis of positive-stranded RNA molecules.[31]

The folding of 333 nt as a reverse complement of the 5'-end (3'-end of the negative-stranded RNA) of TBEV revealed a stem-loop pattern different from the 3'-UTR of positive-stranded RNA. However, 2 nucleotide regions in these 3'-ends are identical and conserved among all TBFVs. One of these, an 11-nt region, forms a loop within the folding pattern at the 3'-end of the negative strand and a stem at the 3'-UTR of the positive strand.[34] These structural motifs at the 5' and 3'-UTR termini could be recognition sites for viral RNA polymerase.[34]

The alignment of the 5'-UTRs of different TBFVs demonstrated an internal hypervariable domain in which Powassan virus has a deletion of 27 bases.[34] The predicted folding of the 5'-UTR sequence produces a stem-loop structure similar for all TBFV, and the 27 nt deletion in the Powassan virus has no effect on the typical 5'-UTR folding.[34] This indicates that the length of stem-loop structure 3 is not critical for virus infectivity.[34]

3'-untranslated region

The alignment of 3'-UTRs of all TBFVs revealed 2 nucleotide regions, 1 about 340 bases in length, of conserved sequence at the extreme 3'-end (designated C3'- UTR) and another hypervariable region placed between the stop codon and the C3'-UTR where even strains from a single species showed deletions of different lengths,[34] whereas some TBEV strains have a 30-250 nt long poly(A) sequence in this region.[39] Deletions or a poly(A) sequence insertion in the variable region were found in strains passaged in mammalian cell culture,[40] and deletions of different lengths were also observed in TBEV strains isolated from human patients.[41-43] It was suggested that the hypervariable region could act as a spacer separating the folded 3'-UTR structure from the rest of the genome that might be necessary for efficient binding of viral RNA polymerase and cellular factors involved in transcription 34 and may play a role in the natural transmission cycle of TBEV.[44-46] A short poly(A) tract is genetically more stable compared with the virus having a long poly(A) tract.[45]

Previous studies reported that the variable region plays no role in viral replication and virulence for laboratory mice.43 However, recent studies revealed that partial deletions and poly(A) insertion in the variable region increases TBEV virulence in the mouse model.[45,46] These data suggested that the variable region of the 3'-UTR might impact neurovirulence and function as a critical virulence factor.[45,46]

All TBFVs share a common folding pattern of secondary structures at the C3'-UTR position. RNA in this region is predicted to fold into a 3' stem-loop and it contains conserved sequence elements. However, these structures are different from those observed in mosquito-borne flaviviruses.[34] Indeed, some RNA sequences within the 3'-UTR clearly distinguish mosquito-borne from TBFVs.[37,39] Modifications within the 3'-UTR of TBEV that affect the conserved structural motifs are known to attenuate the virus without altering their antigenic specificity. Modification of this region might form the basis for live-attenuated vaccines and/or for antiviral therapeutics.[47,48]

Short direct repeat sequences (20-70 nucleotides long) in the 3'-UTR were found to be conserved for each flavivirus group or subgroup.[48] Four R1 repeats, two R2 repeats, and two R3 repeats, approximately 23, 26, and 70 nucleotides long, respectively, apparently arranged randomly, have been described in the 3'-UTR of the TBFVs.[34,47,48] These short repeats apparently originated from at least 6 long repeat sequences (LRS) approximately 200 nucleotides in length, arranged in tandem. Four of these LRS are present in the 3'-UTR and 2 in the 3' region of the ORF. Thus, it seems that evolution of the 3'-UTR and probably the ORF occurred through multiple duplications of LRS that form the

basis for the development of the functionally important secondary RNA structures in the 3'-UTR. Subsequent formation of extended RNA domains evolved as promoters and enhancers of virus replication determined by the selective requirements of the vertebrate and invertebrate hosts.[39,47]

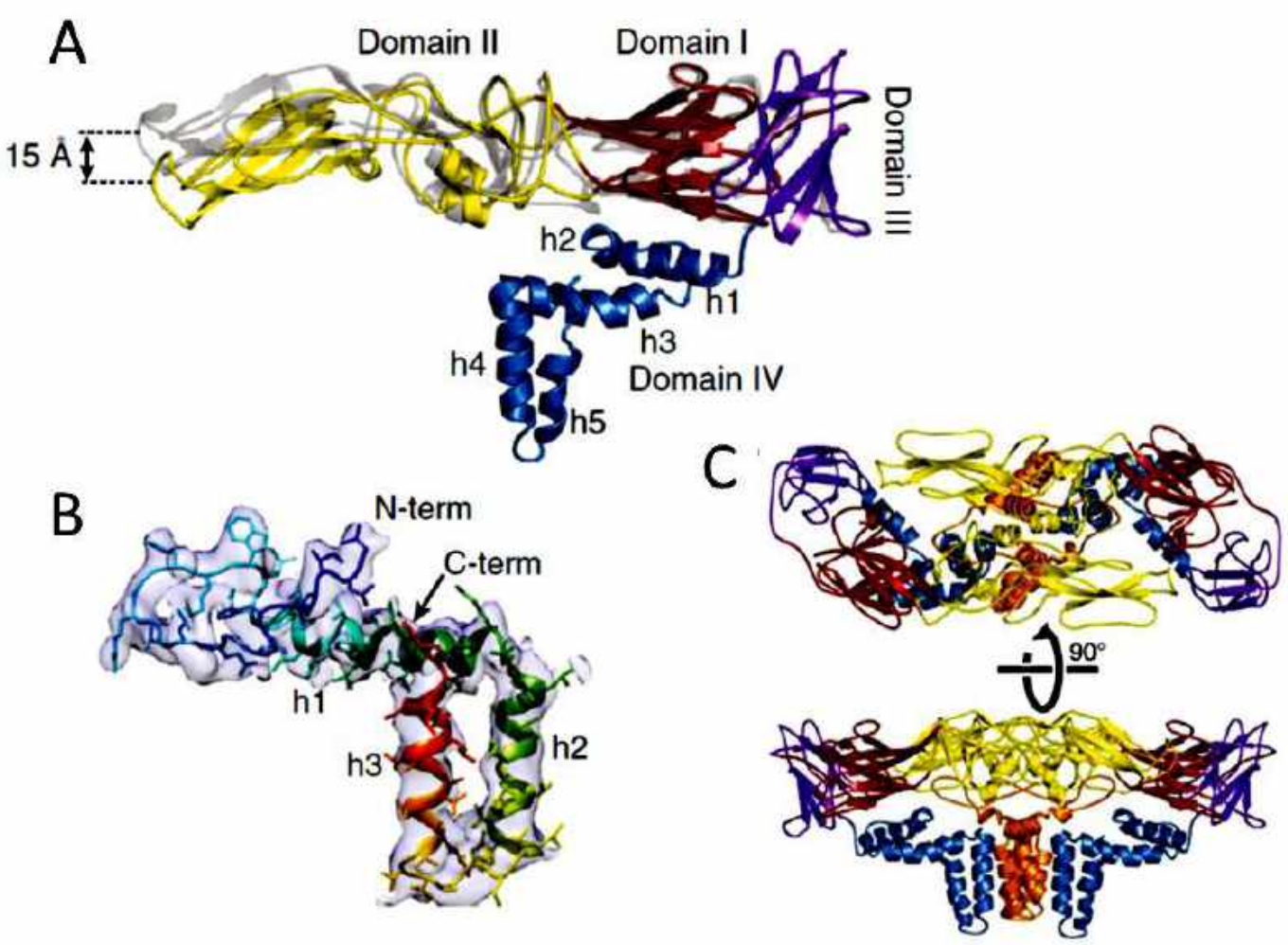
Flaviviruses, including TBFVs, are known to produce unique non-coding subgenomic flaviviral RNA (sfRNA), which is derived from the 3'-UTR. SfRNA results from incomplete degradation of viral RNA by the cellular 5'-3' exoribonuclease XRN1.49 The exoribonuclease activity stops at the highly ordered RNA secondary structures at the beginning of the 3'-UTR. SfRNA is involved in modulating multiple cellular pathways; e.g., inhibiting antiviral activity of type I interferons (IFN) and RNAi pathways, facilitating viral pathogenicity.[50]

Proteins encoded by the virus

Structural proteins

C (Capsid) protein is a relatively small (11 kDa), basic, and highly positively charged protein with low sequence homology between different flaviviruses.[51] Within the ORF

Figure 5



A. Superposition of cryo-EM (colored) and X-ray (gray) E-protein structures. Domain I is colored in red, domain II in yellow, domain III in violet, and domain IV in blue.

B. M-protein rainbow-colored from N-terminus in blue to C-terminus in red with electron density map shown as semi-transparent surface. The M-protein consists of an extended N-terminal loop followed by perimembrane (h1) and two transmembrane helices (h2 and h3).

C. Heterotetramer of two E-proteins and two M-proteins. E-proteins are colored according to domains, and M-proteins are shown in orange.

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that encodes the single polyprotein precursor of all structural and non-structural proteins, protein C is located at the amino-terminal end and is thus synthesized first during translation. The protein interacts with viral RNA genomes and represents a structural component of the nucleocapsid. Despite the low sequence homology among diverse flaviviruses, regions of hydrophobic and hydrophilic amino acids are conserved. The C-terminal hydrophobic domain (this domain is cleaved from mature C protein) is preceded by a hydrophilic region, and a central hydrophobic region. The N-terminus contains a hydrophilic region.[31] The central hydrophobic region mediates membrane association of the protein and the charged residues that cluster at the hydrophilic N- and C-termini presumably mediate the interaction of the protein with viral RNA.[50,51] In flavivirus infected cells, it was found that the mature C protein accumulates on the surface of endoplasmic reticulum (ER)-derived organelles named lipid droplets. The lipid droplets may play multiple roles during the viral life-cycle; i.e., they could sequester the flaviviral capsid protein early during infection and provide a scaffold for genome encapsidation.[52]

The introduction of various deletions into the TBEV genome that removed parts of the central hydrophobic domain of protein C revealed a remarkable structural and functional flexibility of this protein.[53] TBEV mutants carrying deletions in C that extended from residue 28 up to residue 43 were viable in cell culture. The mutants produced substantial amounts of subviral particles lacking capsid, and the deletions impaired the assembly or stability of the virions.[53] However, virus viability was affected when the deletions extended up to residue 48 or when the full hydrophobic domain was removed.[53] Interestingly, these deletions led to spontaneous mutations in other regions of the C protein that generally increased the C protein hydrophobicity and restored infectivity of the virus.[54]

prM protein is a glycosylated precursor of the membrane **protein M**. The carboxyl terminus of C protein serves as an internal signal sequence element leading the structural protein prM into the membrane of the endoplasmic reticulum. The viral protease NS2B-NS3 cleaves this signal sequence, releasing the N-terminus of prM protein.[53] The prM protein shows a chaperone-like activity during the envelope protein E folding.[55] The N-terminus of the pr is mainly hydrophilic and, in TBEV, contains a single N-linked glycosylation site that appears to have an important role during virion assembly and release.[31,51,56] Six cysteine residues, all disulphide-bridged, are highly conserved. The C-terminal region contains an ectodomain and 2 potential membrane-spanning domains.[57] The cleavage of prM into pr and M occurs in the Golgi complex and is mediated by furin or a furin-like enzyme[58,59] leading to a conversion from immature to mature fusogenic and fully infectious viral particles (Figure 3).[58] The pr fragment is then secreted.[51] A conserved region in the prM protein is a critical molecular determinant for the assembly and secretion of the virus.[60] The M-protein consists of an N-terminal loop and three helices (Figure 5B). The first helix is situated as a perimembrane and the last two as trans-membranes; however, the M-protein is not exposed at the surface of the viral particle due to its small size and close association with the viral envelope membrane.[23] Two M-proteins together with two E-proteins form a compact heterotetramer, which is the main building block of the virion, formed by head-to-tail dimerization of two E-M heterodimers (Figure 5C).[23]

The E protein contains the major viral antigens and is the main target for neutralizing antibodies (although antibodies directed against prM/M and NS1 also induce some protective immunity). Moreover, the E protein is responsible for specific binding to a cellular receptor and penetration of the virus into the host cell. It is also believed to be a main determinant of TBEV virulence.[61] The three-dimensional structure of the E protein was studied at the resolution of 2.0 Å by X-ray crystallography[62] (Figure 5). Comparison of the crystal structure of E protein and the structure of E protein in the virion observed by cryoelectron microscopy revealed root-mean-square deviations (RMSD) of 1.7 Å for the corresponding Cα atoms.[23] The most important difference is in the positioning of domains I–III relative to each other. Whereas in the crystal structure the domains I, II, and III are arranged in a line, in the virion the tip of domain II is bent 15 Å towards the virus membrane (Figure 5A).[23] Such a bending of the ectodomain in the virion prevents induction of premature membrane fusion mediated by the E protein.[23] The structure of TBEV E protein was found to be highly similar to E1 glycoprotein from a distantly related virus, Semliki Forest virus (family *Togaviridae*). These proteins were defined as class II virus fusion proteins, distinct from previously characterized class I fusion proteins such as hemagglutinin of influenza virus.[51]

The protein forms 2 monomers anchored in the membrane by their distal parts at physiological pH. After virus uptake by receptor-mediated endocytosis into host cells, acidic pH in endosomes triggers irreversible changes in the E protein structure including its re-arrangement to trimeric forms. This leads to the initiation of the fusion process between the viral and endosomal membrane.[63] Conserved histidines in the E protein function as molecular switches and, by their protonation at acidic pH, control the fusion process.[64]

Each E protein monomer is composed of 3 domains (I- III). Domain I is located in the central part of the protein. It is formed by 8 antiparallel beta sheets, contains the *N*-terminus of the protein, 2 disulphide bridges, and an *N*-glycosylation site. Mass spectrometric analysis was employed to examine the variations in *N*-glycosylation profiles of TBEV cultured in human neural and tick cells. The predominant asparagine-linked oligosaccharides identified on the surface of TBEV derived from human neuronal cells included high-mannose glycan with five mannose residues

($Man_5GlcNAc_2$), a complex biantennary galactosylated structure with core fucose ($Gal_2GlcNAc_2Man_3GlcNAc_2Fuc$), and a group of hybrid glycans with the composition $Gal_{0-1}GlcNAc_1Man_{3-5}GlcNAc_2Fuc_{0-1}$. In contrast, the N-glycosylation profile of TBEV grown in tick cells revealed paucimannose ($Man_{3-4}GlcNAc_2Fuc_{0-1}$) and high-mannose structures containing five and six mannose residues ($Man_{5-6}GlcNAc_2$) as the major glycans present on the viral envelope protein.[65] The function of E protein glycosylation was investigated using recombinant TBEV with or without the E protein *N*-linked glycan. The results suggested that glycosylation of the TBEV E protein is critical for the intracellular secretory process in mammalian cells but cleavage of the *N*-linked glycan after secretion did not affect virion infectivity in these cells. On the other hand, E protein glycosylation seems to play no significant role in virus reproduction in ticks.[66]

Domain II is formed of 2 long loops that extend out of domain I and form a finger-like structure. Domain II contains a number of beta sheets and 3 disulphide bridges.[62,67] Part of the domain responsible for the fusion of viral envelope with the membrane of the endosome is called the fusion peptide; this peptide mediates insertion of the E protein into the endosomal membrane resulting in fusion of viral envelope with the membrane of the endosome.[68] The initiation of fusion is crucially dependent on the protonation of 1 of the conserved histidines (His323), which works as a pH sensor at the interface between domains I and III of E, leading to the dissolution of domain interactions and to the exposure of the fusion peptide.[64]

Domain III has the typical fold of an immunoglobulin constant (IgC) molecule.[67] It contains a beta barrel composed of 7 antiparallel beta sheets. The lateral part of domain III is believed to be responsible for binding to a specific cellular receptor.[62]

Amongst the most conserved parts of the E protein, there are 12 cysteine residues forming 6 disulphide bridges with conserved localization in common with all known flaviviruses.[69]

The E protein is also considered to be a major determinant of TBEV virulence. Amino acid substitutions in E protein often cause a decrease in neuroinvasiveness, although neurovirulence is usually not reduced.[70] The highest number of attenuating mutations in the E protein was revealed in the domain that probably binds to specific cell receptors and participates in membrane fusion.[63] A number of identified substitutions causing escape of the virus from the neutralizing effect of monoclonal antibodies,[71] deficiency in the ability to agglutinate erythrocytes,[72] and a change in virus growth properties in cell cultures, mice, or ticks,[61,73-76] have been described.

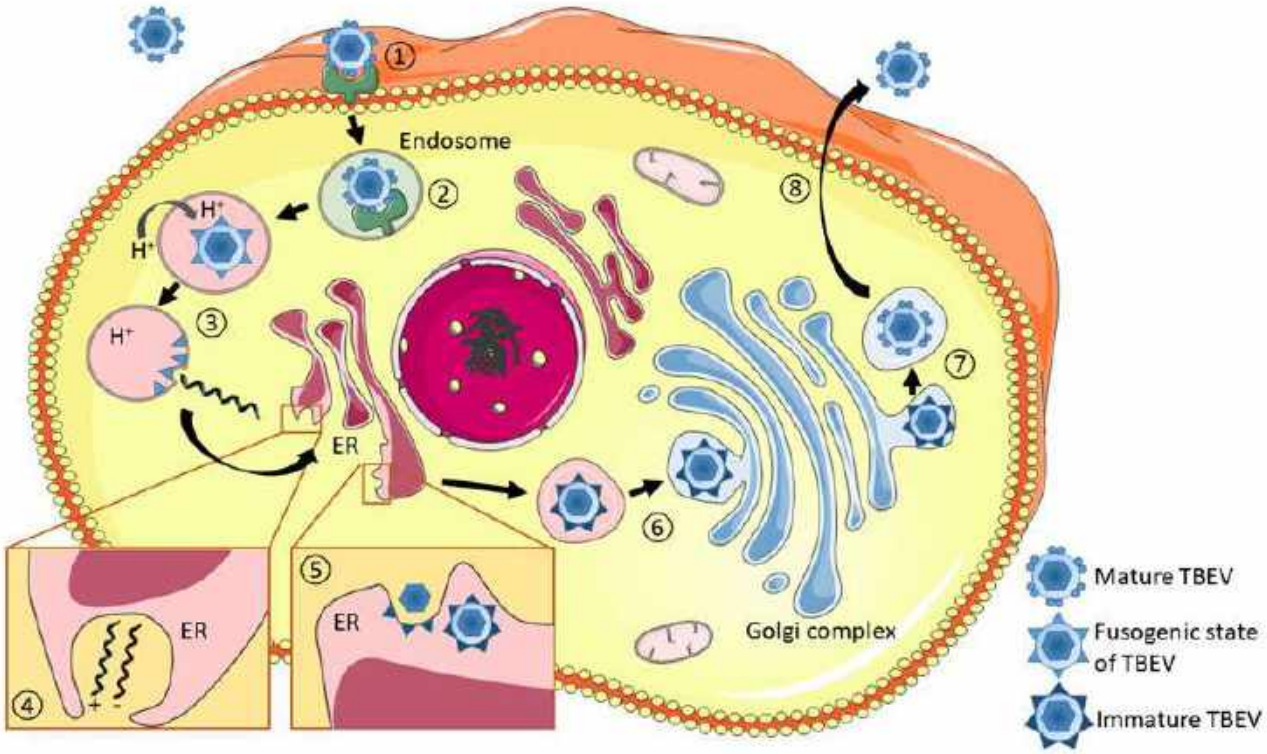
The E protein serves as the primary target and inducer of neutralizing antibodies.[27] Neutralizing antibodies can be elicited by any of the three domains of the E protein, with numerous sites across the particle's surface having the potential to induce potent neutralizing antibodies. These epitopes may include quaternary epitopes, which consist of residues from adjacent domains or adjacent E proteins on the surface.[23,77] The neutralization process by antibodies can occur through inhibition of the interaction between the E protein and the receptor on the host cell surface. Alternatively, it can involve the inhibition of post-entry processes, such as blocking the fusion of the viral envelope with the endosomal membrane. This fusion process necessitates significant reorganization of the E protein domains, which antibodies can impede, thereby preventing viral entry and infection.[23,78]

Recently, highly potent human monoclonal antibodies that target the E protein domain III have been discovered. These antibodies show great promise for use as post-exposure prophylaxis or early therapeutics for TBE.[79] Through the selection of TBEV escape variants by culturing the virus with increasing concentrations of the antibody, it was determined that a combination of two amino acid substitutions in the E protein is necessary. One substitution occurs in domain III, while the other occurs in domain II. The domain III substitution impairs formation of a salt bridge critical for antibody-epitope interaction. The substitution in domain II is not located within the antibody epitope, but it is believed to induce quaternary rearrangements of the virus surface. This rearrangement occurs due to the repulsion of positively charged residues on the adjacent domain I. Consequently, both resistance mechanisms—a substitution in domain III and one in domain II—are required for TBEV to evade neutralization by this antibody.[80]

Antibodies that target the fusion loop of the E protein, a region highly conserved among flaviviruses, often exhibit cross-reactivity across multiple flavivirus species. However, they typically do not neutralize TBEV. This is attributed to their recognition of cryptic epitopes that are not typically exposed on the surface of mature virions. Consequently, these antibodies are unable to access the endosomes where viral fusion occurs, thus limiting their neutralization capability against TBEV.[81]

A unique mechanism of TBEV infection enhancement by antibodies against E protein, which operates independently of interactions with Fcγ receptors, has been described. This mechanism involves the binding of a specific antibody to the E protein on the viral surface, particularly recognizing an epitope located at the interface of the dimeric envelope protein E. This binding event triggers the dissociation of E protein dimers and exposes the fusion loop, facilitating the exposure of a structural element that interacts with the lipids of the cellular plasma membrane. Consequently, this process enhances viral infection by promoting viral entry into host cells.[82]

Figure 6



Schematic illustration of the TBEV life cycle. (1) Infection begins with the binding of viral particles to specific cell-surface receptors, which have not yet been unequivocally identified. (2) Viral particles enter cells via endocytic pathway. (3) Low pH in the late endosome triggers conformational changes in the E proteins, leading to rearrangement of dimers to trimeric forms (fusogenic state) and the subsequent fusion of the viral envelope with endosomal membranes, which leads to virion uncoating. (4) Replication of the virus occurs through the synthesis of anti-sense (negative) RNA, which serves as the template for genome RNA production. Replication complexes are localized in membranous structures within the endoplasmic reticulum (ER). (5) Assembled nucleocapsids acquire lipid envelopes by budding into the ER lumen. (6) Immature particles pass through the Golgi complex. (7) Maturation takes place in the trans-Golgi network, involving the cleavage of prM and the reorganization of E proteins into fusion-competent homodimers, leading to a change from spiky immature to smooth mature particles. (8) Mature particles are transported in cytoplasmic vesicles and released into the extracellular space by exocytosis.

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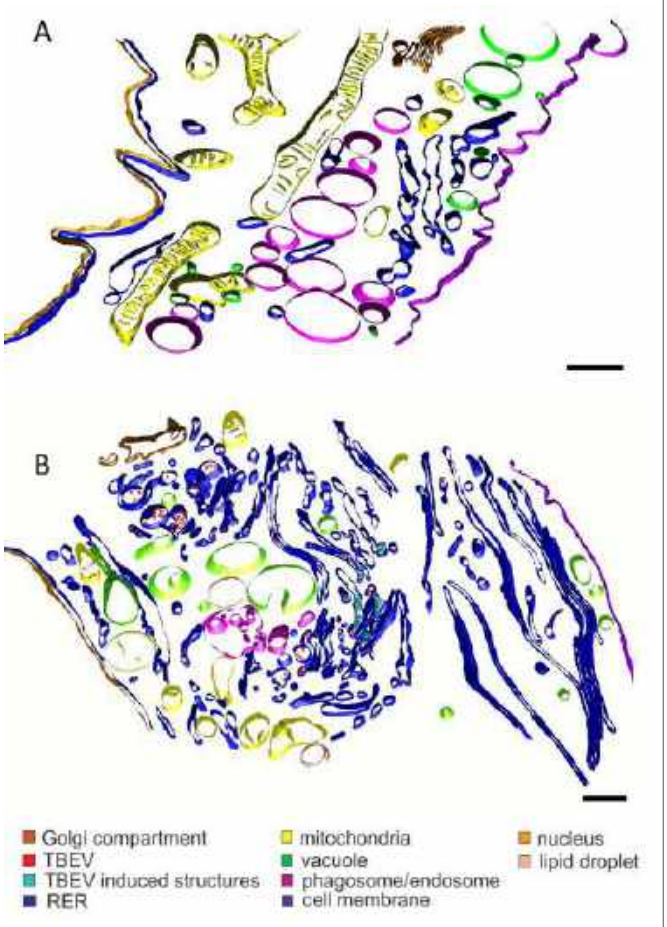
Non-structural proteins

NS1 is a glycoprotein containing 2 or 3 potential glycosylation sites and 12 conserved cysteines forming disulphide bridges.[83] It exists in dimeric forms localized freely in the cytoplasm or associated with membranes. Since the protein is highly hydrophilic and contains no transmembrane domains, its association with membranes remains poorly understood. Probably, dimerization creates a hydrophobic surface of the protein for its peripheral association with membranes.[51,84] Alternatively, some species of the protein could be anchored into the membrane by glycosyl-phosphatidylinositol.[51,85] The intracellular NS1 is central to viral RNA replication. The NS1 protein along with other non-structural proteins (see below) and viral RNA are targeted towards the luminal side of the endoplasmic reticulum, forming a replication complex (RC). Intracellular NS1 also interacts with various host proteins to assist viral replication, translation, and virion production; e.g., interaction of NS1 with 60S ribosomal subunits was described.[86] Secretion of NS1 protein into the extracellular space appears particularly in the form of pentamers or hexamers and occasionally as decamers or dodecamers.[87] This so-called 'soluble antigen', together with membrane-bound NS1 induces a protective immune response in the host.[88,89] NS1 protein is also known to activate the Toll-like receptors (TLRs),[90] and inhibit the complement system.[91,92]

NS2A is a small, hydrophobic protein, currently with no defined function. It is believed to play a role in forming the RC.[51] A small membrane-associated protein, NS2B, serves as a crucial co-factor for protease activity of the NS3 protein. The central hydrophilic domain of the NS2B protein possibly

Figure 7



Morphological changes in TBEV-infected mammalian cells. 3D models of mock-infected (A) and TBEV-infected human astrocytes (B). TBEV infection causes extensive morphological changes, including membrane reorganization of the endoplasmic reticulum; differences are evident in the Golgi complex, mitochondria, and phagosomes. (From[113], with permission).

interacts with the NS3 protein and it is flanked by hydrophobic regions probably anchored in the membrane.[93] The central hydrophilic region of NS2B (40 amino acids that mediate the NS2B co-factor activity) is flanked by hydrophobic regions that mediate membrane association.[51]

NS3, the second largest viral protein, is an enzyme central to virus replication and polyprotein processing. Conserved regions impart functions as a serine protease, helicase, and RNA nucleoside triphosphatase.[51] The protease activity is localized at the N-terminal domain of NS3, and this enzyme cleaves peptide bonds between NS2A-NS2B, NS2B-NS3, NS3-NS4A, and NS4B-NS5. As mentioned above, the protease activity occurs, in association with a 40-amino acid region of NS2B, resulting in the formation of a heterodimeric complex.[51,94] It was found that mutations which were mapped in close proximity to the NS2B-NS3 protease active site may determine the neuro- or non-neuropathogenicity of TBEV.[95] The C-terminal region of the NS3 protein has a helicase activity, utilizing the energy released from ATP to unwind RNA duplexes. Possible functions include elimination of complex secondary structures of viral RNA and/or resolving RNA duplexes formed during replication.[51] The C-terminal region also has RNA triphosphatase and 5'RNA phosphatase activities.[96] Due to the crucial role of NS3 protein in the virus replication process, this protein represents an excellent target for the development of specific antiviral inhibitors.[94,97]

NS4A and **NS4B** are small, hydrophobic proteins. NS4A is probably part of the replication complex.[98] NS4B, a trans-membrane protein localized to the sites of replication and nucleus, partially blocks activation of STAT1 and IFN-stimulated response element (ISRE) promoters in cells stimulated with IFN.[99] NS4A and, to a lesser extent, NS2A also block IFN signaling, and the cumulative effect of these 2 proteins together with NS4B results in robust IFN signaling inhibition.[100]

NS5 is the largest (100 kDa) and most highly conserved viral protein serving as a viral RNA-dependent RNA polymerase.[101] Its C-terminus shares sequence homology with RNA-dependent RNA polymerases of other positive-stranded RNA viruses.[51,102,103] The N-terminal domain has a function as AdoMet-dependent methyltransferase involved in the mRNA capping process, transferring a methyl group from the cofactor S-adenosyl-l-methionine onto the N7 atom of the cap guanine and onto the 2'OH group of the ribose moiety of the first RNA nucleotide.[94] The NS5 proteins form complexes with NS3 proteins, which results in stimulation of the NS3 RNA nucleoside triphosphatase activity.[51,104]

The NS5 protein is a promising target for specific antiviral inhibitors. Indeed, several nucleoside analogues targeting NS5 and causing premature termination of viral RNA synthesis were found to exhibit high inhibitory activity against TBEV.[105,106]

Apart from the main function as RNA-dependent RNA polymerase, the TBEV NS5 protein interferes with type I IFN JAK-STAT signaling.[107,108]

Replication strategy

Infection of the host cell with TBEV begins with the binding of the virus to a cell receptor (Figure 6), which has not yet been unequivocally identified. Interaction of the viral particle with cellular receptors is mediated by viral E glycoprotein. Kopecký et al.[109] identified 2 polypeptides of 35 and 18 kDa as putative vertebrate receptors for TBEV using a viroblot technique with anti-idiotypic monoclonal antibodies directed against antibodies that neutralize the infectivity of TBEV. However, the anti-idiotypic monoclonal antibodies did not bind effectively to tick cells, implying that different receptors are used by vertebrate and invertebrate cells for the binding of TBEV.[109] T-cell immunoglobulin and

mucin domain 1 (TIM-1) was found to act as another cellular entry factor for TBEV.[110] It remains unclear whether TBEV uses single or multiple receptors on susceptible cells. Involvement of highly conserved glycosaminoglycans, such as heparan sulphate, during attachment and entry of flaviviruses has been suggested, but it seems likely that other host-cell receptor(s) can also mediate entry of TBEV into the host cells.[76,111] Apparently, just the ability to use multiple receptors could be responsible for the very wide host range of flaviviruses, which replicate in arthropods and in a broad range of vertebrates.[112]

In addition, in the presence of sub-neutralizing levels of specific immunoglobulins, the attachment and uptake by cells expressing Fc receptors might be enhanced, and this is called antibody-dependent enhancement.

After binding to the receptor, virus is internalized into clathrin-coated vesicles by the process of endocytosis. Acidification within the endosomal vesicle triggers conformational changes of the E proteins leading to rearrangement of the dimers to trimeric forms and subsequent fusion of the viral envelope with the membrane of the vesicle (Figure 6).[114,115] At a pH threshold of 6.5, the acidic environment triggers oligomeric rearrangement of metastable E dimers into stable trimers on the virion surface. This process exposes the fusion loop, located at the tip of domain II of the E protein.[116,117] The fusion loop interacts with the endosomal membrane, thereby mediating the initiation of the membrane fusion process.[117] The viral nucleocapsid is then released into the cytoplasm and viral RNA is uncoated. The exact mechanism of nucleocapsid uncoating remains unknown. The positive-sense viral RNA is the translational template, also functioning as a template for negative-sense RNA synthesis and formation of the double-stranded replicative intermediate.

The ratio of the newly synthesized positive-stranded RNA to negative-stranded RNA is at least 10 or 100 to 1, indicating that some regulatory mechanism must exist to produce higher numbers of positive-stranded RNA molecules.[51] The biological explanation for this is the double function of the genomic positive-strand RNA: it is used as a template both for transcription of the negative strand and translation of the viral polyprotein, while the negative strand is only transcribed into the new positive strands.[36]

The single viral polyprotein is cleaved by viral and cellular proteases into individual viral proteins. The surface structural proteins prM and E (and also NS1) are translocated into the lumen of the ER and their amino termini are liberated through proteolytic cleavage by host signalase. The newly synthesized RNA is condensed by protein C into nucleocapsids on the cytoplasmic site of ER. Viral envelope is acquired by budding of the nucleocapsid into ER.[118]

TBEV replicates in the cytoplasm in close association with virus-induced intracellular membrane structures, also called replication compartments (Figure 6). These compartments provide an optimal microenvironment for viral RNA replication by limiting diffusion of viral/host proteins and viral RNA, thereby increasing the concentration of components required for RNA synthesis, and by providing a scaffold for anchoring the replication complex.[119] These packets of vesicles have a diameter of about 80 nm and are formed as invaginations of the endoplasmic reticulum within a highly-organized network of inter-connected membranes (Figure 6).[119]

Virus assembly takes place in the endoplasmic reticulum, leading to the formation of immature particles. The immature non-infectious virions contain proteins prM and E in heterodimeric association forming spikes at the surface of the particles. These immature “spiky” virions are transported to the Golgi complex, where the pr part of the prM molecule is cleaved by the cellular protease furin, and the E protein is reorganized from trimers to form fusion-competent homodimers. The slightly acidic pH in the trans-Golgi complex leads to the conformational changes that are required for furin cleavage.[59] Interestingly, the low-pH-induced structural changes appear to be irreversible in TBEV in contrast with mosquito-borne flaviviruses, where this change seems to be reversible.[59,120] The function of prM and the pr fragment is to protect the E protein in the acidic Golgi complex and prevent premature membrane fusion at this stage of the viral life cycle.[121] The mature virions pass through the host secretory pathway and are finally released from the host cell by fusion of the transport vesicle membrane with the plasma membrane (Figure 6).[118]

TBEV infection is associated with dramatic morphological changes occurring in the infected cells (Figure 7). These include formation of smooth membrane structures, proliferation of endoplasmic reticulum, reorganization of the Golgi complex, and accumulation and convolution of membranes. Several cellular organelles are often damaged.[113,122-124] The infection is commonly cytocidal; the infected cells often die by apoptosis or necrosis,[122] but some vertebrate cell types survive the lytic crisis and become chronically infected.[125]

It was found that NS3 protein from Langat virus is able to activate cellular caspase-8 and induce apoptosis of the host cell.[109] On the other hand, tick cells do not undergo major inhibition of host macromolecular synthesis caused by the infection. No dramatic cytopathic and ultrastructural changes are seen in the infected tick cells and persistent productive infection is established in these cells.[124,126-129] However, both vertebrate and tick cells activate innate defense mechanisms against the infection.[129]

The TBEV maturation process in tick cells seems, however, to be different from that observed in vertebrate cells. In a cell line derived from the tick *Rhipicephalus appendiculatus*

infected with TBEV, nucleocapsids are found in the cytoplasm and the envelope is acquired by budding on cytoplasmic membranes or into cellular vacuoles.[130]

Concluding remarks

The chapter summarized the major biological features of TBEV, focusing particularly on virus taxonomy, structure, genetics, and replication strategy in host cells. The past 2 decades have witnessed tremendous progress in our understanding of the structural, biochemical, and molecular aspects of a variety of the processes involved in morphogenesis, genome replication, maturation, and genetic basis for virulence of flaviviruses, including TBEV.

This has been made possible by the recent advances in structural and biochemical techniques, and methods of molecular biology, mainly site-directed mutagenesis. However, several key questions related to TBEV molecular biology and individual steps in the TBEV life-cycle remain unresolved. Major gaps in our understanding of the TBEV replication strategy both in mammalian and tick cells still exist. For instance, the nature of the cellular receptor for virus entry into the host cell, mechanisms of viral genome release from nucleocapsid, packaging of viral RNA by the C protein, and virus maturation remain to be identified. Except for the E glycoprotein, no structural data for the other TBEV proteins are available, and indeed the complete functional role of some proteins remains obscure. The role of specific RNA secondary structures present in TBEV untranslated genomic regions in viral RNA replication, capping, and controlling the functions of non-structural proteins, such as NS3 or NS5, need to be established. These and other unresolved problems highlight the necessity for further research into the molecular, genetic, and structural properties of TBEV. Advances in our basic knowledge of TBEV biology should promote the development of more effective methods of controlling this important human pathogen.

Acknowledgments: This chapter is dedicated to the memory of our esteemed co-author, Ernest A. Gould. DR was supported by the Ministry of Health of the Czech Republic, grant No. NU22-05-00659. MEB was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, USA. We gratefully acknowledge the assistance of Dr John Pettersson (Zoonosis Science Center, Uppsala University, Sweden), who prepared and supplied Figure 1, and Dr Tibor Füzik, who provided Figure 3D.

Contact: ruzekd@paru.cas.cz

Citation:
Růžek D, Yoshii K, Bloom ME, Gould EA. TBE virology. Chapter 4. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_4-7

References

1. Postler TS, Beer M, Blitvich BJ, et al. Renaming of the genus Flavivirus to Orthoflavivirus and extension of binomial species names within the family Flaviviridae. *Arch Virol*. Aug 10 2023;168(9):224. doi:10.1007/s00705-023-05835-1

2. Gould EA, Solomon T. Pathogenic flaviviruses. *Lancet.* Feb 9 2008;371(9611):500-9. doi:10.1016/s0140-6736(08)60238-x

3. Hollidge BS, González-Scarano F, Soldan SS. Arboviral encephalitides: transmission, emergence, and pathogenesis. *J Neuroimmune Pharmacol*. Sep 2010;5 (3):428-42. doi:10.1007/s11481-010-9234-7

4. Wilson MR. Emerging viral infections. *Curr Opin Neurol.* Jun 2013;26(3):301-6. doi:10.1097/WCO.0b013e328360dd2b

5. Blitvich BJ, Firth AE. A Review of Flaviviruses that Have No Known Arthropod Vector. *Viruses*. Jun 21 2017;9(6) doi:10.3390/v9060154

6. Blitvich BJ, Firth AE. Insect-specific flaviviruses: a systematic review of their discovery, host range, mode of transmission, superinfection exclusion potential and genomic organization. *Viruses*. Apr 10 2015;7(4):1927-59. doi:10.3390/v7041927

7. Ergunay K, Tkachev S, Kozlova I, Růžek D. A Review of Methods for Detecting Tick-Borne Encephalitis Virus Infection in Tick, Animal, and Human Specimens. *Vector Borne Zoonotic Dis*. Jan 2016;16(1):4-12. doi:10.1089/vbz.2015.1896

8. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet.* May 31 2008;371(9627):1861-71. doi:10.1016/s0140-6736(08)60800-4

9. Yoshii K, Sunden Y, Yokozawa K, et al. A critical determinant of neurological disease associated with highly pathogenic tick-borne flavivirus in mice. *J Virol.* May 2014;88(10):5406-20. doi:10.1128/jvi.00421-14

10. Zlobin VI, Demina TV, Mamaev LV, et al. [Analysis of genetic variability of strains of tick-borne encephalitis virus by primary structure of a fragment of the membrane protein E gene]. *Vopr Virusol*. Jan-Feb 2001;46(1):12-6. Analiz geneticheskoĭ éntsefalita po pervichnoĭ strukture fragmenta gena belka obolochki E.

11. Demina TV, Dzhioev YP, Verkhozina MM, et al. Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. *J Med Virol*. May 2010;82(6):965-76. doi:10.1002/jmv.21765

12. Dai X, Shang G, Lu S, Yang J, Xu J. A new subtype of eastern tick-borne encephalitis virus discovered in Qinghai-Tibet Plateau, China. *Emerg Microbes Infect*. Apr 25 2018;7(1):74. doi:10.1038/s41426-018-0081-6

13. Grard G, Moureau G, Charrel RN, et al. Genetic characterization of tick-borne flaviviruses: new insights into evolution, pathogenetic determinants and taxonomy. *Virology.* Apr 25 2007;361(1):80-92. doi:10.1016/j.virol.2006.09.015

14. Hubálek Z, Pow I, Reid HW, Hussain MH. Antigenic similarity of central European encephalitis and louping-ill viruses. *Acta Virol*. Dec 1995;39(5-6):251-6.

15. Moureau G, Cook S, Lemey P, et al. New insights into flavivirus evolution, taxonomy and biogeographic history, extended by analysis of canonical and alternative coding sequences. *PLoS One*. 2015;10(2):e0117849. doi:10.1371/journal.pone.0117849

16. Zanotto PM, Gao GF, Gritsun T, et al. An arbovirus cline across the northern hemisphere. *Virology*. Jun 20 1995;210 (1):152-9. doi:10.1006/viro.1995.1326

17. Zanotto PM, Gould EA, Gao GF, Harvey PH, Holmes EC. Population dynamics of flaviviruses revealed by molecular phylogenies. *Proc Natl Acad Sci U S A*. Jan 23 1996;93 (2):548-53. doi:10.1073/pnas.93.2.548

18. Gould EA, de Lamballerie X, Zanotto PM, Holmes EC. Evolution, epidemiology, and dispersal of flaviviruses revealed by molecular phylogenies. *Adv Virus Res*. 2001;57:71-103. doi:10.1016/s0065-3527(01)57001-3

19. Pettersson JH, Fiz-Palacios O. Dating the origin of the genus Flavivirus in the light of Beringian biogeography. *J Gen Virol*. Sep 2014;95(Pt 9):1969-1982. doi:10.1099/vir.0.065227-0

20. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus Flavivirus. *J Virol.* Jan 1998;72(1):73 -83. doi:10.1128/jvi.72.1.73-83.1998

21. Marin MS, Zanotto PM, Gritsun TS, Gould EA. Phylogeny of TYU, SRE, and CFA virus: different evolutionary rates in the genus Flavivirus. *Virology*. Feb 1 1995;206(2):1133-9. doi:10.1006/viro.1995.1038

22. Shiu SY, Ayres MD, Gould EA. Genomic sequence of the structural proteins of louping ill virus: comparative analysis with tick-borne encephalitis virus. *Virology.* Jan 1991;180 (1):411-5. doi:10.1016/0042-6822(91)90048-g

23. Füzik T, Formanová P, Růžek D, Yoshii K, Niedrig M, Plevka P. Structure of tick-borne encephalitis virus and its neutralization by a monoclonal antibody. *Nat Commun*. Jan 30 2018;9(1):436. doi:10.1038/s41467-018-02882-0

24. Pulkkinen LIA, Barrass SV, Domanska A, Överby AK, Anastasina M, Butcher SJ. Molecular Organisation of Tick-Borne Encephalitis Virus. *Viruses*. Apr 11 2022;14(4) doi:10.3390/v14040792

25. Vratskikh O, Stiasny K, Zlatkovic J, et al. Dissection of antibody specificities induced by yellow fever vaccination. *PLoS Pathog.* 2013;9(6):e1003458. doi:10.1371/journal.ppat.1003458

26. Anastasina M, Füzik T, Domanska A, et al. The structure of immature tick-borne encephalitis virus. *bioRxiv*. 2023:2023.08.04.551633. doi:10.1101/2023.08.04.551633

27. Heinz FX, Allison SL, Stiasny K, et al. Recombinant and virion -derived soluble and particulate immunogens for vaccination against tick-borne encephalitis. *Vaccine*. Dec 1995;13(17):1636-42. doi:10.1016/0264-410x(95)00133-l

28. Allison SL, Tao YJ, O'Riordain G, Mandl CW, Harrison SC, Heinz FX. Two distinct size classes of immature and mature subviral particles from tick-borne encephalitis virus. *J Virol*. Nov 2003;77(21):11357-66. doi:10.1128/jvi.77.21.11357-11366.2003

29. Heinz FX, Mandl CW. The molecular biology of tick-borne encephalitis virus. Review article. APMIS. Oct 1993;101 (10):735-45. doi:10.1111/j.1699-0463.1993.tb00174.x

30. Černý J, Selinger M, Palus M, et al. Expression of a second open reading frame present in the genome of tick-borne encephalitis virus strain Neudoerfl is not detectable in infected cells. *Virus Genes*. Jun 2016;52(3):309-16. doi:10.1007/s11262-015-1273-y

31. Chambers TJ, Hahn CS, Galler R, Rice CM. Flavivirus genome organization, expression, and replication. *Annu Rev Microbiol*. 1990;44:649-88. doi:10.1146/annurev.mi.44.100190.003245

32. Tuplin A, Evans DJ, Buckley A, Jones IM, Gould EA, Gritsun TS. Replication enhancer elements within the open reading frame of tick-borne encephalitis virus and their evolution within the Flavivirus genus. *Nucleic Acids Res*. Sep 1 2011;39(16):7034-48. doi:10.1093/nar/gkr237

33. Thurner C, Witwer C, Hofacker IL, Stadler PF. Conserved RNA secondary structures in Flaviviridae genomes. *J Gen Virol*. May 2004;85(Pt 5):1113-1124. doi:10.1099/vir.0.19462-0

34. Gritsun TS, Venugopal K, Zanotto PM, et al. Complete sequence of two tick-borne flaviviruses isolated from Siberia and the UK: analysis and significance of the 5' and 3'-UTRs. *Virus Res*. May 1997;49(1):27-39. doi:10.1016/s0168-1702(97)01451-2

35. Proutski V, Gaunt MW, Gould EA, Holmes EC. Secondary structure of the 3'-untranslated region of yellow fever virus: implications for virulence, attenuation and vaccine development. *J Gen Virol.* Jul 1997;78 (Pt 7):1543-9. doi:10.1099/0022-1317-78-7-1543

36. Proutski V, Gould EA, Holmes EC. Secondary structure of

the 3' untranslated region of flaviviruses: similarities and differences. *Nucleic Acids Res*. Mar 15 1997;25(6):1194-202. doi:10.1093/nar/25.6.1194

37. Gritsun TS, Gould EA. Origin and evolution of flavivirus 5'UTRs and panhandles: trans-terminal duplications? *Virology*. Sep 15 2007;366(1):8-15. doi:10.1016/j.virol.2007.04.011

38. Ruzek D, Avšič Županc T, Borde J, et al. Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. *Antiviral Res. Apr* 2019;164:23-51. doi:10.1016/j.antiviral.2019.01.014

39. Wallner G, Mandl CW, Kunz C, Heinz FX. The flavivirus 3'-noncoding region: extensive size heterogeneity independent of evolutionary relationships among strains of tick-borne encephalitis virus. *Virology*. Oct 20 1995;213 (1):169-78. doi:10.1006/viro.1995.1557

40. Mandl CW, Kunz C, Heinz FX. Presence of poly(A) in a flavivirus: significant differences between the 3' noncoding regions of the genomic RNAs of tick-borne encephalitis virus strains. *J Virol*. Aug 1991;65(8):4070-7. doi:10.1128/jvi.65.8.4070-4077.1991

41. Formanová P, Černý J, Bolfíková B, et al. Full genome sequences and molecular characterization of tick-borne encephalitis virus strains isolated from human patients. *Ticks Tick Borne Dis*. Feb 2015;6(1):38-46. doi:10.1016/j.ttbdis.2014.09.002

42. Leonova GN, Belikov SI, Kondratov IG, Takashima I. Comprehensive assessment of the genetics and virulence of tick-borne encephalitis virus strains isolated from patients with inapparent and clinical forms of the infection in the Russian Far East. *Virology*. Aug 15 2013;443(1):89-98. doi:10.1016/j.virol.2013.04.029

43. Mandl CW, Holzmann H, Meixner T, et al. Spontaneous and engineered deletions in the 3' noncoding region of tick-borne encephalitis virus: construction of highly attenuated mutants of a flavivirus. *J Virol.* Mar 1998;72(3):2132-40. doi:10.1128/jvi.72.3.2132-2140.1998

44. Sakai M, Muto M, Hirano M, Kariwa H, Yoshii K. Virulence of tick-borne encephalitis virus is associated with intact conformational viral RNA structures in the variable region of the 3'-UTR. *Virus Res*. May 4 2015;203:36-40. doi:10.1016/j.virusres.2015.03.006

45. Sakai M, Yoshii K, Sunden Y, Yokozawa K, Hirano M, Kariwa H. Variable region of the 3' UTR is a critical virulence factor in the Far-Eastern subtype of tick-borne encephalitis virus in a mouse model. *J Gen Virol*. Apr 2014;95(Pt 4):823-835. doi:10.1099/vir.0.060046-0

46. Asghar N, Lee YP, Nilsson E, et al. The role of the poly(A) tract in the replication and virulence of tick-borne encephalitis virus. *Sci Rep*. Dec 16 2016;6:39265. doi:10.1038/srep39265

47. Gritsun TS, Gould EA. The 3' untranslated region of tick-borne flaviviruses originated by the duplication of long repeat sequences within the open reading frame. *Virology*. Jul 5 2006;350(2):269-75. doi:10.1016/j.virol.2006.03.002

48. Gritsun TS, Gould EA. Origin and evolution of 3'UTR of flaviviruses: long direct repeats as a basis for the formation of secondary structures and their significance for virus transmission. *Adv Virus Res*. 2007;69:203-48. doi:10.1016/s0065-3527(06)69005-2

49. Silva PA, Pereira CF, Dalebout TJ, Spaan WJ, Bredenbeek PJ. An RNA pseudoknot is required for production of yellow fever virus subgenomic RNA by the host nuclease XRN1. *J Virol*. Nov 2010;84(21):11395-406. doi:10.1128/jvi.01047-10

50. Khromykh AA, Westaway EG. RNA binding properties of core protein of the flavivirus Kunjin. *Arch Virol.* 1996;141(3-4):685-99. doi:10.1007/bf01718326

51. Lindenbach BD, Rice CM. Molecular biology of flaviviruses. *Adv Virus Res*. 2003;59:23-61. doi:10.1016/s0065-3527(03)59002-9

52. Samsa MM, Mondotte JA, Iglesias NG, et al. Dengue virus capsid protein usurps lipid droplets for viral particle formation. *PLoS Pathog.* Oct 2009;5(10):e1000632. doi:10.1371/journal.ppat.1000632

53. Kofler RM, Heinz FX, Mandl CW. Capsid protein C of tick-borne encephalitis virus tolerates large internal deletions and is a favorable target for attenuation of virulence. *J Virol*. Apr 2002;76(7):3534-43. doi:10.1128/jvi.76.7.3534-3543.2002

54. Kofler RM, Leitner A, O'Riordain G, Heinz FX, Mandl CW. Spontaneous mutations restore the viability of tick-borne encephalitis virus mutants with large deletions in protein C. *J Virol.* Jan 2003;77(1):443-51. doi:10.1128/jvi.77.1.443-451.2003

55. Lorenz IC, Allison SL, Heinz FX, Helenius A. Folding and dimerization of tick-borne encephalitis virus envelope proteins prM and E in the endoplasmic reticulum. *J Virol.* Jun 2002;76(11):5480-91. doi:10.1128/jvi.76.11.5480-5491.2002

56. Goto A, Yoshii K, Obara M, et al. Role of the N-linked glycans of the prM and E envelope proteins in tick-borne encephalitis virus particle secretion. *Vaccine.* Apr 27 2005;23(23):3043-52. doi:10.1016/j.vaccine.2004.11.068

57. Chambers TJ, Diamond MS. Pathogenesis of flavivirus encephalitis. *Adv Virus Res*. 2003;60:273-342. doi:10.1016/s0065-3527(03)60008-4

58. Elshuber S, Allison SL, Heinz FX, Mandl CW. Cleavage of protein prM is necessary for infection of BHK-21 cells by tick-borne encephalitis virus. *J Gen Virol.* Jan 2003;84(Pt 1):183-191. doi:10.1099/vir.0.18723-0

59. Stadler K, Allison SL, Schalich J, Heinz FX. Proteolytic activation of tick-borne encephalitis virus by furin. *J Virol.* Nov 1997;71(11):8475-81. doi:10.1128/jvi.71.11.8475-8481.1997

60. Yoshii K, Igarashi M, Ichii O, et al. A conserved region in the prM protein is a critical determinant in the assembly of flavivirus particles. *J Gen Virol.* Jan 2012;93(Pt 1):27-38. doi:10.1099/vir.0.035964-0

61. Gritsun TS, Holmes EC, Gould EA. Analysis of flavivirus envelope proteins reveals variable domains that reflect their antigenicity and may determine their pathogenesis. *Virus Res*. Mar 1995;35(3):307-21. doi:10.1016/0168-1702(94)00090-y

62. Rey FA, Heinz FX, Mandl C, Kunz C, Harrison SC. The envelope glycoprotein from tick-borne encephalitis virus at 2 A resolution. *Nature*. May 25 1995;375(6529):291-8. doi:10.1038/375291a0

63. Holzmann H, Stiasny K, York H, Dorner F, Kunz C, Heinz FX. Tick-borne encephalitis virus envelope protein E-specific monoclonal antibodies for the study of low pH-induced conformational changes and immature virions. *Arch Virol*. 1995;140(2):213-21. doi:10.1007/bf01309857

64. Fritz R, Stiasny K, Heinz FX. Identification of specific histidines as pH sensors in flavivirus membrane fusion. *J Cell Biol*. Oct 20 2008;183(2):353-61. doi:10.1083/jcb.200806081

65. Lattová E, Straková P, Pokorná-Formanová P, et al. Comprehensive N-glycosylation mapping of envelope glycoprotein from tick-borne encephalitis virus grown in human and tick cells. *Sci Rep*. Aug 6 2020;10(1):13204. doi:10.1038/s41598-020-70082-2

66. Yoshii K, Yanagihara N, Ishizuka M, Sakai M, Kariwa H. N-linked glycan in tick-borne encephalitis virus envelope protein affects viral secretion in mammalian cells, but not in tick cells. *J Gen Virol*. Oct 2013;94(Pt 10):2249-2258. doi:10.1099/vir.0.055269-0

67. Heinz FX. Molecular aspects of TBE virus research. *Vaccine*. Apr 1 2003;21 Suppl 1:S3-s10. doi:10.1016/s0264-410x(02)00820-4

68. Heinz FX, Allison SL. Flavivirus structure and membrane fusion. *Adv Virus Res*. 2003;59:63-97. doi:10.1016/s0065-3527(03)59003-0

69. Nowak T, Wengler G. Analysis of disulfides present in the membrane proteins of the West Nile flavivirus. *Virology*. Jan 1987;156(1):127-37. doi:10.1016/0042-6822(87)90443-0

70. McMinn PC. The molecular basis of virulence of the encephalitogenic flaviviruses. *J Gen Virol*. Nov 1997;78 (Pt 11):2711-22. doi:10.1099/0022-1317-78-11-2711

71. Holzmann H, Stiasny K, Ecker M, Kunz C, Heinz FX. Characterization of monoclonal antibody-escape mutants of tick-borne encephalitis virus with reduced neuroinvasiveness in mice. *J Gen Virol*. Jan 1997;78 (Pt 1):31-7. doi:10.1099/0022-1317-78-1-31

72. Khasnatinov MA, Ustanikova K, Frolova TV, et al. Non-hemagglutinating flaviviruses: molecular mechanisms for the emergence of new strains via adaptation to European ticks. *PLoS One.* Oct 5 2009;4(10):e7295. doi:10.1371/journal.pone.0007295

73. Labuda M, Jiang WR, Kaluzova M, et al. Change in phenotype of tick-borne encephalitis virus following passage in Ixodes ricinus ticks and associated amino acid substitution in the envelope protein. *Virus Res.* Mar 1994;31(3):305-15. doi:10.1016/0168-1702(94)90024-8

74. Goto A, Hayasaka D, Yoshii K, Mizutani T, Kariwa H, Takashima I. A BHK-21 cell culture-adapted tick-borne encephalitis virus mutant is attenuated for neuroinvasiveness. *Vaccine*. Sep 8 2003;21(25-26):4043-51. doi:10.1016/s0264-410x(03)00269-x

75. Mandl CW, Allison SL, Holzmann H, Meixner T, Heinz FX. Attenuation of tick-borne encephalitis virus by structure-based site-specific mutagenesis of a putative flavivirus receptor binding site. *J Virol.* Oct 2000;74(20):9601-9. doi:10.1128/jvi.74.20.9601-9609.2000

76. Mandl CW, Kroschewski H, Allison SL, et al. Adaptation of tick-borne encephalitis virus to BHK-21 cells results in the formation of multiple heparan sulfate binding sites in the envelope protein and attenuation in vivo. *J Virol*. Jun 2001;75(12):5627-37. doi:10.1128/jvi.75.12.5627-5637.2001

77. Kiermayr S, Stiasny K, Heinz FX. Impact of quaternary organization on the antigenic structure of the tick-borne encephalitis virus envelope glycoprotein E. *J Virol*. Sep 2009;83(17):8482-91. doi:10.1128/jvi.00660-09

78. Stiasny K, Brandler S, Kössl C, Heinz FX. Probing the flavivirus membrane fusion mechanism by using monoclonal antibodies. *J Virol.* Oct 2007;81(20):11526-31. doi:10.1128/jvi.01041-07

79. Agudelo M, Palus M, Keeffe JR, et al. Broad and potent neutralizing human antibodies to tick-borne flaviviruses protect mice from disease. *J Exp Med.* May 3 2021;218(5) doi:10.1084/jem.20210236

80. Svoboda P, Haviernik J, Bednar P, et al. A combination of two resistance mechanisms is critical for tick-borne encephalitis virus escape from a broadly neutralizing human antibody. *Cell Rep.* Sep 26 2023;42(9):113149. doi:10.1016/j.celrep.2023.113149

81. Stiasny K, Kiermayr S, Holzmann H, Heinz FX. Cryptic properties of a cluster of dominant flavivirus cross-reactive antigenic sites. *J Virol*. Oct 2006;80(19):9557-68.

doi:10.1128/jvi.00080-06

82. Haslwanter D, Blaas D, Heinz FX, Stiasny K. A novel mechanism of antibody-mediated enhancement of flavivirus infection. *PLoS Pathog*. Sep 2017;13(9):e1006643. doi:10.1371/journal.ppat.1006643

83. Lee JM, Crooks AJ, Stephenson JR. The synthesis and maturation of a non-structural extracellular antigen from tick-borne encephalitis virus and its relationship to the intracellular NS1 protein. *J Gen Virol*. Feb 1989;70 (Pt 2):335-43. doi:10.1099/0022-1317-70-2-335

84. Muller DA, Young PR. The flavivirus NS1 protein: molecular and structural biology, immunology, role in pathogenesis and application as a diagnostic biomarker. *Antiviral Res*. May 2013;98(2):192-208. doi:10.1016/j.antiviral.2013.03.008

85. Jacobs MG, Robinson PJ, Bletchly C, Mackenzie JM, Young PR. Dengue virus nonstructural protein 1 is expressed in a glycosyl -phosphatidylinositol-linked form that is capable of signal transduction. *FASEB J*. Aug 2000;14(11):1603-10. doi:10.1096/fj.14.11.1603

86. Cervantes-Salazar M, Angel-Ambrocio AH, Soto-Acosta R, et al. Dengue virus NS1 protein interacts with the ribosomal protein RPL18: this interaction is required for viral translation and replication in Huh-7 cells. *Virology*. Oct 2015;484:113-126. doi:10.1016/j.virol.2015.05.017

87. Gritsun TS, Liapustin VN, Shatalov AG, Lashkevich VA. [Multiple forms of the NS1 protein as the main component of the nonvirion ("soluble") antigen of the tick-borne encephalitis virus]. *Vopr Virusol.* Nov-Dec 1990;35(6):471-4. Mnozhestvennye formy belka NS1 kak osnovnogo komponenta nevirionnogo ("rastvorimogo") antigena virusa kleshchevogo éntsefalita.

88. Gould EA, Buckley A, Barrett AD, Cammack N. Neutralizing (54K) and non-neutralizing (54K and 48K) monoclonal antibodies against structural and non-structural yellow fever virus proteins confer immunity in mice. *J Gen Virol*. Mar 1986;67 (Pt 3):591-5. doi:10.1099/0022-1317-67-3-591

89. Salat J, Mikulasek K, Larralde O, et al. Tick-Borne Encephalitis Virus Vaccines Contain Non-Structural Protein 1 Antigen and may Elicit NS1-Specific Antibody Responses in Vaccinated Individuals. *Vaccines (Basel).* Feb 12 2020;8(1)doi:10.3390/vaccines8010081

90. Chen J, Ng MM, Chu JJ. Activation of TLR2 and TLR6 by Dengue NS1 Protein and Its Implications in the Immunopathogenesis of Dengue Virus Infection. *PLoS Pathog.* Jul 2015;11 (7):e1005053. doi:10.1371/journal.ppat.1005053

91. Avirutnan P, Hauhart RE, Somnuke P, Blom AM, Diamond MS, Atkinson JP. Binding of flavivirus nonstructural protein NS1 to C4b binding protein modulates complement activation. *J Immunol*. Jul 1 2011;187(1):424-33. doi:10.4049/jimmunol.1100750

92. Rastogi M, Sharma N, Singh SK. Flavivirus NS1: a multifaceted enigmatic viral protein. *Virol J*. Jul 29 2016;13:131. doi:10.1186/s12985-016-0590-7

93. Chambers TJ, Nestorowicz A, Amberg SM, Rice CM. Mutagenesis of the yellow fever virus NS2B protein: effects on proteolytic processing, NS2B-NS3 complex formation, and viral replication. *J Virol*. Nov 1993;67(11):6797-807. doi:10.1128/jvi.67.11.6797-6807.1993

94. Bollati M, Alvarez K, Assenberg R, et al. Structure and functionality in flavivirus NS-proteins: perspectives for drug design. *Antiviral Res*. Aug 2010;87(2):125-48. doi:10.1016/j.antiviral.2009.11.009

95. Růzek D, Gritsun TS, Forrester NL, et al. Mutations in the NS2B and NS3 genes affect mouse neuroinvasiveness of a Western European field strain of tick-borne encephalitis virus. *Virology*. May 10 2008;374(2):249-55. doi:10.1016/j.virol.2008.01.010

96. Wengler G, Wengler G. The NS 3 nonstructural protein of flaviviruses contains an RNA triphosphatase activity. *Virology*. Nov 1993;197(1):265-73. doi:10.1006/viro.1993.1587

97. Singh V, Somvanshi P. Structural modeling of the NS 3 helicase of Tick-borne encephalitis virus and their virtual screening of potent drugs using molecular docking. *Interdiscip Sci.* Sep 2009;1(3):168-72. doi:10.1007/s12539-009-0039-4

98. Uchil PD, Satchidanandam V. Architecture of the flaviviral replication complex. Protease, nuclease, and detergents reveal encasement within double-layered membrane compartments. *J Biol Chem*. Jul 4 2003;278(27):24388-98. doi:10.1074/jbc.M301717200

99. Muñoz-Jordán JL, Laurent-Rolle M, Ashour J, et al. Inhibition of alpha/beta interferon signaling by the NS4B protein of flaviviruses. *J Virol.* Jul 2005;79(13):8004-13. doi:10.1128/jvi.79.13.8004-8013.2005

100. Muñoz-Jordan JL, Sánchez-Burgos GG, Laurent-Rolle M, García -Sastre A. Inhibition of interferon signaling by dengue virus. *Proc Natl Acad Sci U S A*. Nov 25 2003;100(24):14333-8. doi:10.1073/pnas.2335168100

101. Steffens S, Thiel HJ, Behrens SE. The RNA-dependent RNA polymerases of different members of the family Flaviviridae exhibit similar properties in vitro. *J Gen Virol*. Oct 1999;80 (Pt 10):2583-2590. doi:10.1099/0022-1317-80-10-2583

102. Černý J, Černá Bolfíková B, Valdés JJ, Grubhoffer L, Růžek D. Evolution of tertiary structure of viral RNA dependent polymerases. *PLoS One.* 2014;9(5):e96070. doi:10.1371/journal.pone.0096070

103. Černý J, Černá Bolfíková B, de AZPM, Grubhoffer L, Růžek D. A deep phylogeny of viral and cellular right-hand polymerases. *Infect Genet Evol.* Dec 2015;36:275-286. doi:10.1016/j.meegid.2015.09.026

104. Cui T, Sugrue RJ, Xu Q, Lee AK, Chan YC, Fu J. Recombinant dengue virus type 1 NS3 protein exhibits specific viral RNA binding and NTPase activity regulated by the NS5 protein. *Virology*. Jul 5 1998;246(2):409-17. doi:10.1006/viro.1998.9213

105. Eyer L, Šmídková M, Nencka R, et al. Structure-activity relationships of nucleoside analogues for inhibition of tick-borne encephalitis virus. *Antiviral Res*. Sep 2016;133:119-29. doi:10.1016/j.antiviral.2016.07.018

106. Eyer L, Valdés JJ, Gil VA, et al. Nucleoside inhibitors of tick-borne encephalitis virus. *Antimicrob Agents Chemother.* Sep 2015;59(9):5483-93. doi:10.1128/aac.00807-15

107. Best SM, Morris KL, Shannon JG, et al. Inhibition of interferon-stimulated JAK-STAT signaling by a tick-borne flavivirus and identification of NS5 as an interferon antagonist. *J Virol.* Oct 2005;79(20):12828-39. doi:10.1128/jvi.79.20.12828-12839.2005

108. Werme K, Wigerius M, Johansson M. Tick-borne encephalitis virus NS5 associates with membrane protein scribble and impairs interferon-stimulated JAK-STAT signalling. *Cell Microbiol*. Mar 2008;10(3):696-712. doi:10.1111/j.1462-5822.2007.01076.x

109. Kopecký J, Grubhoffer L, Kovár V, Jindrák L, Vokurková D. A putative host cell receptor for tick-borne encephalitis virus identified by anti-idiotypic antibodies and virus affinoblotting. *Intervirology*. 1999;42(1):9-16. doi:10.1159/000024954

110. Zhang X, Liang C, Wang H, et al. T-Cell Immunoglobulin and Mucin Domain 1 (TIM-1) Is a Functional Entry Factor for Tick-Borne Encephalitis Virus. *mBio.* Feb 22 2022;13(1):e0286021. doi:10.1128/mbio.02860-21

111. Kroschewski H, Allison SL, Heinz FX, Mandl CW. Role of heparan sulfate for attachment and entry of tick-borne encephalitis virus. *Virology*. Mar 30 2003;308(1):92-100. doi:10.1016/s0042-6822(02)00097-1

112. Rodrigues R, Danskog K, Överby AK, Arnberg N. Characterizing the cellular attachment receptor for Langat virus. *PLoS One*. 2019;14(6):e0217359. doi:10.1371/journal.pone.0217359

113. Palus M, Bílý T, Elsterová J, et al. Infection and injury of human astrocytes by tick-borne encephalitis virus. *J Gen Virol*. Nov 2014;95(Pt 11):2411-2426. doi:10.1099/vir.0.068411-0

114. Heinz FX, Stiasny K, Püschner-Auer G, et al. Structural changes and functional control of the tick-borne encephalitis virus glycoprotein E by the heterodimeric association with protein prM. *Virology*. Jan 1994;198(1):109-17. doi:10.1006/viro.1994.1013

115. Guirakhoo F, Heinz FX, Mandl CW, Holzmann H, Kunz C. Fusion activity of flaviviruses: comparison of mature and immature (prM-containing) tick-borne encephalitis virions. *J Gen Virol.* Jun 1991;72 (Pt 6):1323-9. doi:10.1099/0022-1317-72-6-1323

116. Allison SL, Schalich J, Stiasny K, Mandl CW, Heinz FX. Mutational evidence for an internal fusion peptide in flavivirus envelope protein E. *J Virol*. May 2001;75(9):4268-75. doi:10.1128/jvi.75.9.4268-4275.2001

117. Stiasny K, Allison SL, Schalich J, Heinz FX. Membrane interactions of the tick-borne encephalitis virus fusion protein E at low pH. *J Virol*. Apr 2002;76(8):3784-90. doi:10.1128/jvi.76.8.3784-3790.2002

118. Mandl CW. Steps of the tick-borne encephalitis virus replication cycle that affect neuropathogenesis. *Virus Res*. Aug 2005;111(2):161-74. doi:10.1016/j.virusres.2005.04.007

119. Miorin L, Romero-Brey I, Maiuri P, et al. Three-dimensional architecture of tick-borne encephalitis virus replication sites and trafficking of the replicated RNA. *J Virol*. Jun 2013;87(11):6469-81. doi:10.1128/jvi.03456-12

120. Yu IM, Zhang W, Holdaway HA, et al. Structure of the immature dengue virus at low pH primes proteolytic maturation. *Science*. Mar 28 2008;319(5871):1834-7. doi:10.1126/science.1153264

121. Yu IM, Holdaway HA, Chipman PR, Kuhn RJ, Rossmann MG, Chen J. Association of the pr peptides with dengue virus at acidic pH blocks membrane fusion. *J Virol*. Dec 2009;83(23):12101-7. doi:10.1128/jvi.01637-09

122. Růžek D, Vancová M, Tesařová M, Ahantarig A, Kopecký J, Grubhoffer L. Morphological changes in human neural cells following tick-borne encephalitis virus infection. *J Gen Virol*. Jul 2009;90(Pt 7):1649-1658. doi:10.1099/vir.0.010058-0

123. Bílý T, Palus M, Eyer L, Elsterová J, Vancová M, Růžek D. Electron Tomography Analysis of Tick-Borne Encephalitis Virus Infection in Human Neurons. *Sci Rep*. Jun 15 2015;5:10745. doi:10.1038/srep10745

124. Offerdahl DK, Dorward DW, Hansen BT, Bloom ME. A three-dimensional comparison of tick-borne flavivirus infection in mammalian and tick cell lines. *PLoS One.* 2012;7(10):e47912. doi:10.1371/journal.pone.0047912

125. Mlera L, Offerdahl DK, Martens C, Porcella SF, Melik W, Bloom ME. Development of a Model System for Tick-Borne Flavivirus Persistence in HEK 293T Cells. *mBio.* Jun 4 2015;6(3):e00614. doi:10.1128/mBio.00614-15

126. Lawrie CH, Uzcátegui NY, Armesto M, Bell-Sakyi L, Gould EA. Susceptibility of mosquito and tick cell lines to infection with various flaviviruses. *Med Vet Entomol*. Sep 2004;18(3):268-74. doi:10.1111/j.0269-283X.2004.00505.x

127. Růzek D, Bell-Sakyi L, Kopecký J, Grubhoffer L. Growth of tick-borne encephalitis virus (European subtype) in cell lines from vector and non-vector ticks. *Virus Res*. Oct 2008;137(1):142-6. doi:10.1016/j.virusres.2008.05.013

128. Bell-Sakyi L, Růzek D, Gould EA. Cell lines from the soft tick Ornithodoros moubata. *Exp Appl Acarol*. Nov 2009;49(3):209-

19. doi:10.1007/s10493-009-9258-y

129. Weisheit S, Villar M, Tykalová H, et al. Ixodes scapularis and *Ixodes ricinus* tick cell lines respond to infection with tick-borne encephalitis virus: transcriptomic and proteomic analysis. *Parasit Vectors*. Nov 18 2015;8:599. doi:10.1186/s13071-015-1210-x

130. Senigl F, Grubhoffer L, Kopecky J. Differences in maturation of tick-borne encephalitis virus in mammalian and tick cell line. *Intervirology*. 2006;49(4):239-48. doi:10.1159/000091471

Chapter 5

TBEV-transmission and natural cycles

Lidia Chitimia-Dobler

Key points

- The natural cycle of the TBE virus is dependent on vector ticks and reservoir hosts.
- There are differing transmission cycles in varying environments, from cold northern coniferous forests to temperate central European forests.
- Within a natural transmission cycle there are different ways of transmission - tick-to-tick (transovarial, sexual), host-to-tick (viremic), and also tick-to-tick and host-to-host.
- The complexity of natural transmission cycles is inadequately explored and poorly understood.

Introduction

Ticks play a critical role in the transmission of a wide variety of viral, bacterial, and protozoan pathogens to humans and animals.[1,2] In the case of humans, infection is accidental as these transmission cycles are invariably enzootic with the natural hosts most frequently being wild birds and mammals.[1] In order to be tangentially affected by such cycles, humans must be bitten by a vector tick species found in habitats visited by humans, as well as the tick's usual hosts, as the dispersal of ticks not attached to hosts covers only very short distances.[3] In addition, the tick has to accept humans as a suitable host, meaning that the species involved usually have a broad host spectrum.

Nevertheless, these tick species may only be part of the transmission cycle, with eco-epidemiologically significant sub-cycles involving tick species not commonly in contact with humans.[4,5] Thus, the transmission of tick-borne pathogens often comprises a complex network of interactions involving several tick and host species. Below, we provide background to the biology of ticks and how this can influence, specifically, the eco-epidemiological cycle of tick-borne encephalitis virus (TBEV).

Structure and morphology

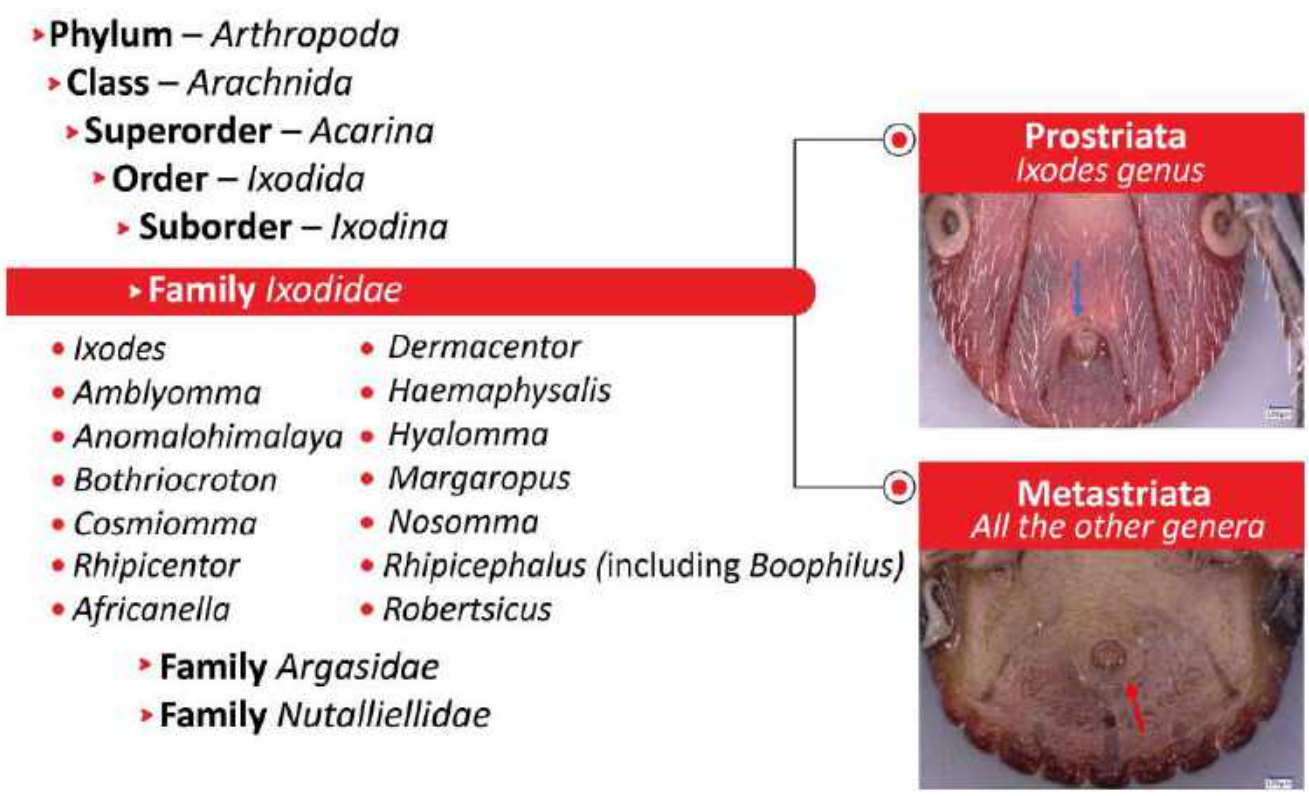
Ticks are a group of hematophagous ectoparasites with about 910 living species.[6] They belong to the phylum Arthropoda, the class Arachnida, the superorder Acarina, and the order Ixodida, and they are exclusively parasitic. The Ixodida contain 3 families: the Ixodidae with 15 genera (hard ticks), the Argasidae with 15 genera (soft ticks), and the Nuttalliellidae, represented by only one species, *Nuttalliella namaqua.*[7,8,9] (Mans et al. 2021) All the tick species involved in the eco-epidemiological cycle of TBEV belong to the Ixodidae. Details of tick biology generally can be found in a variety of publications, for example in Nicholson et al.,[8] Petney et al.,[10] and Sonenshine and Roe,[11] and a list of valid species names in Guglielmone and Nava.[12] The following genera of ticks contain species known to transmit TBEV or in their species TBEV was detected.

Ixodes is the largest tick genus, with 266 described species worldwide.[7] *Ixodes* species are characterized by a distinct groove that encircles the anus anteriorly and a lack of eyes. Males have 7 sclerotized ventral plates that are absent in the males of other genera. The genus *Ixodes* has been subdivided in roughly 15 subgenera (e.g. *Ixodes*, *Pholeoixodes*) on the basis of morphology.[13,14] The genus has a worldwide distribution, including parts of Antarctica.[8,15] Some species are particularly important as vectors of TBEV: *Ixodes ricinus* the castor bean tick or sheep tick in Europe and middle Asia, *Ixodes persulcatus* the taiga tick in northeastern Europe and northern Asia, and *Ixodes ovatus* in the forest belt of middle Asia and Japan.

The genus *Dermacentor* has 44 species worldwide.[7] The basis capitulum appears rectangular when viewed dorsally. A pair of medially directed spurs occurs on the first pair of coxae. The palps are short and thick. The scutum is almost always ornamented. *Dermacentor* species are found mostly in Europe, Asia, and North America.[15] In Europe, TBEV has been recovered from 2 species, *Dermacentor reticulatus* (the ornate dog tick), *Dermacentor marginatus* (the ornate sheep tick), and in Asia from *Dermacentor nuttalli*.

Haemaphysalis is the second largest (176 species) tick genus.[7] This eyeless genus can, in most cases, be identified by a pronounced lateral projection of palpal segment 2, which extends well beyond the basis capitulum. In Europe, TBEV has been recovered from *Haemaphysalis punctata*

Figure 1: The *Ixodidae* family



The *Ixodidae* family is divided in two groups: *Prostriata*, which includes only the genus *Ixodes* and which is characterizad by an anal groove encircling the anus anteriorly (blue arrow); and *Metastriata* , including 14 genera, which all have an anal groove behind the anus (red arrow).

(the red sheep tick), *Haemaphysalis concinna* in Europe and Asia, and from *Haemaphysalis longicornis* in Asia.[8,15]

The genus *Hyalomma* is relatively small with 27 species of small- to large-sized ticks.[16] They are characterized by their elongated palps, which are at least twice as long as wide. The distinct eyes are located in sockets adjacent to the postero-lateral edges of the scutum that is unornamented. The distribution of *Hyalomma* species is limited to the Old World, primarily to arid or semiarid habitats. *Hyalomma marginatum* (the Mediterranean *Hyalomma*) is the only member of this genus from which TBEV has been recovered.

The biology of hard ticks

All the species known to transmit TBEV have a 3-host life cycle (Figure 2). Each postembryonic life stage requires a blood meal from a suitable host, after which the tick detaches and molts in the leaf litter. The arrows with broken lines in the figure show the potential transmission paths to humans. The line from larvae to humans indicates that transovarial transmission from an infected female can happen which results in infective larvae. Infection of the tick can occur when larvae, nymphs, or females feed on an infective host (see below).

The larva, nymph, and adult (female or male – Figures 3a, 3b, 3c, and 3d) are active stages that require a host (this is not the case for males of the genus *Ixodes*, which can mate off-host without feeding).[17] Larvae are easily recognizable by the presence of only 3 pairs of legs, and absent spiracular and genital apertures (Figures 4a and 4b). Nymphs have 4 pairs of legs and spiracles (Figures 5a and 5b). Adult females have 4 pairs of legs, and spiracles, a genital aperture, and porose areas on the dorsal surface of the basis capituli (Figures 3a and 3b). Adult males have 4 pairs of legs, the scutum covers the entire dorsal surface, and 7 hard sclerotized plates cover the ventral body surface of some species (Figures 3c and 3d).

Types of hard ticks

Ixodid ticks fall into 2 behavioral groups. Exophilic or non-nidicolous ixodid ticks occur in the open environment and are associated, with forests, savannahs, second-growth areas of scrub and brush, grassy meadows, semi-desert, or desert areas. These species are usually not very host-specific. Nidicolous or endophilic ixodid ticks live in or near the nests of their hosts, are adapted to highly specialized environments (crevices or other shelters used by their hosts), and tend to be more host-specific.[8,15] Many *Ixodes* species are nidicolous.[14] The main vectors of TBEV, *I. ricinus*

Table 1: Tick species, tick habitats, and involved hosts in relation to the TBEV subtype an distribution

Tick species (subgenus)	Main habitats[6,17,148]	Hosts[6,17,148]	Virus subtype	Vector role	References**
Ixodes (Ixodes Ricinus)[70,78,91,138-145]	deciduous and mixed forests	reptiles, birds, mammals, human	ES, SS	principal vector in Europe	Radda 1973; Kožuch et al. 1967; Alekseev et al. 1996; Demina et al. 2010; Süss 2011; Wojcik-Fatla et al. 2011; Stefanoff et al. 2013; Katargina et al. 2013; Biernat et al. 2014; Drelich et al. 2014; Cuber et al. 2015
Ixodes (Pholeoixodes) arboricola[49,50]	nidicolous, nests and burrows	birds	ES	persistence and transmission to white mice; considered to be a secondary amplifying vector of TBE virus in wild populations	Lichard and Kozuch 1967; Gresikova and Kaluzova 1997
Ixodes (Pholeoixodes) lividus[140]	nests	birds	SS		Demina et al. 2010
Ixodes (Pholeoixodes) hexagonus[62,91,146,147]	nidicolous, nests, burrows, caves, rock shelters, dog kennels and also buildings	hedgehogs, wild carnivores, dogs, rarely human	ES	transstadial and transovarial transmission; TBE virus isolates. Isolated from female and nymph infesting a hedgehog; a pool of 3 females from red fox	Radda 1973; Krivanec et al. 1988; Valarcher et al. 2015; Streissle 1960
Ixodes (Pholeoixodes) canisuga[90,91]	nidicolous, nests, burrows	hedgehogs, wild carnivores, dogs	?	little is known about the vector competence	Radda et al. 1968; Radda 1973
Ixodes (Scaphixodes) frontalis[52,60,61]	nests	birds	ES	detection of TBEV; vector competence and importance in transmission cycle unknown	Hillyard 1996; Labuda and Nuttall 2004; Obsomer et al. 2013
Ixodes (Exopalpiger) trianguliceps[146,148]	endophilic. shady mixed and deciduous forests	small mammals (ca 50 species), birds, and a viviparous lizard	ES	vector and reservoir of TBE virus among the small mammals	Nowak-Chmura and Siuda 2012; Valarcher et al. 2015
Ixodes (Ixodes) persulcatus[139-141]	exophilic, deciduous and mixed forests	polyxenic reptiles, birds, mammals, human	ES, SS, FES	principal vector for the Siberian and Far Eastern subtypes from north-eastern Europe to Russian Far East, China and Japan	Demina et al. 2010; Alekseev et al. 1996; Süss 2011

ES, European subtype (TBEV-EU); FES, Far Eastern subtype (TBEV-FE); SS, Siberian subtype (TBEV-Sib)
** Reference for tick habitat and host: Nowak-Chmura and Siuda, 2012; Petney et al., 2012; Guglielmone et al., 2014*
*** Reference for tick species involved in TBE virus transmission*

and *I. persulcatus* are exophilic and exceptional both in terms of their large variety of hosts they use as well as the habitats they occupy.[18]

Host-finding behavior

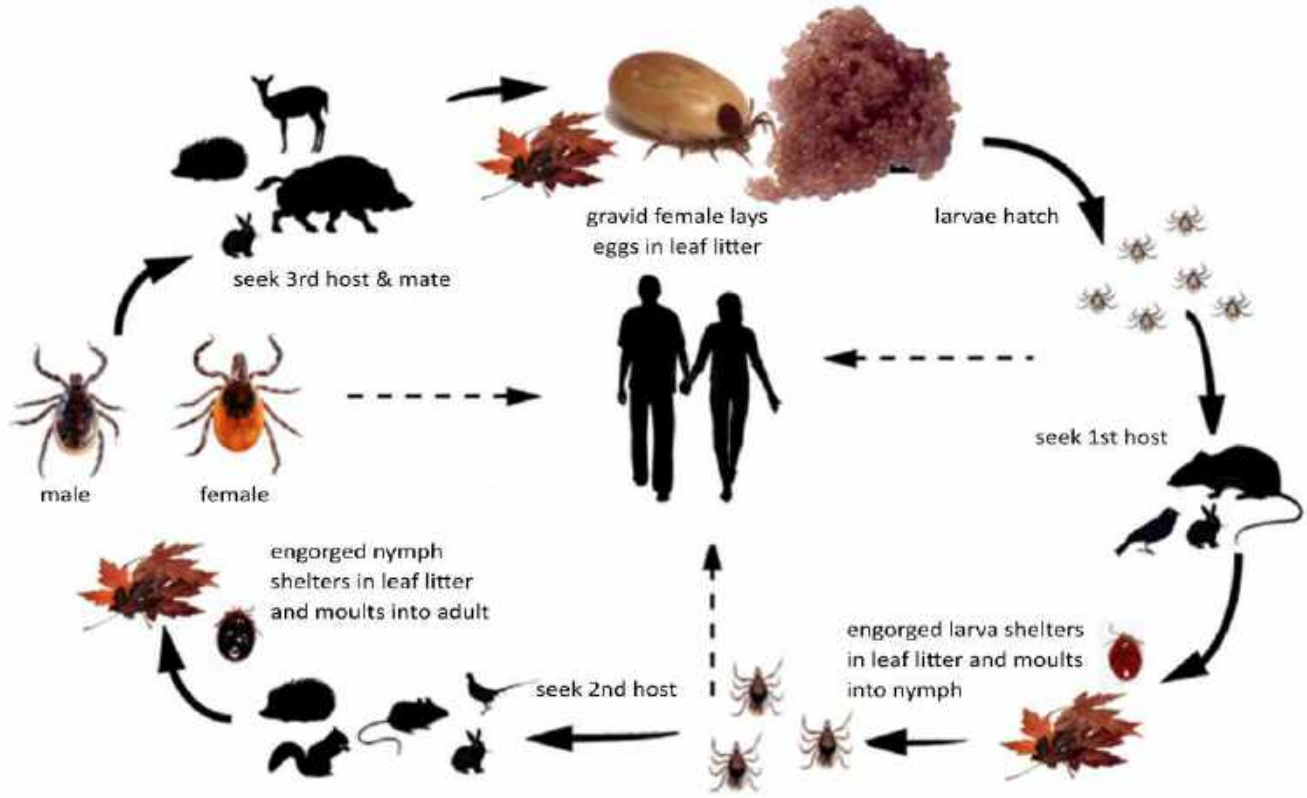
Ixodid ticks' host-seeking behavior is under the control of different abiotic factors that differ according to the region. In temperate and sub-polar regions, seasonal activity is mainly regulated by ambient temperature, changing photoperiod, and incident solar energy, and in the more temperate regions, tick activity is often controlled by saturation deficit and relative humidity, with long-term dry conditions being adverse for survival.[14] Those species involved in the transmission of TBEV tend to quest passively or ambush their hosts by climbing onto weeds, grasses, or other lower vegetation to wait for a host to pass nearby.

Ixodes ricinus adults can climb as high as 1.5 m on brushy vegetation.[19] The immature stages are found lower, up to 70 cm for larvae (O. Kahl, personal communication) and less than 1 m for nymphs.[19] Ticks are able to sense a host with their Haller's organ, which is located on the tarsi I. Haller's organ possesses chemo-, mechano-, and thermoreceptors that also ensures (together with the receptors on the palps) selection of a suitable feeding site on the host body. The most important stimuli are carbon dioxide (CO_2), vibration produced by moving potential hosts, and host temperature. For some species, visual images, host smell, and even noise can stimulate the tick.[15,20-22]

Feeding behavior

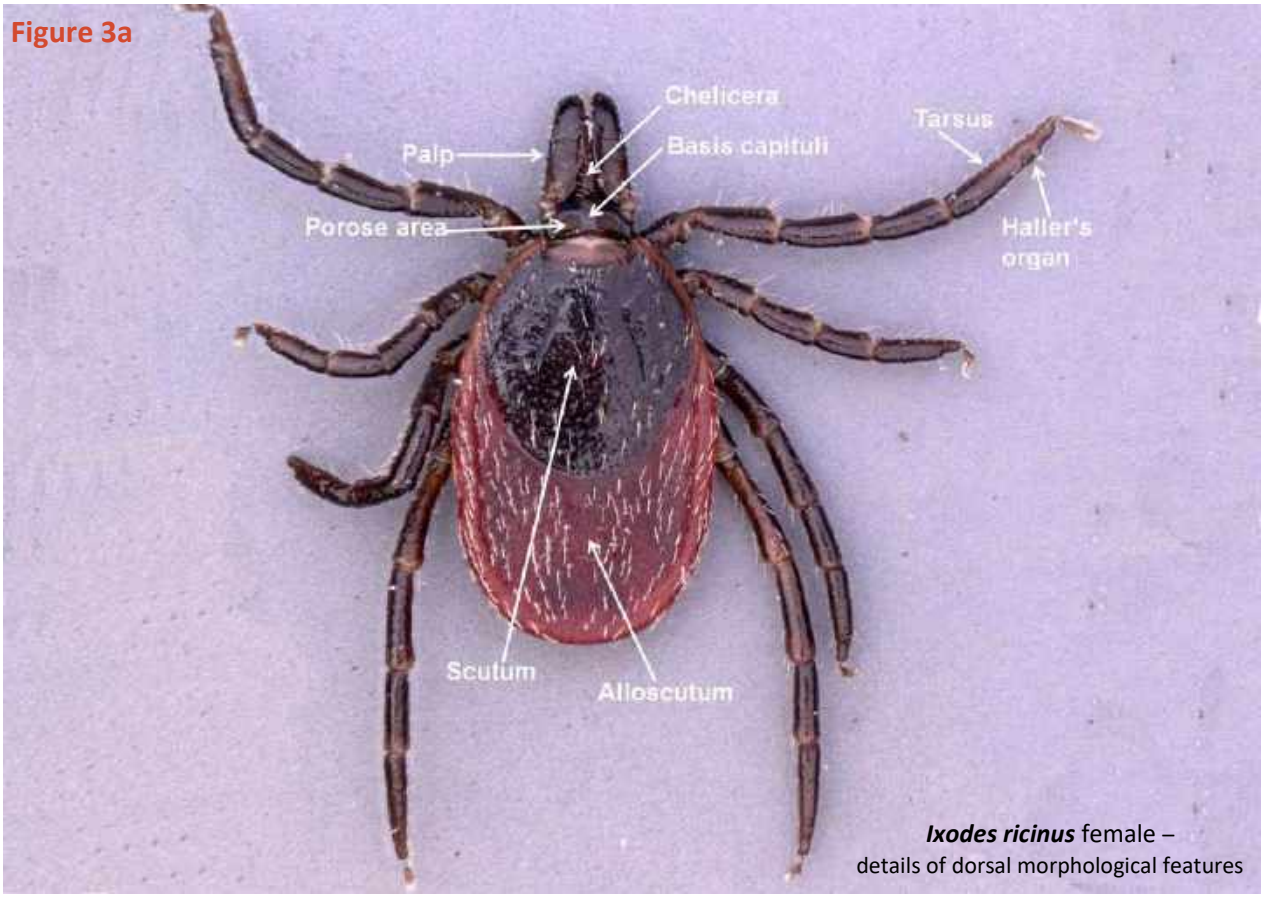
Feeding behavior, even on preferred hosts, is not a uniform process. An ixodid tick may crawl on the host for several hours in search of a suitable feeding site. After attachment, many ixodid ticks secrete cement during the first 1–2 days to secure themselves at the wound site.[22] The feeding tick begins salivating into the developing hematoma and sucking blood; phases of salivation and blood sucking alternate.[8] Saliva not only plays an important role in the feeding tick's osmoregulation[23] but also has a variety of pharmacological effects. There is an extensive array of antihemostatic, anti-inflammatory, and immunomodulatory proteins and lipids in the tick saliva that suppress the host's ability to reject the feeding tick.[8,23–26] Anticoagulant effects, inhibiting factor Xa, were first shown in *I. ricinus* in 1898-1899.[22,23] In addition, many tick species produce proteins that inhibit thrombin directly or inhibit the conversion of prothrombin to thrombin by inhibiting factor V. Other proteins prevent platelet aggregation or bind, antagonize or degrade important host mediators of pain, itching and inflammation, particularly the host's own histamine, serotonin, and bradykinin.[8,25]

Figure 2



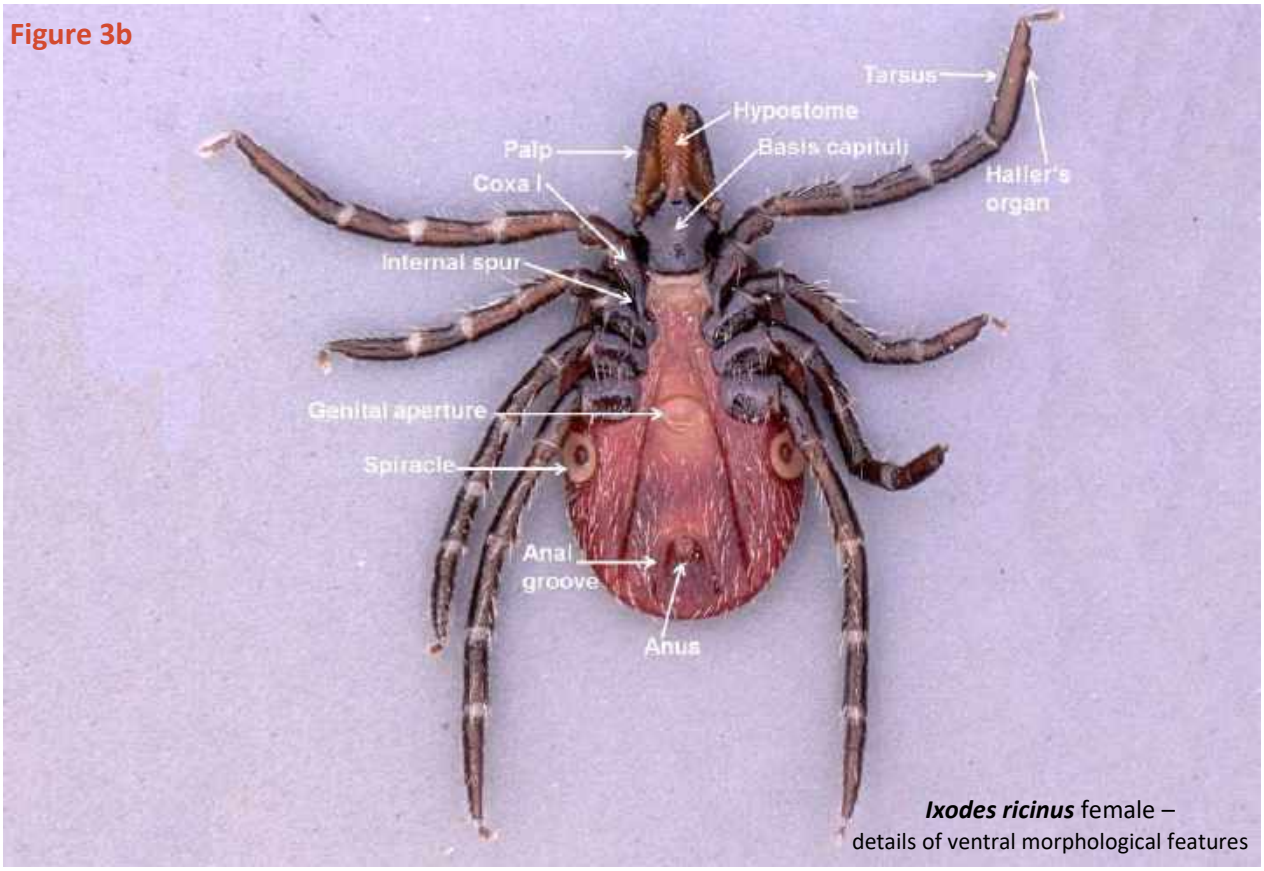
The life-cycle of Ixodes ricinus. The dotted arrows indicate potential transmission to humans. ©Nina Littwin

Figure 3a



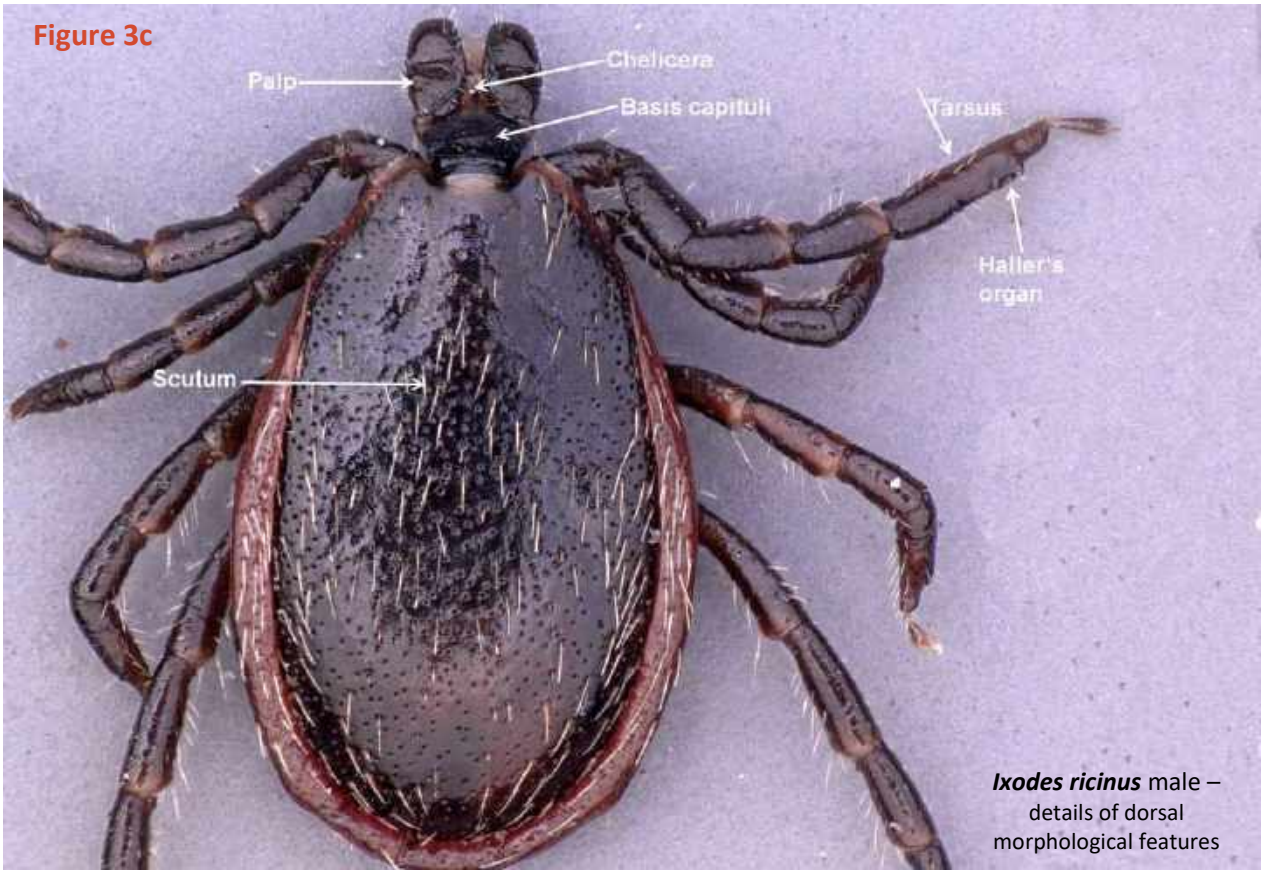
Ixodes ricinus female –
details of dorsal morphological features

Figure 3b



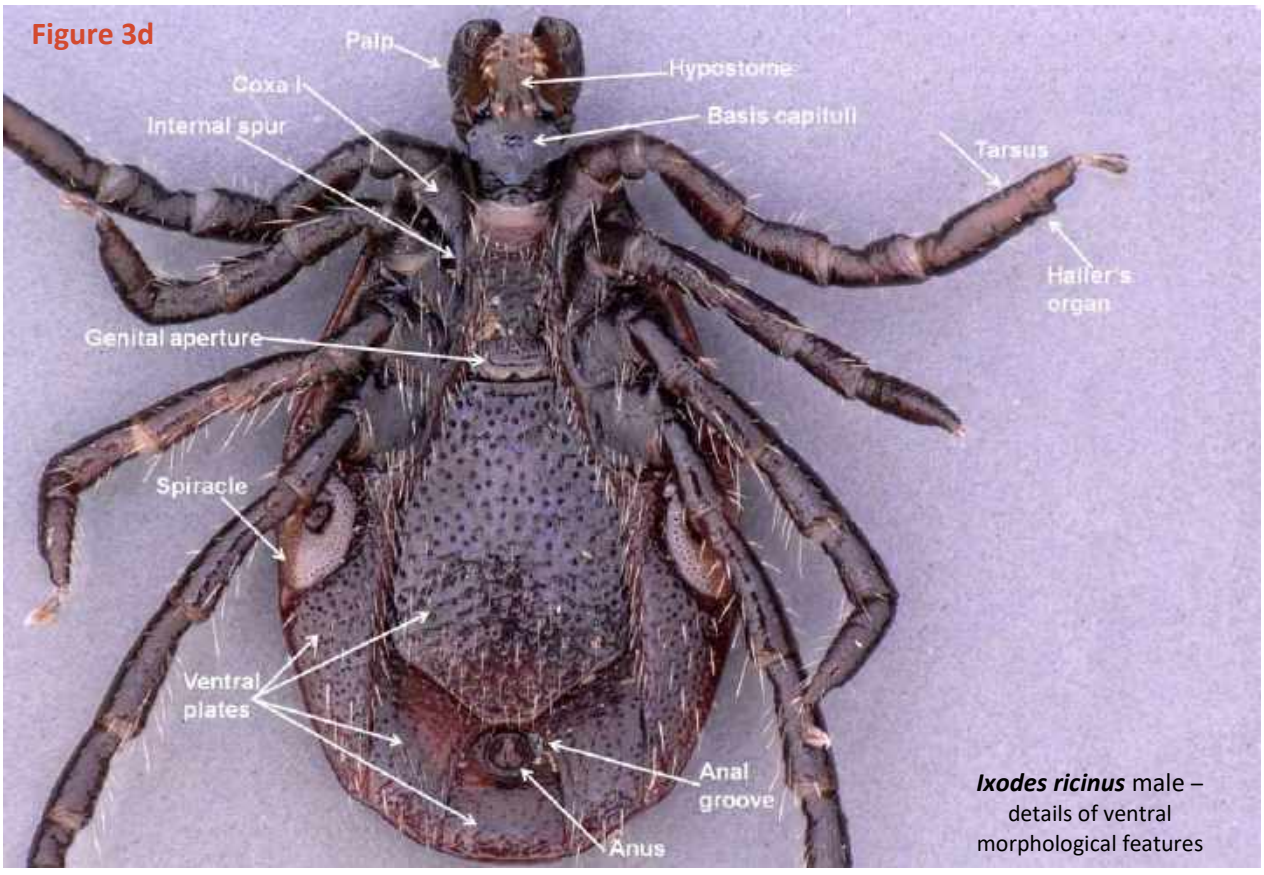
Ixodes ricinus female –
details of ventral morphological features

Figure 3c



Ixodes ricinus male – details of dorsal morphological features

Figure 3d



Ixodes ricinus male – details of ventral morphological features

Figure 4a

Ixodes ricinus larva – dorsal view

Figure 4b

Ixodes ricinus larva – ventral view

Figure 5a

Ixodes ricinus nymph – dorsal view

Figure 5b

Ixodes ricinus nymph – ventral view

Ixodid ticks feed gradually because they must first produce new cuticle to accommodate the massive blood meal.[17] Typical attachment periods range from as few as 2 days for larvae to as long as 13 days for females.[3,15]

An *I. ricinus* female can reach approximately 450 mg at the end of feeding from approximately 2 mg at the beginning of feeding.[21]

Drop-off

The controlled timing of drop-off from the host offers important ecological advantages. For non-nidicolous ticks, such drop-off rhythms are synchronized with host behavioral patterns. This tends to disperse fed ticks in optimal habitats where they can develop and reproduce. Photoperiod appears to be the dominant abiotic exogenous factor affecting drop-off patterns. The daily light:dark cycle induces a regular rhythm of feeding and dropping off. Detachment may occur while hosts are inactive in their nests or burrows or, alternatively, it may be coordinated with the period of high host activity.[15]

Host specificity

Tick species can be either opportunistic or specific with respect to the hosts they choose; both *I. ricinus* and *I. persulcatus* are opportunistic species, especially the immatures. For *I. ricinus*, more than 300 species of vertebrate hosts have been recorded.[15,27] Larvae and nymphs of *I. ricinus* feed readily on lizards, birds, and small mammals, as well as on larger hosts including deer. Adults feed on medium-sized and large mammals, especially ungulates, as well as humans, as do the immature ticks.[15] *Ixodes persulcatus* is more restricted to 46 species of hosts.[28] (Wang et al. 2023)

Questing height is also important. Ticks questing on or near the ground are exposed mostly to small animals, while those questing higher in the vegetation are more likely to encounter larger animals. The extent to which different hosts are utilized depends on host behavior and opportunities for contact, such as foraging range, time of day and time spent foraging, habitats visited, and other factors.[14]

Acceptance of a vertebrate animal is also dependent on physiological factors and the ability of the ticks to recognize it as a host. Host utilization may be influenced by the ability of ticks to evade or suppress host homeostatic systems and avoid rejection.[24]

Hard tick ecology, environmental factors

Ticks occur in many terrestrial habitats ranging from cool, arboreal northern forests to hot, arid deserts. Each species, however, has become adapted to the specific types of habitat where it is generally found in highest abundance. All *I. ricinus* postembryonic stages are exophilic and depend entirely on a suitable combination of climatic variables, making them vulnerable to climate changes and especially to desiccation. Thus, they are mainly found in cool, moist forests.[8,21,29,30]

Water balance is a critical determinant of a tick's ability to wait for hosts. Ticks may quest for weeks or even months while waiting for a host. When they have a body water deficit, they retreat to more sheltered, humid micro-environments, such as the rotting vegetation in a meadow or damp leaf litter on the forest floor. They secrete a hygroscopic salivary secretion onto their external mouthparts that collects atmospheric water at relative humidity = 80-85% (active water vapor sorption).[31] Rehydrated ticks are able to resume host-seeking. Some ticks are able to remain in the questing position for many days without rehydration, while others must return to their humid microenvironments.[32] Dense ecotonal vegetation provides shade, increased moisture, protection from intense solar radiation, and plants that support the tick hosts.

There have been various studies showing the relationship between *I. ricinus* and vegetation type in central Europe[33,34] and the capacity of this species to adapt to a large variety of biotopes with low temperature (e.g., Sweden) and high altitudes, up to 1500 m.[35–37]

Ixodes persulcatus is distributed in 14 countries, between 21° and 66° of northern latitude in Eurasia, mostly with a temperate continental climate (Wang et al. 2023). In a model predicting the suitable habitats for *I. persulcatus*, it was shown that temperature and humidity are the main factors in the distribution of this species (Wang et al. 2023). Vegetation also has an impact on the tick distribution, its requirement is wood and wet biotopes (Wang et al. 2023, Shchuchinova et al. 2015).

Normally, temperature and relative humidity in a burrow, cave, or similar type of shelter are more uniform throughout the year than in the external macro-environment. The higher relative humidity in such microenvironments is due in part to the presence of hosts, their wastes, and the plant materials they use to construct or line their nests.[38] Nidicolous ticks exhibit behavioral patterns that restrict their distribution to these sheltered locations. They avoid bright sunlight and low humidity, the type of conditions prevailing at the entrances of burrows or caves. Confined within these hidden, restricted locations, nidicolous ticks become active when hosts are present. However, when the hosts are absent, they may wait for up to several years for hosts to return, or until they die of starvation.

Diapause

An important physiological trait that enables ticks to survive adverse environmental conditions and conserve energy until conditions improve is diapause as a form of dormancy.[39] Diapause is induced by an external cue before adverse conditions occur. It is not terminated by favorable external conditions – as it is the case with quiescence – but there is some diapause development before its termination. During diapause ticks become inactive, reduce their metabolic rates, and do not feed on hosts even when given the opportunity.[8,21] Diapause can occur in each life stage, whether it is unfed or engorged. This varies, however, between species and can also differ within a tick species in different geographic areas. As an example, oviposition can be delayed in *D. marginatus.* Engorged females that feed in late summer, early fall or in winter oviposit only in the following spring.[8]

Life cycle and seasonal activity

Ixodes persulcatus inhabits mainly coniferous forests of Asia and Eastern Europe, while *I. ricinus* inhabits deciduous and mixed forests in the British Isles, in Continental Europe, and western Asia.[8,28,40–42] *Ixodes persulcatus* adult females and eggs are unable to survive the winter, however, that *I. persulcatus* larvae and nymphs, whether unfed or engorged, are able to overwinter. In contrast, eggs as well as unfed and satiated females of *I. ricinus* are capable of overwintering, a principal difference between the life-cycles of the two tick species. Vector tick activity is well correlated with the seasonal pattern of TBE occurrence. In such a focus, it is common for 2–3% of the ticks to be virus-infected.[43] In Northern and Central Europe, the seasonal activity of *I. ricinus* often has 2 peaks, one in spring (May–June) and the other one at the end of summer (September-October).

For *I. persulcatus* adults four types of seasonal dynamics throughout their distribution area were described, differing in the duration of the active period (Korenberg 2000). In the north-western area of distribution, *I. persulcatus* becomes active immediately after the melting of the snow cover with a rapid increase in abundance in May, followed by a sharp decline in mid-summer. In Karelia (a middle taiga subzone), adult activity lasted on average 74 days. Between 2012 and 2023, the relative abundance of ticks increased significantly in comparison with the 1980s monitoring period, showing a tendency towards an earlier start of the tick activity, as in the 1980s (Bugmyrin and Bespyatova, 2023).

Unfed *Dermacentor reticulatus* adults are mostly active in spring and autumn, occasionally in winter, but usually not in summer (June to early August).[44–46] During periods of snow cover and the driest and hottest weeks of the year *Dermacentor reticulatus* is inactive (Guglielmone et al. 2014). The larvae feed for 3-6 days, nymphs for 5-12 days, and females for 7-16 days, while males may remain in the host for a long time, even in the absence of females (Slovak et al. 2002, Simo et al. 2004). Adults can overwinter unfed or engorged (Kiewra et al. 2016, Drehmann et al. 2020) and are able to survive 2.5 years of starvation (Razumova, 1998). Interestingly, this tick can spend the whole winter on hosts (Karbowiak et al. 2014). *Dermacentor reticulatus* eggs can survive under water for several months and may be spread by floods into new areas (Hoogstraal, 1967). *Dermacentor reticulatus* in immature life stages is assumed to be nidicolous and therefore cannot be collected from vegetation. Nevertheless, Schmuck et al. (2020) collected *D. reticulatus* immatures (47 questing larvae and two nymphs) by flagging in June and July in 2018 and 2019, in two different locations close to the city of Leipzig, Germany. To understand under which circumstances *D. reticulatus* immatures were found outside the burrows of their hosts and can be collected from vegetation needs further investigation (Schmuck et al. 2020).

Tick species involved in TBEV transmission

Of the 54 species of ixodid ticks known from the Western Palearctic,[47] eight species from three genera are known to be able to transmit TBEV, and the virus has been isolated from at least 14 other species (Table 1). *Ixodes ricinus*, the most commonly encountered European tick species, is considered to be the principal vector of TBEV there.[48] Lichard and Kozuch[49] were able to show TBEV persistence and transmission to white mice by *Ixodes arboricola*, which is considered a secondary amplifying vector of TBEV.[50] *Ixodes persulcatus* is also known to transmit TBEV.[51,52] It is the adult female *I. persulcatus*, which infects humans with TBEV and other zoonotic pathogens. Neither the larval nor the nymphal stage often attaches to humans.[8] Both *D. marginatus* and *D. reticulatus* are also vectors of TBEV.[53–55]

Haemaphysalis concinna is a known vector of TBEV as well.[56,57] Evidence for the vectorial capacity of *Haemaphysalis inermis* for TBEV is available from Nosek et al.[58] The virus has been isolated in the Czech Republic from female and nymphal *I. hexagonus* infesting a hedgehog.[61] TBEV also has been detected in *Haemaphysalis punctata*.[62,63]

The role of *Dermacentor* ticks (Table 1) in the circulation of TBEV in the environment is unclear and poorly studied.[64,65] *D. reticulatus* appears to be spreading and population density increasing during recent decades.[66-68] In eastern Poland, the mean prevalence of infection with TBEV found in questing adult *D. reticulatus* was 10.8% (range 7.3–14.3% in infected areas): This is considerably higher than the prevalence found in questing adult *I. ricinus* (1.6%, range 0.7–4.3% in infected areas).[69]

Table 2. Animal hosts from which TBEV* has been recovered

Order/Family	Species	Virus type
Mammalia: Rodentia		
Muridae	*Apodemus agrarius*[85,93,150]	FES
	Apodemus flavicollis[93,138]	ES
	Apodemus sylvaticus[93,138]	ES
	Apodemus speciosus[151]	FES
	Apodemus argenteus[151]	FES
	Myodes rufocanus[151]	FES
	Rattus norvegicus[151]	FES
Cricetidae	*Microtus agrestis*[93]	ES
	Microtus arvalis[93,138]	ES
	Myodes glareolus[93,138,150]	ES
	Myodes rufocanus[85]	
	Myodes rutilus[85]	
Sciuridae	*Sciurus vulgaris*[59,138]	ES
Dipodidae	*Sicista betulina*	
Eulipotyphlya		
Erinaceidae	*Erinaceus concolor*[59]	
	Erinaceus roumanicus[138]	ES
Talpidae	*Talpa europaea*[59]	
Soricidae	*Sorex araneus*[85,138]	ES
Goats	*Capra* sp.[157-159]	
Sheep	*Ovis aries*[158]	
Bovidaes	*Bos taurus*[158]	
Bison	*Bison bonasus*[72]	FES
Carnivora		
Canidae	*Vulpes vulpes*[90,91,152,153]	
	Canis familiaris[160]	FES
Mustelidae	*Mustela putorius*[115]	ES
Artiodactyla		
Cervidae	*Cervus elaphus*[134,154]	
	Capreolus capreolus[134,155,156]	
	Alces alces[134]	
Aves (families)**	Virus isolation[59,82,161,162]: Passeriformes: Acrocephalidae, Bombycillidae, Corvidae, Emberizidae, Frigillidae, Hirundinidae, Laniidae, Motacillidae, Muscicapidae, Paridae, Passeridae, Psylloscopidae, Sittidae, Sturnidae, Sylviidae, Turdidae. Others: Anatidae, Phasianidae, Picidae, Rallidae, Scolopacidae Transovarial transmission[59]: Accipitridae, Charadriidae, Columbidae, Emberizidae, Laniidae, Troglodytidae, Turdidae	

ES, European subtype (TBEV-EU); FES, Far-Eastern subtype (TBEV-FE); SS, Siberian subtype (TBEV-Sib)
**Selected references; **Less information available*

Prevalence of TBEV in questing adult *D. reticulatus* ticks from Białowieża Primeval Forest was similar (1.58%)[70] to that in questing *I. ricinus* (1.30%),[71] as was the case in Moldova (adult *I. ricinus* 3.8%, adult *D. reticulatus* 3.9%, but adult *Haemaphysalis punctata* 8.8%).[72] The natural occurrence of TBEV in a *D. reticulatus* tick population was also proven for Germany during 2016 to 2018 by isolation of several TBEV strains from this tick species in a natural focus.[73]

The differences in TBEV prevalence in the various vector species remain puzzling. Questing *I. ricinus* usually have a very low prevalence of the virus, ranging from no virus in many areas to less than 1% in most others, and rarely reaching 2–5%, in unfed adults.[74–78] Knap and Avsic-Zupanc[77] showed that over a 4-year period, the prevalence was at the expected low level in the 8 areas studied, but that no area was consistently positive for the virus. This may be related to the frequently low sample sizes (14/30 samples had fewer than 300 specimens).

Prevalence of the virus in feeding ticks, although very variable, can be substantially higher.[78] Waldenström et al.[80] showed a low prevalence (0.5%) in nymphs and larvae feeding on migratory birds in Sweden, while Kazarina et al.[81] detected 14% nymphs and 7% larvae of *I. ricinus* on migratory birds infected in Latvia. Data for *I. persulcatus* are more variable. Korenberg and Kovalevskii[82] reported a high TBEV prevalence in unfed adults, ranging from 10.9% to 38.7% over 6 years (mean 26.2%) in unfed adults in the Pre-Ural Region, whereas the prevalence in the Primorskii Region of the Russian Far-East ranged from a little over 1% to over 9% from 1970 to 1990, and in the Khabarovsk Region from 3.4% to 9.4% over 4 years.[83] In the Novosibirsk Region, the prevalence of TBEV in unfed adult *I. persulcatus* was 3.6%, with 0.8% being pathogenic to laboratory mice.[84] In the same study, 3.3% of questing adult *I. pavlovskyi* were infected with the virus with 1.8% of the isolates being pathogenic. Information on less commonly encountered vectors is rarely available and sample sizes are usually low, making such data unreliable (e.g., Kim et al.)[85] Long-term studies and statistical analyses showed that higher average temperatures during the summer-autumn period may lead to higher levels of TBEV found in ticks and consequently increase the risk for humans to develop symptomatic TBE following an infected tick bite.[86]

Vertebrate hosts

The prevalence of antibodies to TBEV in hosts is quite variable.[80] TBEV has been found in numerous mammal species from different families, as well as in a large number of passerine and non-passerine bird species (Table 2). Virus infection was demonstrated by antibodies to the virus or viral ribonucleic acid (RNA) detection in a wide variety of bird species,[80,81,87,88] with virus isolation from *Turdus pilaris* (fieldfare) and *Acrocephalus dumetorum* (Blyth's reed warbler) opening the possibility of virus transfer to new foci during bird dispersal or migration.[87] Viremia has been induced experimentally in birds, reaching levels sufficient to infect feeding ticks.[59] Generally speaking, findings of TBEV in animals, whether indirect or direct, do not mean very much eco-epidemiologically. Only the demonstration of reservoir competence indicates an active role in the perpetuation of TBEV.

Red foxes (*Vulpes vulpes*) are known to be reservoir-competent for TBEV.[89,90] Although *I. hexagonus* is a proven vector of TBEV, little is known about the vector competence of the fox tick *I. canisuga*.

In recent years, the detection of viral RNA in hosts has become possible. Tonteri et al.,[91] in Finland, detected the European (TBEV-EU) and Siberian (TBEV-Sib) subtypes in *M. glareolus*, TBEV-Sib in the shrew *Sorex araneus*, and TBEV-EU in *Microtus agrestis*. Achazi et al.[93] detected TBEV RNA in rodent brain tissue in prevalences up to 20% in TBE non-risk as well as in risk areas in east-German Federal States. In the Novosibirsk region of Siberia, where *I. persulcatus* and *I. pavlovskyi* are the main TBEV vectors, the prevalence of TBEV viral RNA in 5 small mammal species was extremely high.[85] It ranged from 35.3% for *A. agrarius* organs to 82.2% for *Myodes rutilus* blood, with a mean value for all species and tissues of 62.1%. All 3 virus subtypes were represented. In addition to small mammal hosts, larger wild and domestic animals frequently have high antibody prevalences. Because they feed large numbers of vector ticks, they can be used as sentinels for the occurrence of TBEV in a given area.

TBEV transmission

Nuttall et al.[94] noted: "Reciprocal interactions of parasites transmitted by blood-sucking arthropod vectors have been studied primarily at the parasite-host and parasite-vector interface. The third component of this parasite triangle, the vector-host interface, has been largely ignored."

The adult female tick is considered to play only a minor role in virus circulation. Tick males, which either do not feed or feed for only a short time, might also be involved in virus transmission.[96] TBEV invades all tick tissues, including the salivary glands and ovaries,[95] thus it may be transmitted by ticks in the following ways: 1) via saliva, 2) transovarially (vertically), and 3) sexually.[40,97–99]

TBEV transmission from vector ticks to hosts via saliva

Certain species of ticks are vectors and reservoirs of TBEV, and they can transmit the virus already when they start feeding[43,100] with viral particles contained in the saliva,

which the ticks release into the host tissues.[40]

TBEV is present in the alveolar cells of the salivary glands of *D. marginatus* and *H. inermis* females in as few as five days after their feeding on viremic white mice.[55] Also certain vertebrates, so-called reservoir hosts, are important for the amplification of the virus and are together with vector ticks the basis for the heteroxenous TBEV perpetuation.[101]

Viremic transmission from hosts to feeding ticks

Ticks become infected with TBEV while they feed on a viremic host.[98,99,102] Nosek et al.[103,104] proved that a viremia in a host lower than 10^1 mouse LD_{50}./0.03 ml was insufficient to cause infection in ticks. In individual engorged *I. ricinus* ticks, the virus titer was 10^1-10^4 mouse LD_{50}/0.03 ml. Viremic white mice served as virus donors.[103,104] Grešíková and Nosek[105] demonstrated the persistence of TBEV in *H. inermis* (from larva to nymph) and then the transmission from *H. inermis* nymphs to white mice. Viremia surpassing the threshold values of infectivity for tick vectors was also found in some juvenile and adult *Myodes rufocanus*, *M. rutilus*, and *Micromys minutus*. The viremia level depends on the rodent species and age, and exhibits individual variability.[106]

Co-feeding transmission

TBEV transmission is also possible from infected to non-infected ticks during feeding close to each other on a non-viremic host.[98,102] Cellular infiltration of tick feeding sites, and the migration of cells from such sites, can provide a vehicle for transmission between co-feeding ticks that is independent of host viremia.[102] The non-viremic route of transmission between co-feeding ticks can even occur in rodents that are already immune to TBEV.[108] The degree of co-feeding virus transmission may be influenced by local climatic factors that affect the seasonal timing of tick host-seeking activity and, as such, can be used to predict the focal distribution of TBEV.[107,109]

Transovarial transmission

Another possible way for ticks to transmit TBEV involves transovarial transmission and transstadial persistence (see below), which were described for the first time as early as 1940.[110] However, only some eggs in the batch of a TBEV-infected vector tick female become infected.[111] In addition, virus can partly be lost during transition from stage to stage,[112] and not all tick individuals reach the next life stage irrespective of the presence or absence of the pathogen. Danielova and Holubova[113] found that only 0.23% of larvae coming from infected females were TBEV-positive. Other studies showed that 0.58% to 0.75% of the larvae were transovarially infected. Thus, the rate of transovarial transmission remains below 1%. Nuttall et al.[114] suggest that transovarial transmission is important for the maintenance of a natural focus even if it occurs at a very low rate.

Danielova et al.[76] detected TBEV in 2 out of 647 flagged larvae of *I. ricinus*, which indicates transovarial transmission.

Transstadial persistence

TBEV was not detectable in *I. ricinus* nymphs 14 days after molting from larvae that had engorged on viremic *A. flavicollis*, but TBEV was present in these ticks two months post ecdysis. Many nymphs contained the virus, indicating that the latter undergoes an eclipse phase during metamorphosis.

Sexual transmission in ticks

Transmission of TBEV from males to females[116] is successful in only 10% of copulations in infected *I. persulcatus*, but it may provide notable support for the transfer of the virus to the following generation of ticks if transovarial transmission follows. A mathematical model of sexual transmission of the virus[117] was developed long before determining that such a sort of transmission occurs. Virus exchange between a non-engorged female and an infected male of *I. persulcatus* that 'feeds' on (i.e., attaches to) the female before or after copulation is quite probable, and it has been proven that the saliva of starved males contains a fairly large amount of virus, sufficient for infecting not only animals[118] but also humans. The feeding of *I. persulcatus* males on females with which they later copulate can be observed in 2–10% of cases.[118]

Vertical TBEV transmission in vertebrates

TBEV transmission from mother to her offspring in small rodents, e.g., red voles (*M. rutilus*), was shown for naturally infected reservoir hosts as well as after experimental infection with different sublethal doses of the virus.[119] TBEV RNA was detected in up to 90% of the newborn rodents, 240–280 days after experimental infection of their parents, by real-time polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and bioassays. The small amounts of TBEV RNA detected in the embryos, placenta, and blood serve as evidence of prenatal transmission. Postnatal transfer of the virus might occur through the rodent's milk. Vertical virus transmission may occur before, during, and/or after birth of the baby rodents with a high frequency. In natural foci, this could ensure long-term persistence of TBEV in mammal hosts without involving any arthropod vectors.[119] Divé et al. (2020) reported detailed investigation of pre- and postnatal health assessment of three children in the context of severe maternal TBEV infection during pregnancy. The clinical and virological data strongly suggest that fetal TBEV infection did not occur, despite severe manifestations in the

pregnant females. Non-reservoir hosts do not directly participate in virus transmission, but can play an important role in the maintenance of natural foci. The density of reservoir-incompetent hosts may have either a positive effect on virus transmission, by amplifying the tick population, or a negative ('dilution') effect, as tick bites on a non-reservoir host cannot lead to virus transmission.[98,120]

Alimentary route of transmission

Humans mostly become infected with TBEV via tick bites, but viral transmission is also possible via the consumption of unpasteurized goat, cow and sheep milk.[43] Approximately 1% of all TBEV infections in humans are probably acquired by consuming infected unpasteurized milk and milk products from infected livestock, particularly goats.[121]

Outbreaks due to alimentary virus transmission are known from Eastern, Central and Southern Europe,[122,123] and have to be considered particularly in cases of local epidemics.[123–125] Ličková et al. (2022) summarize the history and recent alimentary TBEV infections in Europe. In an alimentary outbreak in Germany, due to consumption of a fresh goat cheese, the virus could be for the first time isolated from naturally infected cheese (Brockmann et al. 2018).

TBEV interhuman transmission of TBEV by breast milk has not been confirmed or ruled out. Kerlik et al. (2022) reported a case of probable transmission of TBEV from an unvaccinated mother to an infant through breast-feeding.

The natural cycle

The natural cycle of TBEV is highly complex, and many details remain obscure. The three prevailing TBEV subtypes overlap in some areas, they all have multiple mammalian reservoir hosts and various tick vectors, and in some areas these subtypes occur sympatrically. Humans are not included in these natural cycles, but may enter those transmission cycles inadvertently.

Small mammals as a reservoir and vector ticks play a central role in the natural cycle of TBEV, but non-reservoir hosts such as birds and large vertebrates, such as wild ungulate species, or foxes, may also indirectly contribute to the spread and maintenance of TBEV. Additionally, changing climatic patterns, as well as changes in ecosystems, may not only affect the spatial distribution of TBEV, but also the maintenance of small natural TBEV foci.[128,129] Small rodents such as *A. flavicollis* are important hosts for the larvae of *I. ricinus*, the probably most important TBEV amplifying host in Central Europe. *Apodemus flavicollis temporarily* develops high virus titers necessary to infect ticks. Detailed studies by Radda et al.,[90,115] who trapped small rodents and collected the engorged ticks in a natural TBE focus for 2 years, showed that given certain prerequisites are fulfilled (high numbers of rodents, vector tick larvae and nymphs feeding on these rodents), such a natural TBEV focus is able to sustain itself without any significant input of other hosts. This may explain why many of these natural foci are stable, but restricted to small areas, and why they harbor TBEV-positive ticks over a long period of time. Forest structure, especially deforestation and reforestation, are known to have a huge impact on ticks and vertebrate reservoir hosts for many tick-borne pathogens.[130,131]

Experimental transstadial maintenance of TBEV in *D. marginatus* and *D. reticulatus* ticks emphasizes the role of both species. TBEV infection and transmission rates in *Dermacentor* species to hosts are somewhat lower than in species of the genera *Ixodes* and *Haemaphysalis*.[54] Feeding larvae and nymphs of *I. persulcatus* may become infected with TBEV if the virus titer in the host blood reaches at least 3.0 $\log_{10}$ LD_{50}/0.03 mL.[132] Such levels of viremia occur only in small rodents and are a critical factor in the virus circulation between vertebrates and ticks in natural foci. In small rodents, the infection is asymptomatic.[91]

TBEV has been isolated from a wide range of birds from many different families, including migratory species, which may be important for the distribution of the virus. A common strategy for migratory birds is to rest at certain stopover sites along their routes. At these sites, the birds can be infested with ticks or engorged ticks can detach after engorgement. Sándor et al.[133] detected 4 different tick species on 11 different bird species in the Danube Delta, including larvae, nymphs, and females of *I. ricinus*.

A high variability is found between areas and years with respect to viral prevalence in both vertebrate hosts and vector tick populations, while consistent differences between vectors. For example the generally higher TBEV prevalences in *I. persulcatus* compared with those in *I. ricinus* may relate to the ecology/biology of the individual vectors. The complexity is well defined by the various mathematical models aimed at exploring the dynamics of TBEV ecology.[98,136,137] Hartemink et al.[137] list 19 parameters based on field data to define the basic reproduction number (R_o) of tick-borne infections, while Rosà et al.[98] list 32 parameters in a more comprehensive model. Unfortunately, no single study has been able to comprehensively measure all the parameters needed to test these models, although approximations are available.

Contact: lydiachitimia@gmail.com

Citation: Chitimia-Dobler L. TBEV-transmission and natural cycles. Chapter 5. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_5-7

References

1. de la Fuente J, Estrada-Pena A, Venzal J, Kocan K, Sonenshine D. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. *Front Biosci.* 2008;13:6938-46.
2. Estrada-Peña A, Jongejan F. Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission. *Exp Appl Acarol.* 1999;23:685-715.
3. Balashov Y. Bloodsucking ticks (Ixodoidea) – vectors of diseases of man and animals. *Misc Publ Entomol Soc.* 1972;8:163-376.
4. Skuballa J, Petney T, Pfäffle M, Taraschewski H. Molecular detection of *Anaplasma phagocytophilum* in the European hedgehog *(Erinaceus europaeus)* and its ticks*. Vector Borne Zoonotic Dis.* 2010;10:1055-7.
5. Pfäffle M, Littwin N, Muders S, Petney T. The ecology of tick-borne diseases. *Int J Parasitol.* 2013;43:1059-77.
6. Beati L, Klompen H. Phylogeography of Ticks (Acari: Ixodida). *Annu Rev Entomol.* 2019; 64:379-397.
7. Guglielmone A, Robbins R, Apanaskevich D, Petney T, Estrada -Peña A, Horak I. *The hard ticks of the world (Acari: Ixodida: Ixodidae). Heidelberg: Spinger; 2014.*
8. Nicholson W, Sonenshine D, Lane R, Uilenberg G. Ticks (Ixodida). In*: Medical and Veterinary Entomology.* 2nd Ed. Eds. Mullen GR, Durden LA. 2009.
9. Barker S C, Burger T D. Two new genera of hard ticks, *Robertsicus* n. gen. and *Archaeocroton* n. gen., and the solution to the mystery of Hoogstraal's and Kaufman's "primitive" tick from the Carpathian Mountains*. Zootaxa.* 2018;4500 4:543-552.
10. Mans J, Kelava S, Pienaar R, Featherston J, de Castro MH, Quetglas J, Reeves WK, Durden LA, Miller MM, Laverty TM, Shao R, Takano A, Kawabata H, Moustafa MAM, Nakao R, Matsuno K, Greay TL, Evasco KL, Barker D, Barke SC. Nuclear (18S-28S rRNA) and mitochondrial genome markers of Carios (Carios) vespertilionis (Argasidae) support Carios Latreile, 1796 as a lineage embedded in the Ornithodorinae: re-classification of the Carios sensu Klompen and Oliver (1993) clade into its respective subgenera. *Tick Tick Born Dis*. 2021;12:101688. https://doi.org/10.1016/j.tbdis.2021.101688
11. Petney T, Robbins R, Guglielmone A, et al. A look at the world of ticks. *Parasitology Research Monographs*. 2011;2:283-96.
12. Sonenshine D, Roe RM. (eds) *Biology of Ticks*, 2nd Ed. Bands 1 and 2. Oxford University Press, Oxford. 2013
13. Guglielmone A, Nava S. Names for Ixodidae (Acari: Ixodoidea): valid, synonyms, incertae sedis, nomina dubia, nomina nuda, lapsus, incorrect and suppressed names–with notes on confusions and misidentifications. *Zootaxa*. 2014;24:1-256.
14. Clifford C.M., Sonenshine D.E., Keirans J.E., Kohls G.M. Systematics of the subfamily Ixodinae (Acarina: Ixodidae) 1. The subgenera of *Ixodes*. *Ann. Entomol. Soc. Am*. 1973;66:489–500.
15. Filippova N.A. *Ixodid Ticks of the Subfamily Amblyomminae*. Leningrad: Izd. Nauka.1977.
16. Sonenshine D, Lane R, Nicholson W. Ticks (Ixodida). *Med Vet Entomol*. 2002;10:517-58.
17. Sands A.F., Apanaskevich D.A., Matthee S., Horak I.G., Harrison A., Karim S., Mohammad M.K., Mumcuoglu K.Y., Rajakaruna R.S., Santos-Silva M.M., Matthee C.A.. Effects of tectonics and large scale climatic changes on the evolutionary history of Hyalomma ticks. *Mol Phylogenet Evol*. 2017;114:153-165.
18. Petney T, Pfäffle M, Skuballa J. An annotated checklist of the ticks (Acari:Ixodida) of Germany. *Syst Appl Acarol*. 2012;17:115-70.
19. Moshkin M, Novikov E, Tkachev S, Vlasov V. Epidemiology of a tick-borne viral infection: theoretical insights and practical implications for public health. *BioEssays*. 2009;31:620–8.
20. Liebisch A, Liebisch G. Biologie und Ökologie der Zecken. In: *Einheimische Zeckenborreliose (Lyme-Krankheit) bei Mensch und Tier*. 4th Ed. Eds Horst H, Liebisch A. Balingen: Spitta; 2003.
21. Waladde S, Rice M. The sensory basis of tick feeding behavior. In: *Physiology of ticks*. Eds Obenchain F, Galun R. Oxford: Pergamon Press; 1982.
22. Guetard M. *Ixodes ricinus: morphologie, biologie élevage, données bibliographique*. Toulouse: Thése dr. vet. ENV; 2001.
23. Mehlhorn H. *Encyclopedic reference of parasitology*. Berlin, Heidelberg: Springer; 2001.
24. Mao H, Kaufman WR. DNA binding properties of the ecdysteroid receptor in the salivary gland of the female ixodid tick, Amblyomma hebraeum. *Insect Biochem Mol Biol*. 1998;28:947-57.
25. Ribeiro J. Role of saliva in blood-feeding by arthropods. *Annu Rev Entomol*. 1987;32:463-78.
26. Ribeiro J. Ribeiro JMC. Role of saliva in tick/host interactions. *Exp Appl Acarol.* 1989;7:15-20.
27. Turni C, Lee R, Jackson L. Effect of salivary gland extracts from the tick, Boophilus microplus, on leucocytes from Brahman and Hereford cattle. *Parasite Immunol*. 2002;24:355-61.
28. Andersson J. Epizootiology of Lyme Borreliosis. *Scand J Infect Dis*. 1991;77:23-34.
29. Balashov Y. Distribution of ixodid ticks (Acarina, Ixodidae) over landscapes within their ranges. *Entomol Rev*. 1997;77:625-37.
30. Wang S-S, Liu J-Y, Wang B-Y, Wang W-J, Cui X-M, Jiang J-F, Sun Y, Guo W-B, Pan Y-S, Zhou Y-H, Lin Z-T, Jiang B-G, Zhao L, Cao W-C. Geographical distribution of *Ixodes persulcatus* and associated pathogens: Analysis of integrated data from a China field survey and global published data. *One Health* 2023;16:100508. https://doi.org/10.1016/j.onehlt.2023.100508
31. Estrada-Peña A, Mihalca A, Petney T, (eds). *Ticks of the Western Palearctic*. Heidelberg: Springer; 2018.

32. Gage K, Burkot T, Eisen R, Hayes E. Climate and vector-borne diseases. *Am J Prev Med*. 2008;35:436-45.

33. Gaede K., Knülle W. On the mechanism of water vapour sorption from unsaturated atmospheres by ticks. *J Exp Biol*. 1997;200:1491–1498.

34. Knülle W., Rudolph R.D. Humidity relationships and water balance of ticks. In *Physiology of Ticks* (ed. F. D. Obenchain. and R. L. Galun), pp. 43–70. Oxford: Pergamon Press. 1982.

35. Daniel M, Kolar J. Using satellite data to forecast the occurrence of the common tick *Ixodes ricinus*. *J Hyg Epidemiol Microbiol Immunol*. 1990;34:243-52.

36. Daniel M, Kolar J; Zeman P, Pavelka K, Sadlo J. Predictive map of *Ixodes ricinus* high incidence habitats and a tick-borne encephalitis risk assessment using satellite data. *Exp Appl Acarol*. 1998;22:417-33.

37. Perez C, Rodhain F. Biologie d' *Ixodes ricinus*, I. Ecologie, cycle evolutif. *Bull Soc Pathol Exot*. 1977;2:187-92.

38. Shchuchinova LD, Kozlova IV, Zlobin VI. Influence of altitude on tick-borne encephalitis infection risk in the natural foci of the Altai Republic, Southern Siberia. *Ticks Tick Born Dis*. 2015;6:322-329 https://doi.org/10.1016/j.ttbdis.2015.02.005.

39. Perez C, Rodhain F. Biologie d'Ixodes ricinus, II. Incidence epidemiologique. *Bull Soc Pathol Exot*. 1977;2:193-201.

40. Tälleklint L, Jaenson T. Tälleklint L, Jaenson TGT. Increasing geographical distribution and density of *Ixodes ricinus* (Acari: Ixodidae) in central and northern Sweden. *J Med Entomol*. 1998;4:521-6.

41. Burda H, Šumbera R, Begall S. Microclimate in burrows of subterranean rodents – revisited. In: Subterranean rodents: *News from underground*. Eds Begall S, Burda H, Schleich C. Berlin Heidelberg: Springer Verlag; 2007.

42. Gray J, Kahl O, Lane RS, Levin ML, Tsao JI. Diapause in ticks of the medically important Ixodes ricinus species complex. *Ticks Tick-Borne Dis*. 2016 7:992-1003.

43. Filippova N. *Taiga tick Ixodes persulcatus Schulze (Acarina, Ixodidae) Morphology, Systematics, Ecology, Medical importance*. [In Russian]. Leningrad: Nauka; 1985.

44. Bugmyrin SV, Bespyatova LA. Seasonal activity of adult ticks *Ixodes persulcatus* (Acari, Ixodidae) in the North-West of the distribution area. Animals 2023;13:3834. https://doi.org/10.3390/ani13243834

45. Korenberg E. Seasonal population dynamics of Ixodes ticks and tick-borne encephalitis virus. *Exp Appl Acarol*. 2000;24:665–81.

46. Naumov R. The longevity of the tick *Ixodes ricinus* (Acari: Ixodidae) in Central Russia. [In Russian]. *Parazitologia*. 2006;40:384-95.

47. Gaidamovich S. Tick-borne Flavivirus infections. *Exotic Viral Infect*. 1995.

48. Guglielmone A, Nava S, Robbins R, Apanaskevich D, Petney T, Estrada-Peña A, Horak I. *The hard ticks of the world (Acari: Ixodida: Ixodidae)*. Heidelberg: Spinger; 2014.

49. Slovák M, Labuda M, Marley SE. Mass laboratory rearing of *Dermacentor reticulatus* ticks (Acarina, Ixodidae). Biologia 2002;57:261-266.

50. Šimo L, Kocáková P, Sláviková M, Kubeš M, Hajnická V, Vančová I, Slovák M. Dermacentor reticulatus (Acari, Ixodidae) female feeding in laboratory. *Biologia* 2004;59:655-660.

51. Kiewra D, Czułowska A, Lonc E. Winter activity of *Dermacentor reticulatus* (Fabricius, 1794) in the newly emerging population of Lower Silesia, south-west Poland. *Tick Tick Borne Dis*. 2016;7:1124-1127. http://dx.doi.org/10.1016/j.ttbdis.2016.08.012

52. Drehmann M, Springer A, Lindau A, Fachet K, Mai S, Thoma D, Schneider CR, Chitimia-Dobler L, Bröker M, Dobler G, Mackenstedt U, Strube C. The spatial distribution of Dermacentor ticks (Ixodidae) in Germany - Evidence of a continuing spread of *Dermacentor reticulatus*. Front. Vet. Sci.2020;7:578220. doi: 10.3389/fvets.2020.578220

53. Razumova I. The activity of Dermacentor reticulatus Fabr. (Ixodidae) ticks in nature. *Med. Parazitol*. 1998;4:8–14. (In Russian)

54. Hoogstraal H. Ticks in relation to human diseases caused by Rickettsia species. *Annu Rev Entomol.* 1967;12:377-420.

55. Schmuck HM, Chitimia-Dobler L, Król N, Kacza J, Pfeffer M. Collection of immature *Dermacentor reticulatus* (Fabricius, 1794) ticks from vegetation and detection of Rickettsia raoultii in them. *Ticks and Tick Borne Dis*. 2020;11:101543. https://doi.org/10.1016/j.ttbdis.2020.101543

56. Chitimia-Dobler L. Spatial distribution of *Dermacentor reticulatus* in Romania. *Vet Parasitol*. 2015;214:219-23.

57. Karbowiak G. The occurrence of the Dermacentor reticulatus tick – its expansion to new areas and possible causes. *Ann Parasitol*. 2014;60:37–47.

58. Kiewra D, Czulowska A, Lonc E. Winter activity of Dermacentor reticulatus (Fabricius, 1794) in the newly emerging population of Lower Silesia, south-west Poland. *Ticks Tick Borne Dis*. 2016;6:1124-7.

59. Süss J. Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine*. 2003;21 Suppl 1:S19-35.

60. Lichard M, Kozuch O. Persistence of tick-borne encephalitis virus in nymphs and adults of *Ixodes arboricola* and its transmission to white mice. *Acta Virol*. 1967;11:480.

61. Grešíková M, Kaluzova M. Biology of tick-borne encephalitis virus. *Acta Virol.* 1997;41:115–24.

62. Nuttall P, Labuda M. Tick-borne encephalitis subgroup. In: *Ecological dynamics of tick-borne zoonoses*. Eds Sonenshine D, Mather T. 1994:351-91.

63. Labuda M, Nuttall P. Tick-borne viruses. *Parasitology*. 2004;129 (S1):S221-45.

64. Hoogstraal H. Ticks in relation to human diseases caused by viruses. *Ann Rev Entomol.* 1966;11:261–308.

65. Kožuch O, Nosek J. Transmission of tick-borne encephalitis (TBE) virus by *Dermacentor marginatus* and *D. reticulatus*

ticks. *Acta Virol*. 1971;15:334.

66. Nosek J. The ecology and public health importance of *Dermacentor marginatus* and *D. reticulatus* ticks in central Europe. *Folia Parasitol*. 1972;19:93-102.
67. Kožuch O, Nosek T. Experimental transmission or tick-borne encephalitis (TBE) virus by *Haemaphysalis concinna* ticks. *Acta Virol*. 1980;24:377.
68. Khazova T, Iastrebov V. Combined focus of tick-borne encephalitis, tick-borne rickettsiosis and tularaemia in the habitat of *Haemaphysalis concinna* in south central Siberia. [In Russian] *Zh Mikrobiol Epidemiol Immunobiol.* 2001;78–80.
69. Nosek J, Ciampor F, Kožuch O, Rajcáni J. Localization of tick-borne encephalitis virus in alveolar cells of salivary glands of *Dermacentor marginatus* and *Haemaphysalis inermis* ticks. *Acta Virol*. 1972;16:493-497.
70. Hubálek Z, Rudolf I. Tick-borne viruses in Europe. *Parasitol Res*. 2012;111:9-36.
71. Obsomer V, Wirtgen M, Linden A, et al. Spatial disaggregation of tick occurrence and ecology at a local scale as a preliminary step for spatial surveillance of tick-borne diseases: general framework and health implications in Belgium. *Parasit Vectors*. 2013;6:1.
72. Krivanec K, Kopecky E, Tomkova E, Grubhoffer L. Isolation of TBE virus from the tick *Ixodes hexagonus*. *Folia Parasitol*. 1988;35:273–6.
73. Grešíková M. Studies on tick-borne arboviruses isolated in Central Europe. Biological works. *Slovak Acad Sci Bratislava*. 1972;p.9.
74. Grešíková M, Calisher Grešiková M, Calisher CH. *The Arboviruses: Epidemiology and ecology*. Vol. IV, CRC Press, Inc., Boca Raton, Florida, 1988;p.177.
75. Karbowiak G. The occurrence of the *Dermacentor reticulatus* tick – its expansion to new areas and possible causes. *Ann Parasitol*. 2014;60:37–47.
76. Karbowiak G, Kiewra D. New locations of *Dermacentor reticulatus* ticks in Western Poland: the first evidence of the merge in *D. reticulatus* occurrence areas? *Wiad Parazytol*. 2010;56:333–40.
77. Dautel H, Dippel C, Oehme R, Hartelt K, Schettler E. Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of Rickettsia sp. *Int J Med Microbiol*. 2006; 296:149-56.
78. Rubel F, Brugger K, Pfeffer M, et al. Geographical distribution of *Dermacentor marginatus* and *Dermacentor reticulatus* in Europe. *Ticks Tick Borne Dis.* 2016;7:224-33.
79. Wojcik-Fatla A, Cisak E, Zając V, Zwoliński J, Dutkiewicz J. Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from the Lublin region (eastern Poland). *Ticks Tick Borne Dis*. 2011;2:16-9.
80. Biernat B, Karbowiak G, Werszko J, Stańczak J. Prevalence of tick-borne encephalitis virus (TBEV) RNA in *Dermacentor reticulatus* ticks from natural and urban environment, Poland. *Exp Appl Acarol*. 2014;64:543-51.
81. Biernat B, Karbowiak G, Stańczak J, Masny A, Werszko J. The first detection of the tick-borne encephalitis virus (TBEV) RNA in *Dermacentor reticulatus* ticks collected from the lowland European bison (*Bison bonasus bonasus L*.). *Acta Parasitol*. 2016;61:130-5.
82. Ponomareva E, Mikryukova T, Gori A, et al. Detection of Far-Eastern subtype of tick-borne encephalitis viral RNA in ticks collected in the Republic of Moldova. *J Vector Borne Dis.* 2015;52:334.
83. Chitimia-Dobler L, Lemhöfer G, Król N, Bestehorn M, Dobler G, Pfeffer M. Continuous isolation of tick-borne encephalitis virus from adult *Dermacentor reticulatus* ticks in an endemic area in Germany. *Parasit Vectors*. 2019;12:90.
84. Nosek J, Kožuch O, Grulich I. The structure of tick-borne encephalitis (TBE) foci in Central Europe. *Oecologia*. 1970;5:61-73.
85. Danielová V, Daniel M, Schwarzová L, et al. Integration of a Tick-Borne Encephalitis Virus and *Borrelia burgdorferi* sensu lato into Mountain Ecosystems, Following a Shift in the Altitudinal Limit of Distribution of Their Vector, *Ixodes ricinus* (Krkonoše Mountains, Czech Republic). *Vector Borne Zoonotic Dis*. 2010;10:223-30.
86. Burri C, Bastic V, Maeder G, Patalas E, Gern L. Microclimate and the zoonotic cycle of tick-borne encephalitis virus in Switzerland. *J Med Entomol*. 2011;48:615-27.
87. Drelich A, Andreassen Å, Vainio K, Kruszyński P, Wąsik T. Prevalence of tick-borne encephalitis virus in highly urbanized and low risk area in Southern Poland. *Ticks Tick Borne Dis*. 2014;5:663-7.
88. Imhoff M, Hagedorn P, Schulze Y, Hellenbrand W, Pfeffer M, Niedrig M. Review: Sentinels of tick-borne encephalitis risk. *Ticks Tick Borne Dis*. 2015;6:592-600.
89. Knap N, Avšič-Županc T. Factors affecting the ecology of tick-borne encephalitis in Slovenia. *Epidemiol Infect*. 357-66.
90. Waldenstrom J, Lundkvist A, Falk K, et al. Migrating birds and tick-borne encephalitis virus. *Emerg Infect Dis*. 2007;13:1215.
91. Kazarina A, Japiņa K, Keišs O, et al. Detection of tick-borne encephalitis virus in *I. ricinus* ticks collected from autumn migratory birds in Latvia. *Ticks Tick Borne Dis*. 2015;2:178-80.
92. Nosek T, Kožuch O, Ernek E, Lichard M. The importance of goats in the maintenance tick-borne encephalitis virus in nature. *Acta Virol*. 1967;11:470.
93. Nosek T, Kožuch O, Ernek E, Lichard M. Ubertragung des Zeckenenzephaliltis Virus durch die Weibchen von *Ixodes ricinus* und Nymphen *Haemaphysalis inermis* auf der Rehkitzen (*Capreolus capreolus*). *Zbl Bakt I Orig*. 1967;203:162.
94. Grešíková M, Nosek J. Isolation of tick-borne encephalitis virus from *Haemaphysalis inermis* ticks. *Acta Virol*. 1966;10:359-61.
95. Kožuch O, Chunikhin S, Grešíková M, et al. Experimental characteristics of viremia caused by two strains of tick-borne encephalitis virus in small rodents. *Acta Virol*. 1981;25:219-

24.

96. Randolph S, Miklisová D, Lysy J, Rogers D, Labuda M. Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. *Parasitology.* 1999;118:177-186.

97. Labuda M, Kozuch O, Zuffova E, Eleckova E, Hails R, Nuttall P. Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology*. 1997;235:138-143.

98. Randolph S, Green R, Peacey M, Rogers D. Seasonal synchrony: the key to tick-borne encephalitis foci identified by satellite data. *Parasitology*. 2000;121:15-23.

99. Pavlovsky E, Soloviev V. Experimental investigation of the tick -borne encephalitis virus circulation in the tick-vector organism (*Ixodes persulcatus*). [In Russian]. *Archiv Biol Nauk*. 1940;59:111-117.

100. Ilienko V, Gorozhankina T, Smorodintsev A. Main reguliers of transovarial transmission of tick-borne encephalitis virus by tick vectors. [In Russian] *Med Parazitol.* 1970;3:263-268.

101. Benda R. The common tick *Ixodes ricinus* L. as a reservoir and vector of tick-borne encephalitis. I. Survival of the virus (strain B3) during the development of the tick under laboratory conditions. *J Hyg Epidemiol Microbiol Immunol*. 1958;2:314-330.

102. Danielova V, Holubova J. Transovarial transmission rate of tick-borne encephalitis virus in *Ixodes ricinus* ticks. *Mod Acarol*. 1991;2:7-10.

103. Tonteri E. Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008–2009. *Emerg Infect Dis.* 2011;17.

104. Nuttall P, Jones L, Labuda M, Kaufmann R. Adaptation of arbovirus to ticks. *J Med Entomol*. 1994;31:1-9.

105. Radda A, Hofmann H, Pretzmann G. Threshold of viraemia in *Apodemus flavicollis* for infection of *Ixodes ricinus* with tick-borne encephalitis virus. *Acta Virol*. 1969;13:74-7.

106. Chunikhin S, Stefutkina L, Korolev M, Reshetnikov I, Khozinskaya G. Sexual transmission of tick-borne encephalitis virus in ixodids (Ixodidae) [In Russian]. *Parazitologia*. 1983;17:214-5.2015;143:2059-67.

107. Nutall P. Displaced tick-parasite interactions at the host interface. *Parasitology*. 1998;116:S65-72.

108. Korenberg E, Kovalevskii Y. Main features of tick-borne encephalitis eco-epidemiology in Russia. *Zentralbl Bakteriol*. 1999;289:525-39.

109. Korenberg E, Horakova M, Kovalevsky J, Hubalek Z, Karavanov A. Probability models of the rate of infection with tick-borne encephalitis virus in *Ixodes persulcatus* ticks. *Folia Parasitol*. 1992;39:85-92.

110. Bakhvalova V, Chicherina G, Potapova O, et al. Tick-borne encephalitis virus diversity in ixodid ticks and small mammals in south-western Siberia, Russia. *Vector Borne Zoonotic Dis*. 2016;16:541-549.

111. Kim S, Yun S, Han M, et al. Isolation of tick-borne encephalitis viruses from wild rodents, South Korea. *Vector Borne Zoonotic Dis*. 2008;8:7-14.

112. Daniel M., Danielová V., Fialová A., Malý M., Kříž B., Nuttall P.A. Increased relative risk of tick-borne encephalitis in warmer weather. *Front Cell Infect Microbiol.* 2018;8: doi: 10.3389/fcimb.2018.00090.

113. Mikryukova T, Moskvitina N, Kononova Y, et al. Surveillance of tick-borne encephalitis virus in wild birds and ticks in Tomsk city and its suburbs (Western Siberia). *Ticks Tick Borne Dis*. 2014;5:145-51.

114. van Tongeren H. Viraemia and antibody response of the mallard (Anas platyrhynchos) to infection with tick-borne encephalitis virus. *J Comp Pathol.* 1983;4:521-30.

115. Radda A, Kunz C, Hofmann H. Nachweis von Antikörpern in Wildseren zur Erfassung von Herden des Virus der Frühsommer-Meningo-Enzephalitis in Niederösterreich. *Zentralbl Bakteriol.* 1968;208:88-93.

116. Radda A. Die Zeckenenzephalitis in Europa. *Angewandte Zool*. 1973;60:409-61.

117. Achazi K, Růžek D, Donoso-Mantke O, et al. Rodents as sentinels for the prevalence of tick-borne encephalitis virus. *Vector Borne Zoonotic Dis*. 2011;11:641-7.

118. Karbowiak G, Biernat B. The role of particular tick development stages in the circulation of tick-borne pathogens affecting humans in Central Europe. 2. Tick-borne encephalitis virus. *Ann Parasitol.* 2016;62:3-9.

119. Korenberg E, Ivanova L, Yurkova E. Epidemicity rate of tick-borne encephalitis natural foci (range of limits). *Med Parazitol*. 1986;2:35-9.

120. Labuda M, Randolph S. Survival of tick-borne encephalitis virus: cellular basis and environmental determinants. *Zentralbl Bakteriol*. 1999;288:51 3-24.

121. Rosa R, Pugliese A, Norman R, Hudson P. Thresholds for disease persistence in models for tick-borne infections including nonviraemic transmission, extended feeding and tick aggregation. *J Theor Biol*. 2003;224:359-76.

122. Divé I, Veje M, Dobler G, Bergström T, Buxmann H, Paul B, Louwen F, Berger A, Jahnke K, Strzelczyk A, Studahl M, Hentz E, Nürnberger L. Tick-borne encephalitis virus (TBEV) infection in pregnancy: Absence of virus transmission to the fetuses despite severe maternal disease – A case study. *Ticks Tick Borne Dis*. 2020,11:101491. https://doi.org/10.1016/j.ttbdis.2020.101491

123. Satz N. *Frühsommermeningenzephalitis (FMSE).* Huber; 2006.

124. Okulova N, Chunikhin S, Vavilova V, Mayorova A. The site of tick's infecting bite and severity of encephalitis. *Med Parazitol.* 1989;5:78-84.

125. Beklemishev W. Some problems of epidemiology and epizootology of tick-borne encephalitis [In Russian]. *Med Parazitol (Moscow)*. 1959;3:310-8.

126. Labuda M, Austyn J, Zuffova E, et al. Importance of localized skin infection in tick-borne encephalitis virus transmission. *Virology*. 1996;219:

127. Rasnitsyn S. Evaluation of the importance of transphase and transovarial transmission for preservation of the causative agent population [In Russian]. *Med Parazitol*. 1976;3:269-74.

128. Alekseev A. Ecology of tick-borne encephalitis virus: part of Ixodidae tick males in its circulation. *Ecolog Parasitol. (*Leningrad, Petrozavodsk). 1991;1:51-62, 100.

129. Brockmann SO, Oehme R, Buckenmaier T, Beer M, Jeffery-Smith A, Spannenkrebs M, Haag-Milz S, Wagner-Wiening C, Schlegel C, Fritz J, Zange S, Bestehorn M, Lindau A, Hoffmann D, Tiberi S, Mackenstedt U, Dobler G. A cluster of two human cases of tick-borne encephalitis (TBE) transmitted by unpasteurised goat milk and cheese in Germany, May 2016. *Euro Surveill*. 2018;23 (15):pii=17-00336. https://doi.org/10.2807/1560-7917.ES.2018.23.15.17-00336

130. Kerlik J, Avdičová M, Musilová M, Bérešová J, Mezencev R. Breast Milk as Route of Tick-Borne Encephalitis Virus Transmission from Mother to Infant. *Emerg Infect Dis*. 2022;28 (5):1060-1061. doi:10.3201/eid2805.212457

131. Bakhvalova V, Potapova O, Panov V, Morozova O. Vertical transmission of tick-borne encephalitis virus between generations of adapted reservoir small rodents. *Virus Res*. 2009;140:172-8.

132. Rosa R, Pugliese A. Effects of tick population dynamics and host densities on the persistence of tick-borne infections. *Math Biosci*. 2007;208:216-40.

133. Bogovic P, Strle F. Tick-borne encephalitis: a review of epidemiology, clinical characteristics, and management. *World J Clin Cases.* 2015;3:430-41.

134. Kriz B, Benes C, Daniel M. Alimentary transmission of tick-borne encephalitis in the Czech Republic (1997-2007). *Epidemiol Mikrobiol Immunol*. 2009;58:98-103.

135. Hudopisk N, Korva M, Janet E, et al. Tick-borne encephalitis associated with consumption of raw goat milk, Slovenia. *Emerg Infect Dis*. 2013;19:806-8.

136. Holzmann H, Aberle S, Stiasny K, et al. Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. *Emerg Infect Dis*. 2009;15:1671-3.

137. Helpert A, Sinnecker H. Ausgewählte Erhebungen zur Zeckenenzephalitis-Epidemie im Kreis Niesky, Bezirk Dresden. Dt Gesundh-Wes. 1966;21:1277-9.

138. Kondrashov aZ, Filippovets R. Infection-rate of Ixodes persulcatus ticks and some aspects of transovarial transmission after their dosed infection with tick-borne encephalitis virus. *Voprosy Virusol*. 1970;6:703-8.

139. Alekseev A, Burenkova L, Chunikhin S. Peculiarities of behaviour of ticks Ixodes persulcatus P.Sch., infected by tick-borne encephalitis virus [In Russian]. *Med Parasitic Di*s. 1988;2:71-5.

140. Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosa R. Forest structure and roe deer abundance predict tick-borne encephalitis risk in Italy. *PLoS One*. 2009;4:e4336.

141. Pretzmann G, Loew J, Radda A. Untersuchungen in einem Naturherd der Frühsommer-Meningoencephalitis (FSME) in Niederösterreich. *Zentralbl Bakteriol Orig*. 1964;194:431-9.

142. Süss J. Tick-borne encephalitis in Europe and beyond – the epidemiological situation as of 2007. *Euro Surveill*. 2008;13:18916.

143. Jaenson T, Hjertqvist M, Bergström T, Lundkvist Å. Why is tick-borne encephalitis increasing? A review of the key factors causing the increasing incidence of human TBE in Sweden. *Parasit Vectors*. 2012;5:184.

144. Chunikhin S. Experimental investigation on tick-borne encephalitis virus ecology. *Vopr Virusol.* 1990;35:183-7.

145. Sándor A, Mărcuțan D, D'Amico G, Gherman C, Dumitrache M, Mihalca A. Do the ticks of birds at an important migratory hotspot reflect the seasonal dynamics of *Ixodes ricinus* at the migration initiation site? A case study in the Danube Delta. *PLoS One*. 2014;9:e89378.

146. Tonteri E, Jokelainen P, Matala J, Pusenius J, Vapalahti O. Serological evidence of tick-borne encephalitis virus infection in moose and deer in Finland: sentinels for virus circulation. *Parasit Vectors*. 2016;9:54.

147. Korenberg E, Kovalevsky Y. General scheme of tick-borne encephalitis virus circulation. *Zool Zhurnal.* 1977;56:1467- 78.

148. Norman R, Bowers R, Begon M, Hudson P. Persistence of tick-borne virus in the presence of multiple host species: tick reservoirs and parasite mediated compete-tion. *J Theoretical Biol*. 1999;200:111-8

149. Hartemink N, Randolph S, Davis S, Heesterbeek J. The basic reproduction number for complex disease systems: Defining R0 for tick-borne infections. *Am Nat*. 2008;171:743-54.

150. Kožuch O, Grešíková M, Nosek J, Lichard M, Sekeyova M. The role of small rodents and hedgehogs in a natural focus of tick-borne encephalitis. *Bull World Health Organ*. 1967;36(Suppl 1):61.

151. Alekseev A, Burenkova L, Vasilieva I, Dubinina H, Chunikhin S. Preliminary studies on virus and spirochete accumulation in the cement plug of ixodid ticks. *Exp Appl Acarol*. 1996;20:713-23.

152. Demina TV, Dzhioev YP, Verkhozina MM, et al. Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. *J Med Virol*. 2010;82:965-76.

153. Süss J. Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asia-an overview. *Ticks Tick Borne Dis*. 2011;2:2-15.

154. Stefanoff P, Pfeffer M, Hellenbrand W, et al. Virus detection in questing ticks is not a sensitive indicator for risk assessment of tick-borne encephalitis in humans. *Zoonoses Public Health*. 2013;60:215-26.

155. Katargina O, Russakova S, Geller J, et al. Detection and characterization of tick-borne encephalitis virus in Baltic Countries and Eastern Poland. *PLoS ONE*. 2013;8:e61374.

156. Biernat B, Cieniuch S, Stańczak J. Detection of TBEV RNA in *Ixodes ricinus* ticks in north-eastern Poland. *Ann Agr Env Med*. 2014;21:689-92.

157. Cuber P, Andreassen Å, Vainio K, et al. Risk of exposure to ticks (Ixodidae) and the prevalence of tick -borne encephalitis virus (TBEV) in ticks in Southern Poland. *Ticks Tick Borne Dis*. 2015;6:356-63.

158. Valarcher J, Haglund S, Juremalm M, et al. Tick-borne encephalitis. *Rev Sci Tech*. 2015;34:453-66.

159. Streissle G. Studies in the transmission of the virus of early summer meningoencephalitis by the tick Ixodes hexagonus Leach. *Zentralbl Bakteriol*. 1960;179:289-297.

160. Novak-Chmura M, Siuda K. Ticks of Poland. Review of contemporary issues and latest research. *Ann Parasitol*. 2012;58:125-55.

161. Golovljova I, Vene S, Sjölander K, Vasilenko V, Plyusnin A, Lundkvist A. Characterization of tick-borne encephalitis virus from Estonia. *J Med Virol*. 2004;74:580-8.

162. Takeda T, Ito T, Osada M, Takahashi K, Takashima I. Isolation of tick-borne encephalitis virus from wild rodents and a seroepizootiologic survey in Hokkaido, Japan. *Am J Trop Med Hyg*. 1999;60:287–91.

163. Rieger M, Nübling M, Müller W, Hasselhorn H, Hofmann F. Foxes as indicators for TBE endemicity a comparative serological investigation. *Zentralbl Bakteri*ol. 1999;289:610-8.

164. Wurm R, Dobler G, Peters M, Kiessig S. Serological Investigations of red foxes (*Vulpes vulpes* L.) for determination of the spread of tick-borne encephalitis in North-Rhine-Westphalia. *J Vet Med*. 2000;47:503-9.

165. Jemeršić L, Dežđek D, Brnić D, et al. Detection and genetic characterization of tick-borne encephalitis virus (TBEV) derived from ticks removed from red foxes (*Vulpes vulpes*) and isolated from spleen samples of red deer (*Cervus elaphus*) in Croatia. *Ticks Tick Borne Dis*. 2014;1:7-13.

166. Gerth H, Grimshandl D, Stage B, Döller G, Kunz C. Roe deer as sentinels for endemicity of tick-borne encephalitis virus. *Epidemiol Infect*. 1995;115:355-65.

167. Skarphédinsson S, Jensen P, Kristiansen K. Survey of tick- borne infections in Denmark. *Emerg Infect Dis*. 2005;11:1055-61.

168. Rizzoli A, Neteler M, Rosa R, Versini W, Cristofolini A, Bregoli M, Buckley A, Gould EA. Early detection of tick-borne encephalitis virus spatial distribution and activity in the province of Trento, northern Italy. *Geospat Health*. 2007;1:169-176.

169. Cisak E, Wójcik-Fatla A, Zajac V, Sroka J, Buczek A, J. D. Prevalence of tick-borne encephalitis virus [TBEV] in samples of raw milk taken randomly from cows, goats and sheep in Eastern Poland. *Ann Agr Env Med*. 2010;17:283-6.

170. Balogh Z, Egyed L, Ferenczi E, Bán E, Szomor KN, Takács M, Berencsi G. Experimental infection of goats with tick-borne encephalitis virus and the possibilities to prevent virus transmission by raw goat milk. *Intervirology.* 2011;3:3:194-200.

171. Takashima I, Morita K, Chiba M, Hayasaka D, Sato T, Takezawa C, Igarashi A, Kariwa H, Yoshimatsu K, Arikawa J, Hashimoto N. A case of tick-borne encephalitis in Japan and isolation of the virus. *J Clin Microbiol*. 1997;35:1943-1947.

172. Lommano E, Dvořák C, Vallotton L, Jenni L, Gern L. Tick-borne pathogens in ticks collected from breeding and migratory birds in Switzerland. *Ticks Tick Borne Dis*. 2014;6:871-82.

173. Csank T, Bhide K, Bencúrová E, Dolinská S, Drzewnioková P, Major P, Korytár Ľ, Bocková E, Bhide M, Pistl J. Detection of West Nile virus and tick-borne encephalitis virus in birds in Slovakia, using a universal primer set. *Arch Virol.* 2016;6:1679-1683.

Chapter 6

Pathogenesis of TBEV-diseases

Anna K Överby, Saravanan Thangamani

Key points

- In this chapter we describe the pathogenesis of tick-borne encephalitis virus (TBEV).
- To cause infection, TBEV needs to cross three different barriers; the physical, the innate and adaptive and the blood-brain barrier.
- TBEV transmission at the skin interface is pro-inflammatory with a marked increase in immune cell infiltrates at the tick-feeding foci.
- The trigger of innate immune and adaptive immune responses, by TBEV is necessary to clear the infection.
- TBEV employs different strategies to evade the innate immune response.
- Both different animal models and reverse genetics will help us understand TBEV pathogenesis.

Transmission and entry:

Tick vectors and tick -host interface

The *Ixodes ricinus* tick serves as the primary carrier of TBEV-Eu in nature, while the *Ixodes persulcatus* tick is the primary vector for TBEV-Sib and TBEV-FE.[1] I. ricinus is widely spread across Europe, reaching into Turkey and northern Iran, whereas *I. persulcatus* is found in the Urals, Siberia, Far-Eastern Russia, as well as parts of China and Japan.[2,3] A zone of sympatry exists in the northern Baltics, western Finland, and northwestern Russia, where the habitats of *I. ricinus* and *I. persulcatus* overlap, leading to the presence of multiple TBEV subtypes.[3-5] TBEV is maintained within natural transmission cycles involving ixodid ticks and wild-living mammalian hosts. Infected ticks are presumed to remain infected throughout their life cycle.[2] While transovarial transmission of TBEV from an infected female tick to the egg mass is possible, this mode of infection is not entirely efficient in sustaining TBEV within the natural tick population.[6]

The transmission of tick-borne encephalitis virus (TBEV) from an infected tick to a host involves a complex interplay between the tick's feeding process and the immunomodulatory properties of its saliva. This process begins shortly after the tick attaches itself to the host. TBEV is transmitted to the vertebrate host along with the tick's saliva as early as one hour after the tick attaches[7] and POWV is transmitted as fast as 15 minutes after attachment.[8] Tick feeding is a sophisticated process, and successful feeding is facilitated by various components present in the tick's saliva, which possess immunomodulatory properties. Notably, tick salivary factors not only aid in blood feeding but also modulate the host environment, thereby promoting the transmission and establishment of TBEV.[9]

Seminal studies conducted by Labuda et al. (1993) demonstrated the significance of saliva-assisted transmission (SAT) of TBEV.[10] They observed that when naïve guinea pigs were inoculated with a mixture of TBEV and salivary gland extract (SGE) obtained from partially fed uninfected female ticks of species like *Ixodes ricinus*, *Dermacentor reticulatus*, or *Rhipicephalus appendiculatus*, and subsequently, uninfected *Rhipicephalus appendiculatus* nymphs fed on these guinea pigs, there was an increased acquisition of the virus by ticks feeding on animals inoculated with the mixture of SGE and virus compared to those inoculated with the virus alone. This research underscores the crucial role of tick saliva in facilitating the transmission of TBEV and sheds light on the mechanisms involved in the transmission dynamics between ticks and hosts. Observations of pathogens being transmitted from infected ticks to uninfected ticks co-feeding on the same host have offered indirect evidence of what is known as "sequential acquisition of tick-borne pathogens," as noted by Nuttall and Labuda in 2004.[9] It is also referred to as co-feeding transmission. In natural environments, it's common for infected ticks to co-feed alongside uninfected ticks on a single host. Labuda et al. conducted experiments where TBEV-infected *I. ricinus* ticks and uninfected ticks co-fed on naïve, natural host species. Intriguingly, they found that the highest numbers of TBEV-infected ticks originated from susceptible host species with very low levels of viremia, providing compelling evidence that non-viremic co-feeding transmission of TBEV is a primary mechanism for maintaining the virus in natural foci.[11,12]

Tick-host-virus interface during TBEV transmission:

Skin acts as the primary barrier against various forms of damage, including mechanical stress, environmental

factors, and potential infections. It serves as the frontline defense between a tick and its host, making it the first point of contact for both TBEV and tick saliva during feeding. Throughout the feeding process, a tick's mouthparts and saliva interact with the host's blood and lymphatic vessels, as well as various cellular components such as fibroblasts, keratinocytes, Langerhans cells, dendritic cells, macrophages, mast cells, natural killer cells, T lymphocytes, and soluble mediators like cytokines, chemokines, complement proteins, and lectins.[13] These cutaneous immune cells play a pivotal role in initiating the host's immune response and inflammatory reactions against tick feeding and potential pathogen transmission.

The significance of skin infection in the transmission of TBEV is paramount. Skin acts as the primary interface where these viruses establish infection in the host.[9] Labuda et al. thoroughly investigated the initial stages of TBEV replication within the skin of two natural host species: bank voles (*Clethrionomys glareolus*) and yellow-necked field mice (*Apodemus flavicollis*). Their experimental setup mirrored natural conditions, with infected and uninfected Ixodes ricinus ticks placed on specific areas of the host's skin. Their findings revealed a correlation between TBEV detection in feeding ticks and the transmission dynamics from infected to uninfected ticks.[14] Additionally, TBEV exhibited a preference for skin sites where ticks were actively feeding. To characterize TBEV-infected cells, Labuda et al. infested laboratory mice with TBEV-infected ticks and cultured skin explants from the infestation sites. They observed the migration of leukocytes from these explants, with viral antigens present in migrating Langerhans cells and neutrophils, indicating their role in viral dissemination.[14] In vitro studies suggest that dendritic cell populations at the tick feeding site are among the early targets of TBEV infection. Recent research indicates that exposure of bone marrow-derived dendritic cells to tick saliva enhances TBEV replication, partly through activation of the pro-survival Akt pathway.[15]

These results underscore the importance of localized skin infection in the early transmission of the virus from infected ticks and its acquisition by uninfected co-feeding ticks.[11,16] Immune cells infiltrating the skin during tick feeding act as carriers for virus transmission between co-feeding ticks, independent of systemic viremia.[14] Langerhans cells, the primary dendritic cell population in the epidermis, likely play a crucial role in virus dissemination, as evidenced by their migration to draining lymph nodes in response to cutaneous infections with other arthropod-borne viruses.[17] Thus, the presence of TBE viral antigen in emigrating Langerhans cells suggests their involvement in transporting TBEV to the lymphatic system, contributing to overall viral dissemination. The importance of virus-infected cells at the tick feeding site and their contribution to initial viral replication and dissemination was further supported by in vitro experiments where *I. ricinus* tick saliva was shown to modulate TBEV infection of dendritic cells. Specifically, when DCs were cultured with TBEV in the presence of *I. ricinus* saliva, the infection rate of the cells was enhanced and there was a decrease in virus-induced TNF- alpha and IL6 production.[18]

A study conducted by Thangamani et al. explored the immune response in the skin to TBEV infection. The study involved allowing TBEV-infected ticks to feed on mice, followed by biopsies of the bite sites at one and three hours post-attachment for RNAseq transcriptome and histochemical analysis. The analysis revealed upregulation of various cytokines (Ccl2, Ccl12, Cxcl1, Cxcl2, Cxcl5, IL6, and IL10) and receptors (CCR1, CCR5, and Sell) after just one hour of TBEV-infected tick feeding, indicating an early activation of the inflammatory response and an increase in immune cell accumulation at the attachment site.[19] Immunohistochemical analysis further confirmed the inflammatory microenvironment at the feeding site, showing an influx of inflammatory cells, especially neutrophils, within one hour of TBEV-infected tick feeding. Among these, TBEV antigens were localized in fibroblasts and mononuclear cells, but not in neutrophils.[19] These findings suggest that TBEV-infected ticks induce rapid inflammation at the cutaneous interface, potentially affecting the transmission of flaviviruses to hosts. This study contributes to our understanding of the early immunological events during tick-borne flavivirus transmission, emphasizing the significance of localized skin infection in this process (Figure 1). Together these studies illustrate the important role of localized skin infection during the early stages of tick-borne flavivirus transmission.

Neuroinvasion and neurotropism:

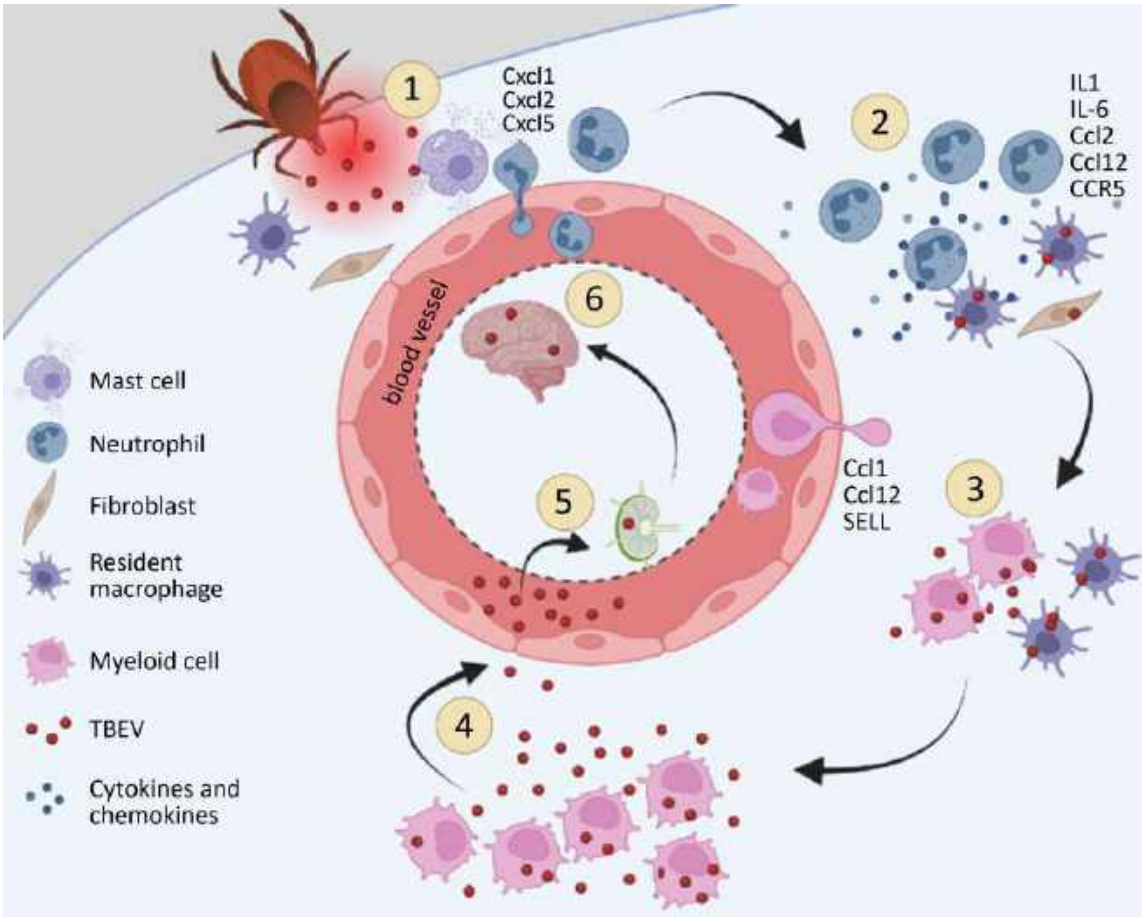
Crossing the brain barriers

It is generally believed that neurotropic flaviviruses can invade the CNS by two main routes; the peripheral nervous system or the hematogenous route via the blood. However, the molecular mechanisms governing the neuroinvasion of TBEV and related tick-borne flaviviruses are not yet clear.

Entry via the peripheral nerves

Some viruses uses the spinal cord to enter the CNS,[20,21] however, during experimental infection of TBEV (strain Torö) and LGTV in mice the spinal cord and brain stem are the last infected areas after sub cutaneous (SC) and intraperitoneal (IP) infection respectively.[22,23] On the other hand, POWV (LB strain) showed spinal cord infection as early as 4 days post-infection and thereafter a caudal to rostral spread within the brain after high viral dose.[24] Indicating that neuroinvasion might depend on the specific virus strain used and the experimental setup. Another report with TBEV (Sofjin) infected mice showed that the autonomic nerves running from the myoenteric plexus were

Figure 1: Proposed overview of the early transmission events of TBEV



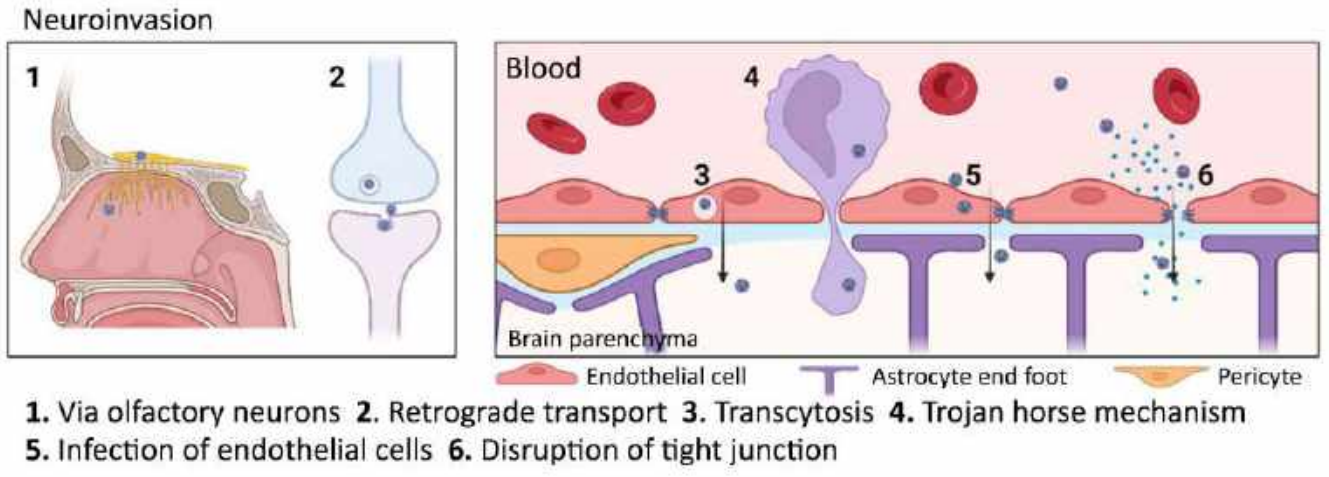
(1) TBEV is transmitted during tick feeding along with tick salivary factors. Mast cells are degranulated as soon as ticks initiate feeding leading to the influx of neutrophils; (2) Release of chemoattractant to recruit immune cells and TBEV establishes infection in permissive cells such as resident fibroblasts, macrophages, and other phagocytes; (3) infiltrating myeloid cells becomes infected with TBEV; (4) replication of TBEV in myeloid cells and release of infectious virus into the blood stream; (5) dissemination of TBEV to the lymphatic tissues; (6) dissemination and establishment of infection in brain. The infographic was generated using Biorender (www.biorender.com).

infected as well as the intestine and intestinal lymph nodes after intravenous infection (IV).[25] There is direct signaling between the gut to the brain via enteroendocrine cells of the mouse gut that form synapses with vagal neurons[26] that may facilitate virus entry. The involvement of the gastrointestinal tract as an important site of infection is supported by the many cases of alimentary TBEV.[27-30] However, in mice the oral route of infection is rather ineffective even in highly immunocompromised interferon alpha receptor (IFNAR) knock out mice[31]. Infection using oral gavage (with feeding needle) is even less efficient.[31] This indicate that the acid environment of the stomach is preventing viral infection, and that the TBEV maybe more likely to establish infection in the mouth or throat. Another possible mechanism for neuroinvasion is via the olfactory sensory neurons in the olfactory bulb. We have seen that the olfactory bulb is the first site of infection after both TBEV (Torö) and LGTV (TP21) after IP and SC infection.[22,32] Also supporting this hypothesis is the reported laboratory-acquired infection with TBEV after high titer exposure of aerosols.[33] However, since a bi-phasic disease course was observed in this case report it indicates viremia before neuroinvasion,[33] and other studies in mice have shown that intranasal infection of mice are less efficient route of infection compared to IP and SC,[31,34] thus neuroinvasion via the olfactory neuron seems less likely for TBEV and LGTV.

Hematogenous route of neuroinvasion

The second plausible route of neuroinvasion is the hematogenous via the blood brain barrier (BBB). The BBB is a very tight barrier that separates the blood from the brain parenchyma and the main function is to prevent free diffusion and toxic molecules to enter the brain. The BBB is lining all capillaries in the brain and to prevent permeability and leakage the endothelial cells have tight junctions. These include the claudines and occludin, which are joined to the cytoskeleton by cytoplasmic proteins, such as zonula occludens (ZO).[35] Lining the endothelial cells are the pericytes and end-feet from nearby astrocytes, and the

Figure 2: Overview of possible routes of TBEV neuroinvasion



The infographic was generated using Biorender (www.biorender.com).

crosstalk between endothelia, pericytes and astrocytes are important to preserve the integrity and function of the barrier. For long it was believed that the breakdown of the BBB was important part of neuroinvasion for TBEV as TBE patients show disruption of the BBB.[36-38] However, virus is detected the brains of mice days before disruption of the BBB,[34,39] and BBB leakage is likely caused by the inflammatory response elicited by the virus in the brain. Microvascular endothelial cells are often used in vitro to mimic the BBB, and infection of these with TBEV (Hypr, Neudoerfl) does not increase permeability or change the key tight junction proteins. Instead the cells become persistently infected and secrete high titers of virus in both directions,[40] indicating that TBEV can cross the BBB via a transcellular pathway without changing permeability. In a more complex in vitro model consisting of both human brain endothelial cells and pericytes POWV (LI9, LI41 linage 2 and LB linage 1) infects both cell types persistently and secrets POWV to the lower chamber without changing the permeabilization.[41] However, no in vivo experiments have verified infection in the vascular endothelial cells of the BBB. Using single nuclei RNA sequencing Chotiwan et al. recently showed that in the cortex of wt mice the pericytes were infected with LGTV but not endothelial cells.[42] The reason for this discrepancy might be that different viral strains and mammalian models were used. Transcytosis is when virus is transported through the cell without productively infecting them. Evidence of transcytosis in vivo through endothelial cells and pericytes has only been shown for Japanese encephalitis (JEV) by electron microscopy.[43] Virus could also traffic through the BBB via so called "Trojan horse" mechanism, where virus infected immune cells infiltrate into the brain. However, even though virus infect different immune cells in the periphery, more research is needed to understand the trafficking behavior of infected cells.[44]

Alternatively, the virus may enter the brain via the blood CSF barrier through the choroid plexus (ChP). ChP is located in the ventricles of the brain and is composed of a monolayer of epithelial cells that contain tight junctions. This epithelial layer rests in a basal lamina surrounding and enclosing a central stroma where dendritic cells, fibroblasts and macrophages can be found. The blood endothelial cells within the ChP central stroma is leaky, thus, the cellular movement of molecules and cells within the CP stroma is not restricted. Both, Zika virus and LGTV have been shown to infect the ChP in vivo, ZIKV targets the pericytes and LGTV targets the ciliated epithelial cells.[34,42,45] However, these observations were made in IFNAR knock out mice and not in WT immunocompetent mice, making these observations difficult to translate into TBEV and human situation. Other factors contributing to neuroinvasion in POWV are, the presence of tick saliva,[24] active replication in macrophages and prolonged viremia, as resistant mice although with similar peak viremia as susceptible mice clear POWV in the periphery.[46]

TBEV tropism in the brain

Viral tropism in the brain is determined by several different factors. First the cellular entry receptor is important for binding and viral entry into cells. For TBEV[47] and LGTV[48] only one entry receptor has been identified, T-Cell Immunoglobulin and Mucin Domain 1 (TIM-1), however it is not likely to be the only one as mice and cells were still susceptible in its absence.[47] We have also seen that cellular tropism of infected wt and IFNAR deficient mice with LGTV is markedly different independent of base line expression of the different brain cells,[42] indicating that host factors, innate immune response and cellular crosstalk are very important for shaping the cellular tropism in the brain.

After neuroinvasion TBEV targets mainly large neurons of the anterior horns, medulla oblongata, pons, dentate nucleus, Purkinje cells, and striatum in humans.[49] Neurons in thalamus, cortex, and Purkinje cells in cerebellum are the main target for TBEV (Hypr) in mice.[50] In POWV lineage-1 the main infected areas are brain stem and spinal cord, and the involvement of spinal cord ventral horn and the brain stem might be the cause of the flaccid paralysis in the mice. Infection can also be detected in the cortex, hippocampus and Purkinje cells in cerebellum.[51] In LGTV infected rats the virus also infects the Purkinje cells, in addition to infection of midbrain, hippocampus, thalamus and frontal lobe.[52] LGTV infection in mice on the other hand does not target the Purkinje cells in the cerebellum but rather excitatory neurons in the entorhinal cortex of the cerebrum.[42] Showing that the experimental systems used are very important. The type I IFN response seem to have a major impact on the cellular tropism in vivo. For LGTV, Lindman et al. showed that RIPK3 is important specifically to restrict infection of the granular cell neurons in the cerebellum. This because it is necessary for upregulation of IFNAR expression and thus upregulation of antiviral Interferon stimulated genes (ISGs).[53] We have shown that both the specific cells and the areas infected with LGTV in the brain is dependent of type I IFN response.[42] In wt mice the excitatory neurons in gray matter of the cerebrum specifically in the entorhinal cortex and audio cortex were infected. Whereas in the absence of IFNAR the tropism shifted to ciliated epithelial cell of the choroid plexus in the ventricles, meninges, and microglia in the white matter tracts of the olfactory.[42] The reasons for this dramatic shift in cellular tropism between the mice are likely to be that the cross talk between cells in the brain, and infiltration of immune cells (CD8 T cells expressing IFNγ) into the brain that activates microglia in WT mice by upregulating CCR1. In the absence of IFNAR the crosstalk between cells are blunted, immune cells are not recruited to the brain, and microglia, which expresses high levels of TIM-1 (Human Protein Atlas), are unable to become activated and thus are susceptible to infection.[42]

Several in vitro studies have shown that primary astrocytes from rat and mouse can be infected with TBEV and they survive and produce virus over many days,[54,55] however, in mice TBEV (Hypr) and LGTV is rarely detected in astrocytes.[42,50] We have also seen that primary mouse astrocytes cultured in vitro become very susceptible to TBEV (Hypr, Aina and Sofjin) in the absence of IFNAR signaling,[56] however, astrocytes are not susceptible in IFNAR knock out mice in vivo,[42] indicating that viral tropism studies should be conducted in vivo not in vitro, as cellular tropism of TBF depends on much more than only the entry receptor.

Immune response to TBEV:

Type I interferon response

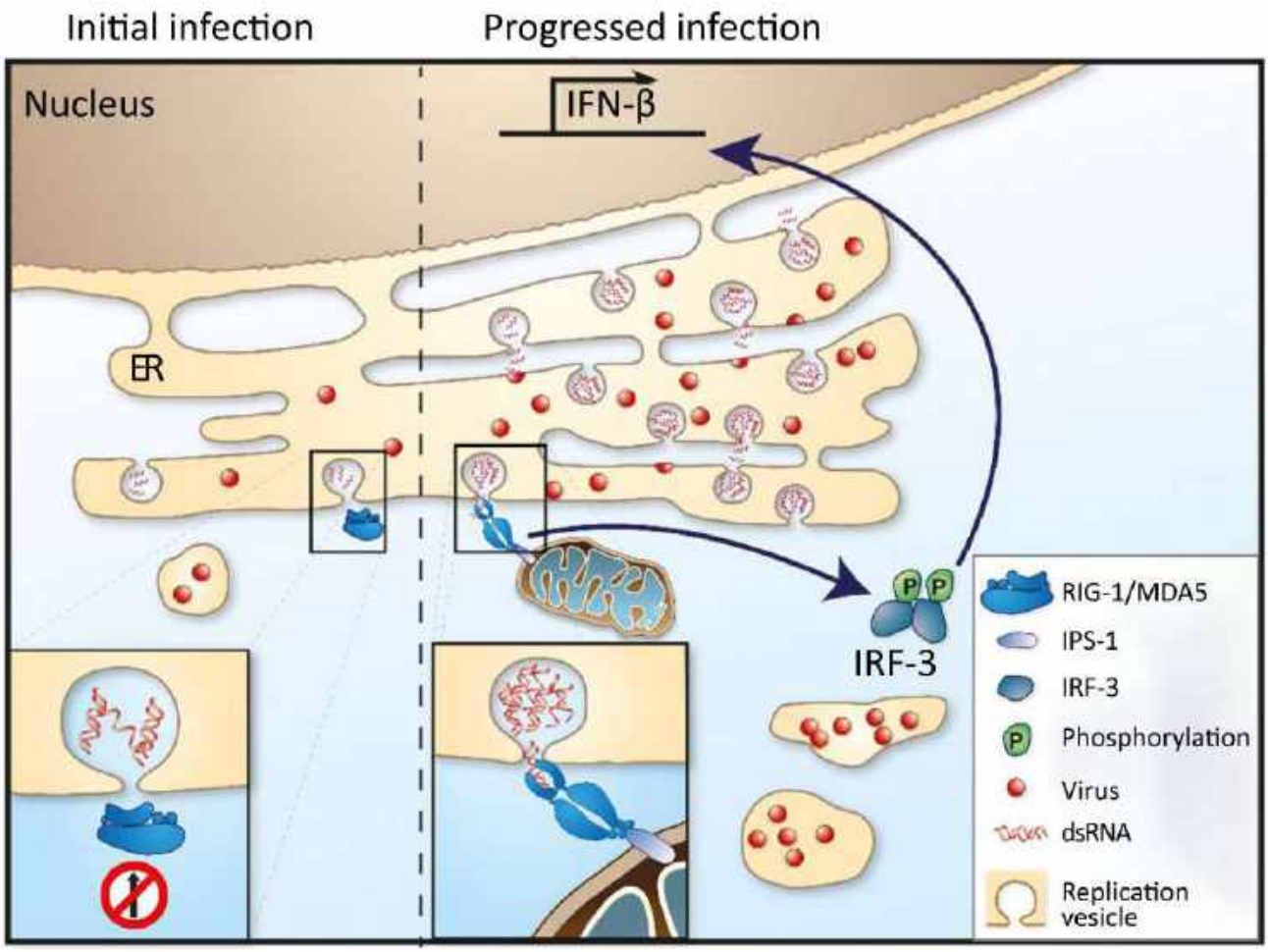
The type I IFN system is the first line of defense against viral infection and an important part of the intrinsic innate immune response that controls virus dissemination and protects against serious disease. This response rapidly detects invading pathogens and upregulates inhibitory effector proteins and cytokines to ensure survival. The detection of pathogens is based on recognition of the non-self pathogen-associated molecular pattern (PAMP) by specific host sensors, the pattern recognition receptors (PRR). This leads to a signaling cascade and the upregulation and secretion of IFN.[57] IFN is a large family of cytokines where the IFNα and -β are type I IFNs and IFNγ is type II IFNs and these are the most studied. Type I IFNs binds to the IFNα receptor (IFNAR), which is expressed on nearly all cell types, in a paracrine and autocrine manner. The IFNAR is composed of a heterodimer of IFNAR1 and IFNAR2. After binding of IFN, the IFNAR activates the Janus kinases, Jak1 and Tyk2, which then phosphorylate the signal transducer and activator of transcription (STAT)-1 and STAT2 proteins, resulting in activation and translocation of the IFN-stimulated gene 3 (ISGF3) transcription factor complex into the nucleus. This ISGF3 induces hundreds of IFN stimulated genes (ISGs), that encode proteins with diverse biological function and some are potent antiviral proteins and part of the response against mammalian viruses.[57]

Recognition of TBEV and induction of IFN

Rapid detection of the pathogen is crucial for mounting a protective response, and several different PRR families have been identified that recognize numerous ligands. The Toll-like receptors (TLRs) are located on the endosome or the plasma membrane, and the retinoic-acid-inducible gene I (RIG-I)-like receptors (RLRs) are in the cytosol. RNA viruses are most likely recognized by TLR3, TLR7, TLR8, or the RLRs (RIG-I and melanoma differentiation-associated gene 5, MDA5), which senses single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA).[58-60]

For TBEV, it is not totally clear which PRRs are dominant. RIG-I, which recognizes short dsRNA and 5’ PPP, has been shown to be important for IFNβ induction in the U2OS (human osteosarcoma) cell line by siRNA depletion,[61] and as MDA5 has been shown to be antagonized by prM of TBEV (Far Eastern subtype) preventing its recruitment to MAVS thus inhibiting IFN upregulation,[62] indicating that both are important for sensing. Both RIG-I and MDA5 bind to the adaptor mitochondria-associated IFNβ promoter stimulator-1 (IPS-1, also called MAVS, VISA or CARDIF) via its caspase recruitment domain after binding to its RNA ligand.[63] IPS-1 is important for IFNβ induction after TBEV (Hypr) infection in mouse embryonic fibroblasts (MEFs); in its absence, no

Figure 3: Viral evasion of IFN induction



TBEV induces vesicles in the Endoplasmatic Reticulum (ER) where the viral RNA synthesis occurs. Early during infection, these vesicles protect the dsRNA from cellular detection by RIG-I and/or MDA5. Later in infection, high amounts of virus particles are produced and the dsRNA leaks out of the vesicles. The pattern recognition receptors (PPRs) RIG-I and/or MDA5 then trigger signalling through IPS-1, phosphorylated IRF3 dimers are transported into the nucleus and IFN-β is upregulated.[64,73]

IFNβ was detected.[64] In addition, mice deficient in IPS-1 succumb to LGTV and TBEV (Hypr) infection earlier. These mice showed lower systemic levels of IFNα, resulting in higher viral titers in the periphery and leading to rapid invasion in the CNS.[23] IPS-1 is also important in the local IFN response within the brain, reducing viral load and spread of LGTV,[23,65,66] indicating an especially important role for RLR in the type I IFN response.

Upon IPS-1 activation, TNF Receptor Associated Factor 3 (TRAF3), TANK Binding Kinase 1 (TBK1) and Inhibitor-κB kinase ε (IKKε) are recruited, leading to phosphorylation and activation of the transcription factor IFN regulatory factor 3 (IRF3). Phosphorylated IRF3, dimerizes and translocate into the nucleus where it binds to the IFNβ gene promoter to initiate transcription and translation.[67,68] IFNβ induction after TBEV infection has been shown to be highly dependent on IRF3 activation in the cells, and IRF3 has been shown to dimerize and translocate into the nucleus after TBEV infection.[64] However, in vivo type I IFN upregulation is not dependent on IRF3 but on IRF7 in the periphery, and IRF7 plays an important role in the CNS to control infection.[69]

Since the type I IFN response is so important in controlling and restricting viral replication, most viruses have developed strategies to prevent upregulation of IFN by antagonizing the different steps in the IFN induction pathway.[74-76] For TBEV (Far Eastern subtype) the prM was recently identified to prevent interaction and signaling between MDA5 and MAVS.[62] TBEV also employ a passive escape mechanism that delays the induction of IFNβ by replicating inside replication vesicles or packets, thereby hiding its dsRNA from RIG-I and other PRRs (Figure 3).[61,64,73,77] Later, during infection, the dsRNA leaks out from the replication vesicles, IRF3 is activated and translocates into the nucleus to transcribe IFNβ, which then is translated and secreted. Thus, the virus is produced and released from

the cell before IFNβ can trigger an antiviral response in neighboring cells (Figure 3).[64,73] Interestingly, different cell types respond to infection in different ways with different kinetic. Primary mouse astrocytes have a very fast type I IFN response and secret IFNs that can protect, astrocytes and primary cortical neurons in culture already 3 to 6 h post infection,[56] and also co-cultured neurons.[78]

Type I IFN signaling and response against TBEV

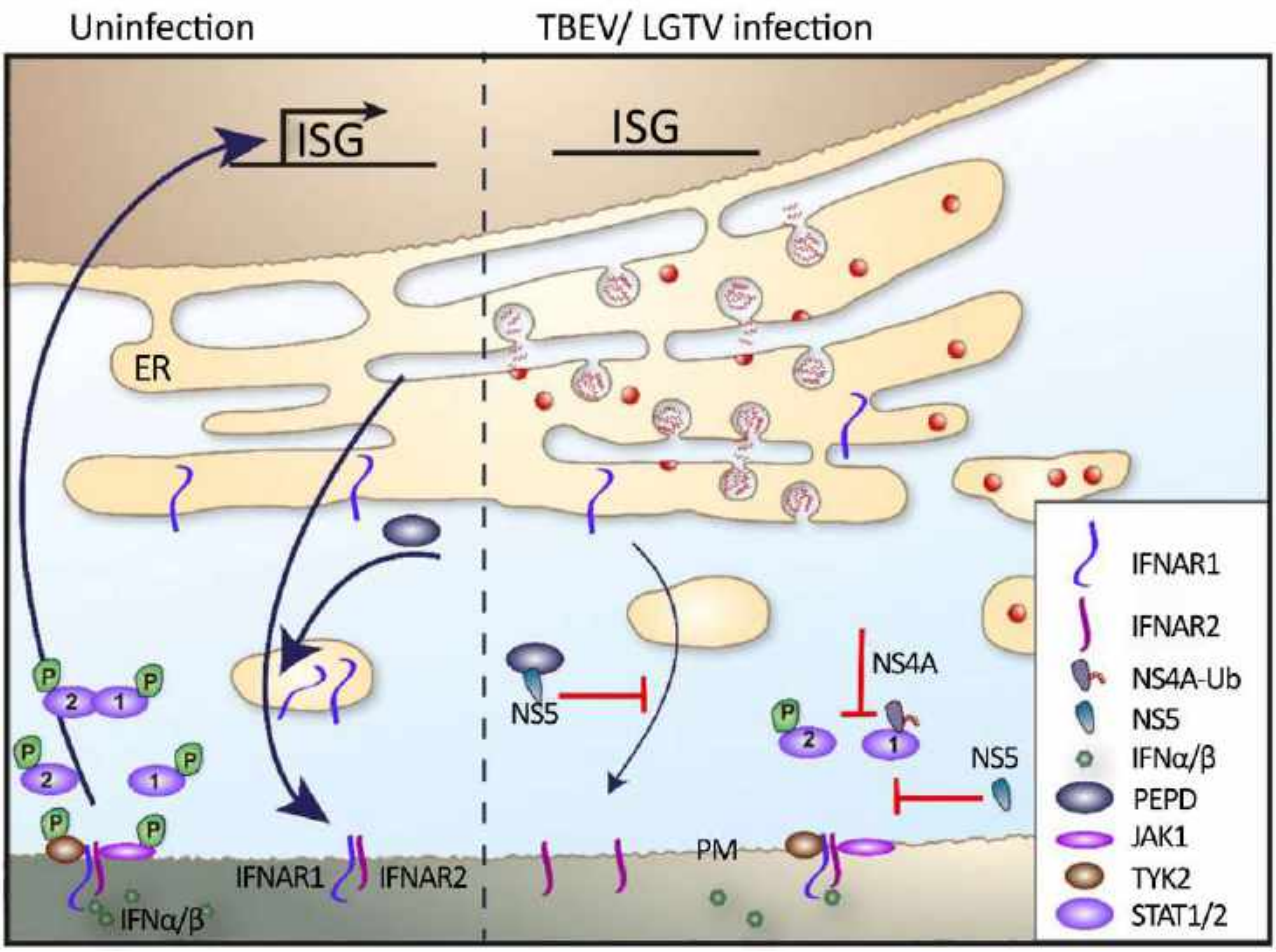
After infection and secretion of IFN, the IFN binds to its receptor the IFNAR1/2 which stimulates the upregulation of hundreds of ISGs that can limit the infection. The ISGs encode for PRR, adaptors and transcription factors to ensure a rapid response after infection. Cytokines and chemokines are also produced which activate and recruit immune cells to limit the infection, as well as antiviral proteins that can target viral replication directly in the cell.[79] The IFNAR is therefore a key molecule in the type I IFN response. The importance of this molecule has been demonstrated for many viruses. For LGTV the type I IFN response determines tropism and can protect mice from lethal infection. In the absence of this response, the virus replicates uncontrollably in all organs, induces a rapid opening of the blood-brain barrier, and the mice succumb very quickly. This research also has shown that IFNAR is important in all cell types; hematopoietic, stroma, neuroectodermal and cells in the periphery.[34]

Most steps in the viral "life" cycle are targeted by 1 or several antiviral proteins encoded by the ISGs. Several ISGs have been identified to have antiviral effect on TBEV the Interferon-induced transmembrane proteins (IFITMs) 1, 2, 3, the rodent tripartite motif (TRIM) protein, TRIM79α, and viperin (virus inhibitory protein, endoplasmic reticulum-associated, IFN-inducible).[80-82] Although all three IFITM proteins are antivirally active IFITM3 is the most potent one and can protect against virus induced cell death, and IFITM proteins are most effective against cell free virus and not against cell to cell virus spread.[80] The antiviral mechanism of TRIM79α is direct targeting of the viral polymerase, the non-structural protein 5 (NS5), an essential component of the replication complex, for lysosomal degradation. TRIM79α seems to be specific for TBEV and LGTV, because mosquito-borne flaiviviruses; WNV and Japanese encephalitis virus (JEV), were shown not to be restricted by this protein.[81]

Viperin, on the other hand, is a highly conserved protein with broad spectrum antiviral activity, which has been shown to restrict a diverse range of viruses from different families. For the Flaviviridae family, viperin restricts hepatitis C, DENV, WNV and TBEV. However, the antiviral mechanism seem to depend on the specific virus. For TBEV, viperin selectively target the positive stranded RNA synthesis. The intracellular location to the ER via viperins N-terminal amphipathic alpha helix is important as it coincides with viral replication. The antiviral activity is depending on the radical S-adenosyl methionine (SAM) domain and the proper iron-sulphur maturation of the protein.[82,83] Recent studies have identified several viral and cellular interaction partners to viperin.[32,83-87] Viperin is able to target TBEV in multiple ways mediating antiviral activity in a cell type-specific manner. Viperin interacts with several TBEV proteins; prM, E, NS2A, NS2B and NS3. The interaction between NS3 and viperin results in proteasome-dependent degradation of NS3[86]. The stability of prM, E, NS2A and NS2B are affected by viperin, but only in the presence of NS3.[86] Interestingly, although viperin do not directly interact with the TBEV C protein, viperin expression induce C particle formation and release from virus infected cells and disturbing the assembly process of TBEV.[87] Viperin mediates this effect by interacting and sequestering the cellular protein Golgi brefeldin A-resistant guanine nucleotide exchange factor 1 (GBF1),[87] which is involved in the vesicular trafficking of the secretory pathway[88,89] and is a pro-viral factor for many different viruses.[90-93] Thus, viperin may target other viruses via its interaction with GBF1. The in vivo importance of viperin during TBEV infection was recently shown in the viperin-/- mice .[32] This study show that specific regions of the brain rely differentially on the antiviral activity of viperin for protection against LGTV. Viperin is important in the olfactory bulb and cerebrum, while viral replication were unchanged in cerebellum and brain stem in the absence of viperin. This effect is due to the different neuronal subtypes, viperin expression is very important in cortical neurons but not at all in granular cell neurons isolated from the cerebellum.[32] Looking at polymorphisms in human TBE have identified several ISGs associated with TBE disease for example Interferon Induced Protein With Tetratricopeptide Repeats 1 (IFIT1),[94] 2'-5'-oligoadenylate synthetase (OAS)2 and OAS3.[95,96]

Even though different ISGs can potently restrict TBEV replication if induced before infection,[56,81,82,98] IFN treatment after infection has limited effect in vitro.[98] The reason for this is the expression of an IFN antagonist, NS4A[100] and NS5.[98,99] TBEV NS4A blocks the phosphorylation and dimerization of STAT1/STAT2 to reduce the type I and type II IFN-mediated signaling.[100] The NS5 protein of LGTV interferes with the phosphorylation of Jak1 and Tyk2 in response to IFNβ, which leads to failure of STAT1/2 phosphorylation and subsequent ISG expression.[98,99] Werme et al. showed that the interaction between Scribble and NS5 is important for plasma membrane targeting and IFN antagonist activity; however, the exact target of NS5 is unclear.[99] In addition, NS5 was shown to block IFN signaling by selectively reducing the level of IFNAR1 expression on the cell surface. This reduction was dependent on NS5 binding to prolidase. Prolidase is needed for IFNAR1 intracellular trafficking, maturation, activation of IFNβ-

Figure 4: Interferon signaling and inhibition



The active IFN receptor is composed of 2 subunits, IFNAR1 and IFNAR2. Prolidase (PEPD) is required for IFNAR1 maturation and intracellular trafficking to the plasma membrane (PM). Once IFNα/β binds to the IFNAR1/2, JAK1 and TYK2 becomes phosphorylated, which then results in phosphorylation of STAT1 and 2. This leads to dimerization of STAT and a signaling cascade that results in upregulation of ISG expression (left panel). In TBEV- and LGTV-infected cells (right panel) the IFN antagonist NS5 binds to PEPD, thus preventing IFNAR1 transport to the PM, and IFNα/β signaling.[97] NS5 also interferes with JAK1, TYK2, and STAT1 phosphorylation upon IFNα/β stimulation, thereby inhibiting ISG production.[98,99] Ubiquitinated NS4A binds to STAT1 and prevent STAT1/STAT2 dimerization and phosphorylation.[100]

stimulated gene induction, and IFN-I-dependent viral control (Figure 4).[97] The relationship between NS5 function and virulence has not been observed for tick-borne flaviviruses, such as TBEV and the low virulence LGTV NS5; both exhibited the same degree of p-STAT inhibition. However, there are most likely other viral proteins that are important for pathogenicity and suppression of innate immune responses, as this has been shown for other flaviviruses. However, for TBEV these mechanisms have yet to be identified.

Adaptive immune response against TBEV

Humoral immunity is an important component of the immune response. As with other flaviviruses, a functional humoral immune response is critically important in controlling infections.[101] Depleting B cells with immunosuppressive treatment of Rituximab lead to severe and fatal TBE.[102] On the other hand, passive transfer of monoclonal or polyclonal TBEV-specific antibodies protects mice in vivo and protection correlates with in vitro neutralization.[103-107] No infectious virus could be detected in the blood or brain of passively protected mice subsequent to TBEV challenge. However, in a vaccination study the antibodies response protected against disease but did not from neuroinvasion, as viral RNA was detected in the CNS.[50] However, antibodies protect not only by neutralization; therefore, because limited virus replication does occur, this indicates that mechanisms of protection from disease exist other than sterilizing immunity.[108]

In addition to effective humoral immunity, the activation of cellular immunity is usually required for clearance of established infection. Distinct T cell subsets play a key role in the induction of protective immune response against TBEV infections. CD4+ T cells are essential in priming the

TBEV-specific antibody response and sustaining the CD8+ T cell response.

For more details about the interplay between TBEV and the humoral immune response, cellular immune response, and different innate immune cells please visit Chapter 7 Immunology of TBEV infection by Zens and Ackermann-Gäumann.

Tools to study pathogenesis:

Overview of relevant animal models

Animal models are pivotal in comprehending the pathogenesis, transmission dynamics, and potential interventions for tick-borne encephalitis virus infection. An optimal animal model should closely emulate the human condition in terms of disease symptoms and underlying mechanisms. Tick-borne viruses exhibit minimal host specificity due to ticks' feeding habits, which vary as they mature and can encompass hosts of various sizes or species without preference. Humans typically become infected incidentally when ticks venture beyond their natural habitats or human ventures into the habitat of ticks. The diverse array of hosts that ticks can feed on renders many tick-borne viruses amenable to investigation using laboratory animals.

Both large and small animal models have been utilized to explore the fundamental aspects of TBEV infection, disease progression, and neuropathogenesis. Early investigations in sheep resulted in a better understanding of the differential neurovirulence and pathogenesis of TBEV.[109] Several species of non-human primates, such as Macaca mulatta (rhesus macaques), Cercopithecus aethiops (African Green monkeys), Macaca fascicularis (Crab-eating macaques), Macaca cynomolgus, and Macaca sylvanus, have been employed to study TBE neuropathogenesis. Though non-human primate models do not mimic human clinical outcomes, they are a good model to understand TBEV infections and to evaluate vaccine efficacy.[110-113]

Small mammals such as Syrian golden hamsters,[114] moles[115] have been used to understand TBEV pathogenesis and disease progression. However, they show reduced susceptibility. Laboratory mice such as ICR, C57BL/6 or BALB/c mice serve as a promising animal model for advancing research into the mechanisms underlying tick-borne virus infections and their pathogenesis.[22,116-120] Due to their closer phylogenetic relationship with humans and notable genomic similarities, especially evident in knock-out mice, where specific genes are deleted to elucidate mammalian genetic factors in infection and disease progression, they offer valuable insights.[23,119] Mice are susceptible to TBEV isolates, resulting in fever and neurological symptoms resembling human encephalitis. Histological examination of infected mice has unveiled substantial brain inflammation and damage, aligning with clinical manifestations observed in human cases.[116,117,119,120]

Kurhade et al. (2018) used C57BL/6 mice to characterize the pathogenesis of TBEV isolated from 2 different transmission foci.[22] The investigators compared the neuroinvasiveness, neurovirulence, and immune response of two European strains (HB171/11 from Germany and Toro-2003 from Sweden) in mice, uncovering distinct differences that enhance our understanding of TBEV pathogenesis. The HB171/11 is low virulent tick isolate from a focus where TBE patients only show gastrointestinal and constitutional symptoms.[121] The Torö-2003 strain is an infectious clone from an island where 32 neurological TBE cases[122] occured. The strain HB171/11 was found to be a low virulent phenotype with low or delayed neuroinvasiveness, and the Toro-2003 strain was found to be highly pathogenic.[22]

In addition, mice have also been used to investigate viral genetic determinants of infection and pathogenesis, and E protein, NS2B, NS3, NS5 protein, and the variable region of the 3' untranslated region have been shown to be important for determining pathogenicity in mice.[118,122-127] However, more studies are needed to fully understand the reason for the different clinical outcomes. Some strains of TBEV and POWV have been suggested to become persistent or chronic however, the mechanism is not clear, but it is interesting that in experimental models of TBEV and related viruses, the virus RNA is found in the brain of rodents[128-132] and in non-human primates[110,113,133,134] for a long time even in the absence of severe disease in the acute phase, although it is not clear if the virus RNA is infectious.

The variety of animal models utilized in research on TBEV underscores the comprehensive strategy needed to grasp and fight this virus, with mice being pivotal in revealing the mechanisms of infection and the progression of the disease.

Reverse genetics systems

Reverse genetics of viruses is the generation and manipulation of viral genomes to investigate the direct effects of changes on virus biology and pathogenesis. For flaviviruses, the first reverse genetic system was developed in 1989 for YFV.[135] Since the genome of flaviviruses is positive stranded, they are infectious if introduced into susceptible cells.[136] There are several different approaches to generate infectious virus. One important step is the generation of a complementary DNA (cDNA) to the RNA genome. The cDNA is often cloned into a plasmid under a specific promoter, which enables the in vitro transcription of viral RNA. This DNA clone enables the introduction of mutations into the genome, and subsequent analysis of the resulting phenotype. Reverse genetics have been used to study virulence, replication, host range, vaccines, and functions of the coding and non-coding regions. However, these clones are laborious and difficult to generate due to

instability and toxicity of some viral sequences in bacteria.[137]

For TBEV 2 separate approaches were used in the beginning; plasmid-based infectious clones[138] and the PCR based methods for constructing recombinant virus.[139,140] Both rely on *in vitro* transcription and transfection of RNA. The most recent technique for generating TBEV clones is the infectious-subgenomic-amplicon (ISA) method. Three PCR amplicons are produced that have a CMV promoter at the 5' non-coding region (NCR) and 70-100 bp overlapping regions; the hepatitis delta ribozyme is followed by the simian virus 40 polyadenylation signal. The amplicons are mixed and introduced into the cells where they recombine and produce infectious virus.[141]

Infectious clone systems have been very useful in studying determinants of replication and biological characteristics as well as to identify pathogenicity factors of TBEV. Two advantages of this approach are that the genome is defined and can be manipulated. In contrast, natural viral isolates of positive stranded RNA viruses are present as a population of different viral types also called quasispecies. This is due to the error prone RNA dependent RNA polymerase. In addition, manipulating natural viral isolates with specific mutagenesis inducing drugs is a very nonspecific approach.

With this technique, several determinates of pathogenicity have been identified. Specifically, the envelope protein responsible for receptor mediated entry,[126] the function of the membrane protein in virus budding,[142] and the importance of different regions in the 3'NCR. Neurovirulence in mice was shown to be dependent on specific amino acid residues in the upper lateral surface of domain III in the envelope (E) protein of TBEV (residues E308, E310 and E311), possibly due to disruption of the receptor binding.[126] The residues S267L, K315E, N389D in LGTV E protein and K46E in the NS3 protein, were shown to be crucial for neuroinvasiveness in immunodeficient mice.[143] The 5' and the 3' NCR contain complementary sequences that help genomic cyclization to form panhandle structures. The NCRs have several conserved structural stem loops that are important for replication, translation initiation and packaging.[144,145] At the beginning of the flavivirus 3' NCR, a secondary structure forms a pseudoknot that protects the terminal 300 to 500 bases from exoribonuclease XRN1 degradation, generating a subgenomic flavivirus RNA (sfRNA).[146-148] The sfRNA has been shown to be critical for WNV induced cytopathic effects[149] and pathogenicity in mice,[149] and is involved in viral subversion of type I IFN response by a yet unknown mechanism.[150] The TBEV sfRNA has been shown to specifically interfere with the RNAi system of ticks.[151] The 3' NCR of TBEV can be divided into a highly conserved core element and a variable region that is both heterogenic in length and sequence.[152] Several European TBEV strains contain an internal poly(A) tract in the variable region of the 3' NCR, which was considered dispensable for replication and virulence in mice.[127,153] However, studies recently showed that the variable region and the poly(A) tract can modulate virulence of the Far Eastern TBEV.[123,154] We have also detected different lengths of the poly(A) tract in a blood feeding tick indicating that the poly(A) might be important for the switch between invertebrate to vertebrate.[155] To investigate this further a long poly(A) Torö-38A and a TBEV Torö with a short poly(A) were cloned and rescued. We were able to show that the viruses with long poly(A) were attenuated in cell culture but more virulent in mice compared with the short poly(A), and the genome with short poly(A) was much more stable compared with the long version, which developed a high quasispecies diversity.[122]

Ongoing challenges and areas for future investigation

Important advances in the identification of molecular and cellular mechanisms of TBEV-induced pathogenesis have been made in recent years. Skin is the interface between a feeding TBEV-infected tick and a host; consequently, the cutaneous immune cells likely play a crucial role in virus transmission. In the earliest stages of TBEV-infected tick feeding, a complex, inflammatory micro-environment exists in the mammalian host's skin, with increased recruitment, migration, and accumulation of Langerhans cells, mononuclear phagocytes, and neutrophils. The dynamic secretion of tick salivary factors at the infected tick feeding foci modulates the cutaneous micro-environment to facilitate TBEV transmission, establishment, and dissemination from the skin to the terminal organs. However, many unanswered questions remain about the function of immune cells at the feeding site of a TBFV-infected tick. Modern single-cell and spatial transcriptomics techniques will allow us to investigate these early transmission events. They will enable us to understand immune processes at a single-cell level. In addition, gaps exist in our current understanding of the dissemination of viruses from the skin to the central nervous system. A better understanding of the virus transmission, establishment, neuroinvasion, dissemination and cellular tropism within the brain will allow us to develop novel countermeasures to prevent TBEV transmission, treat TBEV infections, and reduce disease burden. The interactions between the virus and the innate and adaptive immune response are not fully understood. The use of reverse genetics, specific knock out mouse models, new technologies like whole brain imaging, single cell sequencing and spatial transcriptomics will greatly advance our understanding of TBEV pathogenesis in the future.

Contact: anna.overby@umu.se

Citation: Överby AK, Thangamani S. Pathogenesis of TBEV-diseases. Chapter 6. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_6-7

References

1. Gritsun TS, Nuttall PA, Gould EA. Tick-borne flaviviruses. *Advances in virus research*. 2003;61:317-71.
2. Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. *Antiviral Res*. Jan 2003; 57(1-2):129-46.
3. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet*. May 31 2008;371(9627):1861-71.
4. Kovalev SY, Mukhacheva TA. Tick-borne encephalitis virus subtypes emerged through rapid vector switches rather than gradual evolution. Ecol Evol. Nov 2014;4(22):4307-16. doi:10.1002/ece3.1301
5. Süss J. Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asia-an overview. *Ticks Tick Borne Dis*. Mar 2011;2(1):2-15. doi:10.1016/j.ttbdis.2010.10.007
6. Danielová V, Holubová J, Pejcoch M, Daniel M. Potential significance of transovarial transmission in the circulation of tick-borne encephalitis virus. *Folia Parasitol (Praha).* 2002;49 (4):323-5.
7. Alekseev AN, Burenkova LA, Vasilieva IS, Dubinina HV, Chunikhin SP. Preliminary studies on virus and spirochete accumulation in the cement plug of ixodid ticks. *Exp Appl Acarol*. Dec 1996;20(12):713-23. doi:10.1007/bf00051556
8. Ebel GD, Kramer LD. Short report: duration of tick attachment required for transmission of powassan virus by deer ticks. *Am J Trop Med Hyg*. Sep 2004;71(3):268-71.
9. Nuttall PA, Labuda M. Tick-host interactions: saliva-activated transmission. *Parasitology*. 2004;129 Suppl:S177-89. doi:10.1017/s0031182004005633
10. Labuda M, Jones LD, Williams T, Nuttall PA. Enhancement of tick-borne encephalitis virus transmission by tick salivary gland extracts. *Med Vet Entomol*. Apr 1993;7(2):193-6.
11. Labuda M, Jones LD, Williams T, Danielova V, Nuttall PA. Efficient transmission of tick-borne encephalitis virus between cofeeding ticks. *J Med Entomol.* Jan 1993;30(1):295 -9. doi:10.1093/jmedent/30.1.295
12. Randolph SE. Transmission of tick-borne pathogens between co-feeding ticks: Milan Labuda's enduring paradigm. *Ticks Tick Borne Dis*. Dec 2011;2(4):179-82. doi:10.1016/j.ttbdis.2011.07.004
13. Kazimirova M, Thangamani S, Bartikova P, et al. Tick-Borne Viruses and Biological Processes at the Tick-Host-Virus Interface. *Front Cell Infect Microbiol*. 2017;7:339. doi:10.3389/fcimb.2017.00339
14. Labuda M, Austyn JM, Zuffova E, et al. Importance of localized skin infection in tick-borne encephalitis virus transmission. *Virology.* May 15 1996;219(2):357-66. doi:10.1006/viro.1996.0261
15. Lieskovská J, Páleníková J, Langhansová H, Chmelař J, Kopecký J. Saliva of Ixodes ricinus enhances TBE virus replication in dendritic cells by modulation of pro-survival Akt pathway. *Virology*. Jan 15 2018;514:98-105. doi:10.1016/j.virol.2017.11.008
16. Nuttall PA, Labuda M. Dynamics of infection in tick vectors and at the tick-host interface. *Advances in virus research*. 2003;60:233-72. doi:10.1016/s0065-3527(03)60007-2
17. Johnston LJ, Halliday GM, King NJ. Langerhans cells migrate to local lymph nodes following cutaneous infection with an arbovirus. *J Invest Dermatol*. Mar 2000;114(3):560-8. doi:10.1046/j.1523-1747.2000.00904.x
18. Fialova A, Cimburek Z, Iezzi G, Kopecky J. *Ixodes ricinus* tick saliva modulates tick-borne encephalitis virus infection of dendritic cells. *Microbes and infection / Institut Pasteur*. Jul 2010;12(7):580-5. doi:10.1016/j.micinf.2010.03.015
19. Thangamani S, Hermance ME, Santos RI, et al. Transcriptional Immunoprofiling at the Tick-Virus-Host Interface during Early Stages of Tick-Borne Encephalitis Virus Transmission. Front Cell Infect Microbiol. 2017;7:494. doi:10.3389/fcimb.2017.00494
20. Hixon AM, Clarke P, Tyler KL. Contemporary Circulating Enterovirus D68 Strains Infect and Undergo Retrograde Axonal Transport in Spinal Motor Neurons Independent of Sialic Acid. *Journal of virology.* Aug 15 2019;93(16). doi:10.1128/jvi.00578-19
21. Chen CS, Yao YC, Lin SC, et al. Retrograde axonal transport: a major transmission route of enterovirus 71 in mice. *Journal of virology*. Sep 2007;81(17):8996-9003. doi:10.1128/jvi.00236-07
22. Kurhade C, Schreier S, Lee YP, et al. Correlation of Severity of Human Tick-Borne Encephalitis Virus Disease and Pathogenicity in Mice. *Emerg Infect Dis*. Sep 2018;24 (9):1709-1712. doi:10.3201/eid2409.171825
23. Kurhade C, Zegenhagen L, Weber E, et al. Type I Interferon response in olfactory bulb, the site of tick-borne flavivirus accumulation, is primarily regulated by IPS-1. *J Neuroinflammation*. 2016;13(1):22. doi:10.1186/s12974-016 -0487-9
24. Santos RI, Hermance ME, Reynolds ES, Thangamani S. Salivary gland extract from the deer tick, Ixodes scapularis, facilitates neuroinvasion by Powassan virus in BALB/c mice. *Sci Rep*. Oct 22 2021;11(1):20873. doi:10.1038/s41598-021-00021-2
25. Nagata N, Iwata-Yoshikawa N, Hayasaka D, et al. The pathogenesis of 3 neurotropic flaviviruses in a mouse model depends on the route of neuroinvasion after viremia. *Journal of neuropathology and experimental neurology*. Mar 2015;74(3):250-60. doi:10.1097/NEN.0000000000000166
26. Kaelberer MM, Buchanan KL, Klein ME, et al. A gut-brain neural circuit for nutrient sensory transduction. *Science*. Sep 21 2018;361(6408). doi:10.1126/science.aat5236

27. Buczek AM, Buczek W, Buczek A, Wysokińska-Miszczuk J. Food-Borne Transmission of Tick-Borne Encephalitis Virus-Spread, Consequences, and Prophylaxis. *Int J Environ Res Public Health*. Feb 5 2022;19(3). doi:10.3390/ijerph19031812

28. Gonzalez G, Bournez L, Moraes RA, et al. A One-Health Approach to Investigating an Outbreak of Alimentary Tick-Borne Encephalitis in a Non-endemic Area in France (Ain, Eastern France): A Longitudinal Serological Study in Livestock, Detection in Ticks, and the First Tick-Borne Encephalitis Virus Isolation and Molecular Characterisation. *Front Microbiol*. 2022;13:863725. doi:10.3389/fmicb.2022.863725

29. Kerlik J, Avdičová M, Štefkovičová M, et al. Slovakia reports highest occurrence of alimentary tick-borne encephalitis in Europe: Analysis of tick-borne encephalitis outbreaks in Slovakia during 2007-2016. *Travel Med Infect Dis*. Nov-Dec 2018;26:37-42. doi:10.1016/j.tmaid.2018.07.001

30. Ličková M, Fumačová Havlíková S, Sláviková M, Klempa B. Alimentary Infections by Tick-Borne Encephalitis Virus. *Viruses*. Dec 30 2021;14(1). doi:10.3390/v14010056

31. Schreier S, Cebulski K, Kröger A. Contact-dependent transmission of Langat and tick-borne encephalitis virus in type I interferon receptor-1 deficient mice. *Journal of virology*. Mar 25 2021;95(8). doi:10.1128/jvi.02039-20

32. Lindqvist R, Kurhade C, Gilthorpe JD, Overby AK. Cell-type- and region-specific restriction of neurotropic flavivirus infection by viperin. *J Neuroinflammation*. Mar 15 2018;15 (1):80. doi:10.1186/s12974-018-1119-3

33. Avsic-Zupanc T, Poljak M, Maticic M, et al. Laboratory acquired tick-borne meningoencephalitis: characterisation of virus strains. *Clin Diagn Virol*. Jul 1995;4(1):51-9. doi:10.1016/0928-0197(94)00062-y

34. Weber E, Finsterbusch K, Lindquist R, et al. Type I interferon protects mice from fatal neurotropic infection with Langat virus by systemic and local antiviral responses. *Journal of virology*. Nov 2014;88(21):12202-12. doi:10.1128/JVI.01215-14

35. Persidsky Y, Ramirez SH, Haorah J, Kanmogne GD. Blood-brain barrier: structural components and function under physiologic and pathologic conditions. *J Neuroimmune Pharmacol*. Sep 2006;1(3):223-36. doi:10.1007/s11481-006-9025-3

36. Chekhonin VP, Zhirkov YA, Belyaeva IA, Ryabukhin IA, Gurina OI, Dmitriyeva TB. Serum time course of two brain-specific proteins, alpha(1) brain globulin and neuron-specific enolase, in tick-born encephalitis and Lyme disease. *Clin Chim Acta*. Jun 2002;320(1-2):117-25. doi:10.1016/s0009-8981(02)00057-8

37. Kang X, Li Y, Wei J, et al. Elevation of matrix metalloproteinase-9 level in cerebrospinal fluid of tick-borne encephalitis patients is associated with IgG extravassation and disease severity. *PLoS One*. 2013;8(11):e77427. doi:10.1371/journal.pone.0077427

38. Moniuszko A, Pancewicz S, Czupryna P, et al. ssICAM-1, IL-21 and IL-23 in patients with tick borne encephalitis and neuroborreliosis. *Cytokine*. Nov 2012;60(2):468-72. doi:10.1016/j.cyto.2012.05.007

39. Ruzek D, Salat J, Singh SK, Kopecky J. Breakdown of the blood-brain barrier during tick-borne encephalitis in mice is not dependent on CD8+ T-cells. *PLoS One*. 2011;6(5):e20472. doi:10.1371/journal.pone.0020472

40. Palus M, Vancova M, Sirmarova J, Elsterova J, Perner J, Ruzek D. Tick-borne encephalitis virus infects human brain microvascular endothelial cells without compromising blood -brain barrier integrity. *Virology*. Jul 2017;507:110-122. doi:10.1016/j.virol.2017.04.012

41. Conde JN, Sanchez-Vicente S, Saladino N, et al. Powassan Viruses Spread Cell to Cell during Direct Isolation from Ixodes Ticks and Persistently Infect Human Brain Endothelial Cells and Pericytes. *Journal of virology*. Jan 12 2022;96 (1):e0168221. doi:10.1128/jvi.01682-21

42. Chotiwan N, Rosendal E, Willekens SMA, et al. Type I interferon shapes brain distribution and tropism of tick-borne flavivirus. *Nat Commun*. Apr 10 2023;14(1):2007. doi:10.1038/s41467-023-37698-0

43. Liou ML, Hsu CY. Japanese encephalitis virus is transported across the cerebral blood vessels by endocytosis in mouse brain. *Cell Tissue Res*. Sep 1998;293(3):389-94. doi:10.1007/s004410051130

44. Marshall EM, Koopmans MPG, Rockx B. A Journey to the Central Nervous System: Routes of Flaviviral Neuroinvasion in Human Disease. *Viruses.* Sep 21 2022;14(10). doi:10.3390/v14102096

45. Kim J, Alejandro B, Hetman M, et al. Zika virus infects pericytes in the choroid plexus and enters the central nervous system through the blood-cerebrospinal fluid barrier. *PLoS pathogens*. May 2020;16(5):e1008204. doi:10.1371/journal.ppat.1008204

46. Jasperse BA, Mattocks MD, Noll KE, Ferris MT, Heise MT, Lazear HM. Neuroinvasive Flavivirus Pathogenesis Is Restricted by Host Genetic Factors in Collaborative Cross Mice, Independently of Oas1b. *Journal of virology*. Jul 27 2023;97(7):e0071523. doi:10.1128/jvi.00715-23

47. Zhang X, Liang C, Wang H, et al. T-Cell Immunoglobulin and Mucin Domain 1 (TIM-1) Is a Functional Entry Factor for Tick-Borne Encephalitis Virus. *mBio*. Jan 25 2022;13(1):e0286021. doi:10.1128/mbio.02860-21

48. Rodrigues R, Danskog K, Overby AK, Arnberg N. Characterizing the cellular attachment receptor for Langat virus. *PLoS One*. 2019;14(6):e0217359. doi:10.1371/journal.pone.0217359

49. Gelpi E, Preusser M, Garzuly F, Holzmann H, Heinz FX, Budka H. Visualization of Central European tick-borne encephalitis infection in fatal human cases. *Journal of neuropathology and experimental neurology.* Jun 2005;64(6):506-12.

50. Petry M, Palus M, Leitzen E, et al. Immunity to TBEV Related Flaviviruses with Reduced Pathogenicity Protects Mice from Disease but Not from TBEV Entry into the CNS. *Vaccines*. Feb 26 2021;9(3). doi:10.3390/vaccines9030196

51. Santos RI, Hermance ME, Gelman BB, Thangamani S. Spinal Cord Ventral Horns and Lymphoid Organ Involvement in Powassan Virus Infection in a Mouse Model. *Viruses*. Aug 12 2016;8(8). doi:10.3390/v8080220

52. Maffioli C, Grandgirard D, Engler O, Leib SL. A tick-borne encephalitis model in infant rats infected with langat virus. *Journal of neuropathology and experimental neurology*. Dec 2014;73(12):1107-15. doi:10.1097/nen.0000000000000131

53. Lindman M, Angel JP, Estevez I, et al. RIPK3 promotes brain region-specific interferon signaling and restriction of tick-borne flavivirus infection. *PLoS pathogens*. Nov 2023;19 (11):e1011813. doi:10.1371/journal.ppat.1011813

54. Palus M, Bily T, Elsterova J, et al. Infection and injury of human astrocytes by tick-borne encephalitis virus. *The Journal of general virology*. Nov 2014;95(Pt 11):2411-26. doi:10.1099/vir.0.068411-0

55. Potokar M, Jorgačevski J, Zorec R. Astrocytes in Flavivirus Infections. *Int J Mol Sci*. Feb 6 2019;20(3). doi:10.3390/ijms20030691

56. Lindqvist R, Mundt F, Gilthorpe JD, et al. Fast type I interferon response protects astrocytes from flavivirus infection and virus-induced cytopathic effects. *J Neuroinflammation*. Oct 24 2016;13(1):277. doi:10.1186/s12974-016-0748-7

57. Weber F, Kochs G, Haller O. Inverse interference: how viruses fight the interferon system. *Viral Immunol*. 2004;17 (4):498-515.

58. Nazmi A, Dutta K, Hazra B, Basu A. Role of pattern recognition receptors in flavivirus infections. *Virus research*. Jun 24 2014;185:32-40. doi:10.1016/j.virusres.2014.03.013

59. Yoneyama M, Fujita T. RNA recognition and signal transduction by RIG-I-like receptors. *Immunological reviews*. Jan 2009;227(1):54-65.

60. Akira S, Takeda K. Toll-like receptor signalling. *Nature reviews*. Jul 2004;4(7):499-511. doi:10.1038/nri1391

61. Miorin L, Albornoz A, Baba MM, D'Agaro P, Marcello A. Formation of membrane-defined compartments by tick-borne encephalitis virus contributes to the early delay in interferon signaling. *Virus research*. Feb 2012;163(2):660-6. doi:10.1016/j.virusres.2011.11.020

62. Sui L, Zhao Y, Wang W, et al. Flavivirus prM interacts with MDA5 and MAVS to inhibit RLR antiviral signaling. *Cell Biosci*. Jan 13 2023;13(1):9. doi:10.1186/s13578-023-00957-0

63. Kawai T, Takahashi K, Sato S, et al. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nature immunology*. Oct 2005;6(10):981-8. doi:ni1243 [pii] 10.1038/ni1243

64. Overby AK, Popov VL, Niedrig M, Weber F. Tick-borne encephalitis virus delays interferon induction and hides its double-stranded RNA in intracellular membrane vesicles. *Journal of virology*. Sep 2010;84(17):8470-83. doi:10.1128/jvi.00176-10

65. Zegenhagen L, Kurhade C, Koniszewski N, Overby AK, Kroger A. Brain heterogeneity leads to differential innate immune responses and modulates pathogenesis of viral infections. *Cytokine & growth factor reviews*. 2016. doi:10.1016/j.cytogfr.2016.03.006

66. Zegenhagen L, Kurhade C, Kroger A, Overby AK. Differences in IPS-1 mediated innate immune responses between neurotrophic flavivirus infection. *Journal of Neuroinfectious Diseases*. 2016;7(210). doi:10.4172/2314-7326.1000210

67. Hiscott J. Triggering the innate antiviral response through IRF-3 activation. *The Journal of biological chemistry*. May 25 2007;282(21):15325-9.

68. Yoneyama M, Suhara W, Fukuhara Y, Fukuda M, Nishida E, Fujita T. Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. *The EMBO journal*. Feb 16 1998;17(4):1087-95.

69. Weichert L, Düsedau HP, Fritzsch D, et al. Astrocytes evoke a robust IRF7-independent type I interferon response upon neurotropic viral infection. *J Neuroinflammation*. Sep 22 2023;20(1):213. doi:10.1186/s12974-023-02892-w

70. Ghita L, Breitkopf V, Mulenge F, et al. Sequential MAVS and MyD88/TRIF signaling triggers anti-viral responses of tick-borne encephalitis virus-infected murine astrocytes. *J Neurosci Res*. Oct 2021;99(10):2478-2492. doi:10.1002/jnr.24923

71. Baker DG, Woods TA, Butchi NB, et al. Toll-like receptor 7 suppresses virus replication in neurons but does not affect viral pathogenesis in a mouse model of Langat virus infection. *The Journal of general virology*. Feb 2013;94(Pt 2):336-47. doi:10.1099/vir.0.043984-0 vir.0.043984-0 [pii]

72. Kindberg E, Vene S, Mickiene A, Lundkvist A, Lindquist L, Svensson L. A functional Toll-like receptor 3 gene (TLR3) may be a risk factor for tick-borne encephalitis virus (TBEV) infection. *J Infect Dis.* 2011;203(4):523-528. doi:10.1093/infdis/jiq082

73. Overby AK, Weber F. Hiding from intracellular pattern recognition receptors, a passive strategy of flavivirus immune evasion. *Virulence*. May-Jun 2011;2(3):238-40. doi:10.4161/viru.2.3.16162

74. Rodriguez-Madoz JR, Belicha-Villanueva A, Bernal-Rubio D, Ashour J, Ayllon J, Fernandez-Sesma A. Inhibition of the type I interferon response in human dendritic cells by dengue virus infection requires a catalytically active NS2B3 complex. *Journal of virology*. Oct 2010;84(19):9760-74. doi:10.1128/JVI.01051-10

75. Aguirre S, Maestre AM, Pagni S, et al. DENV inhibits type I IFN production in infected cells by cleaving human STING. *PLoS pathogens*. 2012;8(10):e1002934. doi:10.1371/journal.ppat.1002934

76. Dalrymple NA, Cimica V, Mackow ER. Dengue Virus NS Proteins Inhibit RIG-I/MAVS Signaling by Blocking TBK1/IRF3 Phosphorylation: Dengue Virus Serotype 1 NS4A Is a Unique Interferon-Regulating Virulence Determinant. *MBio*. May 12 2015;6(3):e00553-15. doi:10.1128/mBio.00553-15

77. Miorin L, Romero-Brey I, Maiuri P, et al. Three-dimensional architecture of tick-borne encephalitis virus replication sites and trafficking of the replicated RNA. *Journal of virology*. Jun 2013;87(11):6469-81. doi:10.1128/JVI.03456-12

78. Fares M, Cochet-Bernoin M, Gonzalez G, et al. Pathological modeling of TBEV infection reveals differential innate immune responses in human neurons and astrocytes that correlate with their susceptibility to infection. *J Neuroinflammation*. Mar 3 2020;17(1):76. doi:10.1186/s12974-020-01756-x

79. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. *Nature reviews*. Jul 2008;8(7):559-68.

80. Chmielewska AM, Gómez-Herranz M, Gach P, et al. The Role of IFITM Proteins in Tick-Borne Encephalitis Virus Infection. *Journal of virology*. Jan 12 2022;96(1):e0113021. doi:10.1128/jvi.01130-21

81. Taylor RT, Lubick KJ, Robertson SJ, et al. TRIM79α, an interferon -stimulated gene product, restricts tick-borne encephalitis virus replication by degrading the viral RNA polymerase. *Cell Host Microbe.* 2011;10(3):185-196. doi:10.1016/j.chom.2011.08.004

82. Upadhyay AS, Vonderstein K, Pichlmair A, et al. Viperin is an iron-sulfur protein that inhibits genome synthesis of tick-borne encephalitis virus via radical SAM domain activity. *Cellular microbiology*. Jun 2014;16(6):834-48. doi:10.1111/cmi.12241

83. Upadhyay AS, Stehling O, Panayiotou C, Rosser R, Lill R, Overby AK. Cellular requirements for iron-sulfur cluster insertion into the antiviral radical SAM protein viperin. The *Journal of biological chemistry*. Aug 18 2017;292(33):13879-13889. doi:10.1074/jbc.M117.780122

84. Lindqvist R, Overby AK. The Role of Viperin in Antiflavivirus Responses. *DNA Cell Biol*. Sep 2018;37(9):725-730. doi:10.1089/dna.2018.4328

85. Lindqvist R, Upadhyay A, Overby AK. Tick-Borne Flaviviruses and the Type I Interferon Response. *Viruses*. Jun 21 2018;10(7). doi:10.3390/v10070340

86. Panayiotou C, Lindqvist R, Kurhade C, et al. Viperin restricts Zika virus and tick-borne encephalitis virus replication by targeting NS3 for proteasomal degradation. *Journal of virology*. Jan 10 2018. doi:10.1128/JVI.02054-17

87. Vonderstein K, Nilsson E, Hubel P, et al. Viperin targets flavivirus virulence by inducing assembly of non-infectious capsid particles. *Journal of virology*. Oct 18 2017;92(1). doi:10.1128/JVI.01751-17

88. Claude A, Zhao BP, Kuziemsky CE, et al. GBF1: A novel Golgi-associated BFA-resistant guanine nucleotide exchange factor that displays specificity for ADP-ribosylation factor 5. *The Journal of cell biology*. Jul 12 1999;146(1):71-84.

89. Niu TK, Pfeifer AC, Lippincott-Schwartz J, Jackson CL. Dynamics of GBF1, a Brefeldin A-sensitive Arf1 exchange factor at the Golgi. *Mol Biol Cell*. Mar 2005;16(3):1213-22. doi:10.1091/mbc.E04-07-0599

90. Carpp LN, Rogers RS, Moritz RL, Aitchison JD. Quantitative proteomic analysis of host-virus interactions reveals a role for Golgi brefeldin A resistance factor 1 (GBF1) in dengue infection. *Mol Cell Proteomics*. Nov 2014;13(11):2836-54. doi:10.1074/mcp.M114.038984

91. Lanke KH, van der Schaar HM, Belov GA, et al. GBF1, a guanine nucleotide exchange factor for Arf, is crucial for coxsackievirus B3 RNA replication. *Journal of virology*. Nov 2009;83(22):11940 -9. doi:10.1128/JVI.01244-09

92. Liang W, Zheng M, Bao C, Zhang Y. CSFV proliferation is associated with GBF1 and Rab2. *J Biosci*. Mar 2017;42(1):43-56.

93. Zhang N, Zhang L. Key components of COPI and COPII machineries are required for chikungunya virus replication. Biochemical and biophysical research communications. Nov 25 2017;493(3):1190-1196. doi:10.1016/j.bbrc.2017.09.142

94. Fortova A, Barkhash AV, Pychova M, et al. Genetic polymorphisms in innate immunity genes influence predisposition to tick-borne encephalitis. *J Neurovirol*. Dec 2023;29(6):699-705. doi:10.1007/s13365-023-01182-8

95. Barkhash AV, Babenko VN, Kobzev VF, Romashchenko AG, Voevoda MI. Polymorphism in the human 2'-5'-oligoadenylate synthetase genes (OAS), associated with predisposition to severe forms of tick-borne encephalitis, in populations from North Eurasia. *Mol Biol (Mosk)*. Nov-Dec 2010;44(6):985-93.

96. Barkhash AV, Perelygin AA, Babenko VN, et al. Variability in the 2'-5'-oligoadenylate synthetase gene cluster is associated with human predisposition to tick-borne encephalitis virus-induced disease. *The Journal of infectious diseases*. Dec 15 2010;202 (12):1813-8. doi:10.1086/657418

97. Lubick KJ, Robertson SJ, McNally KL, et al. Flavivirus Antagonism of Type I Interferon Signaling Reveals Prolidase as a Regulator of IFNAR1 Surface Expression. *Cell host & microbe*. Jul 8 2015;18(1):61-74. doi:10.1016/j.chom.2015.06.007

98. Best SM, Morris KL, Shannon JG, et al. Inhibition of interferon-stimulated JAK-STAT signaling by a tick-borne flavivirus and identification of NS5 as an interferon antagonist. *Journal of virology*. Oct 2005;79(20):12828-39.

99. Werme K, Wigerius M, Johansson M. Tick-borne encephalitis virus NS5 associates with membrane protein scribble and impairs interferon-stimulated JAK-STAT signalling. *Cellular microbiology*. Mar 2008;10(3):696-712.

100. Yang Q, You J, Zhou Y, et al. Tick-borne encephalitis virus NS4A ubiquitination antagonizes type I interferon-stimulated STAT1/2 signalling pathway. Emerging microbes & infections. Dec 2020;9(1):714-726. doi:10.1080/22221751.2020.1745094

101. Pierson TC, Fremont DH, Kuhn RJ, Diamond MS. Structural insights into the mechanisms of antibody-mediated neutralization of flavivirus infection: implications for vaccine development. *Cell host & microbe.* Sep 11 2008;4(3):229-38. doi:10.1016/j.chom.2008.08.004

102. Kapadia RK, Staples JE, Gill CM, et al. Severe Arboviral Neuroinvasive Disease in Patients on Rituximab Therapy: A Review. *Clin Infect Dis*. Mar 21 2023;76(6):1142-1148. doi:10.1093/cid/ciac766

103. Agudelo M, Palus M, Keeffe JR, et al. Broad and potent neutralizing human antibodies to tick-borne flaviviruses protect mice from disease. *The Journal of experimental medicine*. May 3 2021;218(5.)doi:10.1084/jem.20210236

104. Kreil TR, Eibl MM. Pre- and postexposure protection by passive immunoglobulin but no enhancement of infection with a flavivirus in a mouse model. *Journal of virology*. Apr 1997;71 (4):2921-7.

105. Heinz FX, Berger R, Tuma W, Kunz C. A topological and functional model of epitopes on the structural glycoprotein of

tick-borne encephalitis virus defined by monoclonal antibodies. Virology. Apr 30 1983;126(2):525-37.

106. Niedrig M, Klockmann U, Lang W, et al. Monoclonal antibodies directed against tick-borne encephalitis virus with neutralizing activity in vivo. Acta virologica. Jun 1994;38(3):141-9.

107. Phillpotts RJ, Stephenson JR, Porterfield JS. Passive immunization of mice with monoclonal antibodies raised against tick-borne encephalitis virus. Brief report. Archives of virology. 1987;93(3-4):295-301.

108. Kreil TR, Maier E, Fraiss S, Eibl MM. Neutralizing antibodies protect against lethal flavivirus challenge but allow for the development of active humoral immunity to a nonstructural virus protein. Journal of virology. Apr 1998;72(4):3076-81.

109. Votiakov VI, Protas, II, Bortkevich VS, Nedz'ved MK. [Experimental study of the pathogenesis of tick-borne encephalitis]. Vopr Virusol. May-Jun 1975;(3):313-7. Eksperimental'noe izuchenie patogeneza kleshchevogo éntsefalita.

110. Zlontnik I, Grant DP, Carter GB. Experimental infection of monkeys with viruses of the tick-borne encephalitis complex: degenerative cerebellar lesions following inapparent forms of the disease or recovery from clinical encephalitis. Br J Exp Pathol. Apr 1976;57(2):200-10.

111. Pripuzova NS, Gmyl LV, Romanova L, et al. Exploring of primate models of tick-borne flaviviruses infection for evaluation of vaccines and drugs efficacy. PLoS One. 2013;8(4):e61094. doi:10.1371/journal.pone.0061094

112. Fokina GI, Malenko GV, Levina LS, et al. Persistence of tick-borne encephalitis virus in monkeys. V. Virus localization after subcutaneous inoculation. *Acta virologica*. Sep 1982;26(5):369-75.

113. Frolova MP, Pogodina VV. Persistence of tick-borne encephalitis virus in monkeys. VI. Pathomorphology of chronic infection in central nervous system. *Acta virologica*. May 1984;28(3):232-9.

114. Gritsun TS, Frolova TV, Zhankov AI, et al. Characterization of a siberian virus isolated from a patient with progressive chronic tick-borne encephalitis. *Journal of virology*. Jan 2003;77(1):25-36.

115. Kozuch O, Grulich I, Nosek J. Experimental infection of the mole with tick-borne encephalitis virus. *J Hyg Epidemiol Microbiol Immunol*. 1966;10(1):120-4.

116. Mandl CW. Steps of the tick-borne encephalitis virus replication cycle that affect neuropathogenesis. *Virus research*. Aug 2005;111(2):161-74. doi:10.1016/j.virusres.2005.04.007

117. Palus M, Vojtiskova J, Salat J, et al. Mice with different susceptibility to tick-borne encephalitis virus infection show selective neutralizing antibody response and inflammatory reaction in the central nervous system. *J Neuroinflammation*. 2013;10:77. doi:10.1186/1742-2094-10-77

118. Růzek D, Gritsun TS, Forrester NL, et al. Mutations in the NS2B and NS3 genes affect mouse neuroinvasiveness of a Western European field strain of tick-borne encephalitis virus. *Virology*. May 10 2008;374(2):249-55. doi:10.1016/j.virol.2008.01.010

119. Ruzek D, Salat J, Palus M, et al. CD8+ T-cells mediate immunopathology in tick-borne encephalitis. *Virology*. Feb 5 2009;384(1):1-6.

120. Engel AR, Rumyantsev AA, Maximova OA, et al. The neurovirulence and neuroinvasiveness of chimeric tick-borne encephalitis/dengue virus can be attenuated by introducing defined mutations into the envelope and NS5 protein genes and the 3' non-coding region of the genome. *Virology*. Sep 15 2010;405(1):243-52. doi:10.1016/j.virol.2010.06.014

121. Dobler G, Bestehorn M, Antwerpen M, Overby-Wernstedt A. Complete Genome Sequence of a Low-Virulence Tick-Borne Encephalitis Virus Strain. *Genome Announc*. Oct 20 2016;4(5). doi:10.1128/genomeA.01145-16

122. Asghar N, Lee YP, Nilsson E, et al. The role of the poly(A) tract in the replication and virulence of tick-borne encephalitis virus. *Sci Rep*. Dec 16 2016;6:39265. doi:10.1038/srep39265

123. Sakai M, Yoshii K, Sunden Y, Yokozawa K, Hirano M, Kariwa H. Variable region of the 3' UTR is a critical virulence factor in the Far-Eastern subtype of tick-borne encephalitis virus in a mouse model. *The Journal of general virology*. Apr 2014;95(Pt 4):823-35. doi:10.1099/vir.0.060046-0

124. Yoshii K, Sunden Y, Yokozawa K, et al. A critical determinant of neurological disease associated with highly pathogenic tick-borne flavivirus in mice. *Journal of virology*. May 2014;88 (10):5406-20. doi:10.1128/jvi.00421-14

125. Lindqvist R, Rosendal E, Weber E, et al. The envelope protein of tick-borne encephalitis virus influence neuron entry, pathogenicity and vaccine protection. *J Neuroinflammation*. Sep 28 2020;17:284. doi:10.1186/s12974-020-01943-w

126. Mandl CW, Allison SL, Holzmann H, Meixner T, Heinz FX. Attenuation of tick-borne encephalitis virus by structure-based site-specific mutagenesis of a putative flavivirus receptor binding site. *Journal of virology*. Oct 2000;74(20):9601-9.

127. Mandl CW, Holzmann H, Meixner T, et al. Spontaneous and engineered deletions in the 3' noncoding region of tick-borne encephalitis virus: construction of highly attenuated mutants of a flavivirus. *Journal of virology*. Mar 1998;72(3):2132-40.

128. Michelitsch A, Fast C, Sick F, et al. Long-term presence of tick-borne encephalitis virus in experimentally infected bank voles (Myodes glareolus). *Ticks Tick Borne Dis*. Jul 2021;12(4):101693. doi:10.1016/j.ttbdis.2021.101693

129. Chiffi G, Grandgirard D, Stöckli S, Valente LG, Adamantidis A, Leib SL. Tick-borne encephalitis affects sleep-wake behavior and locomotion in infant rats. *Cell Biosci*. Aug 2 2022;12(1):121. doi:10.1186/s13578-022-00859-7

130. Scroggs SLP, Offerdahl DK, Stewart PE, Shaia C, Griffin AJ, Bloom ME. Of Murines and Humans: Modeling Persistent Powassan Disease in C57BL/6 Mice. *mBio*. Apr 25 2023;14 (2):e0360622. doi:10.1128/mbio.03606-22

131. Mlera L, Meade-White K, Saturday G, Scott D, Bloom ME. Modeling Powassan virus infection in Peromyscus leucopus, a natural host. *PLoS neglected tropical diseases*. Jan 2017;11 (1):e0005346. doi:10.1371/journal.pntd.0005346

132. Pogodina VV, Frolova TV, Frolova MP, Sobolev SG, Shamanin VA, Pletnev AG. Molecular hybridization with cloned fragments

of tick-borne encephalitis (TBE) virus cDNA in acute and chronic TBE infection. *Acta virologica*. Jan 1991;35(1):71-80.

133. Frolova TV, Pogodina VV, Frolova MP, Karmysheva V. [Characteristics of long-term persisting strains of tick-borne encephalitis virus in different forms of the chronic process in animals]. Vopr Virusol. Jul-Aug 1982;27(4):473-9. Kharakteristika dlitel'no persistiruiushchikh shtammov virusa kleshchevogo éntsefalita pri ralichnykh formakh khronicheskogo protsessa u zhivotnykh.

134. Andzhaparidze OG, Rozina EE, Bogomolova NN, Boriskin YS. Morphological characteristics of the infection of animals with tick-borne encephalitis virus persisting for a long time in cell cultures. *Acta virologica*. May 1978;22(3):218-24.

135. Rice CM, Grakoui A, Galler R, Chambers TJ. Transcription of infectious yellow fever RNA from full-length cDNA templates produced by in vitro ligation. *New Biol*. Dec 1989;1(3):285-96.

136. Boyer JC, Haenni AL. Infectious transcripts and cDNA clones of RNA viruses. *Virology*. Feb 1994;198(2):415-26. doi:10.1006/viro.1994.1053

137. Aubry F, Nougairede A, Gould EA, de Lamballerie X. Flavivirus reverse genetic systems, construction techniques and applications: a historical perspective. *Antiviral Res*. Feb 2015;114:67-85. doi:10.1016/j.antiviral.2014.12.007

138. Mandl CW, Ecker M, Holzmann H, Kunz C, Heinz FX. Infectious cDNA clones of tick-borne encephalitis virus European subtype prototypic strain Neudoerfl and high virulence strain Hypr. *The Journal of general virology*. May 1997;78 (Pt 5):1049-57. doi:10.1099/0022-1317-78-5-1049

139. Gritsun TS, Gould EA. Infectious transcripts of tick-borne encephalitis virus, generated in days by RT-PCR. *Virology*. Dec 20 1995;214(2):611-8. doi:10.1006/viro.1995.0072

140. Gritsun TS, Gould EA. Development and analysis of a tick-borne encephalitis virus infectious clone using a novel and rapid strategy. *Journal of virological methods*. Dec 1998;76(1-2):109-20.

141. Aubry F, Nougairede A, de Fabritus L, Querat G, Gould EA, de Lamballerie X. Single-stranded positive-sense RNA viruses generated in days using infectious subgenomic amplicons. *The Journal of general virology*. Nov 2014;95(Pt 11):2462-7. doi:10.1099/vir.0.068023-0

142. Yoshii K, Konno A, Goto A, et al. Single point mutation in tick-borne encephalitis virus prM protein induces a reduction of virus particle secretion. *The Journal of general virology*. Oct 2004;85(Pt 10):3049-58. doi:10.1099/vir.0.80169-0

143. Rumyantsev AA, Murphy BR, Pletnev AG. A tick-borne Langat virus mutant that is temperature sensitive and host range restricted in neuroblastoma cells and lacks neuroinvasiveness for immunodeficient mice. *Journal of virology*. Feb 2006;80 (3):1427-39. doi:10.1128/JVI.80.3.1427-1439.2006

144. Kofler RM, Hoenninger VM, Thurner C, Mandl CW. Functional analysis of the tick-borne encephalitis virus cyclization elements indicates major differences between mosquito-borne and tick-borne flaviviruses. *Journal of virology*. Apr 2006;80 (8):4099-113.

145. Markoff L. 5'- and 3'-noncoding regions in flavivirus RNA. *Advances in virus research*. 2003;59:177-228.

146. Silva PA, Pereira CF, Dalebout TJ, Spaan WJ, Bredenbeek PJ. An RNA pseudoknot is required for production of yellow fever virus subgenomic RNA by the host nuclease XRN1. *Journal of virology*. Nov 2010;84(21):11395-406. doi:10.1128/JVI.01047-10

147. Funk A, Truong K, Nagasaki T, et al. RNA structures required for production of subgenomic flavivirus RNA. *Journal of virology*. Nov 2010;84(21):11407-17. doi:10.1128/JVI.01159-10

148. Lin KC, Chang HL, Chang RY. Accumulation of a 3'-terminal genome fragment in Japanese encephalitis virus-infected mammalian and mosquito cells. *Journal of virology*. May 2004;78(10):5133-8.

149. Pijlman GP, Funk A, Kondratieva N, et al. A highly structured, nuclease-resistant, noncoding RNA produced by flaviviruses is required for pathogenicity. *Cell host & microbe*. Dec 11 2008;4 (6):579-91. doi:10.1016/j.chom.2008.10.007

150. Roby JA, Pijlman GP, Wilusz J, Khromykh AA. Noncoding subgenomic flavivirus RNA: multiple functions in West Nile virus pathogenesis and modulation of host responses. *Viruses*. Feb 2014;6(2):404-27. doi:10.3390/v6020404

151. Schnettler E, Tykalova H, Watson M, et al. Induction and suppression of tick cell antiviral RNAi responses by tick-borne flaviviruses. *Nucleic Acids Res.* Aug 2014;42(14):9436-46. doi:10.1093/nar/gku657

152. Gritsun TS, Venugopal K, Zanotto PM, et al. Complete sequence of two tick-borne flaviviruses isolated from Siberia and the UK: analysis and significance of the 5' and 3'-UTRs. *Virus research*. May 1997;49(1):27-39.

153. Hoenninger VM, Rouha H, Orlinger KK, et al. Analysis of the effects of alterations in the tick-borne encephalitis virus 3'-noncoding region on translation and RNA replication using reporter replicons. *Virology*. Aug 1 2008;377(2):419-30. doi:10.1016/j.virol.2008.04.035

154. Sakai M, Muto M, Hirano M, Kariwa H, Yoshii K. Virulence of tick-borne encephalitis virus is associated with intact conformational viral RNA structures in the variable region of the 3'-UTR. *Virus research*. May 4 2015;203:36-40. doi:10.1016/j.virusres.2015.03.006

155. Asghar N, Lindblom P, Melik W, et al. Tick-borne encephalitis virus sequenced directly from questing and blood-feeding ticks reveals quasispecies variance. PLoS One. 2014;9(7):e103264. doi:10.1371/journal.pone.0103264

Chapter 7

Immunology of TBEV infection

Kyra D. Zens and Rahel Ackermann-Gäumann

Key points

- The host immune response to Tickborne Encephalitis Virus (TBEV) infection involves the coordination of multiple immune subsets at several distinct tissue sites over time.
- Contributions from both early innate and later adaptive immune responses are critical in controlling TBEV infection.
- Early innate immune responses are driven by Type I interferon-mediated signaling and are dominated by neutrophils and natural killer cells.
- Antibody-mediated humoral responses and T cell-mediated cellular immune responses both contribute to adaptive immune control of TBEV infection.
- The mechanisms of Central Nervous System (CNS) pathogenesis during Tickborne Encephalitis (TBE) remain unclear but may involve a combination of direct viral cytopathic effects and immune-mediated damage.
- An improved understanding of host immune responses during TBE could aid in the development of improved therapies.

Introduction

Tick-borne Encephalitis (TBE) is a severe, vaccine-preventable disease of the Central Nervous System (CNS) caused by the tick-borne encephalitis virus (TBEV). The virus is primarily transmitted to humans through the bite of infected Ixodid ticks, though an estimated 1% of cases occur via alimentary transmission[1,2] and rare cases of transmission through organ or blood donation have been documented [3,4]. An estimated 70% of TBEV exposures are asymptomatic [5-7]. The remaining 30% of individuals experience a brief, asymptomatic incubation phase[1,2,8], followed by a period of viremia accompanied by febrile, influenza-like illness. While most individuals recover without further symptoms, approximately 30% progress to a second phase of illness characterized by CNS involvement[1,2,8,9]. While some individuals transition directly from the first systemic phase to the second CNS phase, referred to as "monophasic" disease, most experience a short symptom-free interval of approximately 1 week between these two phases, which is referred to as "biphasic" disease. Factors driving a monophasic versus biphasic disease course are not completely clear. Data clearly linking viral subtype to clinical disease course are lacking, though it is believed that monophasic disease, as well as a more severe disease course, are more common after infection with the Siberian (TBEV-Sib) and Far Eastern (TBEV-FE) viral subtypes compared to the European (TBEV-Eu) subtype (reviewed in [1,10]). Differences in virulence factors responsible for distinct pathologies between viral subtypes, however, have yet to be described and confounding factors, such as age, chronic conditions, or possibly even regional differences in medical practices could play further roles.

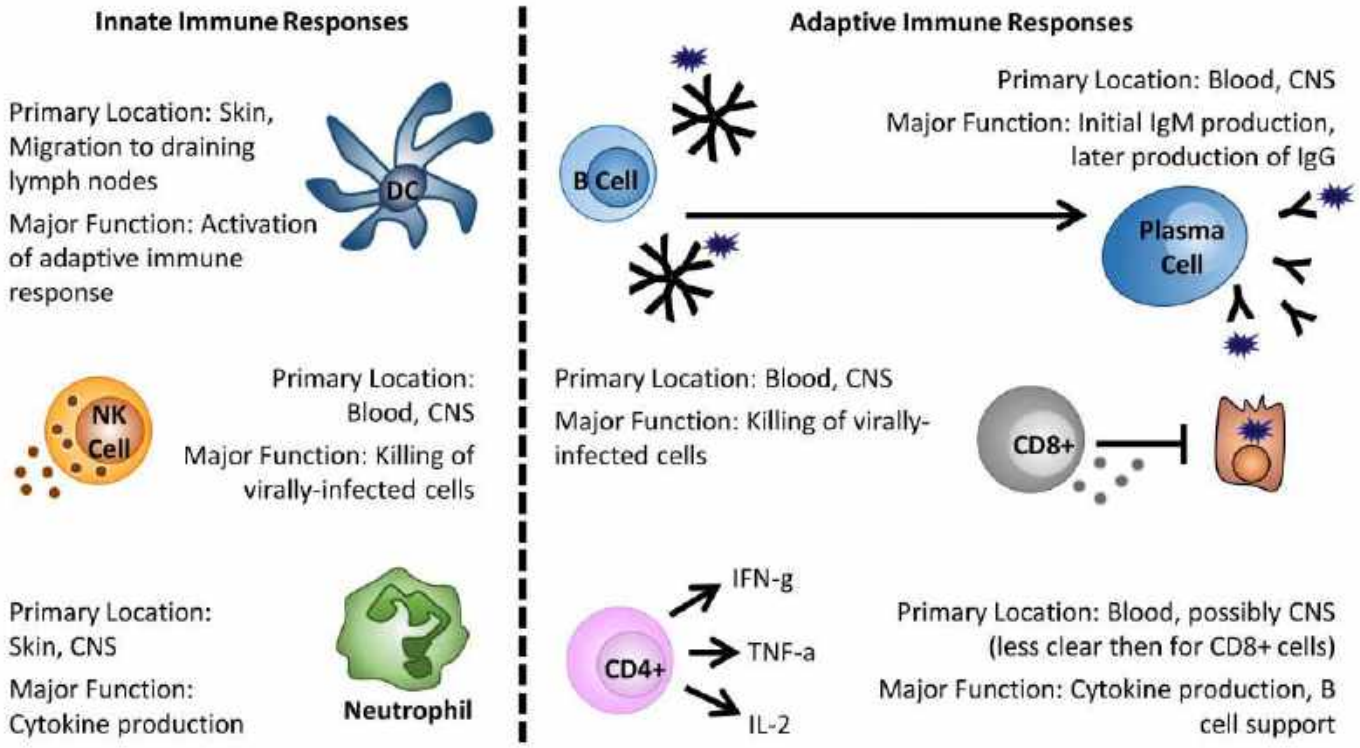
The immune responses which protect individuals against disease represent a complex interplay between many distinct cell types at various times and over different locations. Innate immunity comprises the "first line" defenses following pathogen exposure, acting broadly within the first hours to days following infection to protect against invaders. TBEV belongs to the genus Orthoflavivirus, which also includes the clinically-relevant, arthropod-borne viruses Dengue, West Nile, Yellow Fever, Japanese Encephalitis, and Zika[1,2,11] and early immune responses to TBEV infection share many features with these viruses[12]. Adaptive immune responses, comprised by both humoral (i.e. antibody), and cell-mediated (i.e. T cell) responses, take more time to be established, on the order of days to weeks, as they require the initial activation of the innate immune system. Adaptive immunity, however, provides highly-specific protection against invading pathogens, and further offers immune memory – a subset of cells which are maintained long-term (up to decades), and provide rapid protection upon later re-exposure to the same pathogen.

In this chapter, we summarize the early innate and adaptive immune responses to TBEV infection as well as discuss potential mediators of long-term immune memory protective against later viral reinfection.

TBEV transmission and early local innate immune responses

Skin is perhaps the most important immune organ in that it acts as an initial physical barrier to many infectious organisms. The skin further contains many specialized immune cells, including resident dendritic cell (DC) subsets, natural killer (NK) cells, and T cell subsets, among others

Figure 1: Innate and adaptive immune cells known to be involved in TBE disease

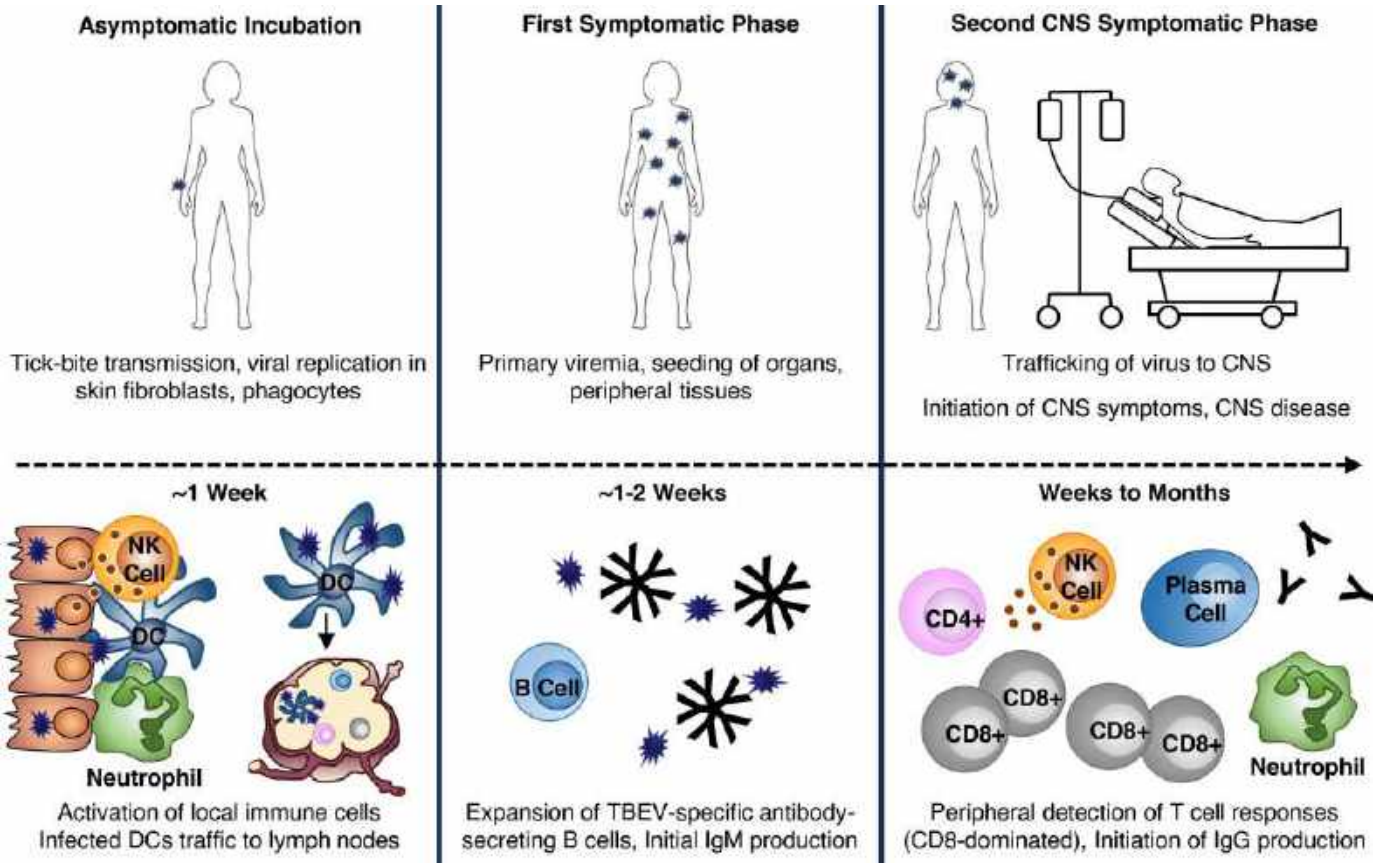


The localization and function of innate and adaptive immune cell subsets described in the context of TBEV infection and TBE disease. DCs are thought to be involved in the initial trafficking of TBEV to the draining lymph nodes following infection. Their major role is in the initiation of later adaptive immune responses. NK cells can be found in the cerebral spinal fluid (CSF) of CNS disease patients and NK cells detected in the blood have an activated (CD57+ CD56dim) phenotype, but lower degranulation and expression of perforin and granzyme B suggesting reduced functionality. Neutrophils are likely among the first cell types at the site of infection and can be infected by TBEV. In CNS disease patients they are present in the CSF and may positively correlate with disease severity. B cells are a key mediator of the adaptive immune response to TBEV as the are responsible for antibody production. Initially IgM is produced, followed by IgG. T cell responses are CD8-biased, though CD4+ T cells are important in providing the B cell help necessary for antibody production.

(Figures 1,2). Transmission of TBEV through tick bites helps the virus to partially circumvent skin's role as a protective physical barrier. Furthermore, factors present within the tick's saliva, including various compounds which help to suppress local innate responses as well as the initiation of adaptive immunity[13-15], further facilitate viral transmission.

The innate immune system is the first line of defense against infection and is especially crucial for so-called "naïve" hosts that have not yet encountered a specific pathogen and developed corresponding adaptive immune memory. Following exposure to TBEV-infected ticks, local skin inflammatory responses begin within 1-3 hours of attachment[16-18]. Pathogen recognition by the innate immune system depends on the host's expression of pattern recognition receptors (PRRs), which identify conserved moieties expressed by invading microorganisms. Toll-Like Receptors (TLRs) and Retinoic Acid-Inducible Gene I (RIG-I)-Like Receptors (RLRs), including RIG-I and Melanoma Differentiation-Associated protein 5 (MDA5), are important in the detection of RNA viruses. Upon activation in this context, PRRs initiate signaling cascades that activate the Interferon (IFN) regulatory factor 3 (IRF-3) signaling pathway, leading to the production of IFN. The role of TLR signaling in protecting against TBEV infection is not well-defined, although TLR-3 and possibly TLR-7, may be involved[19,20]. Roles for RIG-I and MDA5 in the innate immune recognition of TBEV proteins, including non-structural protein 5 (NS5) have been demonstrated[17]. This recognition leads to an early immune response dominated by type I IFN (IFN-a and IFN-b), which seems to be the key mediator of protection during early infection in both in vitro and in vivo models[21,22]. In line with this, mice that lack the IFN-α/β receptor (IFNAR) are unable to control TBEV infection and studies of polymorphisms in innate immune response genes in patients have identified variations in the interferon-induced antiviral proteins oligoadenylate synthetase 2 (OAS2) and 3 (OAS3), which may predispose individuals to the development of clinical TBE[23]. While it has been established that differing strains of TBEV can elicit distinct symptoms in mouse models of disease[20,24] the immunological mechanisms underlying these differences remain incompletely described, though early differences in innate responses due to viral evasion could potentially play an important role.

Figure 2. TBEV transmission and initiation of host immune responses



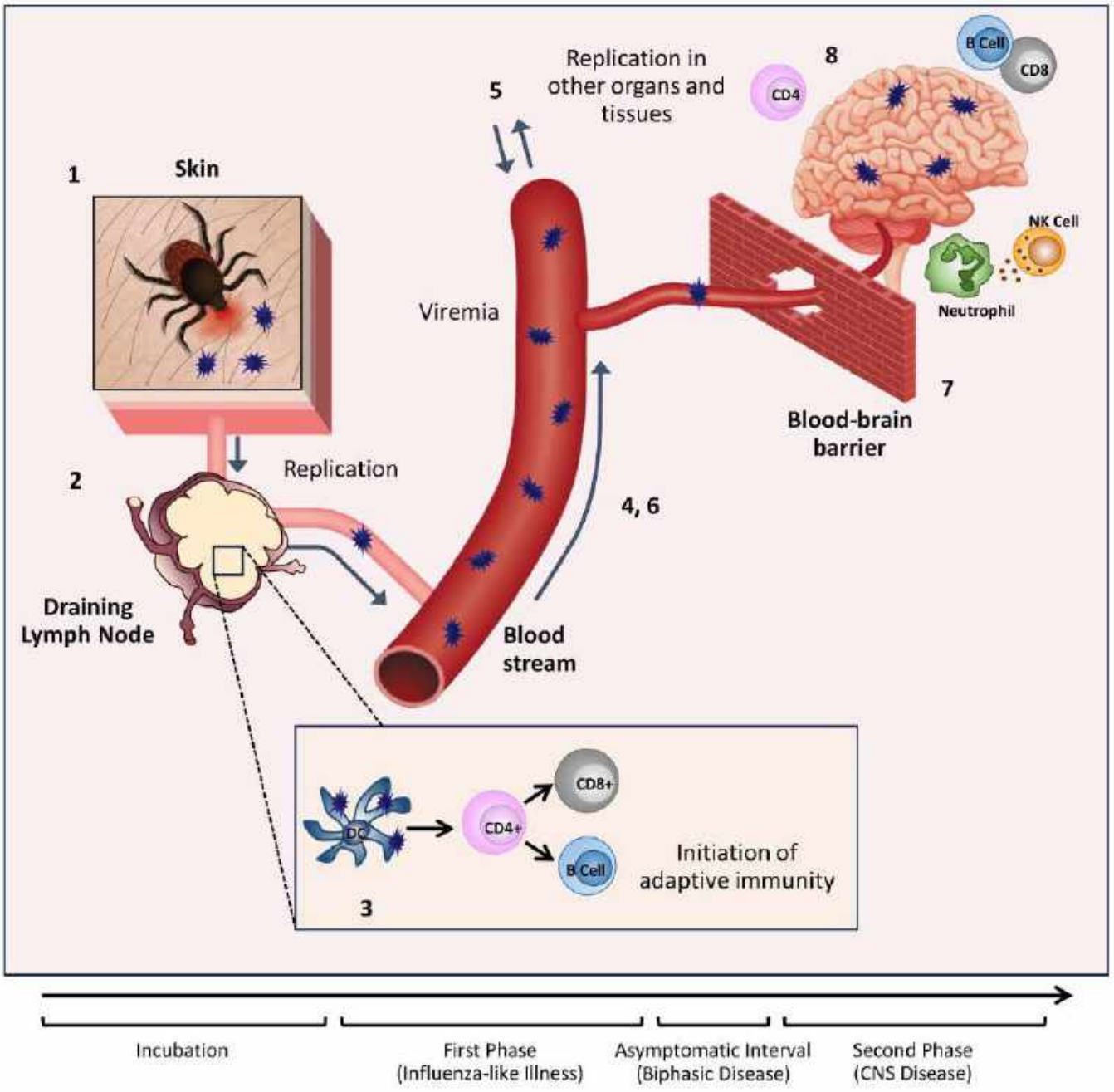
Following tick bite-mediated transmission of TBEV, the virus first infects local skin cells including fibroblasts and phagocytic cells. This leads to the rapid initiation of innate immune responses resulting in the recruitment of additional immune cells to the bite site. Infected DCs are thought to migrate to the draining lymph nodes where they begin to initiate TBEV-specific adaptive immune responses. The virus next disseminates to the organs and peripheral tissues. During this primary viremia, the host experiences the first symptomatic phase of illness. As IgM and antibody-secreting B cells can be detected in patients with biphasic illness upon hospitalization indicates that these responses likely begin during the first phase of illness or short recovery period prior to initiation of CNS symptoms. It is not yet known at what point during the process of viral dissemination that TBEV reaches the CNS. In individuals experiencing biphasic illness with CNS involvement, neutrophils, T cells, NK cells and B cells can be detected in the CNS. Virus-specific T cells and activated NK cells can also be found in peripheral blood. T cell responses, which are strongly CD8-biased, are detected in the blood and peak approximately 1 week after CNS symptom onset. Both anti-TBEV IgM and IgG antibodies are detected in serum during the second phase of TBE. IgM responses peak and begin to transition to IgG responses, which dominate during convalescence. While this figures depicts what is currently known for TBEV infection and the initiation of immune responses during TBE disease, the complete mechanism for this process remains to be understood.

Local dendritic cell (DC) responses

DCs represent a group of cells with a range of functions including acting as a major source of type I IFN during viral infection and playing critical roles in antigen presentation and the activation of adaptive immune responses (Figures 2,3). DCs are often described as the interface between the innate and adaptive immune systems. After TBEV is transmitted, skin-localized DCs are among the first cell types to be infected and they likely play an important role in viral trafficking. In addition, infection of DCs in vitro with Langat virus (LGTV), an attenuated member of the TBE serogroup, has been shown to inhibit type I IFN signaling and reduce IL-12 production – an activator of type 1 adaptive immune responses which are crucial in controlling viral infections[25].

Inhibition of DC type I IFN signaling by the virus, therefore, acts as an important host evasion mechanism and helps to suppress the ensuing immune response. Interestingly, infection of DCs with distinct TBEV strains in vitro has been demonstrated to result in distinct functional capacities, also impacting later activation of CD4+ T cells[20]. In addition, higher viral infectious doses in mice result in delayed DC activation and IFN production, and may impact viral spread to the CNS[20].

Figure 3. TBEV transmission and timeline of viral and host immune response



1) TBEV is transmitted by the bite of an infected tick. 2) The virus infects dendritic cells (DCs) within the kin which traffic to the draining lymph node where the virus replicates further. 3) Presentation of TBEV-derived antigens by infected DCs results in the activation of adaptive immune responses; these take, however weeks to fully develop. 4) The virus is able to spread from the draining lymph node into the blood; during this primary viremia, the host experiences the first symptomatic phase of illness. 5) During primary viremia the virus seeds peripheral organs and replicates further within the tissues. This leads to 6) a second period of virema during which the virus is able to 7) cross the blood brain barrier (BBB). 8) Involvement of the CNS leads to the second phase of disease (in individuals experiencing biphasic illness), neutrophils, T cells, NK cells and B cells can be detected in the CNS.

Primary viremia and seeding of peripheral tissues

In the absence of early immune control within the skin, TBEV next traffics to the draining lymph nodes (Figures 1, 2). This process is not completely understood, but likely occurs during the asymptomatic incubation phase with the migration of virally-infected phagocytes or DCs from the skin playing an important role[26]. Once within the lymph nodes, the virus replicates and eventually seeds peripheral organs (Figures 1, 2). During this viral expansion the host experiences a period of systemic viremia[1,2,8,27,28], which corresponds to the first symptomatic phase of disease. An estimated 70% of individuals control the infection at this stage, though the mechanisms of this control are not clear. Work in a mouse LGTV model has demonstrated a critical role for the type-I IFN response in limiting initial viral replication and systemic spread[29]. This is likely important in the context of TBEV infection as well and suggests a key role for innate immunity in not only early local, but also early systemic immune control of TBEV infection. This is supported by the fact that, due to delayed initiation of adaptive immunity, antibody and T cell responses are absent in the first weeks after pathogen encounter in "naïve" hosts and would, therefore, not be expected to contribute to protection.

Secondary viremia and CNS disease

As described, the remaining 30% of individuals unable to control TBEV during the early local and systemic stages of infection progress to disease which includes CNS involvement. TBEV is neurotropic – preferentially infecting cells of the nervous system. TBEV replication, for example, has been shown to be 10,000-fold higher in human neuronal cells compared to epithelial cells[30]. The ability of the virus to cross the blood brain barrier and invade the CNS is the root cause of clinical disease (Figures 1, 2). In some cases, this progression can directly follow the initial febrile, influenza-like illness (monophasic disease), though most individuals experience a short symptom-free interval prior to CNS disease progression (biphasic disease). In a biphasic disease course, CNS symptoms may occur anywhere from 4 days up to more than 60 days after viral exposure[1,2,8]. Differences in immune control between monophasic and biphasic illness are not well-defined but may also be driven by differences in early innate control rather than differences in later adaptive responses. A recent study comparing monophasic and biphasic disease found that patients with a biphasic disease course were younger and had fewer comorbidities. Levels of proinflammatory cytokines in the CSF were also lower in a biphasic course suggesting less severe disease[31]. In either case, the route by which CNS seeding occurs is not well understood, though breakdown of the blood brain barrier (BBB) does not appear to be necessary for TBEV entry into the brain[32,33] and the virus is no longer present in the blood once CNS involvement is clinically apparent. However, a recent study demonstrating TBEV transmission following organ transplantation brings into question whether the virus may persist in the peripheral tissues for prolonged periods following infection, perhaps even when no longer detectable in the blood[3].

Much of what is known about immune responses to TBEV in humans has been studied during the CNS phase of disease as patients generally present to the clinic only after neurological symptoms have begun. Several studies have evaluated serum cytokine responses in these patients and factors including Chemokine (C-C-motif) Ligand (CCL)5, CCL7, Chemokine (C-X-C-motif) Ligand (CXCL)10, CXCL11, CXCL13, Interferon (IFN)-γ, Interleukin (IL)-1 α, IL-6, IL-15, IL-18, and Tumor Necrosis Factor (TNF)-α have been found to be upregulated, among others[34-40]. A "TBE-specific" cytokine profile, however, which could be useful for diagnostic purposes, has not been defined. Importantly, the entry of immune cells into the brain, which may contribute to immunopathology observed during severe infection in animal studies[33], relies on cytokine-mediated trafficking. In TBE patients, increased levels of CCL5[34] and CXCL10[34,37] in the cerebral spinal fluid (CSF) may be involved in T cell recruitment into the brain during disease through CCR5[34] and CXCR3-mediated[37] trafficking. Similarly, levels of CXCL10 are increased in the sera and brains of mice during TBEV infection[41]. Strong cytokine responses in the brain, coupled with very low neutralizing antibody responses, have been linked to enhanced disease and death[42]. Interestingly, polymorphisms in CCR5, which is an important driver of leukocyte migration, have been implicated in TBE disease susceptibility and severity[19].

Natural killer (NK) cell responses during CNS disease

NK cells (Figure 3) are a subset of cytotoxic innate lymphocytes which play important roles in eliminating virally-infected and tumor cells. While not much is known about the role of NK cells in TBE prior to the development of CNS disease, NK cell-associated cytokines, including IL-12, IL-15, IL-18, IFN-γ, and TNF-α are upregulated in patient sera[43] and NK cells can further be detected in the CSF; indicating their migration to the CNS[44]. Interestingly, while NK cells detected in the peripheral blood of patients have an activated (CD57+ CD56dim) phenotype[43], they appear to be poorly functional, possibly indicating limited protective capacities[43]. Thus, clear roles for NK cells in the context of TBE have not yet been defined, particularly during mild disease where their function may be distinct from that observed in severe disease.

Neutrophil responses during CNS disease

Neutrophils are a critical phagocytic cell subset during the early immune response to viral infections and are major producers of inflammatory cytokines. In tick feeding experiments, neutrophils are attracted to the bite site and can also be infected with TBEV[26]. Like NK cell responses, however, little is known about their role in protection prior to CNS disease. One study found that neutrophils are universally present in the CSF of TBE patients, and, that IL-8, a neutrophil chemoattractant, is the most abundant CSF cytokine[45]. In the same study, neutrophil counts positively correlated with disease severity in patients and their continued detection in CSF samples into convalescence was associated with neurologic sequelae[45]. Supporting this, work in a mouse LGTV model demonstrated increased neutrophil migration into the CNS, and, further, that depletion of neutrophils reduced viral loads, decreased immunopathology, and improved survival[46]. Together these findings suggest that neutrophils may play a role in immunopathology, at least in the context of severe TBE, making them a potential immunotherapeutic target.

Cellular immune responses to TBEV infection

Cellular immunity forms one arm of the so-called "adaptive" immune system (Figure 3). A key feature of adaptive immune responses is the ability to form immune memory following primary pathogen exposure, which is able to provide rapid protective responses upon later pathogen re-encounter. Cellular immunity relies primarily on T cell-mediated immune responses. While T cell responses during TBEV infection are less studied and less understood than humoral responses, T cells seem to play an important role in protection. As with early innate immune responses, a major issue in our understanding of cellular immunity during TBEV infection is that most studies are conducted in patients with relatively severe disease, and late during the disease course – namely after CNS involvement. As a consequence, our understanding of what constitutes "ideal" protective immunity is limited.

CD4+ T cells

Cytokine production is arguably the most important function of CD4+ T cells during antiviral immune responses. These cells are also essential in providing the help necessary for B cells to effectively produce antibodies. Like other orthoflaviviruses, the TBEV genome encodes seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5[1,2,11]), and three structural proteins (capsid (C), two membrane-associated proteins; precursor of membrane/membrane (prM/M), and envelope (E)[1,2,11]). These structural proteins appear to be the major targets of CD4+ T cell responses during TBEV infection[47,48]. In clinical TBE cases, T cell activation has been observed to peak approximately one week after hospitalization, indicating that primary T cell responses are delayed until the CNS phase of illness, at least in severe disease[49,50]. Whether this is the case in mild infections is not clear.

The majority of CD4+ T cells observed during TBEV infection are polyfunctional, producing mainly IL-2, TNF-α, and IFN-γ; the major cytokines of type 1 immune responses (Figure 3)[47,50]. IFN-γ-mediated responses, in particular, are known to be important in the control of viral infections and are often also associated with direct antiviral effector functions in CD4+ T cells. CD4+ T cells appear to have a moderate activation phenotype during TBE infection, suggesting that they may play a less important role in direct viral clearance, but also, may have less immunopathogenic potential, than, for example, CD8+ T cells[51]. In line with their potential protective roles, adoptive transfer of CD4+ T cells has been shown to protect against lethal disease in TBEV-infected Severe Combined Immunodeficiency (SCID; no T or B cells) mice[30].

CD8+ T cells

CD8+ T cells, also known as cytotoxic T cells, play crucial roles in viral infection through their ability to identify and destroy infected host cells, thereby limiting viral replication and spread (Figure 3). In contrast to CD4+ T cells, which appear to target TBEV structural proteins during infection, the CD8+ T cell response appears primarily to target NS proteins; among 6 CD8+ T cell epitopes identified in one study, all were derived from NS proteins[52]. In TBE patients, peak T cell responses are observed approximately 1 week following hospitalization with CD8+ T cell activation substantially increased compared to CD4+ T cells, indicating that responses tend to be CD8-dominated[51]. These CD8+ T cells further displayed an effector phenotype (CD45RA-CCR7) [51,52], and had a highly-activated Eomes+Ki67+T-bet+ transcriptional profile[51]. As patients became convalescent, virus-specific CD8+ T cells transitioned to an Eomes-Ki67-T-bet+ phenotype[51], consistent with a type 1 effector memory (TEM) population.

While immune responses during acute CNS disease are CD8-dominated (Figure 2), the role of these CD8+ T cells in immunopathology versus protection during TBE disease is unclear. Results in animal studies have also been mixed. CCR5-deficient animals experienced a temporal lag in lymphocyte migration into the CNS during LGTV infection which resulted in increased mortality. This was, however, alleviated by adoptive transfer of wildtype (but not CCR5-deficient) T cells, demonstrating the importance to T cell responses in protection from lethal infection[46]. In contrast, survival following lethal TBEV infection in SCID and CD8-knockout mice was increased compared to wildtype or mice with adoptively transferred CD8+ T cells, demonstrating that CD8+ T cells can also contribute to lethal infection[30]. Similarly, CD8+ T cell infiltrates are commonly found in the post-mortem brains of fatal TBE cases[53-55], and a separate

study found that, in severely infected patients, nearly all virus-specific CD8+ T cells expressed a4 and b1 integrins (VLA-4), which are important in lymphocyte homing and can mediate trafficking across the BBB[52]. However, breakdown of the BBB during infection in mice was observed in both wildtype and CD8-knockout animals, indicating that CD8+ T cells themselves are not responsible for BBB permeability during disease[33]. Interestingly, in a mouse model of TBEV infection, TCR CDR3 gene usage differed between lethally and non-lethally infected mice, although no differences in T-cell activation markers or apoptosis-related genes were observed, suggesting that disease severity may be related to antigen specificity, rather than simply the number or activation level of brain-infiltrating T cells[56]. While the mechanism by which TBEV causes CNS destruction remains unclear, a combination of both direct neuronal damage by the virus and indirect damage caused by the immune response may be involved.

Humoral immune responses in TBEV infection

Humoral immunity, mediated by antibodies produced by B cells, is the arm of the adaptive immune response which acts to neutralize and eliminate extracellular microbes and microbial toxins. The humoral immune response plays a critical role in protecting the host from viral infections with antibodies neutralizing virus binding and entry to host cells, as well as coating viral particles to induce their uptake and destruction by phagocytic immune cells; a process termed opsonization. The long-term maintenance of memory B cells enables the immune system to respond more quickly and effectively upon reinfection as these cells rapidly differentiate into antibody-producing plasma cells when they encounter the same pathogen again; in the case of TBEV, helping to eliminate the virus before it can cause widespread infection and disease. Humoral immunity likely plays a crucial role in preventing TBE by generating antibodies that specifically target TBEV. These antibodies neutralize the virus and prevent its spread, helping to limit infection severity and, also, by providing long-term immunity against future viral exposure (Figure 3).

B cells

In contrast to T cells, which, as discussed, peak in their response approximately 1 week post-symptomatic CNS disease, TBEV-specific humoral responses are observed even earlier on during infection (Figure 1). Among TBE patients, activated antibody-producing B cells are already detected at the time of hospital admission. Furthermore, these cells do not appear to expand at this point in time, indicating that these responses are likely initiated prior to CNS-symptomatic disease, perhaps following initial viremia during the asymptomatic interval before CNS symptoms appear[57]. Similarly, in the same study, all patients presented with detectable TBEV-specific IgM and IgG antibodies upon admission which were maintained into convalescence[57]. In comparing immune responses in the peripheral blood and CNS during TBEV infection, several studies have suggested that type 1 cellular immune responses tend to be higher in the CSF[36,38,44,58], while Th17-type responses, dominated by follicular helper T cells which provide help to antibody-producing B cells, and B cell responses are more pronounced in the blood[36,38,44,58]. Together, these findings indicate that B cells and antibody-mediated responses are likely important in controlling the viremic stages of infection where TBEV may spread and seed several peripheral tissues.

Antibody responses

The dynamics of antibody responses following TBEV infection and primary vaccination have been well reviewed [9,10] and humoral immunity is better understood than cellular immunity. While anti-TBEV antibodies are not yet present during the initial viremic phase of TBEV infection[27,28], both IgM, and later on IgG, can be detected in serum during the CNS phase of illness[59] consistent with a limited contribution of adaptive immunity in the early immune control of TBEV during the initial viremic stage of infection. Serum IgM begins to rise within the first six days of CNS symptoms, drops again within six weeks, but remains detectable for several months after infection[59,60]. In contrast, serum IgG levels increase moderately during the CNS symptomatic phase of disease and peak much later - approximately 6 weeks after the onset of the first neurological symptoms [10,59-62]. IgG responses, however, are durable, possibly persisting lifelong following infection, and likely play a major role in protection from reinfection[59,63].

B cell and antibody-mediated responses seem to primarily target the viral E and, to some extent, NS1 proteins. The E glycoprotein mediates viral binding and entry into host cells and is the primary target for neutralizing antibodies during infection as well as in response to TBE vaccination[64]. More than 12 distinct epitopes within E have been identified which elicit antibodies characterized by varying degrees of neutralization potency[64]. In contrast, NS-specific antibodies do not directly neutralize virus infectivity, but likely protect via other mechanisms 64 and several studies have shown that NS1-specific antibodies help to protect against TBE[65-71]. Assessment of anti-NS1 antibody titers may help to distinguish between TBEV infection and previous TBE vaccination, important during vaccine breakthrough infections, as NS proteins are produced mainly during viral replication[72-74]. Low levels of NS1-specific antibodies, however, may also be generated in response to vaccination[75].

Antibody neutralization potential

Neutralizing antibodies are widely considered to be a key mediator of protective immunity against TBE, and, indeed, neutralizing titers of 1:10 or greater are considered a surrogate measurement for the "correlate of protection" against TBE[76,77]. Orthoflaviviral neutralizing antibodies have been shown to interfere with the process of virus-induced membrane fusion, preventing infection of target host cells[78-80]. Other mechanisms of action have been suggested to include blocking the binding of the viral particles to cellular receptors, blocking the interaction of the virion with cellular receptors through steric hindrance, or blocking membrane fusion inside endosomes or phagosomes within the host cells through the cross-linking of E molecules[81]. Importantly, though, orthoflavivirus neutralization appears to be a "multiple hit" phenomenon requiring engagement by more than a single antibody[64]. It is plausible that the mechanism of neutralization of many E-specific antibodies involves both steps of virus entry and is modulated by the composition of antibody populations in polyclonal sera[82].

Epitopes involved in TBEV neutralization have been mapped to each of the three viral E protein domains, to domain-overlapping sites within a single E protein monomer, to E protein dimer-specific sites, and to E protein sites requiring the quaternary arrangement found only within viral particles[82]. The dominance of antibodies to different E domains appears to be heavily impacted by host-species-specific, as well as virus-specific, factors. Many of the most potent orthoflaviviral neutralizing antibodies characterized to date recognize the upper lateral surface of domain III of the E protein (EDIII) that protrudes from the surface of the virion; however these antibodies are major contributors to the neutralizing responses observed in mice but not in humans[64,83]. In contrast, antibodies against domains I and II, EDI and EDII, dominate the human immune response to TBEV[84]. Due to the potent neutralizing activity of anti-EDIII antibodies, though, vaccination or therapeutic strategies focusing on this domain could be beneficial[78].

Cross-neutralization between orthoflaviviruses

While available TBE vaccines designed to protect against the TBEV-Eu subtype have been shown additionally to protect against TBEV-Sib and TBEV-FE subtypes[85-87], antigenic similarities between orthoflaviviruses can also lead to the generation of both species-specific, as well as orthoflavivirus cross-reactive antibodies in response to infection[88]. For instance, a study has demonstrated that individuals who had received vaccinations against Japanese Encephalitis virus, Yellow Fever virus, and TBEV were able to neutralize Louping-ill virus and to a lesser degree West Nile virus and Dengue virus[89]. Similarly, TBEV neutralizing antibodies have been shown to be broadly active against other tick-borne orthoflaviviruses including Louping ill virus, Langat virus, and Omsk Hemorrhagic Fever virus[78], and the immune response generated following TBEV vaccination can protect against Omsk Hemorrhagic Fever virus, Kyasanur Forest Disease virus and Alkhumra virus[90,91]. However, cross-neutralizing antibodies are usually not durable and cross-neutralization is retained only a few months[92]. And while cross-neutralization might provide a certain level of cross-protection from infection, such pre-existing immunity to other orthoflaviviruses may also impair or modulate the immune response to TBEV vaccination. For instance, in a cross-sectional study examining risk factors for seronegativity despite vaccination, individuals being vaccinated against Yellow Fever or Japanese Encephalitis virus were less likely to be seropositive for neutralizing TBEV antibodies[93]. Similarly, both an increase in broadly orthoflavivirus cross-reactive antibodies and an impairment in TBEV-neutralizing activity in individuals with previous vaccination against Yellow Fever virus have been demonstrated[94]. Interestingly, broadly cross-reactive antibodies are more frequently observed in individuals post-vaccination than post-infection[84]. On a molecular basis, cross-reactive antibodies are specific for a cluster of epitopes that are partially occluded in the cage-like assembly of E proteins at the surfaces of infectious virions and involve—but are not restricted to—amino acids of the highly conserved internal fusion peptide loop. The cryptic properties of these sites can provide an explanation for the observed low neutralizing potency of broadly cross-reactive antibodies, despite their specificity for a functionally important structural element in the E protein[88,95-97].

Durability of protection

Following TBEV infection antibody titers remain stable at high levels over many years[98,99]. Titers following infection are also comparable between both older and younger individuals[98,99], in contrast to vaccination where titers tend to be inversely correlated with age. While it is thought that IgG generated in response to infection may possibly persist lifelong, providing continued protection from reinfection[10], a comparison of seroprevalence and average TBE incidence rates from the 1980s through 2001 suggests that this might not be the case[100]. These results suggest that, in order to err on the side of caution, additional booster vaccinations should be considered, even for recovered TBE patients. However, more evidence is necessary to better understand the duration of immunity following TBEV infection to help define best practices for vaccination and ensure continued protection.

Conclusion

TBE is a complex disease which requires the host to respond to viral infection at several distinct tissue sites over a prolonged period of time. Despite considerable insights into innate and adaptive immunity against TBEV infection, numerous questions remain. Early in infection, for example,

the immune response is critically shaped by local responses within the skin. Determining whether local trained innate immune responses or “tissue-resident” T or B cell subsets could protect from TBEV infection, providing rapid control at the initial infection site before viral spread, is an interesting area worth further exploration. Furthermore, understanding and identifying specific cytokine expression profiles contributing either to protection or immunopathology, early in acute TBE disease holds therapeutic promise. In terms of adaptive immunity, while antibody responses have been extensively studied in TBE disease, memory B and T cell responses may also act as important mediators of protection. Additional research focusing on the functions of these adaptive immune subsets, particularly in asymptomatic and mild cases, is crucial to defining "ideal" protective immune responses and establishing a baseline for vaccine-mediated immunity. Ultimately, though, a better understanding of the immune responses involved in protection and possibly also immunopathology of TBE can help in the development of effective strategies for its prevention, diagnosis, and treatment.

Contact: zens@immunology.uzh.ch

Citation: Zens KD. Ackermann-Gäumann R. Immunology of TBEV infection. Chapter 7. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ: *The TBE Book.* 7th ed. Singapore: Global Health Press; 2024. doi: 10.33442/26613980_7-7

References

1. Lindquist L, Vapalahti O. Tick-borne encephalitis. Review. *Lancet*. May 31 2008;371(9627):1861-71. doi:10.1016/s0140-6736(08)60800-4
2. Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. Review. *Antiviral Res*. Jan 2003;57(1-2):129-46. doi:10.1016/s0166-3542(02)00206-1
3. Lipowski D, Popiel M, Perlejewski K, et al. A Cluster of Fatal Tick-borne Encephalitis Virus Infection in Organ Transplant Setting. *J Infect Dis*. Mar 15 2017;215(6):896-901. doi:10.1093/infdis/jix040
4. Wahlberg P, Saikku P, Brummer-Korvenkontio M. Tick-borne viral encephalitis in Finland. The clinical features of Kumlinge disease during 1959–1987. *Journal of Internal Medicine.* 1989/03/01 1989;225(3):173-177. doi:10.1111/j.1365-2796.1989.tb00059.x
5. Kaiser R. Tick-borne encephalitis. *Infect Dis Clin North Am*. Sep 2008;22(3):561-75, x. doi:10.1016/j.idc.2008.03.013
6. Kaiser R. [Tick-borne encephalitis]. *Nervenarzt*. Jun 2016;87 (6):667-80. Frühsommermeningoenzephalitis. doi:10.1007/s00115-016-0134-9
7. Bogovic P, Strle F. Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management. Review. *World J Clin Cases*. May 16 2015;3(5):430-41. doi:10.12998/wjcc.v3.i5.430
8. Haglund M, Günther G. Tick-borne encephalitis--pathogenesis, clinical course and long-term follow-up. Review. *Vaccine*. Apr 1 2003;21 Suppl 1:S11-8. doi:10.1016/s0264-410x(02)00811-3
9. Ruzek D, Avšič Županc T, Borde J, et al. Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. Review. *Antiviral Res*. Apr 2019;164:23-51. doi:10.1016/j.antiviral.2019.01.014
10. Dörrbecker B, Dobler G, Spiegel M, Hufert FT. Tick-borne encephalitis virus and the immune response of the mammalian host. Review. *Travel Med Infect Dis.* Jul 2010;8 (4):213-22. doi:10.1016/j.tmaid.2010.05.010
11. Simmonds P, Becher P, Bukh J, et al. ICTV Virus Taxonomy Profile: Flaviviridae. *J Gen Virol*. Jan 2017;98(1):2-3. doi:10.1099/jgv.0.000672
12. Pan Y, Cai W, Cheng A, Wang M, Yin Z, Jia R. Flaviviruses: Innate Immunity, Inflammasome Activation, Inflammatory Cell Death, and Cytokines. *Front Immunol.* 2022;13:829433. doi:10.3389/fimmu.2022.829433
13. Labuda M, Jones LD, Williams T, Nuttall PA. Enhancement of tick-borne encephalitis virus transmission by tick salivary gland extracts. *Med Vet Entomol.* Apr 1993;7(2):193-6. doi:10.1111/j.1365-2915.1993.tb00674.x
14. Nuttall PA. Tick saliva and its role in pathogen transmission. *Wiener klinische Wochenschrift*. 2023/04/01 2019;135(7):165 -176. doi:10.1007/s00508-019-1500-y
15. Kotál J, Langhansová H, Lieskovská J, et al. Modulation of host immunity by tick saliva. *Journal of Proteomics*. 2015/10/14/2015;128:58-68.doi:10.1016/j.jprot.2015.07.005
16. Thangamani S, Hermance ME, Santos RI, et al. Transcriptional Immunoprofiling at the Tick-Virus-Host Interface during Early Stages of Tick-Borne Encephalitis Virus Transmission. *Front Cell Infect Microbiol*. 2017;7:494. doi:10.3389/fcimb.2017.00494
17. Zheng Z, Yang J, Jiang X, et al. Tick-Borne Encephalitis Virus Nonstructural Protein NS5 Induces RANTES Expression Dependent on the RNA-Dependent RNA Polymerase Activity. *J Immunol.* Jul 1 2018;201(1):53-68. doi:10.4049/jimmunol.1701507
18. Hermance ME, Santos RI, Kelly BC, Valbuena G, Thangamani S. Immune Cell Targets of Infection at the Tick-Skin Interface during Powassan Virus Transmission. *PLoS One*. 2016;11 (5):e0155889. doi:10.1371/journal.pone.0155889
19. Ellwanger JH, Chies JAB. Host immunogenetics in tick-borne encephalitis virus infection—The CCR5 crossroad. Review. *Ticks and Tick-borne Diseases.* 2019;10(4):729-741. doi:10.1016/j.ttbdis.2019.03.005
20. Shevtsova AS, Motuzova OV, Kuragina VM, et al. Lethal Experimental Tick-Borne Encephalitis Infection: Influence of Two Strains with Similar Virulence on the Immune Response. *Front Microbiol.* 2016;7:2172. doi:10.3389/fmicb.2016.02172
21. Kurhade C, Zegenhagen L, Weber E, et al. Type I Interferon response in olfactory bulb, the site of tick-borne flavivirus accumulation, is primarily regulated by IPS-1. *J Neuroinflammation*. Jan 27 2016;13:22. doi:10.1186/s12974-016-0487-9

22. Overby AK, Popov VL, Niedrig M, Weber F. Tick-borne encephalitis virus delays interferon induction and hides its double-stranded RNA in intracellular membrane vesicles. *J Virol*. Sep 2010;84(17):8470-83. doi:10.1128/jvi.00176-10
23. Barkhash AV, Perelygin AA, Babenko VN, et al. Variability in the 2'–5'-Oligoadenylate Synthetase Gene Cluster Is Associated with Human Predisposition to Tick-Borne Encephalitis Virus-Induced Disease. *The Journal of Infectious Diseases*. 2010;202(12):1813-1818. doi:10.1086/657418
24. Kurhade C, Schreier S, Lee YP, et al. Correlation of Severity of Human Tick-Borne Encephalitis Virus Disease and Pathogenicity in Mice. *Emerg Infect Dis*. Sep 2018;24(9):1709-1712. doi:10.3201/eid2409.171825
25. Robertson SJ, Lubick KJ, Freedman BA, Carmody AB, Best SM. Tick-borne flaviviruses antagonize both IRF-1 and type I IFN signaling to inhibit dendritic cell function. *J Immunol*. Mar 15 2014;192(6):2744-55. doi:10.4049/jimmunol.1302110
26. Labuda M, Austyn JM, Zuffova E, et al. Importance of localized skin infection in tick-borne encephalitis virus transmission. *Virology*. May 15 1996;219(2):357-66. doi:10.1006/viro.1996.0261
27. Saksida A, Duh D, Lotrič-Furlan S, Strle F, Petrovec M, Avšič-Županc T. The importance of tick-borne encephalitis virus RNA detection for early differential diagnosis of tick-borne encephalitis. *Journal of Clinical Virology*. 2005/08/01/ 2005;33(4):331-335. doi:https://doi.org/10.1016/j.jcv.2004.07.014
28. Saksida A, Jakopin N, Jelovšek M, et al. Virus RNA Load in Patients with Tick-Borne Encephalitis, Slovenia. *Emerging Infectious Disease journal*. 2018;24(7):1315. doi:10.3201/eid2407.180059
29. Weber E, Finsterbusch K, Lindquist R, et al. Type I interferon protects mice from fatal neurotropic infection with Langat virus by systemic and local antiviral responses. *J Virol*. Nov 2014;88(21):12202-12. doi:10.1128/jvi.01215-14
30. Růzek D, Salát J, Palus M, et al. CD8+ T-cells mediate immunopathology in tick-borne encephalitis. *Virology*. Feb 5 2009;384(1):1-6. doi:10.1016/j.virol.2008.11.023
31. Bogovič P, Lotrič-Furlan S, Avšič-Županc T, et al. Comparison of Clinical, Laboratory and Immune Characteristics of the Monophasic and Biphasic Course of Tick-Borne Encephalitis. *Microorganisms*. Apr 10 2021;9(4)doi:10.3390/microorganisms9040796
32. Palus M, Vancova M, Sirmarova J, Elsterova J, Perner J, Ruzek D. Tick-borne encephalitis virus infects human brain microvascular endothelial cells without compromising blood-brain barrier integrity. *Virology*. Jul 2017;507:110-122. doi:10.1016/j.virol.2017.04.012
33. Růžek D, Salát J, Singh SK, Kopecký J. Breakdown of the blood-brain barrier during tick-borne encephalitis in mice is not dependent on CD8+ T-cells. *PLoS One*. 2011;6(5):e20472. doi:10.1371/journal.pone.0020472
34. Grygorczuk S, Osada J, Toczyłowski K, et al. The lymphocyte populations and their migration into the central nervous system in tick-borne encephalitis. *Ticks Tick Borne Dis*. Sep 2020;11(5):101467. doi:10.1016/j.ttbdis.2020.101467
35. Grygorczuk S, Czupryna P, Pancewicz S, et al. The increased intrathecal expression of the monocyte-attracting chemokines CCL7 and CXCL12 in tick-borne encephalitis. *J Neurovirol*. Jun 2021;27(3):452-462. doi:10.1007/s13365-021-00975-z
36. Toczylowski K, Grygorczuk S, Osada J, et al. Evaluation of cerebrospinal fluid CXCL13 concentrations and lymphocyte subsets in tick-borne encephalitis. *Int J Infect Dis*. Apr 2020;93:40-47. doi:10.1016/j.ijid.2020.01.023
37. Lepej SZ, Misić-Majerus L, Jeren T, et al. Chemokines CXCL10 and CXCL11 in the cerebrospinal fluid of patients with tick-borne encephalitis. *Acta Neurol Scand*. Feb 2007;115(2):109-14. doi:10.1111/j.1600-0404.2006.00726.x
38. Bogovič P, Kastrin A, Lotrič-Furlan S, et al. Comparison of laboratory and immune characteristics of the initial and second phase of tick-borne encephalitis. *Emerg Microbes Infect.* Dec 2022;11(1):1647-1656. doi:10.1080/22221751.2022.2086070
39. Atrasheuskaya AV, Fredeking TM, Ignatyev GM. Changes in immune parameters and their correction in human cases of tick-borne encephalitis. *Clin Exp Immunol*. Jan 2003;131(1):148-54. doi:10.1046/j.1365-2249.2003.02050.x
40. Zidovec-Lepej S, Vilibic-Cavlek T, Ilic M, et al. Quantification of Antiviral Cytokines in Serum, Cerebrospinal Fluid and Urine of Patients with Tick-Borne Encephalitis in Croatia. *Vaccines*. 2022;10(11):1825.
41. Pokorna Formanova P, Palus M, Salat J, et al. Changes in cytokine and chemokine profiles in mouse serum and brain, and in human neural cells, upon tick-borne encephalitis virus infection. *J Neuroinflammation*. Nov 7 2019;16(1):205. doi:10.1186/s12974-019-1596-z
42. Palus M, Vojtíšková J, Salát J, et al. Mice with different susceptibility to tick-borne encephalitis virus infection show selective neutralizing antibody response and inflammatory reaction in the central nervous system. *J Neuroinflammation*. Jun 27 2013;10:77. doi:10.1186/1742-2094-10-77
43. Blom K, Braun M, Pakalniene J, et al. NK Cell Responses to Human Tick-Borne Encephalitis Virus Infection. *J Immunol*. Oct 1 2016;197(7):2762-71. doi:10.4049/jimmunol.1600950
44. Tomazic J, Ihan A. Flow cytometric analysis of lymphocytes in cerebrospinal fluid in patients with tick-borne encephalitis. *Acta Neurol Scand*. Jan 1997;95(1):29-33. doi:10.1111/j.1600-0404.1997.tb00064.x
45. Grygorczuk S, Świerzbińska R, Kondrusik M, et al. The intrathecal expression and pathogenetic role of Th17 cytokines and CXCR2-binding chemokines in tick-borne encephalitis. *J Neuroinflammation*. Apr 20 2018;15(1):115. doi:10.1186/s12974-018-1138-0
46. Michlmayr D, Bardina SV, Rodriguez CA, Pletnev AG, Lim JK. Dual Function of Ccr5 during Langat Virus Encephalitis: Reduction in Neutrophil-Mediated Central Nervous System Inflammation and Increase in T Cell-Mediated Viral Clearance. J Immunol. Jun 1 2016;196(11):4622-31. doi:10.4049/jimmunol.1502452
47. Aberle JH, Schwaiger J, Aberle SW, et al. Human CD4+ T Helper Cell Responses after Tick-Borne Encephalitis Vaccination and Infection. *PLoS One.* 2015;10(10):e0140545. doi:10.1371/journal.pone.0140545

48. Schwaiger J, Aberle JH, Stiasny K, et al. Specificities of human CD4+ T cell responses to an inactivated flavivirus vaccine and infection: correlation with structure and epitope prediction. *J Virol*. Jul 2014;88(14):7828-42. doi:10.1128/jvi.00196-14
49. Blom K, Cuapio A, Sandberg JT, et al. Cell-Mediated Immune Responses and Immunopathogenesis of Human Tick-Borne Encephalitis Virus-Infection. Review. *Front Immunol*. 2018;9:2174. doi:10.3389/fimmu.2018.02174
50. Varnaitė R, Blom K, Lampen MH, et al. Magnitude and Functional Profile of the Human CD4(+) T Cell Response throughout Primary Immunization with Tick-Borne Encephalitis Virus Vaccine. *J Immunol.* Feb 15 2020;204 (4):914-922. doi:10.4049/jimmunol.1901115
51. Blom K, Braun M, Pakalniene J, et al. Specificity and dynamics of effector and memory CD8 T cell responses in human tick-borne encephalitis virus infection. *PLoS Pathog*. Jan 2015;11 (1):e1004622. doi:10.1371/journal.ppat.1004622
52. Lampen MH, Uchtenhagen H, Blom K, et al. Breadth and Dynamics of HLA-A2- and HLA-B7-Restricted CD8(+) T Cell Responses against Nonstructural Viral Proteins in Acute Human Tick-Borne Encephalitis Virus Infection. *Immunohorizons*. Jul 2 2018;2(6):172-184. doi:10.4049/ immunohorizons.1800029
53. Gelpi E, Preusser M, Laggner U, et al. Inflammatory response in human tick-borne encephalitis: analysis of postmortem brain tissue. *J Neurovirol.* Aug 2006;12(4):322-7. doi:10.1080/13550280600848746
54. Gelpi E, Preusser M, Garzuly F, Holzmann H, Heinz FX, Budka H. Visualization of Central European tick-borne encephalitis infection in fatal human cases. *J Neuropathol Exp Neurol*. Jun 2005;64(6):506-12. doi:10.1093/jnen/64.6.506
55. Sendi P, Hirzel C, Pfister S, et al. Fatal Outcome of European Tick-borne Encephalitis after Vaccine Failure. *Front Neurol*. 2017;8:119. doi:10.3389/fneur.2017.00119
56. Fujii Y, Hayasaka D, Kitaura K, Takasaki T, Suzuki R, Kurane I. T -cell clones expressing different T-cell receptors accumulate in the brains of dying and surviving mice after peripheral infection with far eastern strain of tick-borne encephalitis virus. Viral Immunol. Aug 2011;24(4):291-302. doi:10.1089/ vim.2011.0017
57. Varnaitė R. Adaptive Immune Responses to Tick-Borne Encephalitis Virus and SARS-COV-2. Karolinska Institutet (Sweden); 2022.
58. Bogovič P, Lusa L, Korva M, et al. Inflammatory Immune Responses in the Pathogenesis of Tick-Borne Encephalitis. J Clin Med. May 22 2019;8(5)doi:10.3390/jcm8050731
59. Holzmann H. Diagnosis of tick-borne encephalitis. Review. Vaccine. Apr 1 2003;21 Suppl 1:S36-40. doi:10.1016/s0264-410x(02)00819-8
60. Růžek D, Dobler G, Donoso Mantke O. Tick-borne encephalitis: pathogenesis and clinical implications. Travel Med Infect Dis. Jul 2010;8(4):223-32. doi:10.1016/ j.tmaid.2010.06.004
61. Günther G, Haglund M, Lindquist L, Sköldenberg B, Forsgren M. Intrathecal IgM, IgA and IgG antibody response in tick-borne encephalitis. Long-term follow-up related to clinical course and outcome. Clin Diagn Virol. May 1997;8(1):17-29. doi:10.1016/s0928-0197(97)00273-0
62. Stiasny K, Holzmann H, Heinz FX. Characteristics of antibody responses in tick-borne encephalitis vaccination breakthroughs. Vaccine. Nov 23 2009;27(50):7021-6. doi:10.1016/j.vaccine.2009.09.069
63. Taba P, Schmutzhard E, Forsberg P, et al. EAN consensus review on prevention, diagnosis and management of tick-borne encephalitis. Eur J Neurol. Oct 2017;24(10):1214-e61. doi:10.1111/ene.13356
64. Pierson TC, Diamond MS. Molecular mechanisms of antibody -mediated neutralisation of flavivirus infection. Review. Expert Rev Mol Med. May 12 2008;10:e12. doi:10.1017/ s1462399408000665
65. Aleshin SE, Timofeev AV, Khoretonenko MV, et al. Combined prime-boost vaccination against tick-borne encephalitis (TBE) using a recombinant vaccinia virus and a bacterial plasmid both expressing TBE virus non-structural NS1 protein. BMC Microbiology. 2005/08/02 2005;5(1):45. doi:10.1186/1471-2180-5-45
66. Jacobs SC, Stephenson JR, Wilkinson GWG. Protection elicited by a replication-defective adenovirus vector expressing the tick-borne encephalitis virus non-structural glycoprotein NS1. Journal of General Virology. 1994;75(9):2399-2402. doi:https://doi.org/10.1099/0022-1317-75-9-2399
67. Khoretonenko MV, Vorovitch MF, Zakharova LG, et al. Vaccinia virus recombinant expressing gene of tick-borne encephalitis virus non-structural NS1 protein elicits protective activity in mice. Immunology Letters. 2003/12/15/ 2003;90(2):161-163. doi:https://doi.org/10.1016/ j.imlet.2003.09.002
68. Kuzmenko YV, Starodubova ES, Shevtsova AS, et al. Intracellular degradation and localization of NS1 of tick-borne encephalitis virus affect its protective properties. Journal of General Virology. 2017;98(1):50-55. doi:https:// doi.org/10.1099/jgv.0.000700
69. Salat J, Mikulasek K, Larralde O, et al. Tick-Borne Encephalitis Virus Vaccines Contain Non-Structural Protein 1 Antigen and may Elicit NS1-Specific Antibody Responses in Vaccinated Individuals. Vaccines (Basel). Feb 12 2020;8(1)doi:10.3390/ vaccines8010081
70. Timofeev AV, Butenko VM, Stephenson JR. Genetic Vaccination of Mice with Plasmids Encoding the NS1 Non-structural Protein from Tick-borne Encephalitis Virus and Dengue 2 Virus. Virus Genes. 2004/01/01 2004;28(1):85-97. doi:10.1023/B:VIRU.0000012266.04871.ce
71. Volpina OM, Volkova TD, Koroev DO, et al. A synthetic peptide based on the NS1 non-structural protein of tick-borne encephalitis virus induces a protective immune response against fatal encephalitis in an experimental animal model. Virus Res. Sep 2005;112(1-2):95-9. doi:10.1016/ j.virusres.2005.03.026
72. Girl P, Bestehorn-Willmann M, Zange S, Borde JP, Dobler G, von Buttlar H. Tick-Borne Encephalitis Virus Nonstructural Protein 1 IgG Enzyme-Linked Immunosorbent Assay for Differentiating Infection versus Vaccination Antibody

Responses. J Clin Microbiol. Mar 25 2020;58(4)doi:10.1128/jcm.01783-19
73. Stiasny K, Leitner A, Holzmann H, Heinz FX. Dynamics and Extent of Non-Structural Protein 1-Antibody Responses in Tick-Borne Encephalitis Vaccination Breakthroughs and Unvaccinated Patients. Viruses. May 27 2021;13(6) doi:10.3390/v13061007
74. Albinsson B, Rönnberg B, Vene S, Lundkvist Å. Antibody responses to tick-borne encephalitis virus non-structural protein 1 and whole virus antigen–a new tool in the assessment of suspected vaccine failure patients. Infection Ecology & Epidemiology. 2019/01/01 2019;9(1):1696132. doi:10.1080/20008686.2019.1696132
75. Ackermann-Gäumann R, Brêchet A, Smetana J, et al. Vaccination against tick-borne encephalitis elicits a detectable NS1 IgG antibody response. J Virol Methods. Dec 2023;322:114831. doi:10.1016/j.jviromet.2023.114831
76. Vaccines against tick-borne encephalitis: WHO position paper - Recommendations. Conference Paper. Vaccine. 2011;29 (48):8769-8770. doi:10.1016/j.vaccine.2011.07.024
77. Holzmann H, Kundi M, Stiasny K, et al. Correlation between ELISA, hemagglutination inhibition, and neutralization tests after vaccination against tick-borne encephalitis. J Med Virol. Jan 1996;48(1):102-7. doi:10.1002/(sici)1096-9071(199601)48:1<102::Aid-jmv16>3.0.Co;2-i
78. Agudelo M, Palus M, Keeffe JR, et al. Broad and potent neutralizing human antibodies to tick-borne flaviviruses protect mice from disease. J Exp Med. May 3 2021;218(5) doi:10.1084/jem.20210236
79. Füzik T, Formanová P, Růžek D, Yoshii K, Niedrig M, Plevka P. Structure of tick-borne encephalitis virus and its neutralization by a monoclonal antibody. Nat Commun. Jan 30 2018;9(1):436. doi:10.1038/s41467-018-02882-0
80. Yang X, Qi J, Peng R, et al. Molecular Basis of a Protective/Neutralizing Monoclonal Antibody Targeting Envelope Proteins of both Tick-Borne Encephalitis Virus and Louping Ill Virus. *J Virol.* Apr 15 2019;93(8)doi:10.1128/jvi.02132-18
81. Baykov IK, Chojnowski G, Pachl P, et al. Structural insights into tick-borne encephalitis virus neutralization and animal protection by a therapeutic antibody. *bioRxiv*. 2021;
82. Heinz FX, Stiasny K. Flaviviruses and their antigenic structure. Review. *J Clin Virol*. Dec 2012;55(4):289-95. doi:10.1016/j.jcv.2012.08.024
83. Tsouchnikas G, Zlatkovic J, Jarmer J, et al. Immunization with Immune Complexes Modulates the Fine Specificity of Antibody Responses to a Flavivirus Antigen. *J Virol*. Aug 2015;89(15):7970-8. doi:10.1128/jvi.00938-15
84. Jarmer J, Zlatkovic J, Tsouchnikas G, et al. Variation of the specificity of the human antibody responses after tick-borne encephalitis virus infection and vaccination. *J Virol.* Dec 2014;88(23):13845-57. doi:10.1128/jvi.02086-14
85. Holzmann H, Vorobyova MS, Ladyzhenskaya IP, et al. Molecular epidemiology of tick-borne encephalitis virus: cross-protection between European and Far Eastern subtypes. *Vaccine*. 1992/01/01/ 1992;10(5):345-349. doi:https://doi.org/10.1016/0264-410X(92)90376-U
86. Hayasaka D, Goto A, Yoshii K, Mizutani T, Kariwa H, Takashima I. Evaluation of European tick-borne encephalitis virus vaccine against recent Siberian and far-eastern subtype strains. *Vaccine.* 2001/09/14/ 2001;19(32):4774-4779. doi:https://doi.org/10.1016/S0264-410X(01)00218-3
87. Takashima I, Hayasaka D, Goto A, Kariwa H, Mizutani T. Epidemiology of tick-borne encephalitis (TBE) and phylogenetic analysis of TBE viruses in Japan and Far Eastern Russia. *Jpn J Infect Dis*. 2001/02// 2001;54(1):1-11.
88. Rathore APS, St John AL. Cross-Reactive Immunity Among Flaviviruses. Review. *Front Immunol.* 2020;11:334. doi:10.3389/fimmu.2020.00334
89. Mansfield KL, Horton DL, Johnson N, et al. Flavivirus-induced antibody cross-reactivity. *J Gen Virol*. Dec 2011;92(Pt 12):2821-2829. doi:10.1099/vir.0.031641-0
90. Chidumayo NN, Yoshii K, Kariwa H. Evaluation of the European tick-borne encephalitis vaccine against Omsk hemorrhagic fever virus. Microbiol Immunol. Feb 2014;58 (2):112-8. doi:10.1111/1348-0421.12122
91. Fritz R, Orlinger KK, Hofmeister Y, et al. Quantitative comparison of the cross-protection induced by tick-borne encephalitis virus vaccines based on European and Far Eastern virus subtypes. *Vaccine.* Feb 1 2012;30(6):1165-9. doi:10.1016/j.vaccine.2011.12.013
92. Collins MH, McGowan E, Jadi R, et al. Lack of Durable Cross-Neutralizing Antibodies Against Zika Virus from Dengue Virus Infection. *Emerg Infect Dis.* May 2017;23(5):773-781. doi:10.3201/eid2305.161630
93. Lindblom P, Wilhelmsson P, Fryland L, et al. Factors determining immunological response to vaccination against tick-borne encephalitis virus in older individuals. *PLoS O*ne. 2014;9(6):e100860. doi:https://doi.org/10.1371/journal.pone.0100860
94. Bradt V, Malafa S, von Braun A, et al. Pre-existing yellow fever immunity impairs and modulates the antibody response to tick-borne encephalitis vaccination. *NPJ Vaccines*. 2019;4:38. doi:10.1038/s41541-019-0133-5
95. Rey FA, Stiasny K, Vaney MC, Dellarole M, Heinz FX. The bright and the dark side of human antibody responses to flaviviruses: lessons for vaccine design. *EMBO Rep*. Feb 2018;19(2):206-224. doi:10.15252/embr.201745302
96. Stiasny K, Medits I, Roßbacher L, Heinz FX. Impact of structural dynamics on biological functions of flaviviruses. Review. *Febs j*. Mar 5 2022;doi:10.1111/febs.16419
97. Stiasny K, Kiermayr S, Holzmann H, Heinz FX. Cryptic properties of a cluster of dominant flavivirus cross-reactive antigenic sites. *J Virol.* Oct 2006;80(19):9557-68. doi:10.1128/jvi.00080-06
98. Baldovin T, Mel R, Bertoncello C, et al. Persistence of immunity to tick-borne encephalitis after vaccination and natural infection. *J Med Virol.* Aug 2012;84(8):1274-8. doi:10.1002/jmv.23313
99. Remoli ME, Marchi A, Fortuna C, et al. Anti-tick-borne encephalitis (TBE) virus neutralizing antibodies dynamics in natural infections versus vaccination. *Pathog Dis*. Mar 2015;73(2):1-3. doi:10.1093/femspd/ftu002
100. Kriz B, Hubalek Z, Marek M, Daniel M, Strakova P, Betasova L. Results of the Screening of Tick-Borne Encephalitis Virus Antibodies in Human Sera from Eight Districts Collected Two Decades Apart. *Vector Borne Zoonotic Dis*. Aug 2015;15 (8):489-93. doi:10.1089/vbz.2014.1747

Chapter 8

TBE in children

Malin Veje, Zane Freimane, Dace Zavadska

Key points

- TBE follows a similar clinical course in children and adults, manifesting mainly as meningitis. However, a broader clinical picture is seen in children, especially in preschool age.
- Laboratory evaluation may show elevated blood inflammatory indices, but cerebrospinal fluid analysis and anti-TBEV serology are still crucial for establishing the TBE diagnosis.
- The case fatality in pediatric TBE is overall very low. However, severe cases also occur in the pediatric population.
- Long-term somatic sequelae occur also after childhood TBE. Yet, long-term symptoms and neurodevelopmental/cognitive deficits are seen in 10–40% of infected children.
- Protective immunity can be effectively elicited in children by TBE vaccines as of 1 year of age.

Children, ticks, and TBE

Compared to TBE in adults, data on TBE in children is relatively sparse. It used to be generally accepted that TBE in childhood was rare and followed a milder course compared to adults. However, during the past two decades, this notion has been challenged. Various European countries such as Sweden and Latvia have reported severe cases and neurological sequelae after TBE also in children.

In general, the clinical picture of children with TBE is similar to the one described in adults. In both children and adults, TBE manifests as a neurological illness, most commonly meningitis. However, children and adolescents as a group tend to have milder neurological symptoms, and the disease less frequently has severe and lethal consequences. Children have a better long-term prognosis, compared to adults.[1-3] The largest multicenter study performed in Europe, showed that meningitis is more common among children compared to adults.[4] A large retrospective study from Poland, comparing 68 pediatric to 601 adult TBE cases, concluded that the disease was milder in children.[5] In this cohort, 97% of the cases in children were classified as meningitis. A nationwide prospective study in Latvia identified 40 TBEV-infected children 1–15 years of age and 90% of children had symptoms of CNS inflammation and all were hospitalized. In this cohort, 83% of the cases in children were classified as meningitis and 17% as meningoencephalitis, 33% of them with a moderate clinical course.[6] Another recent large cohort study from Germany, including 66 pediatric and 515 adult cases, confirmed that children as a group have milder disease manifestations compared with adults.[7] However, the same study noted that 56% of the children had a moderate or severe disease.

Children with TBE initially present with non-specific symptoms such as headache, fever, malaise/fatigue and because of that, cases may be overlooked. This idea was substantiated by a prospective Swedish study on children seeking medical care for neurological complaints[8] and confirmed by a Swiss case series.[9] Initial clinical diagnosis of TBE in children can be challenging due to a lack of specific symptoms. TBE can disguise as other common infectious diseases. TBEV infection should therefore be considered in all children with or without tick bite history presenting with non-specific symptoms during tick activity season in endemic countries.

Although rare, cases in newborns and children a few months old have been published.[10-13] A case from Slovakia described TBEV transmitted via breastfeeding to an eight-month old infant.[14] As concluded in the recent review article by Parfut et al, the incidence of TBE in children seems to peak at around nine years of age and increases continually with age.[1,15-19] TBE in childhood naturally affects both boys and girls, but approximately twice as many cases are seen in boys. Boys also tend to have a more severe disease.[1-3,20,21]

Tick-bites have been recalled in 48-76% of childhood TBE cases.[2,8,16,17,19,22,23] A biphasic course is reported in around 70 (20-100) % of cases.[1,2,5,7,8,16,18,19,24,25] Cases presenting with only fever are rarely studied, but do exist.[19,26] In the majority of reports on pediatric TBE, fever is present in virtually all cases at diagnosis.[1,2,16,19] However, both retrospective data from a fairly large cohort[22] and prospective data from a study with broad inclusion criteria,[8] show that fever >38.5° C is not always observed in pediatric TBE. In addition to fever, headache and vomiting have been reported as central features of childhood TBE at rates of approximately 90–100% and 50–90%, respectively. Self-reported fatigue/malaise, behavioral changes, photophobia, muscle pain, etc. are commonly reported, but occur at varying frequencies.[1-3,7,8,16,17,19,22] Meningeal signs (nausea,

vomiting, and nuchal rigidity) are prevalent findings, noted in >80% of infected children,[1,2,7,16,19,23] but young children have a less-pronounced clinical presentation.[8]

The clinical picture of pediatric TBE usually manifests as meningitis in 63–79% of cases, meningoencephalitis in 21–38%, and meningoencephalomyelitis in 0–4%. A study from Latvia reported that a mild disease course was seen more often in children than adults: 67% in 1-15 years of age and 60.5% in adults. However, none of the children had severe disease compared to 9% of the adults6. A recent Lithuanian study showed that milder disease manifestations were more common in children aged 1-8 years than in those 9-17 years old.[1,4,16,17,23] Clinical findings in childhood TBE include tremor, ataxia, impaired general appearance, somnolence, lymphadenopathy, apatheia, hyperesthesia, speech disorders, sensation disorders, and confusion/cognitive dysfunction.[1,2,5,8,16,17,19,22,24] Though uncommon, some children present with seizures, hemiparesis, paresis of the limbs, or cranial nerve pareses.[1,5,7,22,27] The largest clinical studies on TBE in children report median hospital stays ranging between 5-18 days, similar numbers or slightly shorter than what is described in the adult population.[1-3,7,16,19,22,28,29]

TBE without signs of CNS inflammation are not mandatorily reported and included in official surveillance, therefore the non-CNS TBE form is not well recognized and investigated. In the literature this TBE clinical picture has been described as “fever form”, “non-CNS cases of TBE” or “Febrile illness” and is characterized by the presence of fever and constitutional symptoms, and the absence of clinical signs of CNS involvement at the time of illness. According to the published data, up to 50% of symptomatic TBEV infections manifest without CNS involvement.[30,31] However, a recent population-based study reported less frequent non-CNS TBE cases among children than in adults, 8.7% and 18.7%, respectively.[6] This may indicate higher TBE awareness in children, especially towards excluding neurological involvement of the disease.

Diagnosis

For TBE diagnosis, detection of TBEV-specific IgM and IgG is required to prove TBEV infection, see ECDC criteria.[32] Lumbar puncture should be performed to confirm CNS inflammation and shows an elevated leukocyte count with predominantly mononuclear cells[1-3,16,19,22,24]. Increased CSF protein/ albumin levels seem to be more common in adults than in children with TBE.[2,5,22] CRP and leukocyte counts are often elevated, but in analogy with the adult population, no laboratory tests can discriminate TBE from other viral infections.[1-3,5,16,19,22]

Electroencephalogram (EEG) results can help confirm the diagnosis, but are not specific for TBE. The EEG abnormalities seen include mild to moderate, generalized, slowing background activity, but also sharp waves in contrast, though seldom generalized spike wave activity.[1,3,11,16,18,24] Magnetic resonance imaging (MRI) has been used infrequently in children with TBE. Similar to findings in adults, the most commonly reported finding is alterations in the thalami.[3,24,27,33-35] MRI changes have also been detected in cerebellar structures, putamen, and caudate nucleus, as well as the cortex. Of note, some children present with a normal MRI.[24,27] In a review of the spectrum of MRI findings in childhood TBE, von Stülpnagel et al reported poor outcomes, i.e., long-term neurologic disabilities and death, in children with MRI changes.[27] However, these data were retrospective and there might be a selection bias towards more severe cases undergoing MRI. Nonetheless, it can be concluded that pronounced CNS damage in pediatric TBE exists.

Short and long-term consequences of childhood TBE

Although most cases have a favorable outcome, a large proportion of children with TBE still have symptoms at discharge,[14,17,23] which contrasts with children with some other CNS infections.[36] Engman et al. reported significantly more days of acute illness in childhood TBE compared to children with neuroborreliosis or other infections with CNS symptoms. Additionally, they found a prolonged period of convalescence and more days of sick leave in the TBE cases.[37] Reported rates of admission into intensive care units range from 0 % to the very high 22% of TBE cases in children.[1,7,16,19,21] Compared with adults, fatal cases of TBE are reported only infrequently.[4,21,22,38]

While the occurrence of long-term neurologic and neuro-psychological sequelae in adults after TBE infection now is well-established,[3,4] the literature is inconsistent when it comes to the risk for long-term residua of childhood TBE. There are also considerable methodology differences between studies, both regarding methods and time-point for evaluation.[15]

For many years, but also recently, some studies have concluded that pediatric TBE has a more favorable outcome.[7,16,17,19] However, defining the complications of TBE is important. Only determining the gross neurologic status and a superficial assessment of health and cognitive functioning, leads to the conclusion that childhood TBE is not a long-term problem for most patients. But emerging data support the premise that pediatric TBE carries a risk of incomplete recovery, especially in terms of well-being and cognitive functions.

One of the first studies addressing the issue of incomplete neurocognitive recovery was published in 2005 by Schmolck et al. Over a mean of 3.2 years (range 6 months–11 years) after acute TBE illness, 19 pediatric subjects were evaluated

in comparison with healthy controls. Children who had suffered from TBE displayed lower scores in a structured neurologic examination and had significantly impaired attention and psycho-motor speed. Additionally, only 1/14 children in the TBE group had a normal EEG during hospitalization, whereas the remaining children were found to display pathological symptoms (mainly background slowing) without clinical disease. At follow-up, 8/19 EEGs were normal.[24] Later, in a Swiss study, researchers concluded that permanent residua (i.e., severe mental and physical handicap) after pediatric TBE were rare (1 child out of 55, approximately 2%), but no specific assessment of cognitive functions was performed.[17]

By administering validated questionnaires, Fowler et al. showed that 4 out of 6 children had residual symptoms, not always obvious, several years after TBE was diagnosed.[39] The occurrence of residual symptoms was later confirmed by Engman et al. Pediatric TBE patients, recruited from a previous prospective study, followed up 1 year after their acute disease, reported significantly more fatigue, headache, and irritability than did children after neuroborreliosis or control subjects. Additionally, the children were screened for neuro-developmental problems (e.g., executive functions, memory, motor skills, behavior, etc.) using a validated questionnaire. Children in the TBE group had significantly more difficulties (5 out of 7), mainly with memory, executive function, and perception.[37]

In a larger study by Fowler et al., the findings of residual symptoms and neurodevelopmental/cognitive problems in childhood TBE were consolidated. Of note, the severity of the acute phase of disease did not influence the risk of long-term disease burden. More than three residual symptoms (e.g., headache, fatigue, memory problems, irritability, concentration problems, etc.) were seen in approximately 70% of the children at follow-up on average 4.2 years after the acute disease. Clinically significant problems with executive functioning were noted in approximately 40% of the children. Additionally, a significant decrease in working memory index, but not global IQ, was seen using the Wechsler Intelligence Scale for Children-IV.[18]

Prominent deficits in working memory capacity and increased task-related functional MRI signal in working memory-related cortical areas during working memory testing have been shown in pediatric patients after TBE. These functional MRI abnormalities suggest diffuse neuronal damage behind the development of neurodevelopmental/cognitive problems seen in childhood TBE.[40]

Krbková et al. also described cognitive problems (memory problems and lowered school grades) at follow-up in a large study; however, they found such deficits to a somewhat lower extent (11%).[19] Fatigue is a common residual symptom after TBE. A recent Swiss review on sleep-related symptoms concluded that 73,9% of children suffer from fatigue at long-term follow up (≥12 months) after TBE,[41] and sleep disorders have also been reported after TBE in adults.[42] Using phone interviews at 18 months post TBE, a recently published German cohort study including 59 children concluded a more favorable outcome for children compared to adults. The most common remaining self-reported symptom in the whole cohort consisting of both children and adults at 18 months´ follow-up was fatigue.[43]

Long-term sequelae of a more somatic nature are less frequently reported in childhood TBE. However, such cases occur. Fritsch et al. reported severe neurologic residua (hemiparesis and epilepsia) at a rate of 1.7% in their large pediatric cohort[1]. Others have also reported on neurologic sequelae, mainly hemiparesis, in children with TBE.[11,19,21,33] However, the frequency of paralysis and paresis in pediatric TBE is only reported up to approximately 2%, which is lower than the rate seen in adults.[2-4,16,17,21,33] While rare, such neurologic residua constitute a significant handicap in those affected, disrupting quality of life for many years. That TBE in childhood can be associated with altered cerebral electrophysiologic processes, i.e., pathologic EEGs and development of epilepsia,[1,11,19,24,33] is further substantiated by a report by Mukhin et al. Rather treatment-resistant epilepsia partialis continua was seen in 10 Russian children (predominantly boys) days to years after TBE. This cohort also suffered from oculomotor dysfunction, varying degree of paresis, dysarthria, cerebellar signs, and cognitive dysfunction.[44]

To conclude, pediatric TBE carries a high risk for subjective sequelae, which to some extent can be objectively assessed by using structured questionnaires and interviews.[18,21,36] The early findings by Schmolck et al[24] that TBE in childhood can be associated with neurodevelopmental/cognitive difficulties have now been verified.[18,19,37] As summarized in a review by R. Steffen; Although larger studies may be required to determine the incidence of these sequelae, the individual child's long-term disease burden cannot be neglected.[45] In contrast to somatic residua and epilepsy, which of course are rare but more easily diagnosed, neurodevelopmental/cognitive problems may elude diagnosis due to young children's difficulties in verbalizing their problems and for their parents to recognize them. Hence, an opportunity exists to advocate for structured follow-up of children diagnosed with TBE so that early actions can be taken.

TBE immunity and vaccination in children

Children, from the age of 1 year, as well as adults, can elicit highly effective protective immunity to TBEV (i.e., response to the viral E protein) by immunization with the two TBE vaccines available in the EU46. These vaccines are based on the European TBEV strains Neudörfl (FSME-IMMUN® Junior)

and K23 (Encepur® Children).[47] The field effectiveness in children less than 15 years of age is reported to be 97% after immunization with either of the two vaccines; however, it should be noted that the vaccine based on the Neudörfl strain had a higher market share at the time of the study (>96%).[48] TBE vaccination effectiveness has also been demonstrated by the nearly complete disappearance of TBE in a highly endemic area with implementation of a general vaccination program.[49]

Vaccination breakthroughs, although rare, occur in children. In the multicenter study by Kohlmaier et al, 16 of the 546 patients where data could be obtained were previously vaccinated, and 9 of these 16 patients where younger than 20 years.[4] Among the many publications on immunization in children, it is important to note that the vaccines marketed within the EU have been shown to be safe and effective in eliciting antibody titers, that the booster interval can be expanded, and that rapid immunization schedules have worked well.[50] Previous recommendations stated that the primary TBE vaccination (i.e., the first 3 doses) preferably should be accomplished with the same vaccine because of differences in each vaccine's immunologic properties.[50-52] However, more recent data suggest that the vaccines may be interchangeable and even point out advantages with administration of vaccine shots from the two different brands.[53,54]

Natural immunity to TBE seems to persist over time and as children age, according to Baldovin et al., but with the reservation that their cohort was small.[55] Truly long-term data on natural immunity (for example, follow-up of now-older adults after TBE in childhood years) have not yet been reported.

Contact: malin.veje@gu.se

Citation:
Veje M, Freimane Z, Zavadska D. TBE in children. Chapter 8. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi: 10.33442/26613980_8-7

References

1. Fritsch P, Gruber-Sedlmayr U, Pansi H, et al. Tick-borne encephalitis in Styrian children from 1981 to 2005: a retrospective study and a review of the literature. *Acta Paediatr*. 2008;97(5):535-8. doi:10.1111/j.1651-2227.2008.00763.x
2. Logar M, Arnez M, Kolbl J, Avsic-Zupanc T, Strle F. Comparison of the epidemiological and clinical features of tick-borne encephalitis in children and adults. *Infection*. 2000;28(2):74-7. doi:10.1007/s150100050050
3. Kaiser R. The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994-98: a prospective study of 656 patients. Brain. 1999;122(11):2067-78.
4. Kohlmaier B, Schweintzger NA, Sagmeister MG, et al. Clinical Characteristics of Patients with Tick-Borne Encephalitis (TBE): A European Multicentre Study from 2010 to 2017. *Microorganisms.* Jun 30 2021;9(7)doi:10.3390/microorganisms9071420
5. Krawczuk K, Czupryna P, Pancewicz S, Ołdak E, Moniuszko-Malinowska A. Comparison of tick-borne encephalitis between children and adults-analysis of 669 patients. *J Neurovirol*. Aug 2020;26(4):565-571. doi:10.1007/s13365-020-00856-x
6. Zavadska D, Freimane Z, Karelis G, et al. Effectiveness of Tick-borne Encephalitis Vaccines in Children, Latvia, 2018–2020. *Pediatr Infect Dis J*. 2023;42(10):927-931. doi:10.1097/inf.0000000000004034
7. Nygren TM, Pilic A, Böhmer MM, et al. Tick-borne encephalitis: Acute clinical manifestations and severity in 581 cases from Germany, 2018–2020. *J Infection*. 2023;86(4):369-375. doi:10.1016/j.jinf.2023.02.018
8. Sundin M, Hansson ME, Engman ML, et al. Pediatric tick-borne infections of the central nervous system in an endemic region of Sweden: a prospective evaluation of clinical manifestations. *Eur J Pediatr*. 2012;171(2):347-52. doi:10.1007/s00431-011-1542-2
9. Meyer PM, Zimmermann H, Goetschel P. Tick-borne encephalitis presenting as fever without localising signs—a case series. *Eur J Pediatr.* 2010/06/01 2010;169(6):767-769. doi:10.1007/s00431-009-1097-7
10. Grubbauer HM, Dornbusch HJ, Spork D, Zobel G, Trop M, Zenz W. Tick-borne encephalitis in a 3-month-old child. *Eur J Pediatr*. 1992;151(10):743-4. doi:10.1007/bf01959081
11. Jones N, Sperl W, Koch J, Holzmann H, Radauer W. Tick-borne encephalitis in a 17-day-old newborn resulting in severe neurologic impairment. *Pediatr Infect Dis J*. 2007;26(2):185-6. doi:10.1097/01.inf.0000253056.34894.5f
12. Kosina P, Plisek S, Krausova J, Kracmarova R. Tick-borne encephalitis virus – a rare cause of encephalitis in infants. *Wien Klin Wochenschr*. 2008;120(21):710-711. doi:10.1007/s00508-008-1069-3
13. Iff T, Meier R, Olah E, Schneider JFL, Tibussek D, Berger C. Tick-borne meningo-encephalitis in a 6-week-old infant. *Eur J Pediatr*. 2005;164(12):787-788. doi:10.1007/s00431-005-1753-5
14. Kerlik J, Avdičová M, Musilová M, Bérešová J, Mezencev R. Breast Milk as Route of Tick-Borne Encephalitis Virus Transmission from Mother to Infant. *Emerg Infect Dis.* 2022;28(5):1060-1061. doi:10.3201/eid2805.212457
15. Parfut A, Laugel E, Baer S, et al. Tick-borne encephalitis in pediatrics: An often overlooked diagnosis. *Infect Dis Now*. 2023;53(2):104645. doi:10.1016/j.idnow.2023.01.005
16. Lesnicar G, Poljak M, Seme K, Lesnicar J. Pediatric tick-borne encephalitis in 371 cases from an endemic region in Slovenia, 1959 to 2000. *Pediatr Infect Dis J*. 2003;22(7):612-7. doi:10.1097/01.inf.0000073202.39700.a0
17. Stähelin-Massik J, Zimmermann H, Gnehm HE. Tick-Borne Encephalitis in Swiss Children 2000–2004: Five-Year Nationwide Surveillance of Epidemiologic Characteristics and Clinical Course. *Pediatr Infect Dis J.* 2008;27(6):555-557. doi:10.1097/INF.0b013e318165c195

18. Fowler Å, Forsman L, Eriksson M, Wickström R. Tick-borne encephalitis carries a high risk of incomplete recovery in children. *J Pediatr.* 2013;163(2):555-60. doi:10.1016/j.jpeds.2013.01.037
19. Krbková L, Štroblová H, Bednářová J. Clinical course and sequelae for tick-borne encephalitis among children in South Moravia (Czech Republic). *Eur J Pediatr*. 2015;174(4):449-58. doi:10.1007/s00431-014-2401-8
20. Zöldi V, Juhász A, Nagy C, Papp Z, Egyed L. Tick-borne encephalitis and Lyme disease in Hungary: the epidemiological situation between 1998 and 2008. *Vector Borne Zoonotic Dis*. 2013;13(4):256-65. doi:10.1089/vbz.2011.0905
21. Cizman M, Rakar R, Zakotnik B, Pokorn M, Arnez M. Severe forms of tick-borne encephalitis in children. *Wien Klin Wochenschr*. 1999;111(12):484-7.
22. Hansson ME, Orvell C, Engman ML, et al. Tick-borne encephalitis in childhood: rare or missed? *Pediatr Infect Dis J*. 2011;30(4):355-7. doi:10.1097/INF.0b013e3181fe3b5a
23. Bogdanavičienė K, Gudavičiūtė G, Šeškutė M. A Retrospective Analysis of Tick-borne Encephalitis in Children Treated in Kaunas Hospital During 2012 to 2019. *Pediatr Infect Dis J*. 2022;41(9):702-705. doi:10.1097/INF.0000000000003595
24. Schmolck H, Maritz E, Kletzin I, Korinthenberg R. Neurologic, Neuropsychologic, and Electroencephalographic Findings After European Tick-borne Encephalitis in Children. *J Child Neurol*. 2005;20(6):500-508. doi:10.1177/088307380502000606
25. Kaiser R. Frühsommermeningoenzephalitis im Kindes- und Jugendalter. Monatsschrift Kinderheilkunde. 2006;154(11):1111-1116. doi:10.1007/s00112-005-1184-4
26. Meyer PM, Zimmermann H, Goetschel P. Tick-borne encephalitis presenting as fever without localising signs–a case series. *Eur J Pediatr*.2010;169(6):767-9. doi:10.1007/s00431-009-1097-7
27. von Stülpnagel C, Winkler P, Koch J, et al. MRI-imaging and clinical findings of eleven children with tick-borne encephalitis and review of the literature. *Eur J Paediatr Neurol*. Jan 2016;20(1):45-52. doi:10.1016/j.ejpn.2015.10.008
28. Bogovič P, Stupica D, Rojko T, et al. The long-term outcome of tick-borne encephalitis in Central Europe. *Ticks Tick Borne Dis*. 2018;9(2):369-378. doi:10.1016/j.ttbdis.2017.12.001
29. Günther G, Haglund M, Lindquist L, Forsgren M, Sköldenberg B. Tick-bone encephalitis in Sweden in relation to aseptic meningo-encephalitis of other etiology: a prospective study of clinical course and outcome. *J Neurol*. 1997;244(4):230-238. doi:10.1007/s004150050077
30. Bogovič P, Kastrin A, Lotrič-Furlan S, et al. Clinical and Laboratory Characteristics and Outcome of Illness Caused by Tick-Borne Encephalitis Virus without Central Nervous System Involvement. *Emerg Infect Dis*. 2022;28(2):291-301. doi:10.3201/eid2802.211661
31. Dumpis U, Crook D, Oksi J. Tick-Borne Encephalitis. *Clinical Infectious Diseases*. 1999;28(4):882-890. doi:10.1086/515195
32. ECDC Meeting Report 2011. Second expert consultation on tick-borne diseases with emphasis on Lyme borreliosis and tick-borne encephalitis. 2012. Accessed 28 March, 2024. https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/Tick-borne-diseases-meeting-report.pdf.
33. Kluger G, Schöttler A, Waldvogel K, et al. Tickborne encephalitis despite specific immunoglobulin prophylaxis. *Lancet*. 1995;346(8988):1502. doi:10.1016/s0140-6736(95)92527-9
34. Zawadzki R, Garkowski A, Kubas B, et al. Evaluation of Imaging Methods in Tick-Borne Encephalitis. *Pol J Radiol*. 2017;82:742-747. doi:10.12659/pjr.903940
35. Palyga-Bysiecka I, Kręcisz B, Szczepańska B. Clinical course and neurological sequels after tick-borne encephalitis in children – case report. journal article. *Ann Agric Environ Med*. 2022;29(1):162-167. doi:10.26444/aaem/133206
36. Fowler A, Stödberg T, Eriksson M, Wickström R. Childhood encephalitis in Sweden: etiology, clinical presentation and outcome. *Eur J Paediatr Neurol*. 2008;12(6):484-90. doi:10.1016/j.ejpn.2007.12.009
37. Engman ML, Lindström K, Sallamba M, et al. One-year follow-up of tick-borne central nervous system infections in childhood. *Pediatr Infect Dis J*. 2012;31(6):570-4. doi:10.1097/INF.0b013e31824f23c0
38. Varnaitė R, Gredmark-Russ S, Klingström J. Deaths from Tick-Borne Encephalitis, Sweden. *Emerg Infect Dis.* 2022;28(7):1471-1474. doi:10.3201/eid2807.220010
39. Fowler Å, Stödberg T, Eriksson M, Wickström R. Long-term Outcomes of Acute Encephalitis in Childhood. *Pediatrics*. 2010;126(4):e828. doi:10.1542/peds.2009-3188
40. Henrik U, Åsa F, Ronny W. Increased working memory related fMRI signal in children following Tick Borne Encephalitis. *Eur J Paediatr Neurol*. 2016;20(1):125-30. doi:10.1016/j.ejpn.2015.09.004
41. Chiffi G, Grandgirard D, Sendi P, Dietmann A, Bassetti CLA, Leib SL. Sleep-Wake and Circadian Disorders after Tick-Borne Encephalitis. *Microorganisms*. 2022;10(2)doi:10.3390/microorganisms10020304
42. Veje M, Studahl M, Thunström E, et al. Sleep architecture, obstructive sleep apnea and functional outcomes in adults with a history of Tick-borne encephalitis. *PloS one*. 2021;16(2):e0246767. doi:10.1371/journal.pone.0246767
43. Nygren TM, Pilic A, Böhmer MM, Wagner-Wiening C, Wichmann O, Hellenbrand W. Recovery and sequelae in 523 adults and children with tick-borne encephalitis in Germany. *Infection*. 2023;51(5):1503-1511. doi:10.1007/s15010-023-02023-w
44. Mukhin KY, Mameniškienė R, Mironov MB, et al. Epilepsia partialis continua in tick-borne Russian spring-summer encephalitis. *Acta Neurol Scand*. 2012;125(5):345-52. doi:10.1111/j.1600-0404.2011.01575.x
45. Steffen R. Tick-borne encephalitis (TBE) in children in Europe: Epidemiology, clinical outcome and comparison of vaccination recommendations. *Ticks Tick Borne Dis*. 2019;10(1):100-110. doi:10.1016/j.ttbdis.2018.08.003
46. Angulo FJ, Zhang P, Halsby K, et al. A systematic literature

review of the effectiveness of tick-borne encephalitis vaccines in Europe. *Vaccine*. 2023;41(47):6914-6921. doi:10.1016/j.vaccine.2023.10.014
47. Heinz FX, Holzmann H, Essl A, Kundi M. Field effectiveness of vaccination against tick-borne encephalitis. *Vaccine*. 2007;25 (43):7559-7567. doi:10.1016/j.vaccine.2007.08.024
48. Pöllabauer EM, Pavlova BG, Löw-Baselli A, et al. Comparison of immunogenicity and safety between two paediatric TBE vaccines. *Vaccine*. Jun 23 2010;28(29):4680-5. doi:10.1016/j.vaccine.2010.04.047
49. Zenz W, Pansi H, Zoehrer B, et al. Tick-borne encephalitis in children in Styria and Slovenia between 1980 and 2003. *Pediatr Infect Dis J*. Oct 2005;24(10):892-6. doi:10.1097/01.inf.0000180506.76201.43
50. Wittermann C, Petri E, Zent O. Long-term persistence of tick-borne encephalitis antibodies in children 5 years after first booster vaccination with Encepur Children. *Vaccine*. 2009;27 (10):1585-8. doi:10.1016/j.vaccine.2008.12.057
51. Prymula R, Pöllabauer EM, Pavlova BG, et al. Antibody persistence after two vaccinations with either FSME-IMMUN® Junior or ENCEPUR® Children followed by third vaccination with FSME-IMMUN® Junior. *Hum Vaccin Immunother*. 2012;8(6):736-42. doi:10.4161/hv.20058
52. Wittermann C, Schöndorf I, Gniel D. Antibody response following administration of two paediatric tick-borne encephalitis vaccines using two different vaccination schedules. *Vaccine*. 2009;27(10):1661-6. doi:10.1016/j.vaccine.2008.10.003
53. Bestehorn-Willmann M, Girl P, Greiner F, Mackenstedt U, Dobler G, Lang D. Increased Vaccination Diversity Leads to Higher and Less-Variable Neutralization of TBE Viruses of the European Subtype. *Vaccines*. 2023;11(6):1044. doi:10.3390/vaccines11061044
54. Vaccines against tick-borne encephalitis: WHO position paper – 2011. Accessed 28 March, 2024. https://www.who.int/teams/immunization-vaccines-and-biologicals/policies/position-papers/tick-borne-encephalitis
55. Baldovin T, Mel R, Bertoncello C, et al. Persistence of immunity to tick-borne encephalitis after vaccination and natural infection. *J Med Virol*. 2012;84(8):1274-1278. doi:10.1002/jmv.23313

Chapter 9

Tick-borne encephalitis in adults

Johann Sellner, Petra Bogovic, Joanna Zajkowska

Key points:

- Tick-borne encephalitis (TBE) is a viral infectious disease in humans that involves the nervous system.
- Frequently, there is a febrile illness phase 1-21 days before the onset of neurological and neuropsychiatric symptoms.
- The most common neurological manifestations include meningitis, encephalitis, myelitis, radiculitis, or a combination thereof.
- Long-term sequelae are present in almost every second person with nervous system involvement in this vaccine-preventable disease.

Introduction

Tick-borne encephalitis (TBE) encompasses various disorders caused by infection with the TBE virus (TBEV). TBEV is a positive-strand RNA virus in the genus Flaviviridae, which is primarily transmitted by infected ticks (primarily genus Ixodes) and occasionally by consuming unpasteurized dairy products from infected ruminants.[1] Among the several viral subtypes of TBEV, the European subtype (TBEV-Eur) is predominantly found in Europe. Siberian (**TBEV**-Sib) and Far Eastern (**TBEV**-FE) are additional prominent subtypes.

An overall increase in TBE cases in the European Union (EU)/European Economic Area (EEA) was observed between 2012 and 2020, according to the European Centre for Disease Control (ECDC).[2] In 2021, there was a slight decrease of cases compared to 2020. The drivers of the rising incidence remain unclear.[3] For 2021, 22 EU/EEA countries reported 2.949 confirmed cases, with Czechia (n=589), Sweden (n=533), and Germany (n=417) as the front runners. The notification rate was highest in Lithuania (13.1 cases per 100,000 population), followed by Latvia (11.7) and Estonia (6.2). Among the confirmed cases in which information for vaccination was available, 93.2% were not vaccinated against TBE. There is a seasonal pattern for occurrence. In 2021, 90% of confirmed cases occurred between June and November in the EU/EEA, with July being the month with the highest number of reported cases.[2]

The clinical manifestation of TBE depends on the virulence of the pathogen and the immune status of the host. The majority of the infected people remain asymptomatic or suffer from a self-limiting febrile illness. Some patients develop neurological and neuropsychiatric disturbances caused by meningitis, encephalitis, myelitis, radiculitis, or combinations thereof.[4] Cases of nervous system manifestation are more frequently reported among men (male-to-female ratio 1.5:1) and in the age group 45–64 years.[2] While the mortality of acute infection with TBEV-Eu is in the range of 0.5-2%, involvement of the nervous system is associated with long-term sequelae in almost every second survivor.[5] Clinical course and long-term outcome vary by TBE virus subtype, although some of the reported differences could be related to access to medical care or testing or methodologic biases.[6] Preventive strategies include vaccination and avoiding tick bites; no antiviral medication has been approved.

Risk factors

Ecological variables

TBE virus transmission is affected by place, time, and tick population density. However, infection rates in TBE virus–endemic areas are inconsistent, which impedes risk assessments.[6] People with outdoor occupations, e.g., farmers, forestry workers, and training in forested areas, are at increased risk for contracting TBE. The risk for TBE virus infection for an individual traveler is greatly affected by their itinerary and activities. Among the ECDC cases of 2021, only 1.6% were associated with travel.[2] Most infections result from tick bites acquired in forested areas while bicycling, birdwatching, camping, fishing, hiking, or collecting berries, flowers, or mushrooms.[6] In contrast, the risk is negligible for people who remain in urban or unforested areas and do not consume unpasteurized dairy products.

Epidemiological data from different European countries demonstrate that the incidence of TBE is higher in older adults than in younger age groups. More than half of the patients are ≥50 years of age.[7-9] Both a decline in adaptive and innate immunity and changed lifestyle habits may contribute to this observation.[10] This age distribution is also present among TBE cases in vaccinated people.[11]

Risk factors for severe or protracted course

The most endangered groups for severe clinical manifestation are older adults.[12-15] Immunosuppression is another risk factor for unfavorable outcomes. The case fatality rate for TBE is higher in these patient groups.[16] A recently published cluster of TBE in organ transplant recipients underscores the association between host immune suppression and fatal outcomes.[17] Whether vaccination breakthrough TBE is associated with more severe disease is a matter of investigation.[18] A recent study reported that a protracted disease course was associated with a low serum TBEV-specific IgG antibody response at the time of onset of the neurologic phase of the disease.[19] Another factor that may result in a more severe clinical picture of TBE is the relatively rare occurrence of co-infection with other tick-borne pathogens like *Borrelia burgdorferi*, *Anaplasma phagocytopilum*, *Rickettsia* spp. or *Listeria monocytogenes*.[20,21]

Host genetic risk factors

Clinical and epidemiological data indicate that human susceptibility to clinical TBEV infection greatly varies according to age and gender. Mouse models of TBE corroborate that genetic control influences the clinical course of TBE. In this regard, a robust neutralizing antibody response might be crucial for preventing host fatality. In addition, high expression of various cytokines/chemokines during TBE can mediate immunopathology and be associated with a more severe course of infection and increased fatality.[22] Genetic polymorphisms and immune signatures that may predispose to TBEV infection and its severity are covered in the following sections.

The CCR5 plays a crucial role in leukocyte migration and attraction. In human immunodeficiency virus (HIV) infections, the CCR5Δ32 mutation is crucial for invading CD4 cells by HIV particles with a CCR5 tropism.[23] In mouse models for flaviviral infections, homozygote CCR5-deficient (-/-) mice died in almost 100% of all infections with West Nile virus (WNV), whereas CCR5 (-/+) heterozygote mice, and homozygote mice with a wildtype CCR5 receptor, had a significantly lower mortality rate.[22] These observations from animal studies could be corroborated during a WNV outbreak by identifying the CCR5Δ32 mutation as a strong predictor for a severe clinical disease course in humans. Following the epidemiological results from WNV research, a potential effect of the CCR5Δ32 mutation on TBE was investigated. A clinical study from Lithuania analyzed the incidence of the CCR5Δ32 mutation in different patient populations and found individuals homozygous for CCR5D32 only among patients with TBE.[24] Moreover, the CCR5D32 allele prevalence also increased with the clinical severity of the disease. In another study by this author group, the prevalence of CCR5Δ32 homozygotes was higher in children (2.5%), in adults with severe TBE (1.9%), and in the combined cohort of TBE patients (2.3%) than in controls (0%).[25] In a Polish study, the blood expression of CCR5 neither differed between the groups nor did it change in the course of TBE.[26] The cerebrospinal fluid (CSF) concentration of the CCR ligand CCL5 was increased in TBE, the highest in the most severe presentation and correlated with pleocytosis. In another Polish study, there were 17.6% CCR5Δ32 heterozygotes and 1.5% homozygotes in the TBE cohort, with no statistically significant difference compared to the controls.[27]

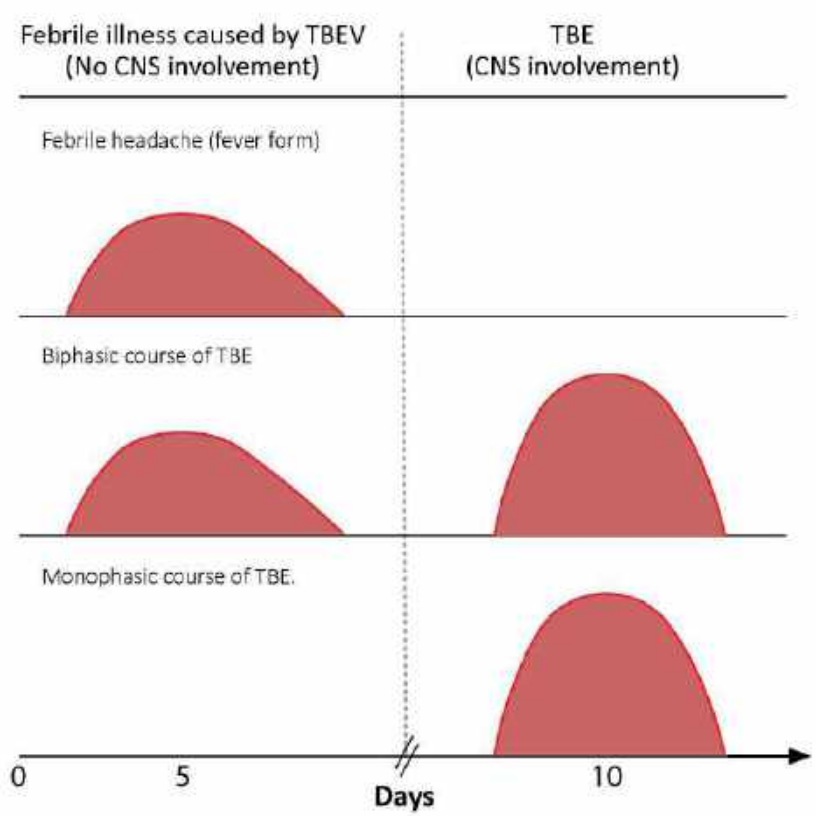
2'-5'-oligoadenylate synthetases (OAS) are a family of interferon-induced enzymes that play an essential role in mammal antiviral defense. Several polymorphisms in the OAS genes correlated with susceptibility and severe forms of Russian TBE.[28,29] The authors of these studies also analyzed OAS polymorphisms in different ethnic populations of the Russian Federation.[30] The studies revealed that the frequency of these SNPs correlated with the probability of disease after exposure to TBEV. Very low SNP frequencies were detected in Altaians, Khakasses, Tuvinians, and Shorians, groups with a high exposure risk for TBEV in their native habitats. These findings implicate that TBE risk SNPs may have served as selection factors.

A Czech study evaluated whether innate immunity genes predispose to TBE in humans.[31] The analysis showed an association of IFIT1 rs304478 SNP and DDX58 rs3739674 and rs17217280 SNPs and TBE in the Czech population.

The IL-28B polymorphism (rs12979860) is associated with an improved sustained virological response upon treatment with antivirals against Hepatitis C virus (HCV).[32] Given the close genetic relationship of flaviviral pathogens like HCV and TBEV, the role of the IL-28B and IL-10 polymorphism was investigated in TBEV infections.[33] In a study from the Novosibirsk region of Russia, the IL-28B polymorphism (rs8103142, rs12980275) and the IL-10 polymorphism (rs1800872) were associated with higher risk for severe TBE.

Dendritic cell (DC)-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) is a C-type lectin, expressed by DCs and a subpopulation of macrophages, involved in the detection of pathogen-associated molecular patterns (PAMPs), cell migration, and interaction with T lymphocytes, potentially contributing to an early response to TBEV at the site of tick feeding and initiation of a specific immune response.[34] Findings in the context of dengue virus and HCV infections pointed to an increased risk of dengue hemorrhagic fever and advanced hepatic injury in hepatitis C when there is an underlying SNP (rs4804803) located in the promoter region of the CD209 gene.[30] DCs in the skin and gut may play an important role as antigen-presenting cells and virus spread early in TBEV infection.[35] A study from Russia of presumably TBEV-Sib cases showed a correlation between the presence of 2 SNPs (rs4804803, rs2287886) in

Figure 1: Timelines of clinical manifestations of illness caused by TBEV



the promotor region of the CD209 gene and the severity of the TBE disease course.[30]

MMP-9 directly degrades extracellular matrix proteins and activates cytokines and chemokines to regulate tissue remodeling. In a study of Russian TBE cases, the frequency of the rs17576 G allele of MMP-9 was significantly higher in TBE cases with severe CNS diseases.[36]

Taken together, several studies disclosed a potential role for various gene polymorphisms in the susceptibility and severity of TBE. These findings need to be corroborated in independent cohorts with appropriate controls, using uniform criteria for disease severity and characterization of the virus strain, as there are also trials that could not confirm these observations.[37]

Clinical course

Definitions of the clinical presentations and time frames

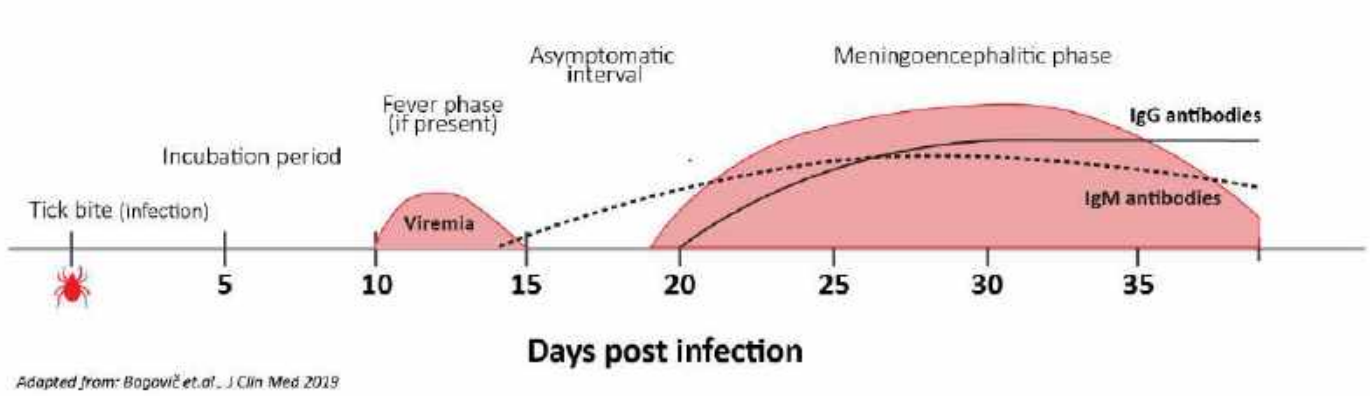
Infection with TBEV may be symptomatic or asymptomatic. A symptomatic infection may manifest as a febrile illness without nervous system involvement or as TBE (Figure 1).[38]

Asymptomatic infection with TBEV is defined as TBEV IgG antibody seroconversion in an asymptomatic person.

Febrile illness resulting from infection with TBEV is defined by the presence of fever and constitutional symptoms, the absence of signs/symptoms of CNS involvement at the time of actual illness, and the presence of TBEV RNA in serum and/or later seroconversion to TBEV. According to the later appearance (or absence) of neurologic involvement, the febrile illness is further sub-classified as either the **initial phase of TBE** (defined as a febrile illness that, after a clinical improvement, is followed by neurologic involvement occurring within at least a 1-month follow-up period and fulfilling criteria for TBE) or as **febrile illness resulting from infection with TBEV in a narrow sense** (abortive form of TBE, febrile headache, summer flu, fever form) when no signs/symptoms of CNS involvement are present at the time of actual illness or within at a least 1-month follow-up period.[38]

TBE is defined as the presence of clinical signs or symptoms of central or peripheral nervous system involvement (.e. meningitis, encephalitis, myelitis, radiculitis, or a combination), with increased CSF leukocyte counts (>5 × 106 cells/L), and demonstration of a recent infection with TBEV indicated by serum specific IgM and IgG antibodies or IgG seroconversion in paired serum samples.[13,39] This definition partly contradicts the ECDC case definition for TBE, which does not explicitly require CSF pleocytosis to diagnose TBE;[40] however ECDC definitions are intended for epidemiological monitoring and are not necessarily optimal

Figure 2: Scheme of clinical events and antibody evolution post TBEV infection



for clinical use. The approximate time course of TBE is shown in Figure 2.[41]

Pathogenesis - clinical highlights

After the bite of an infected tick, TBEV replication occurs locally in the subcutaneous tissue. DCs of the skin (Langerhans cells) play an essential role since they bind with antigens and subsequently induce an immune response by producing proinflammatory cytokines. Langerhans cells are the most relevant cell group for local viral replication, transporting the virus to the regional where further replication occurs. After release into the bloodstream from lymph nodes, TBEV disseminates to other organs, particularly the reticulo-endothelial system (mainly bone marrow, spleen, and liver), where the virus continues to multiply and maintain viremia for several days. Probably during the second viremic phase (which clinically matches with febrile illness without CNS involvement), the virus reaches the brain.[42,43] The precise mechanism of viral passage through the blood-brain barrier is unclear but depends on the presence of viremia. There are four candidate routes:

i) direct axonal retrograde transport from infected peripheral nerves;

ii) infection of highly susceptible olfactory neurons;

iii) virus entry into vascular endothelial cells of brain capillaries, transcytosis, and release of virus into the brain parenchyma; and

iv) diffusion of virus between capillary endothelial cells.

There is also a so-called "Trojan horse" mechanism, which assumes that the virus is transported by infected immune cells to the CNS.[42,44,45] The primary targets of TBEV infection in CNS are neurons. Rarely, oligodendrocytes are infected.[42]

The pathogenesis of asymptomatic infections in humans is poorly defined. It seems logical that, on the one hand, the virus enters the body similarly to symptomatic infections and, on the other hand, does not enter the CNS. Still, it is not clear whether the development of the disease is deterred or interrupted after multiplication in the lymph nodes before or following penetration into the blood.

The characteristics of the TBEV subtype, the quantity of virus copies, and the host immune response influence the pathogenesis. The immune response is necessary not only for controlling TBEV infection but is also thought essential for the resulting clinical manifestations, but knowledge of such responses is incomplete.[41,46] Immune responses during TBEV infection are described in a separate chapter.

Presentations of tick-borne virus infection

Asymptomatic infections

Seroepidemiological studies suggest that most TBEV infections (70%–98%) are asymptomatic; however, the exact proportion of such cases is unknown because partly those with mild clinical presentation may remain below the diagnostic threshold.[47-49]

Symptomatic infections

The time interval from a tick bite to the beginning of the illness is usually 7–14 days, but it may be as short as two days and as long as four weeks. With the alimentary route of infection, there is usually a shorter incubation period of 3 to 4 days; however, the reports are not unanimous.[50-55]

Febrile illness due to TBEV infection (abortive form of TBE, febrile headache, summer flu, fever form)

Information on febrile illness due to TBEV infection also called the abortive form of TBE, febrile headache, summer flu, or fever form, is limited. Clinically and serologically, the initial phase of TBE has been postulated to match the initial phase of TBE, except that subsequent CNS involvement does not occur. Because clinical symptoms and signs of the

illness are non-specific, and because, in parallel to the initial phase of TBE, serum antibodies to TBEV are not yet expected to have developed, the only option for diagnosis at the time of actual illness is demonstrating the presence of TBEV RNA in the blood. However, this approach is not routine and might have a low diagnostic yield owing to several other known or unknown causes of fever, even in a highly endemic region for TBE. Therefore, the possibility that a febrile illness results from TBEV infection is usually tested for and established only after signs or symptoms of CNS involvement appear, which does not happen in the case of the fever form. In that case (and if PCR detection of viral RNA in blood is unavailable), further clinical and microbiologic (serologic) follow-up after improvement is needed to establish the diagnosis.

Data on the frequency of this clinical manifestation of the disease caused by European TBEV subtype are conflicting. TBEV infection manifesting as febrile illness without later CNS involvement is considered frequent[55-57], although not in all reports.[52,58-60], but the scientific basis for such a conclusion is unclear. According to some reports, it represents more than half of all clinically manifested TBEV infections.[55,56] However, this is not confirmed by the results of prospective clinical trials on the etiology of acute febrile illness after a tick bite. In the study by Lotric-Furlan and co-workers, among 56 patients diagnosed with TBEV infection by the presence of TBEV RNA in blood by PCR during febrile illness that developed after a tick bite, in 55 (98.2%) CNS involvement with pleocytosis later appeared.[61,62] In contrast, only one (1.8%) had an isolated febrile illness without later CNS involvement. A more recent, similarly designed study from Slovenia revealed that illness progressed to TBE in 52/62 (84%) adult patients within 18 days after defervescence.[38] In the Russian literature, this clinical manifestation is named “fever form” and is reported to represent up to 50% of all clinical presentations of TBEV infections.[63]

The current view is that febrile illness caused by TBEV infection most frequently presents as a moderate fever, headache, fatigue, and other non-specific symptoms and clinically corresponds to the initial phase of the TBE. The fever usually resolves in a few days, and the disease does not have long-term consequences.[38,64,65] The outcome of symptomatic TBEV infection without CNS involvement is believed to be favorable; however, very little reliable information on the outcome has been published.[38]

Tick-borne encephalitis

In 56–87% of symptomatic patients infected with the European subtype of TBEV, CNS inflammation is preceded by a febrile illness, resulting in a biphasic course of the disease.[4,12,19,52,53,60,66-69] The initial illness (first phase of TBE), which corresponds to viremia, presents with fever, fatigue, malaise, headache, and muscle and joint pain that occurs without CNS inflammation. It usually lasts less than one week, followed by improvement lasting several days.[38,53,70] The hallmark of the second phase of TBE is CNS involvement: in approximately 50% of adult patients, it presents as meningitis, in about 40% as meningoencephalitis, and around 10% as meningoencephalomyelitis.[49] The frequency of different neurological presentations has been somewhat variable.[9,53,60,68,71]

Some patients with TBE have no (obvious) initial phase of the disease and present directly with central nervous system involvement. Data on the monophasic course of the disease are incomplete. Some studies showed that patients with monophasic presentation of TBE have a more severe clinical course of the disease than those with biphasic course.[12,52,53] In addition, some reports on patients with severe TBE who needed intensive care management show an unusually high proportion of those with monophasic course (15/31, 48.4% and 21/33, 63.6%, respectively).[72,73] A direct comparison of the clinical presentation and laboratory findings in 705 adult TBE patients, of whom 283 had monophasic and 422 had biphasic course, revealed that patients with the monophasic course were significantly older (Figure 3xy), more often vaccinated against TBE (7.4% vs. 0.9%), more often had comorbidities (52% vs. 37%), and were more often treated in the intensive care unit (12.4% vs. 5.2%). However, the long-term outcome 2–7 years after TBE was comparable[74]

Case fatality rate in TBE caused by the European subtype of TBEV is 0.5–2% and generally increases with age.[49,70]

TBE caused by Far-Eastern TBEV subtype has been characterized with more severe disease and a case fatality rate of up to 40%, while in TBE caused by Siberian virus subtype the reported case fatality rate is 2–3%, and cases of chronic and progressive forms have been described.[48,70,75,76]

The initial phase of tick-borne encephalitis

Information on the initial phase of TBE is limited. Characterization of 98 adult patients who had TBEV RNA in their blood but no CNS involvement at the time of evaluation revealed that incubation (time from tick bite to onset of the illness) was six days, median duration of illness was seven days, and that 37 (38%) patients were hospitalized for a median three days. The most frequent findings were malaise or fatigue (98%), fever (97%), headache (86%), and myalgia (54%), followed by arthralgia (43%), gastrointestinal symptoms (46%; abdominal pain 2%, nausea/vomiting 38%, loose stools 16%), respiratory symptoms (18%; sore throat 11%, cough 10%) and chills (19%). Typical laboratory findings were leukopenia (88%), thrombocytopenia (59%), and abnormal liver function test results (63%). At the time of positive PCR findings, 0/98

patients had serum IgG TBEV and seven serum IgM TBEV; all patients later seroconverted. Viral RNA load was higher in hospitalized patients with more severe illness than in those who did not need hospitalization but did not differ substantially according to age, sex, duration of illness before testing, or total duration of the actual febrile illness, or for patients with undetectable viral IgM in serum samples when compared with patients in whom antibodies were detectable. Illness progressed to TBE in 84% within 18 days after defervescence.[38] Clinical and laboratory findings in patients with TBEV febrile illness do not distinguish between patients in whom TBE later develops and those in whom it does not.

Clinical spectrum of neurological manifestations

Meningitis is characterized by fever, headache, nausea, vomiting, and meningeal signs. These symptoms and signs are present in most patients but not all. In a study encompassing 448 adult patients with TBE from Slovenia, almost all reported headaches and had fever, more than 50% suffered from nausea and/or vomiting, and 70% had clearly expressed meningeal signs.[68]

Encephalitis may manifest by a variety of neurological symptoms and signs, most often with tremor (especially of the fingers of the upper extremities and tongue), sometimes with nystagmus, speech disorder, ataxia, and movement disorders, occasionally with seizures, and rarely with brain stem symptoms and/or cranial nerve abnormalities. Impaired consciousness, ranging from mild to severe, concentration disturbances, and cognitive function disturbances are rather frequent; amnesia, behavioral changes, psychosis, and delirium may also occur.

Myelitis manifests with flaccid paralyses that are occasionally preceded by severe pain in the affected muscle groups. The involvement is usually asymmetrical. Most often, the extremities are affected, more frequently the upper than the lower limbs, and more often the proximal segments of the extremities than the distal ones. Patients with pareses of respiratory muscles usually require artificial ventilatory support.[13,39,52,53,60]

Radiculitis is a rare manifestation of TBE.[77] In patients with TBE who have radiculitis it is reasonable to look for concomitant Borrelia infection.

Other manifestations in the acute phase of tick-borne encephalitis

Involvement of cranial nerves. Involvement of cranial nerves is rare (usually in less than 5% of patients), mainly asymmetrical, often associated with severe acute illness, and usually has a favorable outcome. Ocular, facial, and pharyngeal muscles are most often affected, but hearing and vestibular defects are also encountered.[4,9,52,53,60] In a series of 1218 adult patients diagnosed with TBE at a single center, 11 (0.9%) developed peripheral facial palsy (two bilateral, nine unilateral); however, 3 out of 11 patients had associated borrelial infection. The latter finding suggests that in patients who develop peripheral facial palsy in the course of TBE, and who had been exposed to ticks in the region where both TBE and Lyme borreliosis are endemic, coexistent infection with Lyme borreliosis has to be taken into account.[78]

Autonomic nervous system disorders.[79,80] Occasionally, autonomic nervous system disorders occur in patients with TBE. These include cardiac and enteric nervous system disturbances.

Encephalitis with normal CSF cell count

There are a few on a serologically confirmed TBEV infection in TBE but without CSF pleocytosis.[81,82] This disagrees with the large series of serologically proven TBE patients in which CSF pleocytosis was found in all cases.[13,39,53] However, the latter findings might result from a selection bias because CSF pleocytosis was one of the essential inclusion criteria for the diagnosis of TBE.

Chronic progressive tick-borne encephalitis

There is no agreement on the existence of chronic TBE. Cases of a chronic progressive form of TBE were reported from Siberia and the Russian Far East, caused by the Siberian TBEV subtype. Both mutations in the TBEV NS1 gene and an inappropriate T-cell immune response are implicated in chronic progressive disease.70 According to information from Western Siberia, 1.7% of patients with acute TBE develop a chronic progressive form of the disease.[83] Clinical presentations include Kozshevnikov's epilepsy, lateral sclerosis, progressive neuritis, progressive muscle atrophy, and a Parkinson-like disease. A broad spectrum of incubation periods, time to the onset of individual neurological signs/symptoms, and survival after the onset of the disease have been reported.[84,85] Progressive TBE is probably not present or uncommon in diseases caused by European TBEV subtype. In the study carried out in Lithuania, where only European TBEV subtype has been recorded , the progressive course was noted in two out of 133 consecutive patients with acute TBE.[53,86]

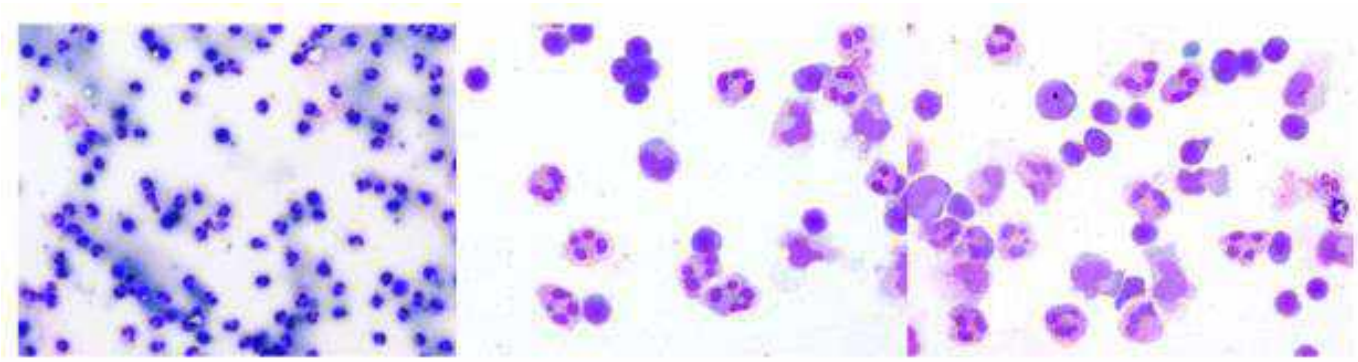
TBE in particular situations (in immunocompromised persons, during pregnancy, in persons vaccinated against the disease) is presented in another chapter (s).

Laboratory findings

CSF pleocytosis

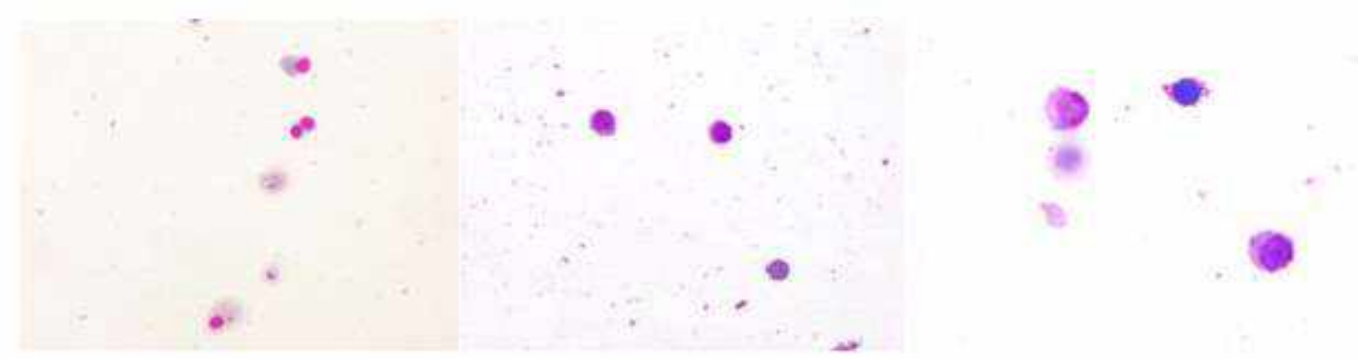
CSF pleocytosis is a dominant laboratory finding in patients with TBE. In 2 large studies, encompassing 731 and 717

Figure 3: Evaluating pleocytosis in TBE (early)



First evaluation of pleocytosis in TBE. The cell preparations cerebrospinal fluid of patients with TBE observed a plurality of cells. In all microscopic views there are cells that occur singly or in small clusters, neutrophils with different numbers of lobes nuclear and clearly visible large monocytes. (1 x 100; 1 x 400; 1 x 400.)

Figure 4: Evaluating pleocytosis in TBE (later)



During recovery, after acute phase, control LP. x 100, single lymphocytes, some monocytes, lack of granulocytes x 200 x 400.

adult patients with TBE, respectively, the median leukocyte values were 60 × 106/L and 86 × 106/L, with a maximal count of 1200 ×106/L.13,87 Some studies indicate that CSF leukocyte count is lower in persons with TBE who are older than 60 years than in younger adults.68 Lymphocytic predominance in CSF is typical for TBE; however, granulocytes may prevail during the first few days (Figures 3 and 4). Most patients have mild to moderately elevated protein and albumin concentrations in CSF and elevated albumin and IgG indexes, indicating disruption of blood-brain barrier.[13,68,70,88]

Peripheral blood

Laboratory abnormalities in the blood are more pronounced in the initial phase of TBE (and in the abortive form of the disease) than in the meningoencephalitic phase. In the first phase of TBE, the number of leucocytes in the peripheral blood is frequently reduced, while in the second phase, it is normal or slightly elevated. Furthermore, the initial phase is characterized by thrombocytopenia and elevated liver enzymes, while the second phase is not; moreover, inflammatory markers are usually within normal limits in the first phase of the disease but may be slightly elevated in some patients in the second phase.[38,39,52,70,89,90] The differences are best shown by comparing the results in patients assessed for laboratory abnormalities in the first and second phases of the disease. An example of such an approach is an analysis of 88 patients with biphasic course of TBE, in whom TBEV RNA in blood was established during the initial phase of illness and who later developed CNS inflammation and seroconversion. Comparison of laboratory findings in the initial and the second (meningoencephalitic) phase of TBE in this study revealed significant differences in peripheral blood leukocyte counts (including neutrophil, lymphocyte, and monocyte counts) and platelet counts, as well as serum concentrations of C-reactive protein, aspartate aminotransferase, and gamma-glutamyl transferase but not for alanine aminotransferase (Table 1).[89] A recent study exposed that in addition to previously known leukopenia, thrombocytopenia, and increased liver enzymes, the initial phase of TBE is relatively often associated also with elevated muscle enzyme activities: 33% of patients had elevated serum creatine kinase, 26% myoglobin and 22% troponin activity; at least one of the muscle enzymes was elevated in 42% of patients. Leukopenia, thrombocytopenia, elevated liver enzymes, and elevations of creatine kinase and myoglobin were

Table 1: Overview of TBE long-sequelae in prospective and retrospective studies

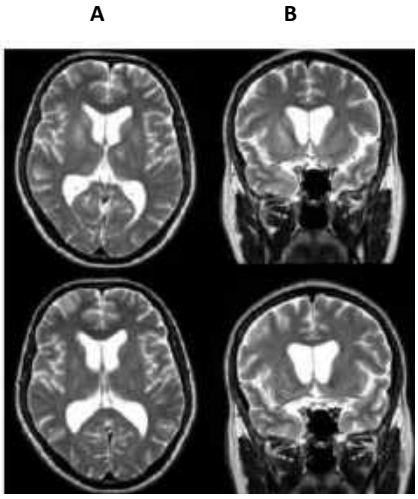
Study	Patients	Follow-up period	Findings
Kaiser R, 1997[71]*	63/70	11-44 months	Unable to work for up to 3 months: **32%** Persistent hearing loss: **11%** Severe dysphagia/dysarthria: **6%** Cognitive deficits: **11%** 1/9 patients with radiculitis and paresis and 15/15 with myelitis had residual paresis CFR: **6.3%**
Mišić-Majerus L, et al. 2009[74]	124	≥ 3 years	Postencephalitic syndrome (PES): **52%** Mild PES symptoms of short duration: **12%** Moderate or severe PES symptoms lasting 3-18 months: **40%** Permanent sequelae: **17%** Spinal nerve paresis: **4%** Hearing impairment: 6% Dysarthria: **2%** Severe mental disorder: **1%** CFR: **2.5%**
Günther G, et al., 1997[56]*	85	1 year	Persistent CNS dysfunction: **40%** Tetraparesis: 2 patients Bilateral paralysis of shoulder muscles: 3 patients
Kaiser R, 1999[43]*	230/656	up to 4 years	Transitory mild paretic complaints: **38%** Sequelae lasting 3 months or longer: **27%** (n=62) 9/62: mild sequelae, not affecting daily life 23/62: moderate sequelae, affecting daily life 30/62: severe sequelae, serious impact on daily life 47/53 with moderate or severe sequelae had paresis of extremities CFR: **1.2%**
Mickiene A, et al., 2002[8]*	117	1 year	Permanent sequelae: **46%**
Czupryna P, et al., 2011[72]	687	1993-2008	Neurological sequelae at discharge from the hospital: **23%** Required further psychiatric treatment: **44%** Long-term sequelae requiring further hospitalizations: **6%** CFR: **0.6%**
Kaiser R, 2011[73]	57	10 years	Only patients included in the study described in Kaiser 1999 and who had a myelitic course were included. Recovered: **19%** Moderate or severe sequelae: **51%** CFR: **30%**

present in the initial phase but resolved later, while mild troponin abnormalities were also found in the second phase of TBE.[91]

Neuroimaging

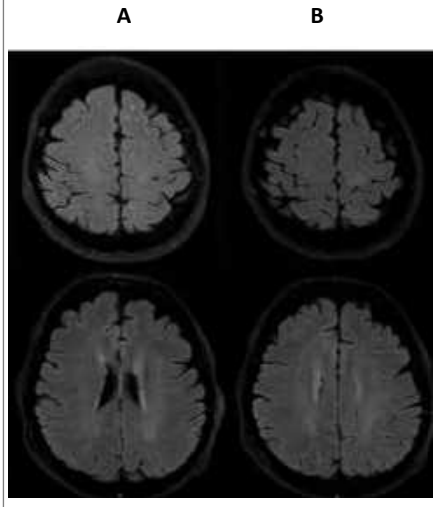
Neuroimaging enables rapid, non-invasive visualization of the central and peripheral nervous system. In clinical practice, neuroimaging is indispensable to corroborate clinical suspicion of nervous system inflammation, rule out mimics, provide hints for the causative pathogen, and assess for complications. Magnetic resonance imaging (MRI), with its excellent soft tissue contrast, is superior to computed tomography (CT). CT is used for exploratory examination of the brain on admission, in case of rapid clinical deterioration, and before lumbar puncture.

Figure 5: MRI visualization of TBE-related abnormalities



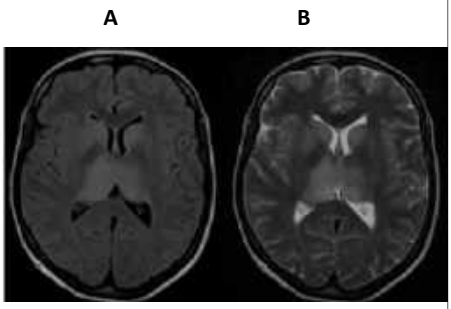
Axial (A) and coronal (B) T2-weighted MRI images show high signal intensity in the basal ganglia and thalami. The second scans (C, D) obtained several months later, show partial resolution of the lesions. Patient with chorea presentation.

Figure 6: Further visualization of TBE-related abnormalities



Axial FLAIR images. There is abnormal signal intensity in the left frontal (A) and left parietal lobe (B) and confluent, poorly visible abnormal bilateral hyperintensity in the periventricular white matter (C) and in the centrum semiovale (D). Parkinsonism as residual sequelae.

Figure 7: Additional visualization of TBE-related abnormalities



Axial fluid-attenuated inversion recovery (FLAIR) image (A) and T2-weighted MR image (B) show bilateral hyperintensity of the caudate nuclei, putamina and thalamus. The right side is slightly more involved than the left side. Patient with immunosuppression.

The nervous system manifestations of TBEV infection include meningitis, encephalitis, myelitis, and radiculitis.4 Most changes in neuroimaging of viral encephalitis are unspecific. They can be observed with several other pathogens and neurological disorders.[92] some radiological features are shared across infectious, immune-mediated, and non-inflammatory causes of nervous system disorders.[93] Moreover, radiological signs may be absent despite clinical signs and symptoms of meningeal, parenchymal, spinal cord, or peripheral nervous system dysfunction. Studies on the correlation of clinical severity with imaging findings are not available in TBE.

Meningitis

Clinical features of meningitis encompass the classic triad of fever, nuchal rigidity, and altered mental status. Meningitis primarily involves the leptomeninges, which consist of the inner arachnoid and the pial meningeal layers. Unenhanced CT can display mild dilatation of the ventricles with effaced subarachnoid spaces, suggesting diffuse cerebral swelling.[94] MRI is more sensitive for detecting radiological features of meningitis than CT.[95] T1-weighted MR imaging may show obliteration of the basilar cisterns. Fluid-attenuated inversion recovery (FLAIR) sequences may demonstrate hyperintensity in the subarachnoid space, even when T1-weighted images appear normal. Postcontrast T1-weighted images may show linear continuous sulcal or cisternal enhancement, with predilection at the basal meninges and cerebellar folia.[96] Enhanced and thickened cranial nerves may also be observed.[97]

Encephalitis

Encephalitis is defined as inflammation of the brain parenchyma associated with neurologic dysfunction. MRI is essential in diagnosing encephalitis, evaluating the disease course and complications, and prognosis.[98] Encephalitic lesions of TBE are present as areas of increased signal intensity on T2/FLAIR-imaging (Figures 5, 6 and 7), which may also enhance upon administration of contrast agents.[99,100] In TBE, the enhancement is mainly restricted to the lesion margins.[96]

The sensitivity of MRI to detect brain lesions despite clinical symptoms of encephalitis due to TBEV infection is low. In a Swiss study of patients with encephalitis or meningoencephalitis by TBE and MR imaging performed after a median of 10 days, 27% had lesions on FLAIR and 6% diffusion restrictions.100 Leptomeningeal enhancement was detected in 44% and brain hemorrhage in 5%. Even with repeated scans, the yield for detecting parenchymal damage in patients with an encephalitic syndrome was 46%, according to an Austrian study.[18] The time point of imaging

could play a significant role in this regard. Brain lesions were detected in two patients on day 21 from hospital admission in the latter study, whereas these were not present on the scans on days 5 and 8, respectively. Contrast enhancement is found only in the minority of patients.[18]

The predilection sites of brain lesions in TBE on FLAIR were the thalamus (50%) and the pontine area (29%) in the Swiss study.100 Thalamic lesions can be uni- or bilateral. Lesions were less frequent in the limbic regions (amygdala and hippocampus, each 21%), the mesencephalon, and the cerebellum (each 21%). In the Austrian study, the predilection sites were the periaqueductal grey (17%), the thalamus, and the brainstem (each 12%).[18] Among the patients in whom a brain lesion was detected, the median number of lesions was 2. In a pilot study of patients with an encephalitic TBE course, glucose hypometabolism was present in 7 out of 10 TBE patients at sites prone to lesion development.[101] Glucose hypometabolism reflects neuronal dysfunction and did not correlate with MRI brain lesions due to TBEV. In line, MR spectroscopy of TBE lesions during the acute phase of the disease shows changes indicative of necrosis. The presence of brain lesions on MRI and lesion expansion may determine prognosis.[18,100] The persistence of lesions over time has not been studied systematically so far. There is anecdotal evidence of a complete resolution of cerebral, brainstem, and spinal cord lesions within six months.[102] A Polish study of patients with encephalitic lesions during acute TBE studied structural brain changes 12 months later.[103] On follow-up, there was marked brain atrophy with a widening of the anterior horns and lateral ventricles, indicating grey and white matter loss.

Myelitis and radiculitis

Myelitis and radiculitis with TBEV infection can occur isolated or in combination. Spinal cord and nerve root MRI findings were studied only in smaller patient series and case reports. TBEV has a propensity for the anterior horn cells of the grey matter in the spinal cord.[96] These lesions are commonly longitudinally extensive, defined as an expansion over three or more vertebral segments, and can expand to the brainstem.[104] Both uni- and bilateral lesions of the grey matter have been reported and are associated with a Polio-like syndrome characterized by acute flaccid paresis.[105,106] There can be a swelling of the grey matter and lesional and leptomeningeal contrast enhancement.[104] Spinal cord lesions often enhance markedly.[96] Rarely, the posterior horns may also be involved.[96] In radiculitis, the roots of the spinal nerves may be thickened and display contrast enhancement.[77,107]

Electroencephalography (EEG)

For viral encephalitis, electroencephalography (EEG) is a valuable adjunct to clinical neurological examination. It can detect subtle or subclinical disturbances of cerebral function and enables the detection and monitoring of seizure activity over time.[108] In most cases, the EEG findings are non-specific and denote global compromise of the brain function but may also provide information about prognosis and therapeutic response. Abnormal EEG findings were reported in 77% of patients with TBE.[69] In most cases, an initially abnormal EEG normalizes within a few weeks. However, a small study of children with TBE reported a higher likelihood of impaired attention and psychomotor speed and that the EEGs were significantly slower on follow -up than control EEGs.[109]

Epileptic seizures can occur as the initial manifestation or during TBE.[71,110] Continuous EEG monitoring for at least 48 hours is recommended in patients with persistent unconsciousness to evaluate intermittent non-convulsive seizures or even persistent non-convulsive status epilepticus.39 The 10-year risk of epilepsy after TBE is 1.7% (95% CI 0.7-2.7).[111]

Prognosis and long-term sequelae

The analysis of the standardized mortality ratio (SMR) in Sweden from 2004–2017 revealed a mortality rate for TBEV infection to be ≈4-fold higher than that of the matched control population.[112] The SMR was 3.96 (95% CI 2.55–5.90). The case fatality rate (CFR) was 0.75% in this study, and in the range of previously reported rates of 0.5% in Europe.[113] No cases in patients <40 years of age were fatal. CFR for diseases caused by the two non-European TBEV subtypes is generally higher, but the data are very limited. In lethal cases, death occurs within 5–10 days after the onset of neurological symptoms in the context of diffuse brain edema or bulbar involvement.

TBE is associated with individual and societal disease burden. The need for hospital care is increased, with protracted in-hospital stays and admission to the intensive care unit during acute TBE.[39] Moreover, the study of the Swedish National Health Data Register for TBE cases diagnosed during 1998-2014 revealed that patients with TBE were hospitalized for more days during the first year after disease onset (11.5 vs. 1.1 days) and had more specialist outpatient visits (3.6 vs. 1.2 visits).[114] They also had more sick leave days (66 vs. 10.7 days) than a reference cohort without TBE, indicating significant productivity losses.

The high proportion of patients with persistent post-TBE symptoms is another strong argument for preventive strategies. Sequelae can be categorized as neurological (e.g., paresis, limb paresis, aphasia, ataxia, sensory impairment, epilepsy, tremor, hearing disorder), neuropsychiatric symptoms (e.g., concentration and memory deficits), and general/unspecific (fatigue, headache, general weakness, poor sleep quality, sweating

disturbances). Previous prospective studies disclosed that neurological and neuropsychological sequelae persist in 40–46% of the patients one year after the acute phase of the disease.[53,60] A study from Slovenia reported that the rate of persistent symptoms was higher at six months than at 12 months, which points to some improvement and regenerative capacity within the first year after TBE.[115] Recent studies corroborate the rate of incomplete recovery beyond 12 months. A study from Southern Germany performed telephone interviews after 18 months from TBEV infection; the period was 2018 to 2020.[14] Full recovery was reported by 67.3% (children: 94.9%, adults: 63.8%). Sequelae included fatigue (17.0%), weakness (13.4%), concentration deficit (13.0%), and impaired balance (12.0%). The recovery rate was 64% lower after severe TBE (compared to mild; HR: 0.36, 95%CI 0.25-0.52) and 22% lower with comorbidities (HR: 0.78, 95%CI 0.62-0.99). Substantial healthcare use was reported (90.1% hospitalization, 39.8% rehabilitation). A study from Lithuania evaluated long-term neurological and neurocognitive sequelae after TBE in adults.116 This prospective study from 2018-2019 revealed that 25.5% of the patients had moderate or major impairment (Glasgow Outcome Scale, GOS) and various levels of disability in 34.7% (Rankin-Scale, RS) at discharge. Up to 18 months from the onset of TBE, over 20% remained with slight to moderate disability (modified RS, mRS). GOS, RS, and mRS scores correlated with disease severity.

There is also evidence for the development of post-encephalitic syndrome (PES). Some authors define PES as the presence of ≥ 2 subjective symptoms that developed or worsened since the onset of TBE and had no other known medical explanation and/or ≥ 1 objective neurological sign.[115]

The reporting of sequelae is affected by a lack of standardized reporting. Consensus criteria for classifying sequelae of TBE and its severity are eagerly awaited. Such a reporting system should include neurological and neuropsychological examinations for the evaluation of cerebral symptoms as well as a scoring system for spinal cord and peripheral nervous system disturbances. A harmonized classification system would also be helpful for a better understanding and monitoring of PES.

Treatment

No specific antiviral therapy is currently available and approved for TBEV infections. Some antiviral agents, specific immunoglobulins, and other potentially protective substances are under investigation for their anti-TBEV efficacy[117]; however, a detailed review of these 'pipeline' agents is beyond the scope of this chapter.

Treatment is supportive and symptomatic. Fever is associated with increased metabolic consumption and dehydratation. Antipyretics, or other physical measures like cooling blankets, or infusion of cooled fluids, should be employed to reduce body temperature. TBE can be accompanied by hypovolemia due to a decreased intake and a secondary loss of fluids. Hyponatremia is a common condition in patients with TBE, including the syndrome of inappropriate antidiuretic hormone secretion (SIADH), cerebral saltwasting syndrome, and reduced sodium supplementation.[118] Mental and behavioral disturbances, delirium, and psychotic signs and symptoms may justify treatment with neuroleptics. In line with other types of brain injury, primary prophylaxis of seizures is currently not recommended, and treatment of clinical seizures is based on general guidelines for the management of seizures/ status epilepticus. Pain and arousal cause intracranial pressure peaks by increasing the cerebral blood flow; therefore, sedatives and careful clinical monitoring are key factors in the prevention of intracranial hypertension and its complications.

Encephalitis often requires ICU admission to ensure oxygenation, airway protection, circulatory support, and prevention and treatment of secondary complications that may impact outcomes. These include cerebral edema, seizures/status epilepticus, and systemic complications, such as fever, aspiration pneumonia, and respiratory failure requiring mechanical ventilation.[119] Early recognition of complications and admission to the ICU is crucial for improving prognosis.

Most survivors do not recover fully and often require extended posthospitalization rehabilitation and care to regain their functional abilities.[5] A comprehensive assessment of neurological, cognitive, and psychiatric functions after hospital discharge is mandatory. Moreover, referral to rehabilitation services and psychiatric support, as with other neurological disorders, is indicated to improve the quality of life of both the patient and their caregivers.

Contact: petra.bogovic@kclj.si

Citation:
Sellner J, Bogovis P, Zajkowska. TBE in adults. Chapter 9. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_9-7

References

1. Chiffi G, Grandgirard D, Leib SL, Chrdle A, Ruzek D. Tick-borne encephalitis: A comprehensive review of the epidemiology, virology, and clinical picture. *Rev Med Virol*. Sep 2023;33(5):e2470. doi:10.1002/rmv.2470

2. ECDC. Tick-borne encephalitis. *ECDC Annual epidemiological report*. European Centre for Disease Prevention and Control; 2024.

3. Saegerman C, Humblet MF, Leandri M, et al. First Expert Elicitation of Knowledge on Possible Drivers of Observed Increasing Human Cases of Tick-Borne Encephalitis in Europe. *Viruses*. Mar 20 2023;15(3) doi:10.3390/v15030791

4. Kohlmaier B, Schweintzger NA, Sagmeister MG, et al. Clinical Characteristics of Patients with Tick-Borne Encephalitis (TBE): A European Multicentre Study from 2010 to 2017. *Microorganisms*. Jun 30 2021;9(7) doi:10.3390/microorganisms9071420

5. Kvam KA, Stahl JP, Chow FC, et al. Outcome and Sequelae of Autoimmune Encephalitis. *J Clin Neurol*. Jan 2024;20(1):3-22. doi:10.3988/jcn.2023.0242

6. Hills S, Gould C, Cossaboom c. Tick-Borne Encephalitis. In: Nemhauser JB, ed. *CDC Yellow Book 2024 - Health Information for International Travel*. Oxford University Press; 2024.

7. Vilibic-Cavlek T, Krcmar S, Bogdanic M, et al. An Overview of Tick-Borne Encephalitis Epidemiology in Endemic Regions of Continental Croatia, 2017-2023. *Microorganisms*. Feb 13 2024;12(2)doi:10.3390/microorganisms12020386

8. Steininger P, Ensser A, Knoll A, Korn K. Results of Tick-Borne Encephalitis Virus (TBEV) Diagnostics in an Endemic Area in Southern Germany, 2007 to 2022. *Viruses.* Nov 30 2023;15(12)doi:10.3390/v15122357

9. Czupryna P, Moniuszko A, Pancewicz SA, Grygorczuk S, Kondrusik M, Zajkowska J. Tick-borne encephalitis in Poland in years 1993-2008--epidemiology and clinical presentation. A retrospective study of 687 patients. *Eur J Neurol*. May 2011;18(5):673-9. doi:10.1111/j.1468-1331.2010.03278.x

10. Jelenik Z, Keller M, Briggs B, et al. Tick-borne encephalitis and golden agers: position paper of the International Scientific Working Group on Tick-borne encephalitis (ISW-TBE). *Wien Med Wochenschr*. May 2010;160(9-10):247-51. doi:10.1007/s10354-010-0758-5

11. Santonja I, Stiasny K, Essl A, Heinz FX, Kundi M, Holzmann H. Tick-Borne Encephalitis in Vaccinated Patients: A Retrospective Case-Control Study and Analysis of Vaccination Field Effectiveness in Austria From 2000 to 2018. *J Infect Dis*. Feb 14 2023;227 (4):512-521. doi:10.1093/infdis/jiac075

12. Radzisauskiene D, Urboniene J, Kaubrys G, et al. The epidemiology, clinical presentation, and predictors of severe Tick-borne encephalitis in Lithuania, a highly endemic country: A retrospective study of 1040 patients. *PLoS One*. 2020;15(11):e0241587. doi:10.1371/journal.pone.0241587

13. Bogovic P, Lotric-Furlan S, Avsic-Zupanc T, Lusa L, Strle F. Factors associated with severity of tick-borne encephalitis: A prospective observational study. *Travel Med Infect Dis*. Nov-Dec 2018;26:25-31. doi:10.1016/j.tmaid.2018.10.003

14. Nygren TM, Pilic A, Bohmer MM, Wagner-Wiening C, Wichmann O, Hellenbrand W. Recovery and sequelae in 523 adults and children with tick-borne encephalitis in Germany. *Infection*. Oct 2023;51(5):1503-1511. doi:10.1007/s15010-023-02023-w

15. Lenhard T, Ott D, Jakob NJ, et al. Predictors, Neuroimaging Characteristics and Long-Term Outcome of Severe European Tick-Borne Encephalitis: A Prospective Cohort Study. *PLoS One.* 2016;11 (4):e0154143. doi:10.1371/journal.pone.0154143

16. Czarnowska A, Groth M, Okrzeja J, et al. A fatal case of tick-borne encephalitis in an immunocompromised patient: case report from Northeastern Poland and review of literature. *Ticks Tick Borne Dis*. Jan 2024;15 (1):102273. doi:10.1016/j.ttbdis.2023.102273

17. Lipowski D, Popiel M, Perlejewski K, et al. A Cluster of Fatal Tick-borne Encephalitis Virus Infection in Organ Transplant Setting. *J Infect Dis.* Mar 15 2017;215(6):896-901. doi:10.1093/infdis/jix040

18. Wagner JN, Sonnberger M, Troescher A, et al. Patients with breakthrough tick-borne encephalitis suffer a more severe clinical course and display extensive magnetic resonance imaging changes. *Eur J Neurol*. Jul 2020;27(7):1201-1209. doi:10.1111/ene.14276

19. Bogovic P, Lotric-Furlan S, Avsic-Zupanc T, et al. Low Virus-Specific IgG Antibodies in Adverse Clinical Course and Outcome of Tick-Borne Encephalitis. *Microorganisms*. Feb 7 2021;9(2)doi:10.3390/microorganisms9020332

20. Carlstromer Berthen N, Tompa E, Olausson S, et al. The AxBioTick Study: Borrelia Species and Tick-Borne Encephalitis Virus in Ticks, and Clinical Responses in Tick-Bitten Individuals on the Aland Islands, Finland. *Microorganisms*. Apr 22 2023;11(5)doi:10.3390/microorganisms11051100

21. Moniuszko A, Dunaj J, Swiecicka I, et al. Co-infections with Borrelia species, Anaplasma phagocytophilum and Babesia spp. in patients with tick-borne encephalitis. *Eur J Clin Microbiol Infect Dis.* Oct 2014;33(10):1835-41. doi:10.1007/s10096-014-2134-7

22. Palus M, Vojtiskova J, Salat J, et al. Mice with different susceptibility to tick-borne encephalitis virus infection show selective neutralizing antibody response and inflammatory reaction in the central nervous system. *J Neuroinflammation*. Jun 27 2013;10:77. doi:10.1186/1742-2094-10-77

23. Faivre N, Verollet C, Dumas F. The chemokine receptor

CCR5: multi-faceted hook for HIV-1. *Retrovirology.* Jan 23 2024;21(1):2. doi:10.1186/s12977-024-00634-1

24. Kindberg E, Mickiene A, Ax C, et al. A deletion in the chemokine receptor 5 (CCR5) gene is associated with tickborne encephalitis. *J Infect Dis*. Jan 15 2008;197 (2):266-9. doi:10.1086/524709

25. Mickiene A, Pakalniene J, Nordgren J, et al. Polymorphisms in chemokine receptor 5 and Toll-like receptor 3 genes are risk factors for clinical tick-borne encephalitis in the Lithuanian population. *PLoS One*. 2014;9(9):e106798. doi:10.1371/journal.pone.0106798

26. Grygorczuk S, Osada J, Parczewski M, et al. The expression of the chemokine receptor CCR5 in tick-borne encephalitis. *J Neuroinflammation*. Feb 22 2016;13:45. doi:10.1186/s12974-016-0511-0

27. Grygorczuk S, Dunaj-Malyszko J, Sulik A, et al. The Lack of the Association of the CCR5 Genotype with the Clinical Presentation and Frequency of Tick-Borne Encephalitis in the Polish Population. *Pathogens*. Mar 4 2022;11(3) doi:10.3390/pathogens11030318

28. Barkhash AV, Perelygin AA, Babenko VN, et al. Variability in the 2'-5'-oligoadenylate synthetase gene cluster is associated with human predisposition to tick-borne encephalitis virus-induced disease. *J Infect Dis*. Dec 15 2010;202(12):1813-8. doi:10.1086/657418

29. Barkhash AV, Babenko VN, Kobzev VF, Romaschenko AG, Voevoda MI. Polymorphism of 2'-5'-oligoadenylate synthetase (OAS) genes, associated with predisposition to severe forms of tick-borne encephalitis, in human populations of North Eurasia. *Mol Biol*. 2010;44(6):875-882. doi:10.1134/S002689331006004X

30. Barkhash AV, Perelygin AA, Babenko VN, Brinton MA, Voevoda MI. Single nucleotide polymorphism in the promoter region of the CD209 gene is associated with human predisposition to severe forms of tick-borne encephalitis. *Antiviral Res*. Jan 2012;93(1):64-8. doi:10.1016/j.antiviral.2011.10.017

31. Fortova A, Barkhash AV, Pychova M, et al. Genetic polymorphisms in innate immunity genes influence predisposition to tick-borne encephalitis. *J Neurovirol*. Dec 2023;29(6):699-705. doi:10.1007/s13365-023-01182-8

32. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*. Sep 17 2009;461(7262):399-401. doi:10.1038/nature08309

33. Barkhash AV, Babenko VN, Voevoda MI, Romaschenko AG. Association of IL28B and IL10 gene polymorphism with predisposition to tick-borne encephalitis in a Russian population. *Ticks Tick Borne Dis*. Jul 2016;7(5):808-812. doi:10.1016/j.ttbdis.2016.03.019

34. Prehaud C, Megret F, Lafage M, Lafon M. Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon. *J Virol*. Oct 2005;79 (20):12893-904. doi:10.1128/JVI.79.20.12893-12904.2005

35. Labuda M, Randolph SE. Survival strategy of tick-borne encephalitis virus: cellular basis and environmental determinants. *Zentralbl Bakteriol*. Dec 1999;289(5-7):513-24. doi:10.1016/s0934-8840(99)80005-x

36. Barkhash AV, Yurchenko AA, Yudin NS, et al. A matrix metalloproteinase 9 (MMP9) gene single nucleotide polymorphism is associated with predisposition to tick-borne encephalitis virus-induced severe central nervous system disease. *Ticks Tick Borne Dis*. May 2018;9(4):763-767. doi:10.1016/j.ttbdis.2018.02.010

37. Czupryna P, Parczewski M, Grygorczuk S, et al. Analysis of the relationship between single nucleotide polymorphism of the CD209, IL-10, IL-28 and CCR5 D32 genes with the human predisposition to developing tick-borne encephalitis. *Postepy Hig Med Dosw (Online)*. Jan 4 2017;71(1):788-796. doi:10.5604/01.3001.0010.3856

38. Bogovic P, Kastrin A, Lotric-Furlan S, et al. Clinical and Laboratory Characteristics and Outcome of Illness Caused by Tick-Borne Encephalitis Virus without Central Nervous System Involvement. *Emerg Infect Dis*. Feb 2022;28 (2):291-301. doi:10.3201/eid2802.211661

39. Taba P, Schmutzhard E, Forsberg P, et al. EAN consensus review on prevention, diagnosis and management of tick-borne encephalitis. *Eur J Neurol*. Oct 2017;24(10):1214-e61. doi:10.1111/ene.13356

40. ECDC. Factsheet. Tick-borne encephalitis. *Annual epidemiological report for 2018*. 2019.

41. Bogovic P, Lusa L, Korva M, et al. Inflammatory Immune Responses in the Pathogenesis of Tick-Borne Encephalitis. *J Clin Med.* May 22 2019;8(5)doi:10.3390/jcm8050731

42. Ruzek D, Dobler G, Donoso Mantke O. Tick-borne encephalitis: pathogenesis and clinical implications. *Travel Med Infect Dis*. Jul 2010;8(4):223-32. doi:10.1016/ j.tmaid.2010.06.004

43. Mandl CW. Steps of the tick-borne encephalitis virus replication cycle that affect neuropathogenesis. *Virus Res*. Aug 2005;111(2):161-74. doi:10.1016/ j.virusres.2005.04.007

44. Palus M, Vancova M, Sirmarova J, Elsterova J, Perner J, Ruzek D. Tick-borne encephalitis virus infects human brain microvascular endothelial cells without compromising blood-brain barrier integrity. *Virology*. Jul 2017;507:110-122. doi:10.1016/j.virol.2017.04.012

45. Ruzek D, Avsic Zupanc T, Borde J, et al. Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines.

Antiviral Res. Apr 2019;164:23-51. doi:10.1016/j.antiviral.2019.01.014

46. Blom K, Cuapio A, Sandberg JT, et al. Cell-Mediated Immune Responses and Immunopathogenesis of Human Tick-Borne Encephalitis Virus-Infection. *Front Immunol*. 2018;9:2174. doi:10.3389/fimmu.2018.02174

47. Gustafson R, Forsgren M, Gardulf A, Granstrom M, Svenungsson B. Clinical manifestations and antibody prevalence of Lyme borreliosis and tick-borne encephalitis in Sweden: a study in five endemic areas close to Stockholm. *Scand J Infect Dis*. 1993;25(5):595-603. doi:10.3109/00365549309008548

48. Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. *Antiviral Res*. Jan 2003;57(1-2):129-46. doi:10.1016/s0166-3542(02)00206-1

49. Kaiser R. Tick-borne encephalitis. *Infect Dis Clin North Am.* Sep 2008;22(3):561-75, x. doi:10.1016/j.idc.2008.03.013

50. Holzmann H, Aberle SW, Stiasny K, et al. Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. *Emerg Infect Dis*. Oct 2009;15(10):1671-3. doi:10.3201/eid1510.090743

51. Hudopisk N, Korva M, Janet E, et al. Tick-borne encephalitis associated with consumption of raw goat milk, Slovenia, 2012. *Emerg Infect Dis*. May 2013;19 (5):806-8. doi:10.3201/eid1905.121442

52. Kaiser R. The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994-98: a prospective study of 656 patients. *Brain*. Nov 1999;122 (Pt 11):2067-78. doi:10.1093/brain/122.11.2067

53. Mickiene A, Laiskonis A, Gunther G, Vene S, Lundkvist A, Lindquist L. Tickborne encephalitis in an area of high endemicity in lithuania: disease severity and long-term prognosis. *Clin Infect Dis*. Sep 15 2002;35(6):650-8. doi:10.1086/342059

54. Bogovic P, Strle F. Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management. *World J Clin Cases*. May 16 2015;3(5):430-41. doi:10.12998/wjcc.v3.i5.430

55. Dumpis U, Crook D, Oksi J. Tick-borne encephalitis. *Clin Infect Dis*. Apr 1999;28(4):882-90. doi:10.1086/515195

56. Kunz C. Tick-borne encephalitis in Europe. *Acta Leiden*. 1992;60(2):1-14.

57. Granstrom M. Tick-borne zoonoses in Europe. Clin Microbiol Infect. Apr 1997;3(2):156-169. doi:10.1111/j.1469-0691.1997.tb00592.x

58. Lotric-Furlan S, Petrovec M, Avsic-Zupanc T, Strle F. Clinical distinction between human granulocytic ehrlichiosis and the initial phase of tick-borne encephalitis. *J Infect*. Jan 2000;40(1):55-8. doi:10.1053/jinf.1999.0587

59. Schultze D, Dollenmaier G, Rohner A, Guidi T, Cassinotti P. Benefit of detecting tick-borne encephalitis viremia in the first phase of illness. *J Clin Virol*. Feb 2007;38(2):172-5. doi:10.1016/j.jcv.2006.11.008

60. Gunther G, Haglund M, Lindquist L, Forsgren M, Skoldenberg B. Tick-bone encephalitis in Sweden in relation to aseptic meningo-encephalitis of other etiology: a prospective study of clinical course and outcome. *J Neurol.* Apr 1997;244(4):230-8. doi:10.1007/s004150050077

61. Lotric-Furlan S, Avsic-Zupanc T, Strle F. Is an isolated initial phase of a tick-borne encephalitis a common event? *Clin Infect Dis*. Jun 2000;30(6):987-8. doi:10.1086/313838

62. Lotric-Furlan S, Avsic-Zupanc T, Strle F. An abortive form of tick-borne encephalitis (TBE)--a rare clinical manifestation of infection with TBE virus. *Wien Klin Wochenschr*. Jul 31 2002;114(13-14):627-9.

63. Ustinova O, Volechova GM, Deviatkov MI, Gusmanova AI. [The clinico-epidemiological characteristics of tick-borne encephalitis in Perm Province]. *Zh Mikrobiol Epidemiol Immunobiol*. May-Jun 1997;(3):33-6. Kliniko-epidemiologicheskie osobennosti kleshchevogo entsefalita v Permskoi oblasti.

64. Meyer PM, Zimmermann H, Goetschel P. Tick-borne encephalitis presenting as fever without localising signs--a case series. *Eur J Pediatr*. Jun 2010;169(6):767-9. doi:10.1007/s00431-009-1097-7

65. Misic-Majerus L, Bujic N, Madaric V, Avsic-Zupanc T. [An abortive type of tick-borne meningoencephalitis]. *Acta Med Croatica*. 2003;57(2):111-6. Abortivni oblik krpeljnog meningoencefalitisa.

66. Barp N, Trentini A, Di Nuzzo M, Mondardini V, Francavilla E, Contini C. Clinical and laboratory findings in tick-borne encephalitis virus infection. *Parasite Epidemiol Control*. Aug 2020;10:e00160. doi:10.1016/j.parepi.2020.e00160

67. Misic Majerus L, Dakovic Rode O, Ruzic Sabljic E. [Post-encephalitic syndrome in patients with tick-borne encephalitis]. *Acta Med Croatica*. Oct 2009;63(4):269-78. Postencefaliticki sindrom u bolesnika s krpeljnim meningoencefalitisom.

68. Logar M, Bogovic P, Cerar D, Avsic-Zupanc T, Strle F. Tick-borne encephalitis in Slovenia from 2000 to 2004: comparison of the course in adult and elderly patients. *Wien Klin Wochenschr*. Nov 2006;118(21-22):702-7. doi:10.1007/s00508-006-0699-6

69. Kaiser R. Tick-borne encephalitis (TBE) in Germany and clinical course of the disease. *Int J Med Microbiol*. Jun 2002;291 Suppl 33:58-61. doi:10.1016/s1438-4221(02)

80012-1

70. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet*. May 31 2008;371(9627):1861-71. doi:10.1016/S0140-6736(08)60800-4

71. Karelis G, Bormane A, Logina I, et al. Tick-borne encephalitis in Latvia 1973-2009: epidemiology, clinical features and sequelae. *Eur J Neurol*. Jan 2012;19(1):62-8. doi:10.1111/j.1468-1331.2011.03434.x

72. Jereb M, Muzlovic I, Avsic-Zupanc T, Karner P. Severe tick-borne encephalitis in Slovenia: epidemiological, clinical and laboratory findings. *Wien Klin Wochenschr*. Jul 31 2002;114(13-14):623-6.

73. Jereb M, Karner P, Muzlovic I, Jurca T. Severe tick-borne encephalitis in Slovenia in the years 2001-2005: time for a mass vaccination campaign? *Wien Klin Wochenschr*. Dec 2006;118(23-24):765-8. doi:10.1007/s00508-006-0728-5

74. Bogovic P, Lotric-Furlan S, Avsic-Zupanc T, et al. Comparison of Clinical, Laboratory and Immune Characteristics of the Monophasic and Biphasic Course of Tick-Borne Encephalitis. *Microorganisms*. Apr 10 2021;9 (4)doi:10.3390/microorganisms9040796

75. Bannova GG, Sarmanova ES, Karavanov AS, Bychkova MV, Pivanova GP. [Biological properties of tick-borne encephalitis strains isolated in different parts of its geographic range]. *Vopr Virusol.* Jan-Feb 1982;(1):41-5. Izuchenie biologicheskikh svoistv shtammov virusa kleshchevogo entsefalita, vydelennykh v raznykh chastiakh ego areala.

76. Dekonenko EP, Umanskii KG. [Sequelae of different clinical forms of the acute stage of tick-borne encephalitis]. *Zh Nevropatol Psikhiatr Im S S Korsakova*. 1984;84(2):202-7. Posledstviia razlichnykh klinicheskikh form ostrogo perioda kleshchevogo entsefalita.

77. Enzinger C, Melisch B, Reischl A, Simbrunner J, Fazekas F. Polyradiculitis as a predominant symptom of tick-borne encephalitis virus infection. *Arch Neurol*. Jul 2009;66 (7):904-5. doi:10.1001/archneurol.2009.117

78. Lotric-Furlan S, Strle F. Peripheral facial palsy in patients with tick-borne encephalitis. *Clin Microbiol Infect*. Oct 2012;18(10):1027-32. doi:10.1111/j.1469-0691.2011.03719.x

79. Kleiter I, Steinbrecher A, Flugel D, Bogdahn U, Schulte-Mattler W. Autonomic involvement in tick-borne encephalitis (TBE): report of five cases. *Eur J Med Res*. Jun 30 2006;11(6):261-5.

80. Neumann B, Schulte-Mattler W, Brix S, et al. Autonomic and peripheral nervous system function in acute tick-borne encephalitis. *Brain Behav.* Aug 2016;6(8):e00485. doi:10.1002/brb3.485

81. Poschl P, Kleiter I, Grubwinkler S, et al. [Severe tick-borne encephalomyelitis with lack of cerebrospinal fluid pleocytosis]. *Fortschr Neurol Psychiatr*. Oct 2009;77 (10):591-3. Schwere Fruhsommer-Meningo-Enzephalomyelitis ohne Liquor-Pleozytose. doi:10.1055/s-0028-1109768

82. Stupica D, Strle F, Avsic-Zupanc T, Logar M, Pecavar B, Bajrovic FF. Tick borne encephalitis without cerebrospinal fluid pleocytosis. *BMC Infect Dis*. Nov 18 2014;14:614. doi:10.1186/s12879-014-0614-0

83. Poponnikova TV. Specific clinical and epidemiological features of tick-borne encephalitis in Western Siberia. *Int J Med Microbiol*. May 2006;296 Suppl 40:59-62. doi:10.1016/j.ijmm.2006.01.023

84. Mukhin KY, Mameniskiene R, Mironov MB, et al. Epilepsia partialis continua in tick-borne Russian spring-summer encephalitis. *Acta Neurol Scand*. May 2012;125(5):345-52. doi:10.1111/j.1600-0404.2011.01575.x

85. Mansfield KL, Johnson N, Phipps LP, Stephenson JR, Fooks AR, Solomon T. Tick-borne encephalitis virus - a review of an emerging zoonosis. *J Gen Virol*. Aug 2009;90(Pt 8):1781 -1794. doi:10.1099/vir.0.011437-0

86. Sidorenko M, Radzijevskaja J, Mickevicius S, et al. Phylogenetic characterisation of tick-borne encephalitis virus from Lithuania. *PLoS One*. 2024;19(2):e0296472. doi:10.1371/journal.pone.0296472

87. Kaiser R. [Epidemiology and progress of early summer meningoencephalitis in Baden-Wurttemberg between 1994 and 1999. A prospective study of 731 patients]. *Dtsch Med Wochenschr*. Sep 29 2000;125(39):1147-53. Epidemiologie und Verlauf der Fruhsommer-Meningoenzephalitis in Baden-Wurttemberg zwischen 1994 und 1999. Eine prospektive Studie an 731 Patienten. doi:10.1055/s-2000-7668

88. Grygorczuk S, Mierzynska D, Zdrodowska A, et al. Tick-borne encephalitis in north-eastern Poland in 1997-2001: a retrospective study. *Scand J Infect Dis*. 2002;34(12):904-9. doi:10.1080/0036554021000026979

89. Bogovic P, Kastrin A, Lotric-Furlan S, et al. Comparison of laboratory and immune characteristics of the initial and second phase of tick-borne encephalitis. *Emerg Microbes Infect*. Dec 2022;11(1):1647-1656. doi:10.1080/22221751.2022.2086070

90. Lotric-Furlan S, Strle F. Thrombocytopenia--a common finding in the initial phase of tick-borne encephalitis. *Infection*. Jul-Aug 1995;23(4):203-6. doi:10.1007/BF01781197

91. Bogovic P, Lotric-Furlan S, Ogrinc K, et al. Elevated levels of serum muscle enzymes in the initial phase of tick-borne encephalitis. *Infect Dis (Lond).* Jun 2024;56(6):504-509. doi:10.1080/23744235.2024.2335349

92. Condos AM, Wangaryattawanich P, Rath TJ. Bacterial, Viral, and Prion Infectious Diseases of the Brain. *Magn Reson Imaging Clin N Am*. May 2024;32(2):289-311. doi:10.1016/j.mric.2023.11.001

93. Fjordside L, Nissen MS, Florescu AM, et al. Validation of a risk score to differentiate autoimmune and viral encephalitis: a Nationwide Cohort Study in Denmark. *J Neurol*. May 18 2024;doi:10.1007/s00415-024-12392-3

94. Mohan S, Jain KK, Arabi M, Shah GV. Imaging of meningitis and ventriculitis. *Neuroimaging Clin N Am*. Nov 2012;22 (4):557-83. doi:10.1016/j.nic.2012.04.003

95. Duong MT, Rudie JD, Mohan S. Neuroimaging Patterns of Intracranial Infections: Meningitis, Cerebritis, and Their Complications. *Neuroimaging Clin N Am*. Feb 2023;33 (1):11-41. doi:10.1016/j.nic.2022.07.001

96. Horger M, Beck R, Fenchel M, et al. Imaging findings in tick-borne encephalitis with differential diagnostic considerations. *AJR Am J Roentgenol*. Aug 2012;199 (2):420-7. doi:10.2214/AJR.11.7911

97. Saremi F, Helmy M, Farzin S, Zee CS, Go JL. MRI of cranial nerve enhancement. *AJR Am J Roentgenol*. Dec 2005;185 (6):1487-97. doi:10.2214/AJR.04.1518

98. Bloch KC, Glaser C, Gaston D, Venkatesan A. State of the Art: Acute Encephalitis. *Clin Infect Dis*. Sep 11 2023;77 (5):e14-e33. doi:10.1093/cid/ciad306

99. Pichler A, Sellner J, Harutyunyan G, et al. Magnetic resonance imaging and clinical findings in adults with tick-borne encephalitis. *J Neurol Sci*. Apr 15 2017;375:266-269. doi:10.1016/j.jns.2017.02.003

100. Abbuehl LS, Branca M, Ungureanu A, et al. Magnetic resonance imaging in acute meningoencephalitis of viral and unknown origin: frequent findings and prognostic potential. *Front Neurol.* 2024;15:1359437. doi:10.3389/ fneur.2024.1359437

101. Dietmann A, Putzer D, Beer R, et al. Cerebral glucose hypometabolism in Tick-Borne Encephalitis, a pilot study in 10 Patients. *Int J Infect Dis*. Oct 2016;51:73-77. doi:10.1016/j.ijid.2016.06.022

102. Czarnowska A, Kapica-Topczewska K, Garkowski A, et al. Severe tick-borne encephalitis in a patient recovered from COVID 19. *Ticks Tick Borne Dis*. Jul 2022;13(4):101940. doi:10.1016/j.ttbdis.2022.101940

103. Czupryna P, Tarasow E, Moniuszko-Malinowska A, et al. MRI and planimetric CT follow-up study of patients with severe tick-borne encephalitis. *Infect Dis (Lond)*. 2016;48 (1):74-81. doi:10.3109/23744235.2015.1083119

104. Neill L, Checkley AM, Benjamin LA, et al. Rhombencephalitis and Myeloradiculitis Caused by a European Subtype of Tick-Borne Encephalitis Virus. *Emerg Infect Dis*. Dec 2019;25(12):2317-2319. doi:10.3201/ eid2512.191017

105. Schellinger PD, Schmutzhard E, Fiebach JB, Pfausler B, Maier H, Schwab S. Poliomyelitic-like illness in central European encephalitis. *Neurology*. Jul 25 2000;55(2):299-302. doi:10.1212/wnl.55.2.299

106. Feige J, Moser T, Hauer L, Pikija S, Sellner J. Clinical Challenges in a 49-Year-Old Patient with Severe Tick-Borne Myeloradiculitis Despite Complete Active Vaccination. *Vaccines (Basel)*. Feb 20 2020;8(1) doi:10.3390/vaccines8010093

107. Racz A, Schaller G, Lunkenheimer J, et al. Isolated meningomyeloradiculitis following infection with tick borne encephalitis virus. *Clin Neurol Neurosurg*. Nov 2012;114(9):1263-5. doi:10.1016/j.clineuro.2012.02.047

108. Sellner J, Trinka E. Epilepsy associated with viral encephalitis. In: Shorvon S, Guerrini R, Schachter S, Trinka E, eds. *The causes of epilepsy*. Cambridge University Press; 2019.

109. Schmolck H, Maritz E, Kletzin I, Korinthenberg R. Neurologic, neuropsychologic, and electroencephalographic findings after European tick-borne encephalitis in children. *J Child Neurol*. Jun 2005;20 (6):500-8. doi:10.1177/088307380502000606

110. Sipila JOT. Adult-onset encephalitis over two decades in easternmost Finland. *Neuroepidemiology*. Feb 28 2024;doi:10.1159/000538020

111. Zelano J, Westman G. Epilepsy after brain infection in adults: A register-based population-wide study. *Neurology*. Dec 15 2020;95(24):e3213-e3220. doi:10.1212/WNL.0000000000010954

112. Varnaite R, Gredmark-Russ S, Klingstrom J. Deaths from Tick-Borne Encephalitis, Sweden. *Emerg Infect Dis*. Jul 2022;28(7):1471-1474. doi:10.3201/eid2807.220010

113. Beaute J, Spiteri G, Warns-Petit E, Zeller H. Tick-borne encephalitis in Europe, 2012 to 2016. *Euro Surveill*. Nov 2018;23(45)doi:10.2807/1560-7917.ES.2018.23.45.1800201

114. Slunge D, Boman A, Studahl M. Burden of Tick-Borne Encephalitis, Sweden. *Emerg Infect Dis*. Feb 2022;28 (2):314-322. doi:10.3201/eid2802.204324

115. Bogovic P, Stupica D, Rojko T, et al. The long-term outcome of tick-borne encephalitis in Central Europe. *Ticks Tick Borne Dis*. Feb 2018;9(2):369-378. doi:10.1016/ j.ttbdis.2017.12.001

116. Griska V, Pranckeviciene A, Pakalniene J, et al. Long-term neurological and neurocognitive impairments after tick-borne encephalitis in Lithuania - a prospective study. *Infect Dis (Lond).* May 6 2024:1-11.

doi:10.1080/23744235.2024.2346793

117. Eyer L, Seley-Radtke K, Ruzek D. New directions in the experimental therapy of tick-borne encephalitis. *Antiviral Res*. Feb 2023;210:105504. doi:10.1016/j.antiviral.2022.105504

118. Czupryna P, Moniuszko A, Garkowski A, Pancewicz S, Zajkowska J. Comparison of hyponatremia and SIADH frequency in patients with tick borne encephalitis and meningitis of other origin. *Scand J Clin Lab Invest*. 2016;76 (2):159-64. doi:10.3109/00365513.2015.1129669

119. Sonneville R, Jaquet P, Vellieux G, de Montmollin E, Visseaux B. Intensive care management of patients with viral encephalitis. *Rev Neurol (Paris)*. Jan-Feb 2022;178(1-2):48-56. doi:10.1016/j.neurol.2021.12.002

Chapter 10

TBE in animals

Martin Pfeffer, Hannah M. Schmuck and Michael Leschnik

Key points

- TBEV-infection can cause symptomatic disease in dogs and horses, similar to the TBE in humans.
- Microbiological confirmation of TBEV infection in animals is similar to diagnostics in humans.
- Domestic ruminants may serve source of human infection via the alimentary routs (dairy products).
- Small mammals play the major role as the reservoir for the TBEV and are thus of utmost epidemiological relevance.
- Other species like cervids and wild boar are of interest for sentinel surveillance, as their seropositivity in a specific region indicates the presence of a natural TBEV-focus

Introduction

While tick-borne encephalitis (TBE) is well documented as a public health threat, the veterinary aspects of this zoonotic disease are little recognized. TBE in animals has, for very long, been considered to be a problem exclusive to domestic ruminants due to their known potential to transmit tick-borne encephalitis virus (TBEV) via raw milk and raw milk products to consumers. While clusters of such cases continuously declined with the invention of milk pasteurization and overall improvements in hygiene management in cattle farming, goats and sheep flocks are still kept in traditional grazing farms where they are exposed to TBEV-infected ticks.[1,2] In other words, even in industrialized countries, consumption of raw milk products continues to be a risk factor to acquire a TBEV infection. As society continues to exhibit a trend towards a preference for "natural products" (assuming consumers can afford these), alimentary TBEV infections may be observed more frequently in the future. While this is a 'direct' zoonotic aspect of TBE (besides the tick bite of course), animals play a role in TBEV transmission in many other ways; either as diseased dead-end hosts, as infected animals without obvious burden of disease, or in maintaining and spreading the virus itself.

Dogs

Canine TBEV infection is a frequent event in endemic areas, with a calculated annual risk of about 11.6%.[3] Total seroprevalence in the canine population has been examined in several countries: Switzerland 3.6–5.9%,[4] Greece 1–8%,[5] Germany 2.1– 42.7%,[6,7] Belgium 0.1%,[8] Denmark 4.8–30%,[9] Czech Republic 3.3–11.3%,[10,11] Norway 16.4%,[12] Finland 6–40%,[13] and Austria 13.3–24%.[3,14] Since inclusion criteria were different regarding the presence of clinical symptoms, residence, and tick-exposure of the examined dogs, results are difficult to compare (Table 1). Different test systems (enzyme-linked immunosorbent assay [ELISA], serum neutralization test [SNT]) used in these studies clearly influenced the results too. TBE has always been stated to be a tick-borne infection, mainly transmitted by ticks of the genus *Ixodes*; however, *Dermacentor reticulatus* ticks may play an important role in transmission to dogs.[15] There has been one single case of a dog from the Czech Republic with a TBE-infection suspected to be due to consumption of raw goat milk.[10] Regardless of the way dogs get infected, a recent study showed that walking a dog is a risk factor for human infections.[16]

Course of TBE

Despite frequent TBEV infections in dogs, most of them do not develop any clinical signs.[17] Dogs seem to be less susceptible than humans, although a lethal outcome within the first week of disease is documented in 16–50% of clinically symptomatic cases . Infection may lead to an acute course of the disease, with complete remission of symptoms within 1–2 weeks (31–59%). Infrequently, prolonged disease courses are described with long time period to remission (12–25%). These dogs frequently suffer from late sequela–like paresis, muscle atrophy, epileptic seizures, or blindness (Figure 1).[10,18,19,27,28]

Clinical pictures

After an estimated incubation period of 5–9 days, first clinical symptoms occur and develop to a maximum level within 48 hours. Initially, most dogs are depressed and show non-specific signs such as salivation and vomiting (25%), refusal to eat, and are reluctant to move due to generalized weakness, although some dogs show compulsive walking, circling to one side (25%), unusual behavior (70–91%), and head pressing (Figure 2).[10,27-30]

Table 1: Serosurveillance studies for TBE virus and TBE virus antibodies in dogs since 2010

Year	Country	Number of dogs	Clinical signs	Virus detection	Reference	Results
2011	Austria	90 dogs	not observed	n.d.	3	repeated testing within one year: 9.8% - 13.4% seropositive
2011 - 2012	Czech Republic	159 dogs	in 7/20 viremic dogs	by PCR	11	11.3% seropositive dogs, viremic dogs 12.6%
2011 - 2012	Finland	148 dogs	not observed	n.d.	13	6% - 40% seropositive
2012 - 2014	Germany	331 healthy dogs	not observed	n.d.	6	2.1% seropositive dogs (ELISA and SNT)
2013 - 2015	Spain	815 healthy dogs	not observed	n.d.	131	1.7% seropositive dogs (ELISA and SNT)
2014 - 2015	Serbia	40 healthy dogs	Not observed	n.d.	20	17.5% (ELISA)
2018 - 2019	Germany	208 healthy dogs	Not observed	n.d.	17	22.1% seropositive dogs * (ELISA and SNT)
2016-2020	Czech Republic	130 healthy dogs	Not observed	n.d.	21	17 (13.1%) seropositive dogs (ELISA and SNT)
2016-2020	Czech Republic	323 dogs with various clinical signs	incl. 171 with neuro-logical disorder	n.d.	21	41 (12.7%) seropositive dogs (ELISA and SNT)
2019 - 2021	Germany	1,317 healthy dogs	Not observed	n.d.	22	1.1% seropositive dogs * (ELISA and SNT)
2020-2021	Lithuania	473 with various disease from two clinics	Symptoms suggestive for TBE were found in 13.7%	PCR from blood	23	102 (21.6%) seropositive dogs (ELISA and SNT). 88 (18.6%) PCR-positive (see Table 2)

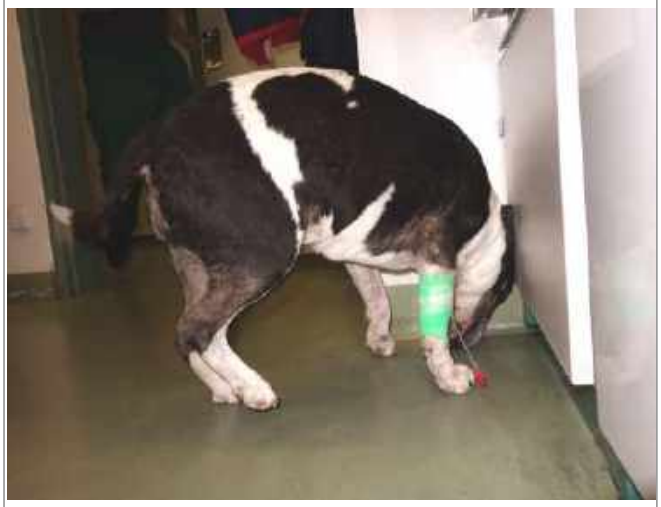
n.d. = not determined, SNT = serum neutralization test, Ab = antibodies,

** showing the difference between nonendemic areas (1.1% in northern Germany) and endemic areas (22.1% in southern Germany)*

Figure 1

A Rottweiler during recovery after chronic disease over 3 months – remarkable weight loss due to systemic muscle atrophy.

Figure 2



Acute head pressing with concurrent compulsive walking and disorientation on day 2 of a dog with TBE.

The elevated body temperature (42–66%) may initially be classed as fever; later on, it is more likely a result of non-voluntary excessive muscle contraction (e.g., seizures, loss of inhibition by upper motor neuron damage). Seizures are a principal result of cerebral damage due to TBEV infection and are observed in 12–33% of canine cases.[28,30] Neurological symptoms like paresis (8–38%), vocalization due to painful perception of active and passive back movement (21–66%), and deficits of the cranial nerves (16–50%) (Figure 3) develop within a few hours thereafter.[28,30,31]

Blindness due to papillitis, optic nerve inflammation, or chiasma opticus neuritis may become the dominant symptom and systemic signs may diminish. Visual deficits may be the major clinical sign of disease and result from detachment of peripapillary retina, peripapillary hemorrhages, and inflammatory edema.[32,33] Degeneration and demyelination of cranial nerves is certainly initiated by the virus' neurotropism. Later on, secondary immune reaction to neural tissue may prolong the period of damage and lead to irreversible symptoms such as retinal and optic disc atrophy. Other cranial nerve deficits like trigeminal dysfunction, resulting in reduced facial sensation and chewing muscle atrophy, vestibular signs (nystagmus and positional strabismus, Figure 4), and facial palsy, are observed.

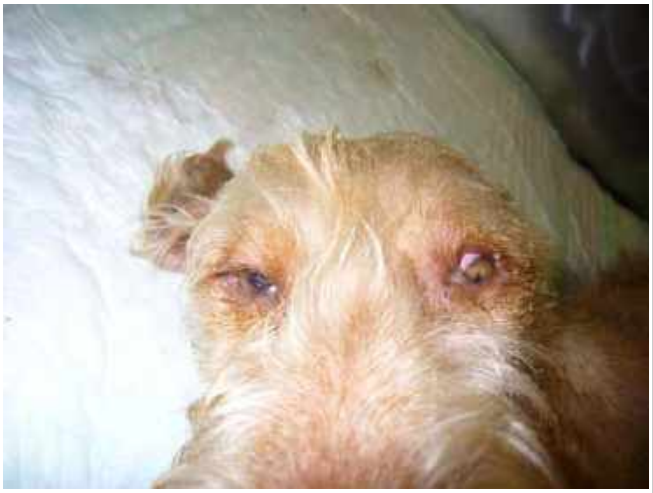
Major involvement of the spinal cord results in mostly symmetrical paresis, muscle twitching, and proprioceptive dysfunction (38-50%), which may also be present as an exclusive symptom and may occur asymmetrically (Figure 5).[10,28,30,31]

There is no significant breed, gender, or age predisposition, although most cases are described in adult middle- to large-breed dogs. Rottweilers and Huskies are overrepresented in the literature[14,31,32] (Table 2).

Figure 3

A male Spitz with central vestibular dysfunction and left-sided Horner syndrome during acute TBE.

Figure 4



A comatose dog in lateral recumbency with severe brain stem encephalitis leading to anisocoria and left-sided strabismus.

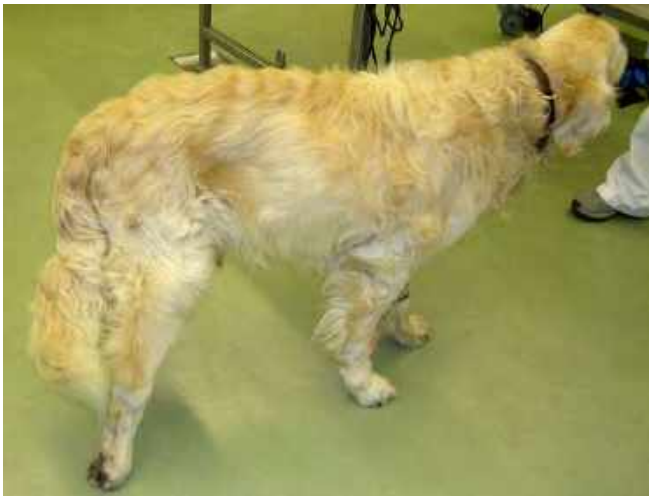
Brainstem symptoms like arrhythmical breathing pattern may be present in comatose dogs, especially in severe cases with guarded prognosis (see Video — https://id-ea.org/tbe/wp-content/uploads/2017/08/VIDEO_TBE_breathing-dog.mp4)

Video: Comatose dog of Figure 3 with arhythmical breathing indicative of brain stem lesion

Involvement of the brainstem may result in symptoms like arrhythmical breathing and disorder of other vital functions. Prognosis of such severe cases is very guarded. Major involvement of the spinal cord results in mostly symmetrical paresis, muscle twitching, and proprioceptive dysfunction (38-50%), which may also be present as an exclusive symptom and may occur asymmetrically (Figure 5).[10,28,30,31]

There is no significant breed, gender, or age predisposition, although most cases are described in adult middle- to large-breed dogs. Rottweilers and Huskies are over-represented in the literature[14,31,32] (Table 2).

Figure 5



A case of canine TBE with hemiparesis and spontaneous dorsal paw placement.

Laboratory findings and diagnosis

A definite diagnosis in dogs with TBE is rarely achieved *intra vitam* as it has to be supposed to be very unlikely to detect the virus in the blood or in the cerebrospinal fluid (CSF). In one study from the Czech Republic, 12.6% of canine blood samples tested positive for TBEV by nested RT-polymerase chain reaction (PCR), although only one-third of these dogs suffered from neurological symptoms.[11]

Whether the other dogs were in an asymptomatic carrier status, or just happened to be tested during their viremic phase with uncharacteristic symptoms, as reported in humans, remained unclear. Virus detection in the CSF has been achieved only in single cases within the first 3 days of disease.[30] Immunological rapid virus clearance in the dog's brain and CSF seems to be very fast and completed before most diagnostic procedures are performed. The inability of the central nervous system's (CNS) local immune system to eliminate the virus within a few days is probably the reason for a fatal outcome, as in most of these cases no specific intrathecal antibody production and no increased cell count in the CSF were detected prior to death.[28] CSF analysis in affected dogs with clinical signs mostly reveals elevated leukocyte count, with predominantly mononuclear cells and elevated total protein. CSF changes are concomitant to virus elimination and rising antibody titers.

Specific antibodies are detectable in the serum of affected dogs within a few days.[7,28,29,31] Comparison of a commercially available all species ELISA, indirect IFT and SNT using a panel of 208 dog sera revealed a sensitivity of 78.3% and 84.8% when compared to SNT and a specificity of 98.8% and 99.4%. IIFT an ELISA are thus good in case of confirming clinical cases with suspicion of TBE but due to the deficits in sensitivity the SNT is superior in

Table 2: Case Reports and Case Series of Tick-borne Encephalitis in Dogs since 2010

Year	Country	Dog breed	Clinical symptoms	Reference	Antigen detection	Antibody response	Confirmation	Outcome
2011-2012	Czech Republic	7 dogs	seizures, disorientation, central vestibular syndrome, paraparesis, cranial nerve deficits	11	yes, PCR from blood	yes	Virus detection, Antibody response	
2012-2014	Switzerland	12 dogs (including 2 Labrador, 1 Rottweiler, 1 Husky, 1 Newfoundland dog)	behavioural changes, ataxia, seizures, paresis, cranial nerve deficits, hyperesthesia	36	n.d.	Yes in 11 dogs	Antibody response, IHC in 5 dogs	6 euthanized, 6 fully recovered
1999-2016	Switzerland	54 dogs	behavioural changes, ataxia, seizures, paresis, cranial nerve deficits, hyperesthesia	19	n.d.	Yes (all dogs)	CSF antibodies (ELISA)	64% survival rate, but long-term sequelae in 17% of these
2019	Sweden	1 Pointer Labrador cross	Ataxia, seizures	24	yes	n.d.	Histopathology and PCR in the brain	euthanized
2020	Germany	1 mixed breed (2years old neutered)	Hyperthermia (39.6°C), hyperaesthesie, head-neck-tremor, cervical pain	25	negative	yes	CSF antibodies (ELISA 133 VIEU)	4 months symptomatic care until restitutio ad integrum
2020-2021	Lithuania	473 with various disease from two clinics	Symptoms suggestive for TBE were found in 13.7%	23	yes	yes	102 (21.6%) seropositive dogs. 88 (18.6%) PCR-positive	18.2% lethality in PCR-positive dogs
2022	Greece	1 mixed breed	Tetraparesis, hyperalgesia, behavioural change, high fever (41.3°C)	26	n.d.	yes	ELISA IgM and IgG	survived

** one dogs was also published in a previous paper, IHC = immunohistochemistry, n.d. = not determined, CSF = cerebrospinal fluid*

epidemiological studies.[17] As TBEV is a biosafety level 3 agent in many countries, production of the antigen used for any serological test is limited to facilities with an appropriate safety level. In order to circumvent this obstacle two assays have been developed using prME subviral particles expressed through a Semliki Forest Virus-based expression system. In one assay this antigen is used in a Vero cells system analogous to an IFT, while the other one is a capture ELISA using a monoclonal antibody (MAB1418) which specifically binds to domain III of glycoprotein E of TBEV. Specificity was thus raised to 100% for both assays making it suitable for epidemiological applications.[33] For clinical confirmation the detection of positive CSF IgG antibodies is recommended.[34] Cross-reactivity to Louping ill virus, West Nile virus, and Usutu virus should be taken into consideration in endemic areas.[10,35] Magnetic resonance imaging findings included bilateral and symmetrical gray matter lesions involving the thalamus, hippocampus, brain stem, basal nuclei, and ventral horn on the spinal cord.

All lesions had minimal or no mass effect, or perilesional edema.[24] These findings are comparable to the distribution of lesions in the canine brain detected by necropsy and immunohistochemistry.[25] Proton magnetic resonance spectroscopy, to evaluate metabolic abnormalities in dogs with TBE, revealed significant differences with dogs with immune mediated meningoencephalitis and healthy dogs.[26]

A tentative diagnosis of TBE in dogs should fulfill the following criteria: tick exposure or observed tick infestation, neurological signs indicative for a diffuse or multifocal CNS disease, (mostly mononuclear) pleocytosis in the CSF, a positive antibody titer in serum or CSF, or in the case of fatal outcome a positive virus confirmation within the brain or spinal cord. In the future, highly sensitive PCR techniques may include virus detection in the diagnostic work-up in early stages of disease. Increasing serum titers may be detected, but more often rapidly decreasing titers are observed when dogs reach partial or complete remission of clinical signs.[17,26]

Possible differential diagnoses include other viral meningoencephalitis such as distemper, rabies, pseudo-rabies, as well as protozoal, bacterial, or fungal meningo-encephalitis, and paraneoplastic and immune-mediated meningoencephalitis.

Treatment

Symptomatic therapy is strongly recommended for dogs with TBE. Water and food maintenance orally, by constant rate infusion, or by gastric tubes and supportive care is essential. Sedation and relaxation are necessary in the case of seizures. Steroid use is controversial, as immunosuppression may prolong the presence of the virus. In dogs with marked CSF pleocytosis, steroids seem to be mandatory to effectively protect the brain tissue from further fulminant immune response. In cases of muscle atrophy and paresis, physiotherapy (Figure 6) as early as possible has been shown to improve the general outcome and shorten the time of rehabilitation.[30,31]

Figure 6

An old Labrador Retriever during rehabilitation. Water training over months improved muscle strength and coordination.

Prevention

There is no licensed anti-TBE vaccine for dogs, although they develop detectable antibody titers after vaccination with a human vaccine.[39] In a recent study dogs were infected either with 10^8 pfu or 10^6 pfu TBEV strain 9001 isolated from *Ixodes ricinus* ticks in the Czech Republic in 1978 (back than Czechoslovakia). All animals developed no overt clinical signs but high IgG antibody titers in ELISA and high SNT antibody titers demonstrating that dogs are capable to mount protectable Immune response upon infection.[21] With the new European Animal Health Law vaccines for animals are handled in the EU like other animal therapeutics. In case no licensed vaccine against TBE is available for dogs the so-called cascade can be used, which in this case means that human vaccine can be rededicated by the treating veterinarian upon request at the responsible veterinary authorities. Depending on the size (and weight) of the dog, the pediatric formulation is recommend. Recently, colleagues tested whole virus inactivated TBEV strain Hypr as vaccine for dogs and found it well tolerated and to elicit a protective immunity.[132]

Tick protection is the most important measure to avoid transmission and infection, mainly performed by regular administration of acaricidal substances (spot on, tablets, shampoos, collars) and immediate tick removal after detection by the owner.[3] Regular anti-tick measures are essential to reduce transmission risk all through the year as single canine cases have been reported even during the

cold seasons of the year.[32]

Horses

Although the first clinical case of laboratory-confirmed TBE in a horse was published more than 35 years ago,[40] our knowledge about the impact of TBEV in horse populations is still scarce. There are only few published studies where clinical signs of neurological disorder could be traced to the TBEV as etiology. After the aforementioned initial published case from Switzerland, 8 horses with clinical symptoms were described in Austria, 2 of which were severely ill;[41] 1 out of 3 diseased animals from a study in Germany had to be euthanized;[42] and again in Germany, some years later, an infected animal had to be euthanized.[43] A case in Austria with the same outcome was worked up in a very thorough way, excellently describing the symptoms and laboratory finding. The authors in addition provide a video as supplementary to the manuscript which shows the 16 years old horse with its neurological symptoms.[44] The clinical picture in horses mirrors that which we described for dogs, displaying a broad spectrum of neurological symptoms: ataxia, tonic-clonic seizures, apathy and stupor, inappetence, mydriasis, convulsions of the legs, skittishness, bruxism, and altered reactions to environmental stimuli. Regarding therapeutic options and prognosis, a horse with recumbent status due to TBE has a poor prognosis as long as it is not possible to force the horse to stand up again.

The few case reports available suggest that clinical TBE in horses is a rare event, although basic horse population-based data are missing. Looking at the few seroprevalence studies in horses, the prevalence of anti-TBE-antibodies ranged from 26.1% and 13% in Austria[43,48] to 2.9% in central Germany,[42] 0.8% in northern Germany[22], 3.7% and 5.6% in eastern Germany[46,47], and 5.2% and 23.4% in southern Germany[43,48] to 0 of 40 horses investigated in Hungary[49] or 0 of 2349 horses from the Czech Republic.[50] Even in Spain a seroprevalence of 3.1% was reported in horses.[51] In Serbia and Croatia 5% and 12.2% of horses showed specific antibodies against TBEV.[20,52] The highest prevalence in horses was reported in a cross-section study from Lithuania with 37.5% reflecting the high human incidence there. Remarkably in this study was that 3.9% of theses horses also had a viremia based on the detection of viral RNA by RT -PCR, but none of the horses showed any overt signs of sickness.[53] Cross-reactivity to other flavivirus may influence these results.[50,54] Horses have been suggested to be good sentinel animals for human TBEV infection risk, because they readily seroconvert upon infection, but they stick more to a given territory in comparison to dogs who, as family members, travel more.

Domestic ruminants

For more than half a century, grazing cattle, goats, and sheep have been known to be susceptible to TBEV infection. Interestingly, these ruminants do not develop any clinical symptoms, and even after experimental infection, a slight elevation of body temperature is a rare finding.[55,56] However, in 2015, a five-month-old lamb in Bavaria displayed neurological symptoms , and after euthanasia, TBEV infection was diagnosed.[57] Whether this case was the result of an unknown underlying disease or immunosuppressive factors cannot be determined. TBE in domestic ruminants, if it occurs at all, appears to be an extreme exception. Nevertheless, infected animals develop viremia with a duration of up to 19 days.[58] A study in the Swiss canton of Valais found 4.25% of the tested goats to be seropositive according to an ELISA test, with 40.4% of these testing positive on a serum neutralization test (SNT).[59] In the canton of Ticino, officially labelled as non-TBE-endemic , SNT-positive goats were found in 10 out of 37 herds (14.6% out of 662 sera).[60] In Germany the intra-herd prevalence in sheep and goat herds was between 2.3% and 25%, but antibodies were found in some of the districts not considered TBE endemic areas, thus reflecting the human situation and arguing for small ruminant as good sentinel animals for human infection risk.[61] In Sweden serology from sheep milk was successfully used to map what they called “TBEV hotspots”.[62]

Even if viremia is shorter than 1 week, virus is shed via milk and remains infectious in cheese or other products prepared from unpasteurized milk. Consumption of such products may have led to an alimentary infection of a group of individuals who became infected through the same batch of contaminated food, resulting in clusters of human cases.[63] Such clusters of cases have recently been reviewed[2] and were thought to be restricted to nations in Eastern Europe with Slovakia having the highest occurrence of alimentary TBE outbreaks in Europe.[64] But alimentary infections due to the consumption of raw milk products are also reported from countries with rather low tick-borne incidences, like Croatia.[65,66] However, alimentary TBEV infection with clinical TBE occurred recently in Germany as a result of consumption of fresh raw goat milk[67]. As there is a growing trend towards consumption of natural food products in the industrialized nations of Western Europe, such scenarios may be witnessed

more frequently in the future. One study in an endemic region in Poland found TBEV in milk from sheep (22.2%), goats (14.8%), and cows (11.1%).68 In Norway, a study found TBEV RNA in 5.4% of tested raw milk samples. Positive blood serum samples only occurred in one municipality, where 88.2% of tested cows had specific antibodies. Remarkably, none of the cows with a positive milk sample had detectable antibodies and vice versa.[69] Domestic ruminants do develop an antibody response, which in the case of goats and sheep is measurable for at least 28 months or even up to 6 years and 10 months.[35,39,70] Exposure to TBEV seems not to result uniformly in seroconversion of the entire flock of animals.[71,72] Whether this indicates that not all animals of the same herd were exposed and infected or that some animals did not mount an immune response is not known. Also, the extent of antibody response seems to vary between the species.[73]

Game animals (wild boar, cervids, foxes)

Roe deer (*Capreolus capreolus*) are the most abundant cervids in Germany, sharing their habitat with ticks everywhere. They are well known as hosts for nymphs and adult ticks and thus are as important to maintenance of the tick population as the small mammals are for larvae and nymphs (see below). It is common to find hundreds of ticks per individual and, consequently, the odds of roe deer becoming infected in TBE-endemic areas are rather high.[74] Therefore, they can be a useful tool to identify endemic areas as could be seen in the Netherlands, where TBE was regarded as an imported disease until 2016. Serologic screening there showed TBEV-neutralizing antibodies with a seroprevalence of 2% in roe deer.[75] Clinical or pathological signs that raise suspicions of an overt TBEV infection have never been described for roe deer until recently with a single case in Italy.[76] Seroconversion after infection seems to be the rule, and this fact has been widely used to estimate TBE prevalence in certain areas. As roe deer are territorial animals, many researchers claim that this serological data could be very useful in finding and describing possible TBE-endemic areas, in particular in low-endemic areas or regions in which TBE cases in humans are reported only sporadically.[77-84] The discrepancy of often double-digit percentages of seroprevalence in roe deer and no, or almost no, human cases is puzzling, and needs to be investigated further. As TBEV is known to be circulating in such areas, an understanding of why only few or no human cases occur could be key to developing strategies aimed at reducing TBE incidence in high-endemic areas (as defined by the number of human cases).

Likewise, the wild boar (*Sus scrofa*) is present all over Europe and is commonly infested with ticks. There are no records of a possible TBE-like disease in wild boar and only 2 studies investigated the seroprevalence against TBEV in wild boar. Nevertheless, these studies demonstrated a surprisingly high percentage of animals with antibodies against TBEV in areas with no notified human TBE cases.[82] A sero-survey of wild boar in Belgium revealed the presence of TBEV, with 2.9% of the 238 wild boar investigated having specific neutralizing antibodies against TBEV.[85] As Belgium is considered to be traditionally free of autochthonous TBE,[2,86,87] this study demonstrates the power of using animal surveillance data for pinpointing TBE-endemic areas. A similar approach was applied in France using wild boar and roe deer sera with similar results, i.e. 2.9% and 0.3% seropositive animals.[88] Like the roe deer described above, wild boars are rather territorial, allowing the geographical allocation of such data. Only the renegade wild sows are known to travel across large areas when they are searching for a new herd. A study from the Czech Republic, traditionally a country with a high TBE incidence, found a positive association between the number of hunted wild boar and human cases. Consequently, the authors concluded that wild boar must play a role in TBEV transmission either directly or indirectly.[89]

In Finland, moose (*Alces alces*) and white-tailed deer (*Odocoileus virginianus*) were found to harbor TBEV-specific antibodies (0.74%) and the use of such seroprevalence data as an indicator for local risk of human TBE infection is recommended.[90] In Norway, 9.4% of 286 moose, 1.4% in red deer and 0.7% in roe deer led to an overall seroprevalence of 4.6% in cervids. Interestingly none of the 83 investigated reindeer showed antibodies against TBEV.[91] One single case report describes the pathological and immunohistological findings in a mouflon (*Ovis ammon musimon*) with marked encephalitis due to TBEV.[92] A Polish study analyzed *D. reticulatus* collected from the lowland European bison (*Bison bonasus bonasus*) in a known endemic focus and found 18.42% of these ticks to be positive for TBEV RNA.[93] Seroprevalence in the bison themselves was found to be >60%.[94] In Japan, the seroprevalence in raccoons varied between 0.8% and 5.9% in eastern and central Hokkaido province while sika deer (*Cervus Nippon*) showed in TBEV-neutralizing antibodies in 0.8% and 2.4% there.[95] A recent case report of a sickened chamois in Italy is further challenging our view that wild animals do not suffer upon infection with TBEV and only seroconvert.[96] It might well be that we have not looked close enough in the past in sick and deceased wildlife. Interestingly, not much is known about the role of foxes (*Vulpes vulpes*) in natural TBE foci. Although it is a highly prevalent predator of small mammals (see below), and is regularly infested with *Ixodes* ticks, there are no recent studies investigating virus or antibodies against TBEV in foxes. Older studies from Germany were mostly performed in non-endemic areas on the German-Dutch border and Brandenburg, and consequently revealed no seroprevalence or a single sero-reactive serum sample

only.[97,98] However, the latter report found every third fox in South-Western Germany to have antibodies against TBEV.[98] In another study in Germany with a large sample size of more than 1200 fox sera, an overall prevalence >20% was found, again correlating with TBE endemic areas (>30% positive samples) versus non-endemic areas with just 13% seropositive fox samples.[99] In Croatia, a study found at least 1.6% of ticks on red foxes and 1.1% of spleen samples of red deer (*Cervus elaphus*) to be positive for TBEV-RNA.[100] It would be interesting and necessary to perform a seroprevalence study in a known endemic area to shed light on the role of the fox in the natural transmission cycle of TBEV and to prove the putative positive correlation between fox abundance and TBE incidence.[98,101]

Studies trying to detect a correlation between human TBE incidence and abundance of certain animals are contradictory. A Swedish study revealed that, with one year of time-lag, the abundance of roe deer, red deer, mountain hare (*Lepus timidus*) and European hare (*Lepus europaeus*) showed positive covariance with the incidence of human TBE.[102] In contrast, moose and fallow deer (*Dama dama*) showed negative covariance and wild boar, lynx (*Lynx lynx*) and red fox showed no significant covariance with human TBE incidence.[99] In Slovenia, red deer abundance was correlated with human TBE incidence when including a three-year time-lag, whereas roe deer showed no significant correlation.[103] An Italian study found roe deer abundance to have a better predictive value for a model explaining the increasing human TBE incidence than roe deer abundance.[104]

Small mammals

Small mammals have an essential role in the maintenance of TBE foci in 2 ways. Firstly, rodents and shrews are the main hosts for *Ixodes* larvae. Without this first blood meal, a tick population would die out over time. They are also, to a lesser extent, hosts for nymphs when they take their blood meal, which is needed before they can molt into adult ticks. Secondly, they are reservoir hosts for TBEV and thus responsible for infections of ticks. The reservoir function, however, has large implications for the longevity of a natural focus. As outlined earlier, in the chapter on transmission and natural cycle, infection of a tick can occur via a viremic host, but another phenomenon has been described which also applies to the infection of ticks while feeding on small mammals. The so-called co-feeding allows the infection of *Ixodes* larvae when an infected *Ixodes* nymph feeds in close proximity. In this case, the rodent does not have to be infected, because the virus finds its way from the nymph directly to the larva.[105] So, it is safe to say that, in many ways, rodents are as necessary as *Ixodes* ticks for maintaining the TBEV life-cycle. In particular bank voles (*Myodes glareolus*) appear to be well adapted to TBEV, leading to long-lasting viremias and infiltration of the brain without causing visible neurological symptoms.[106]

Recent publications have reviewed the prevalence of either viral ribonucleic acid (RNA) or specific antibodies against TBEV in rodents in various countries.[107-110] The antibody prevalence in endemic areas was found to range between 0% and 5.9%. However, seroprevalence rates up to 12.5% were found in some rodent species (e.g., the bank vole, *Myodes glareolus*),[111] suggesting a differing role of particular rodent species in a TBE focus. Viral RNA can also be found in wild rodents, with an even higher prevalence of up to 15%.[112] Studies from Hungary identified TBEV-RNA in 4.2%[113] and TBEV-specific anti-bodies in 5.2% and 4.9% of the tested small rodents.[114] Recently, TBEV-positive bank voles (and ticks) were found in a forest within the city borders of Moscow, Russia.[115] Experimentally infected common voles (*Microtus arvalis*) harbored infectious TBEV for at least 3 months.[112] Viral RNA could be found in the brain tissue of experimentally infected bank voles for up to 168 days.[116] This has important implications, as the brain (and to a lesser extent other organs such as kidney and spleen) seems to be the prime site of virus persistence in rodents. Indeed, TBE viral RNA was found in the brain tissue of naturally infected field voles (*Microtus agrestis*) and bank voles in Finland, after the winter but before the tick season started.[117] Seroprevalence in *Microtus* rodents were found to be 4% in Poland.[118] Thus rodents seem, along with transstadially-infected ticks, to play a role in the 'overwintering' of the TBEV.

Other mammals and birds

As most animals do not develop overt disease upon infection with TBEV, many mammal species have never been investigated as to whether or not they are susceptible to an infection or capable of developing an immune response in terms of measurable antibody titers. According to the broad geographic distribution of TBE covering most of Europe and northern Asia, we consider that there may be many mammal species not yet investigated that react to an infection in a similar manner as described above for wild boars or roe deer, i.e., seroconversion without clinical disease. One exception is the Barbary macaque (*Macaca sylvanus*), a monkey species not native to Eurasia, despite a small population in Gibraltar, the southernmost tip of Spain. An individual of a small group of these animals kept in southwest Germany in an outdoor area fell severely ill with central nervous symptoms and was euthanized for ethical reasons. A pan-encephalitis was diagnosed and TBEV was demonstrated by immunohistochemistry, real-time RT-PCR, and virus isolation.[119,120] Other individuals of this monkey group sero-converted without showing clinical signs.[121] Thus far, we are not aware of further case reports of non-native species kept in semi-free holdings or zoos.

Birds are known to be readily infested with ixodid ticks and are prime suspects for long-distance transportation of ticks.[122] The first studies investigating the prevalence of TBEV-harboring ticks on birds came from the Ottenby Bird

Observatory at the southern tip of the island Öland in Sweden. During the annual ringing, more than 1000 *Ixodes spp*. ticks were collected from birds, with 0.52% showing TBEV RNA.[123] Subsequent studies from Estonia (0.4% positive nymphs[124]), Switzerland (0.27% TBE viral RNA positive[125]), Latvia (14%[126]), Germany (no TBE virus found in almost 2500 *Ixodes ricinus* ticks collected from birds[122]) and Slovakia[127] (a brain sample in a buzzard, *Buteo buteo*) demonstrated the possibility that TBEV can be transported over rather long distances via infected ticks attached to birds.

Studies from the 1960s failed to demonstrate both viremia and clinical illness in great tits (*Parus major*), pheasants (*Phasianus colchicus*), falcons (*Falco tinnunculus*), and buzzards (*Buteo buteo*[51]). Only a small fraction of infected animals seroconverted. Other birds, such as the house sparrow (*Passer domesticus*), common redpoll (*Acanthis flammea*), quail (*Coturnix coturnix*), and duck (*Anas platyrhynchos*), showed either detect-able virus or even moderate viremia after infection.[128] Another study demonstrated that the presence of TBEV seems to vary according to season and bird species. Prevalence rates above 50% indicate that particular bird species like fieldfares (*Turdus pilaris*), bramblings (*Fringilla montifrigilla*), and the common redstart (*Phoenicurus phoenicurus*) may well play a role as a reservoir, or at least amplifying host, for TBEV.[129]

Veterinary diagnostic aspects

In general, the same diagnostic tests and methods are applied for animals as those that are currently in use for diagnostic purposes in humans (see Chapter 11: Diagnosis). With the exception of diseased dogs and horses, which are usually under tight supervision by their owner, the time window to use any direct detection method for TBEV – isolation or real-time RT-PCR – is usually too short to be of any practical relevance. Immuno-histochemistry may be used in euthanized animals. In epidemiological studies using rodents, these methods may be applied as virus and viral RNA can be detected in the brain tissue of infected animals for months (see above). In contrast, serology can be easily applied in any animal species. Three test formats are frequently used for this purpose, i.e., ELISA, IFA (immunofluorescence assay), and SNT. The ELISA can be performed with a species-specific conjugate, which is available for dogs, cattle, sheep, goats, swine (works also for wild boar), cervids, and mouse (works also for voles and mice). However, there is a commercially available, species-independent ELISA which uses protein G-coupled enzyme. Although this test is also available for immunoglobulin (Ig) M antibodies, the IgG version should be used because of the reasons mentioned above. The IFA usually uses a mixture of uninfected and TBEV-infected Vero cells fixed on slides and the antibody-conjugates described for the ELISA. Finally, the SNT is the gold standard and is needed in order to verify results of the other 2 assays. According to the European Centre for Disease Prevention and Control, an SNT titer =1:10 confirms the diagnosis.[35,39,130]

Concluding remarks

Infections of various animals with TBEV are common in TBE -endemic areas, although they are barely noticed due to the lack of overt disease. The known exceptions are dogs and horses, which can become severely ill with the same panel of clinical symptoms, as the same neurological regions in the CNS are affected. Domestic ruminants are a risk for human health as they can shed TBEV through their milk for many days. If unpasteurized, TBEV-contaminated milk or milk products are ingested by consumers, and clusters of human cases may be the consequence. Many wild animal species become infected and develop an antibody response, but they do not appear to be harmed. However, some recently published cases of clinical TBE in a single roe dear and a single chamois challenge this view. Future research may address the potential use of antibody prevalence rates of particular animal species in order to complement the current risk definition for human infections, which at the moment is largely based on the count of human cases alone. Finally, birds seem to play a role in long-distance transportation of TBEV-infected ticks and thus the geographic spread, while small mammals, in particular rodents, are the key players in maintaining a TBE focus in nature.

Contact: pfeffer@vetmed.uni-leipzig.de

Citation:
Pfeffer M, Schmuck HM, Leschnik M. TBE in animals. Chapter 10. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_10-7

References

1. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet*. 2008;371:1861-71.
2. Dobler G, Gniel D, Petermann R, Pfeffer M. Epidemiology and distribution of tick-borne encephalitis. *Wien Med Wochenschr*. 2012;162:230-8.
3. Leschnik M, Feiler A, Duscher G, Joachim A. Effect of owner-controlled acaricidal treatment on tick infestation and immune response to tick-borne pathogens in naturally infested dogs from Eastern Austria. *Parasit Vectors*. 2013;6:62.
4. Matile H, Ferrari E, Aeschlimann, A, Wyler R. Die Verbreitung der Zecken-enzephalitis in der Schweiz. *Schweiz Med Wochenschr*. 1981;111:1262-9.
5. Chambouris R, Sixl W, Stunzer D, Köck M. Antibodies in dogs to the virus of tick-borne encephalitis (early summer

encephalomyelitis/tick-borne encephalitis) in Greece. *Geogr Med*. 1989;3:11-14.

6. Balling A, Beer M, Gniel D, Pfeffer M. Prevalence of antibodies against tick-borne encephalitis virus in dogs from Saxony, Germany. *Berl Münch Tierärztl Wochenschr*. 2015;128:297-303.

7. Reiner B, Grasmück S, Steffen F, et al. Prevalence of TBE antibodies in serum and CSF of dogs with inflammatory and non-inflammatory CNS disease. *Int J Med Microbiol*. 2002;291 (Suppl. 33):234.

8. Roelandt S, Heyman P, Filette MD, et al. Tick-Borne Encephalitis Virus Seropositive Dog Detected in Belgium: Screening of the Canine Population as Sentinels for Public Health. *Vector Borne Zoonotic Dis.* 2011;11:1371-6.

9. Lindhe KES, Meldgaard DS, Jensen PM, Houser GA, Berendt M. Prevalence of tick-borne encephalitis virus antibodies in dogs from Denmark. *Acta Vet Scand*. 2009;51:56.

10. Klimeš J, Juřicová Z, Literák I, Schánilec P, Trachta e Silva E. Prevalence of antibodies to tick-borne encephalitis and West Nile flaviviruses and the clinical signs of tick-borne encephalitis in dogs in the Czech Republic. *Vet Rec*. 2001;148:17-20.

11. Hekrlová A, Kubíček O, Lány P, Rosenbergová K, Schánilec P. Tick-borne encephalitis in dogs: application of 'nested real-time RT-PCR' for intraviral virus detection. *Berl Münch Tierärztl Wochenschr*. 2015;128:397-401.

12. Csángó PA, Blakstad E, Kirtz GC, Pedersen JE, Czettel B. Tick-borne Encephalitis in Southern Norway. *Emerg Infect Dis*. 2004;10:533-4.

13. Levanov L, Perez Vera C, Vapalahti O. Prevalence estimation of tick-borne encephalitis virus (TBEV) antibodies in dogs from Finland using novel dog anti-TBVE IgG Mab-capture and IgG immunofluorescence assays based on recombinant TBEV subviral particles. *Ticks Tick Borne Dis*. 2016;7:979-82.

14. Kirtz G, Kölbl S, Czettel B, Thalhammer JG Frühsommer-Meningoenzephalitis (FSME, Zetraleuropäische Zeckenenzephalitis) beim Hund in Österreich: eine Seroprävalenz-Studie. *Kleintierpraxis.* 2003;48:133-40.

15. Wójcik-Fatla A, Cisak E, Zajac V, Zwoliński J, Dutkiewicz J. Prevalence of tick-borne encephalitis virus in Ixodes ricinus and Dermacentor reticulatus ticks collected from the Lublin region (eastern Poland). *Ticks Tick Borne Dis*. 2011;2:16-19.

16. Nygren TM, Pilic A, Böhmer MM, Wagner-Wiening C, Wichmann O, Harder T, Hellenbrand W Tick-Borne Encephalitis Risk Increases with Dog Ownership, Frequent Walks, and Gardening: A Case-Control Study in Germany 2018-2020. *Microorganisms*. 2022 Mar 23;10(4):690. doi: 10.3390/microorganisms10040690.

17. Girl P, Haut M, Riederer S, Pfeffer M, Dobler G. Comparison of Three Serological Methods for the Epidemiological Investigation of TBE in Dogs. *Microorganisms*. 2021 Feb 15;9 (2):399. doi: 10.3390/microorganisms9020399.

18. Pfeffer M, Dobler G. Tick-borne encephalitis virus in dogs--is this an issue? *Parasit Vectors*. 2011 Apr 13;4:59. doi: 10.1186/1756-3305-4-59.

19. Kleeb C, Golini L, Beckmann K, Torgerson P, Steffen F. Canine Tick-Borne Encephalitis: Clinical Features, Survival Rate and Neurological Sequelae: A Retrospective Study of 54 Cases (1999-2016). *Front Vet Sci*. 2021 Nov 10;8:782044. doi: 10.3389/fvets.2021.782044. eCollection 2021.

20. Potkonjak A, Petrović T, Ristanović E, Lalić I, Vračar V, Savić S, Turkulov V, Čanak G, Milošević V, Vidanović D, Jurišić A, Petrović A, Petrović V. Molecular Detection and Serological Evidence of Tick-Borne Encephalitis Virus in Serbia. *Vector Borne Zoonotic Dis*. 2017 Dec;17(12):813-820. doi: 10.1089/vbz.2017.2167.

21. Salat J, Hunady M, Schanilec P, Strakova P, Stefanik M, Svoboda P, Strelcova L, Bojcukova J, Palus M, Růžek D. Experimental and Natural Infections of Tick-Borne Encephalitis Virus in Dogs. *Viruses*. 2021 Oct 9;13(10):2039. doi: 10.3390/v13102039.

22. Topp AK, Springer A, Mischke R, Rieder J, Feige K, Ganter M, Nagel-Kohl U, Nordhoff M, Boelke M, Becker S, Pachnicke S, Schunack B, Dobler G, Strube C. Seroprevalence of tick-borne encephalitis virus in wild and domestic animals in northern Germany. *Ticks Tick Borne Dis*. 2023 Nov;14 (6):102220. doi: 10.1016/j.ttbdis.2023.102220.

23. Simkute E, Pautienius A, Grigas J, Urbute P, Stankevicius A. The Prevalence, Seroprevalence, and Risk Factors of Tick-Borne Encephalitis Virus in Dogs in Lithuania, a Highly Endemic State. *Viruses*. 2023 Nov 17;15(11):2265. doi: 10.3390/v15112265.

24. Andersson E, Kendall A, Url A, Auer A, Leschnik M. The first RT-qPCR confirmed case of tick-borne encephalitis in a dog in Scandinavia. *Acta Vet Scand*. 2020 Sep 10;62(1):51. doi: 10.1186/s13028-020-00550-2.

25. Dultz R, Goldhammer M. Tick-borne encephalitis in a dog. Tierarztl Prax Ausg K Kleintiere Heimtiere. 2021 Oct;49 (5):377-381. doi: 10.1055/a-1580-8386. [in German]

26. Sioutas G, Tsakou K, Top C, Jongejan F, Papadopoulos E. First clinical case of tick-borne encephalitis (TBE) in a dog in Greece. *Ticks Tick Borne Dis*. 2023 Nov;14(6):102226. doi: 10.1016/j.ttbdis.2023.102226.

27. Leschnik MW, Kirtz GC, Thalhammer JG. Tick-borne encephalitis (TBE) in dogs. *Int J Med Microbiol*. 2002;291 (Suppl. 33):66-9.

28. Leschnik MW, Benetka V, Url A, et al. Virale Enzephalitiden beim Hund in Österreich: diagnostische und epidemiologische Aspekte. *Vet Med Austria / Wien Tierärztl Monatsschr*. 2008;95:190-9.

29. Reiner B, Fischer A, Gödde T, Müller W. Clinical diagnosis of canine tick-borne encephalitis (tbe): Contribution of cerebrospinal fluid analysis (csf) and csf antibody titers. *Zent Bl Bakteriol*. 1999;289:605-9.

30. Leschnik M. *Tick-borne encephalitis in dogs*. Proc. Abildgaard Symposium, Kopenhagen, Dänemark. 2005:43-5.

31. Tipold A, Fatzer R, Holzmann H. Zentraleuropäische Zeckenencephalitis beim Hund. *Kleintierpraxis*. 1993;38:619-28.

32. Stadtbäumer K, Leschnik MW, Nell B. Tick-borne encephalitis virus as a possible etiologic agent for optic neuritis in a dog. *Vet Ophthal*. 2004;7:271-7.

33. Levanov L, Vera CP, Vapalahti O. Prevalence estimation of tick-borne encephalitis virus (TBEV) antibodies in dogs from Finland using novel dog anti-TBEV IgG MAb-capture and IgG immunofluorescence assays based on recombinant TBEV subviral particles. *Ticks Tick Borne Dis*. 2016 Jul;7(5):979-982. doi: 10.1016/j.ttbdis.2016.05.002.

34. Alnefelt Y, Van Meervenne S, Varjonen K, Tidholm A, Rohdin C. Evaluation of antibodies in cerebrospinal fluid for the diagnosis of tick-borne encephalitis in dogs. *Acta Vet Scand*. 2021 Aug 26;63(1):32. doi: 10.1186/s13028-021-00597-9.

35. Klaus C, Ziegler U, Kalthoff D, Hoffmann B, Beer M. Tick-borne encephalitis virus (TBEV) – findings on cross reactivity and longevity of TBEV antibodies in animal sera. *BMC Vet Res*. 2014;10:78.

36. Beckmann K, Steffen F, Ohlerth S, Kircher PR, Carrera I. Three tesla magnetic resonance imaging findings in 12 cases of canine central European tick-borne meningoencephalomyelitis. *Vet Radiol Ultrasound*. 2016;57:41-8.

37. Weissenböck H, Suchy A, Holzmann H. Tick-borne encephalitis in dogs: neuropathological findings and distribution of antigen. *Acta Neuropathol*. 1998;95: 361-6.

38. Sievert C, Hening R, Beckmann K, Kircher PR, Carrera I. Comparison between proton magnetic resonance spectroscopy findings in dogs with tick-borne encephalitis and clinically normal dogs. *Vet Radiol Ultrasound*. 2016;58:53-61.

39. Klaus C, Beer M, Saier R, Schubert H, Bischoff S, Süss J. Evaluation of serological tests for detecting tick-borne encephalitis virus (TBEV) antibodies in animals. *Berl Münch Tierärztl Wochenschr*. 2011;124:443-9.

40. Waldvogel K, Matile H, Wegmann C, Wyler R, Kunz C. Zeckenenzephalitis beim Pferd. *Schweiz Arch Tierheilkd*. 1981;123:227-33.

41. Luckschander N, Kölbl S, Enzensberger O, Zipko HT, Thalhammer JG. Tick-borne encephalitis (TBE) in an Austrian horse population. *Tierärztl Prax Ausgabe G*. 1999;27:235-8.

42. Müller K, König M, Thiel HJ. [Tick-borne encephalitis (TBE) with special emphasis on infection in horses]. *Dtsch Tierärztl Wochenschr*. 2006;113:147-51 [in German].

43. Klaus C, Hörügel U, Beer M. Tick-borne encephalitis virus (TBEV) infection in horses: Clinical and laboratory findings and epidemiological investigations. *Vet Microbiol.* 2013;163:368- 72.

44. de Heus P, Bagó Z, Weidinger P, Lale D, Trachsel DS, Revilla-Fernández S, Matiasek K, Nowotny N. Severe Neurologic Disease in a Horse Caused by Tick-Borne Encephalitis Virus, Austria, 2021. *Viruses*. 2023 Sep 29;15(10):2022. doi: 10.3390/v15102022.

45. Rushton JO, Lecollinet S, Hubálek Z, et al. Tick-borne encephalitis virus in horses, Austria, 2011. *Emerg Infect Dis*. 2013;19:635-7.

46. Ganzenberg S, Sieg M, Ziegler U, Pfeffer M, Vahlenkamp TW, Hörügel U, Groschup MH, Lohmann KL. Seroprevalence and Risk Factors for Equine West Nile Virus Infections in Eastern Germany, 2020. *Viruses*. 2022 May 30;14(6):1191. doi: 10.3390/v14061191.

47. Gothe LMR, Ganzenberg S, Ziegler U, Obiegala A, Lohmann KL, Sieg M, Vahlenkamp TW, Groschup MH, Hörügel U, Pfeffer M. Horses as Sentinels for the Circulation of Flaviviruses in Eastern-Central Germany. *Viruses*. 2023 Apr 30;15(5):1108. doi: 10.3390/v15051108.

48. Janitza-Futterer D. Serologische Untersuchungen zur endemischen Situation der Infektion mit dem FSME-Virus in einer südbadischen Pferde- und Hundepopulation. *Vetmed Diss Munich*. 2003.

49. Sikutova S, Hornok S, Hubalek Z, et al. Serological survey of domestic animals for tick-borne encephalitis and Bhanja viruses in northeastern Hungary. *Vet Microbiol*. 2009;135:267 -71.

50. Sedlák K, Zelená H, Křivda V, Šatrán P. Surveillance of West Nile fever in horses in the Czech Republic from 2011 to 2013. *Epidemiol Mikrobiol Immunol*. 2014; 63:307-11.

51. Camino E, Schmid S, Weber F, Pozo P, de Juan L, König M, Cruz-Lopez F. Detection of antibodies against tick-borne encephalitis flaviviruses in breeding and sport horses from Spain. *Ticks Tick Borne Dis*. 2020 Sep;11(5):101487. doi: 10.1016/j.ttbdis.2020.101487.

52. Vilibic-Cavlek T, Krcmar S, Bogdanic M, Tomljenovic M, Barbic L, Roncevic D, Sabadi D, Vucelja M, Santini M, Hunjak B, Stevanovic V, Boljfetic M, Bjedov L, Masovic V, Potocnik-Hunjadi T, Lakoseljac D, Al-Mufleh M, Savic V. An Overview of Tick-Borne Encephalitis Epidemiology in Endemic Regions of Continental Croatia, 2017-2023. *Microorganisms*. 2024 Feb 13;12(2):386. doi: 10.3390/microorganisms12020386.

53. Pautienius A, Armonaite A, Simkute E, Zagrabskaite R, Buitkuviene J, Alpizar-Jara R, Grigas J, Zakiene I, Zienius D, Salomskas A, Stankevicius A. Cross-Sectional Study on the Prevalence and Factors Influencing Occurrence of Tick-Borne Encephalitis in Horses in Lithuania. *Pathogens*. 2021 Jan 31;10(2):140. doi: 10.3390/pathogens10020140.

54. Cleton NB, van Maanen K, Bergervoet SA, et al. A serological protein microarray for detection of multiple cross-reactive flavivirus infections in horses for veterinary and public health surveillance. *Transbound Emerg Dis*. 2017; 64:1801–12

55. Gresiková M. Excretion of the tick-borne encephalitis virus in the milk of subcutaneously infected cows. *Acta Virol*. 1958;2:188-92.

56. Nosek J, Kozuch O, Ernek E, Lichard M. The importance of goats in the maintenance of tick-borne encephalitis virus in nature. *Acta Virol*. 1967;11:470-82.

57. Böhm B, Schade B, Bauer B, Hoffmann B, Hoffmann D, Ziegler U, Beer M, Klaus C, Weissenböck H, Böttcher J. Tick-borne encephalitis in a naturally infected sheep. *BMC Vet Res*. 2017;13:267.

58. Balogh Z, Egyed I, Ferenczi E, et al. Experimental infection of goats with tick-borne encephalitis virus and possibilities to

prevent virus transmission by raw goat milk. *Intervirology*. 2012;55:194-200.

59. Rieille N, Klaus C, Hoffmann D, Péter O, Voordouw M. Goats as sentinel hosts for the detection of tick-borne encephalitis risk areas in the Canton of Valais, Switzerland, *BMC Vet Res*. 2017;13:217.

60. Casati Pagani S, Frigerio Alossa S, Klasu C, Hoffmann D, Beretta O, Bomio-Pacciorini N, Lazzaro M, Merlani G, Ackermann R, Beuret C. First detection of TBE virus in ticks and sero-reactivity in goats in a non-endemic region in the southern part of Switzerland (Canton of Ticino). *Ticks Tick-Borne Dis*. 2019;10:868-874.

61. Bauer BU, Könenkamp L, Stöter M, Wolf A, Ganter M, Steffen I, Runge M. Increasing awareness for tick-borne encephalitis virus using small ruminants as suitable sentinels: Preliminary observations. *One Health*. 2021 Feb 20;12:100227. doi: 10.1016/j.onehlt.2021.100227.

62. Wallenhammar A, Lindqvist R, Asghar N, Gunaltay S, Fredlund H, Davidsson Å, Andersson S, Överby AK, Johansson M. Revealing new tick-borne encephalitis virus foci by screening antibodies in sheep milk. *Parasit Vectors*. 2020 Apr 8;13(1):185. doi: 10.1186/s13071-020-04030-4.

63. Dorko E, Hockicko J, Rimárová K, Bušová A, Popad'ák P, Popad'áková J, Schréter J. Milk outbreaks of tick-borne encephalitis in Slovakia, 2012-2016. *Cent Eur J Public Health* 2018;26(Suppl):S47-S50.

64. Kerlik J, Avdičová M, Štefkovičová M, Tarkovská V, Pántiková Valachová M, Molčányi T, Mezencev R. Slovakia reports highest occurrence of alimentary tick-borne encephalitis in Europe: Analysis of tick-borne encephalitis outbreaks in Slovakia during 2007-2016. *Travel Med Infect Dis*. 2018;26:37-42.

65. Markovinović L, Kosanović Ličina ML, Tešić V, Vojvodić D, Vladušić Lucić I, Kniewald T, Vukas T, Kutleša M, Krajinović LC. An outbreak of tick-borne encephalitis associated with raw goat milk and cheese consumption, Croatia, 2015. *Infection.* 2016 Oct;44(5):661-5. doi: 10.1007/s15010-016-0917-8.

66. Ilic M, Barbic L, Bogdanic M, Tabain I, Savic V, Kosanovic Licina ML, Kaic B, Jungic A, Vucelja M, Angelov V, Kovacevic M, Roncevic D, Knezevic S, Stevanovic V, Slavuljica I, Lakoseljac D, Vickovic N, Bubonja-Sonje M, Hansen L, Vilibic-Cavlek T. Tick-borne encephalitis outbreak following raw goat milk consumption in a new micro-location, Croatia, June 2019. *Ticks Tick Borne Dis*. 2020 Nov;11(6):101513. doi: 10.1016/j.ttbdis.2020.101513.

67. Brockmann SO, Oehme R, Buckenmaier T, Beer M, Jeffery-Smith A, Spannenkrebs M, Haag-Milz S, Wagner-Wiening C, Schlegel C, Fritz J, Zange S, Bestehorn M, Lindau A, Hoffmann D, Tiberi S, Mackenstedt U, Dobler G. A cluster of two human cases of tick-borne encephalitis (TBE) transmitted by unpasteurised goat milk and cheese in Germany, May 2016. *Euro Surveill*. 2018;23(15):pii=17-00336.

68. Cisak E, Wójcik-Fatla A, Zajac V, et al. Prevalence of tick-borne encephalitis virus (TBEV) in samples of raw milk taken randomly from cows, goats and sheep in eastern Poland. *Ann Agric Env Med*. 2010;17:283-6.

69. Paulsen KM, Stuen S, das Neves CG, Suhel F, Gurung D, Soleng A, Stiasny K, Vikse R, Andreassen ÅK, Granquist EG. Tick-borne encephalitis virus in cows and unpasteurized cow milk from Norway. *Zoonoses Public Health*. 2019;66:212-222.

70. Klaus C, Ziegler U, Hoffmann D, Press F, Fast C, Beer M. Tick-borne encephalitis virus (TBEV) antibodies in animal sera – occurence in goat flocks in Germany, longevits and ability to recall immunological information after more than six years. *BMC Veterinary Res*. 2019;15:399.

71. Klaus C, Hoffmann B, Moog U, et al. Can goats be used as sentinels for Tick-borne encephalitis (TBE) in non-endemic areas? Experimental studies and epizootiological observations. *Berl Münch Tierärztl Wochenschr*. 2010;123:441-5.

72. Klaus C, Beer M, Saier R, et al. Goats and sheep as sentinels for tick-borne encephalitis (TBE) virus – Epidemiological studies in areas endemic and non-endemic for TBE virus in Germany. *Ticks Tick-Borne Dis*. 2012;3:27-37.

73. Klaus C, Hoffmann D, Hoffmann B, Beer M. Frühsommer-Meningoenzephalitis-Virus-Infektionen bei Tieren – Klinik, Diagnostik und epidemiologische Bedeutung. *Berl Münch Tierärztl Wochenschr*. 2016;130:102-12.

74. Vor T, Kiffner C, Hagedorn P, Niedrig M, Ruhe F. Tick burden on European roe deer (Capreolus capreolus). *Exp Appl Acarol*. 2010;51:405-17.

75. Jahfari S, de Vries A, Rijks JM, van Gucht S, Vennema H, Sprong H, Rockx B. Tick-Borne Encephalitis Virus in Ticks and Roe Deer, the Netherlands, Emerg Infect Dis. 2017;23:1028–1030.

76. Da Rold G, Obber F, Monne I, Milani A, Ravagnan S, Toniolo F, Sgubin S, Zamperin G, Foiani G, Vascellari M, Drzewniokova P, Castellan M, De Benedictis P, Citterio CV. Clinical Tick-Borne Encephalitis in a Roe Deer (Capreolus capreolus L.). *Viruses*. 2022 Jan 31;14(2):300. doi: 10.3390/v14020300.

77. Gerth HJ, Grimshandl D, Stage B, Döller G, Kunz C. Roe deer as sentinels for endemicity of tick-borne encephalitis virus. *Epidemiol Infect*. 1995;115:355-65.

78. Van der Poel WH, Van der Heide R, Bakker D, et al. Attempt to detect evidence for tick-borne encephalitis virus in ticks and mammalian wildlife in the Netherlands. *Vector Borne Zoonotic Dis*. 2005;5:58-64.

79. Skarphédinsson S, Jensen PM, Kristiansen K. Survey of tick-borne infections in Denmark. *Emerg Infect Dis*. 2005;11:1055-61.

80. Kiffner C, Vor T, Hagedorn P, Niedrig M, Rühe F. Determinants of tick-borne encephalitis virus antibody presence in roe deer (*Capreolus capreolus*) sera. *Med Vet Entomol*. 2012;26:18-25.

81. Ytrehus B, Vainio K, Dudman SG, Gilray J. Tick-borne encephalitis virus and Louping-Ill virus may co-circulate in southern Norway. *Vector Borne Zoonotic Dis*. 2013;13:762-8.

82. Balling A, Plessow U, Beer M, Pfeffer M. Prevalence of antibodies against tick-borne encephalitis virus in wild game from Saxony, Germany. Ticks Tick Borne Dis. 2014;5:805-9.

83. Duscher GG, Wetscher M, Baumgartner R. Roe deer sera used for TBE surveillance in Austria. *Ticks Tick Borne Dis*. 2015;6:489-93.

84. Frimmel S, Leister M, Löbermann M, Feldhusen F, Seelmann M, Süss J, Reisinger EC. Seroprevalence of tick-borne-encephalitis virus in wild game in Mecklenburg-Western Pomerania (north-eastern Germany). *Ticks Tick Borne Dis*. 2016;7:1151–4.

85. Roelandt S, Suin V, Van der Stede Y, et al. First TBEV serological screening in Flemish wild boar. *Infect Ecol Epidemiol*. 2016;6:31099.

86. Donoso Mantke O, Escadafal C, Niedrig M, Pfeffer M. Tick-borne encephalitis in Europe, 2007 to 2009. *Euro Surveill*. 2011;16:pii=19976.

87. Kunze U, the ISW-TBE. Tick-borne encephalitis – still on the map. Report of the 18th annual meeting of the international scientific working group on tick-borne encephalitis (ISW-TBE). *Ticks Tick Borne Dis*. 2016;7:911-4.

88. Bournez L, Umhang G, Faure E, Boucher J-M, Boué F, Jourdain E, Sarasa M, Llorente F, Jiménez-Clavero MA, Moutailler S, Lacour SA, Lecollinet S, Beck C. Exposure of wild ungulates to the Usutu and Tick-borne encephalitis viruses in France in 2009-2014: Evidence of undetected flavivirus circulation a decade ago. *Viruses*. 2020;12:10.

89. Kríz B, Daniel M, Benes C. The role of game (wild boar and roe deer) in the spread of tick-borne encephalitis in the Czech Republic. *Vector Borne Zoonotic Dis*. 2014;14:801-7.

90. Tonteri E, Jokelainen P, Matala J, Pusenius J, Vapalahti O. Serological evidence of tick-borne encephalitis virus infection in moose and deer in Finland: sentinels for virus circulation. *Parasit Vectors.* 2016;9:54.

91. Paulsen KM, das Neves CG, Granquist EG, Madslien K, Stuen S, Pedersen BN, Viske R, Rocchi M, Laming E, Stiasny K, Andreassen ÅK. Cervids as sentinel-species for tick-borne encephalitis virus in Norway – a serological study. *Zoonoses Public Health*. 2019 Dec 19;[online ahead of print]

92. Bagó Z, Bauder B, Kolodziejek J, Nowotny N, Weissenböck H. Tick-borne encephalitis in a mouflon (Ovis ammon musimon). Vet Rec. 2002;150:218-20.

93. Biernat B, Karbowiak G, Stańczak J, Masny A, Werszko J. The first detection of the tick-borne encephalitis virus (TBEV) RNA in *Dermacentor reticulatus* ticks collected from the lowland European bison (Bison bonasus L.). *Acta Parasitologica*. 2016;61:130–5.

94. Krzysiak MK, Anusz K, Konieczny A, Rola J, Salat J, Strakova P, Olech W, Larska M. The European bison (Bison bonasus) as an indicatory species for the circulation of tick-borne encephalitis virus (TBEV) in natural foci in Poland. *Ticks Tick Borne Dis*. 2021 Nov;12(6):101799. doi: 10.1016/j.ttbdis.2021.101799.

95. Jamsransuren D, Kentaro Y, Hiroaki K, Mitsuhiko A, Kei O, Kei F, et al. Epidemiological survey of tick-borne encephalitis virus infection in wild animals an Hokkaido and Honshu islands, Japan. *Japanese J Veterinary Res*. 2019;67:163-172.

96. Gaffuri A, Sassera D, Calzolari M, Gibelli L, Lelli D, Tebaldi A, Vicari N, Bianchi A, Pigoli C, Cerioli M, Zandonà L, Varisco G, Bertoletti I, Prati P. Tick-Borne Encephalitis, Lombardy, Italy. *Emerg Infect Dis*. 2024 Feb;30(2):341-344. doi: 10.3201/eid3002.231016.

97. Wurm R, Dobler G, Peters M, Kiessig ST. Serological investigations of red foxes (Vulpes vulpes L.) for determination of the spread of tick-borne encephalitis in North Rhine-Westphalia. *J Vet Med B Infect Vet Public Health*. 2000;47:503-9.

98. Rieger MA, Nübling M, Müller W, Hasselhorn HM, Hofmann F. Foxes as indicators for TBE endemicity – a comparative serological and investigation. *Zentralbl Bakteriol*. 1999;289:610-18.

99. Haut M, Girl P, Oswald B, Romig T, Obiegala A, Dobler G, Pfeffer M. The Red Fox (Vulpes vulpes) as Sentinel for Tick-Borne Encephalitis Virus in Endemic and Non-Endemic Areas. *Microorganisms*. 2020 Nov 18;8(11):1817. doi: 10.3390/microorganisms8111817.

100. Jemeršić L, Dežđek D, Brnić D, Prpić J, Janicki Z, Keros T, Roić B, Slavica A, Terzić S, Konjević D, Beck R. Detection and genetic characterization of tick-borne encephalitis virus (TBEV) derived from ticks removed from red foxes (Vulpes vulpes) and isolated from spleen samples of red deer (*Cervus elaphus*) in Croatia. *Ticks Tick Borne Dis*. 2014;5:7-13.

101. Haemig PD, Lithner S, Sjöstedt de Luna, S, et al. Red fox and tick-borne encephalitis (TBE) in humans: Can predators influence public health. *Scand J Infect Dis*. 2008;40:527-32.

102. Jaenson TGT, Petersson EH, Jaenson DGE, Kindberg J, Pettersson JH, Hjertqvist M, Medlock JM, Bengtsson H. The importance of wildlife in the ecology and epidemiology of the TBE virus in Sweden: incidence of human TBE correlates with abundance of deer and hares, *Parasit Vectors*. 2018;11:477.

103. Knap N, Avšič-Županc T. Correlation of TBE Incidence with Red Deer and Roe Deer Abundance in Slovenia. *PLoS ONE*. 2013;8:e66380.

104. Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosà R Forest Structure and Roe Deer Abundance Predict Tick-Borne Encephalitis Risk in Italy. *PLoS ONE*. 2009;4:e4336.

105. Labuda M, Kozuch O, Zuffová E, et al. Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology*. 1997; 235:138-43.

106. Michelitsch A, Tews BA, Klaus C, Bestehorn-Willmann M, Dobler G, Beers M, Wernike K. In vivo characterization of tick-borne encephalitis virus in bank voles (*Myodes glareolus*). *Viruses*. 2019;11:1069.

107. Imhoff M, Hagedorn P, Schulze Y, et al. Review: Sentinels of tick-borne encephalitis risk. *Ticks Tick Borne Dis*. 2015;6:592 - 600.

108. Jääskeläinen A, Tonteri E, Pieninkeroinen I, Sironen T, Voutilainen L, Kuusi M, Vaheri A, Vapalahti O. Siberian subtype tick-borne encephalitis virus in *Ixodes ricinus* in a

newly emerged focus, Finland. *Ticks Tick borne Dis*. 2016;7:216-23.

109. Smura T, Tonteri E, Jääskeläinen A, vonTroil G, Kuivanen S, Huitu O, Kareinen L, Uusitalo J, Uusitalo R, Hannila-Handelberg T, Voutilainen L, Nikkari S, Sironen T, Sane J, Castén J, Vapalahti O. Recent establishment of tick-borne encephalitisfoci with distinct viral lineages in the Helsinki area, Finland. *Emerging Microbes & Infect*. 2019;8:675-683.

110. Brandenburg PJ, Obiegala A, Schmuck HM, Dobler G, Chitimia-Dobler L, Pfeffer M. Seroprevalence of Tick-Borne Encephalitis (TBE) Virus Antibodies in Wild Rodents from Two Natural TBE Foci in Bavaria, Germany. *Pathogens.* 2023 Jan 25;12(2):185. doi: 10.3390/pathogens12020185.

111. Knap N, Korva M, Dolinšek V, et al. Patterns of tick-borne encephalitis virus infection in rodents in Slovenia. *Zoonotic Dis*. 2012;12:236-42.

112. Achazi K, Ruzek D, Donoso-Mantke, O, et al. Rodents as Sentinels for the Prevalence of Tick-Borne Encephalitis Virus. *Vector-Borne Zoonotic Dis.* 2011;11: 641-7.

113. Pintér R, Madai M, Horváth G, Németh V, Oldal M, Kemenesi G, Dallos B, Bányai K, Jakab F. Molecular detection and phylogenetic analysis of tick-borne encephalitis virus in rodents captured in the transdanubian region of Hungary. *Vector Borne Zoonotic Dis*. 2014;14:621-4.

114. Zöldi V, Papp T, Rigó K, Farkas J, Egyed L. A 4-Year Study of a Natural Tick-Borne Encephalitis Virus Focus in Hungary. 2010–2013. *EcoHealth*. 2015;12:174–82.

115. Makenov M, Karan L, Shashina N, Akhmentshina M, Zhurenkova O, Kholodilov I, Karganova G, Smirnova N, Grigoreva Y, Yankovskaya Y, Fyodorova M. First detection of tick-borne encephalitis virus in Ixodes ricinus ticks and their rodent hosts in Moscow, Russia. *Ticks Tick-Borne Dis.* 2019;10:101265.

116. Tonteri E, Kipar A, Voutilainen L, et al. The three subtypes of tick-borne encephalitis virus induce encephalitis in a natural host, the bank vole (Myodes glareolus). *PLoS ONE*. 2013;8:e81214.

117. Tonteri E, Jääskeläinen AE, Tikkakoski T, et al. Tick-borne encephalitis virus in wild rodents in winter, Finland (2008-2009). *Emerg Infect Dis*. 2011;17:72-5.

118. Grzybek M, Tolkacz K, Alsarraf M, Dwużnik D, Szczepaniak K, Tomczuk K, Biernat B, Behnke JM, Bajer A. Seroprevalence of tick-borne encephalitis virus in three species of voles (Microtus spp.) in Poland. *J Wildlife Dis*. 2020;56;in press.

119. Süss J, Gelpi E, Klaus C, et al. Tick-borne encephalitis in naturally exposed monkey (Macaca sylvanus). *Emerg Infect Dis*. 2007;13:905-7.

120. Süss J, Dobler G, Zöller G, et al. Genetic characterization of a tick-borne encephalitis virus isolated from the brain of a naturally exposed monkey (Macaca sylvanus). *Int J Med Microbiol*. 2008;298(Suppl. 44):295-300.

121. Klaus C, Hoffmann B, Beer M, et al. Seroprevalence of tick-borne encephalitis (TBE) in naturally exposed monkeys (Macaca sylvanus) and sheep and prevalence of TBE virus in ticks in a TBE endemic area in Germany. *Ticks Tick Borne Dis*. 2010;1:141-4.

122. Klaus C, Gethmann J, Hoffmann B, et al. Tick infestation in birds and prevalence of pathogens in ticks collected from different places in Germany. *Parasitol Res*. 2016;115:2729-40.

123. Waldenström J, Lundkvist A, Falk KI, et al. Migrating birds and tick-borne encephalitis virus. *Emerg Infect Dis*. 2007;13:1215- 8.

124. Geller J, Nazarova L, Katargina O, et al. Tick-borne pathogens in ticks feeding on migratory passerines in western part of Estonia. *Vector Borne Zoonotic Dis.* 2013;13:443-8.

125. Lommano E, Dvořák C, Vallotton L, Jenni L, Gern L. Tick-borne pathogens in ticks collected from breeding and migratory birds in Switzerland. *Ticks Tick Borne Dis*. 2014;5:871-82.

126. Kazarina A, Japina K, Keiss O, et al. Detection of tick-borne encephalitis virus in I. ricinus ticks collected from autumn migratory birds in Latvia. *Ticks Tick Borne Dis.* 2015;6:178-80.

127. Csank T, Bhide K, Bencúrová E, Dolinská S, Drzewnioková P, Major P, Korytár Ľ, Bocková E, Bhide M, Pistl J. Detection of West Nile virus and tick-borne encephalitis virus in birds in Slovakia, using a universal primer set. *Arch Virol.* 2016; 161:1679–1683.

128. Hubálek Z, Rudolf I. Tick-borne viruses in Europe. *Parasitol Res*. 2012;111:9-36.

129. Mikryukova TP, Moskvitina NS, Kononova YV, et al. Surveillance of tick-borne encephalitis virus in wild birds and ticks in Tomsk city and its suburbs (Western Siberia). *Ticks Tick Borne Dis*. 2014;5:145-51.

130. ECDC (European Centre for Disease Prevention and Control) *Epidemiological situation of tick – borne encephalitis in the European Union and European Free Trade Association countries*. Stockholm: ECDC; http://www.ecdc.europa.eu/en/ publications/Publications/TBE-in-EU-EFTA.pdf. 2012.

131. García-Bocanegra I, Jurado-Tarifa E, Cano-Terriza D, Martínez R, Pérez-Marín JE, Lecollinet S. Exposure to West Nile virus and tick-borne encephalitis virus in dogs in Spain. *Transbound Emerg Dis.* 2018;65:765-772.

132. Salat J, Hunady M, Svoboda P, Strelova L,Strakova P, Fortova A, Paulus M, Ruzek D. Efficacy and immunogenicity of a veterinary vaccine candidate against tick-borne encephalitis in dogs. *Vaccine*. 2023; 41:6150-55.

Chapter 11

Diagnostic of TBEV-infections

Gerhard Dobler

Key points

- TBE appears with non-characteristic clinical symptoms, which cannot be distinguished from other forms of viral meningitis or encephalitis or other diseases.
- Cerebrospinal fluid and neuro-imaging may give some evidence of TBE, but ultimately cannot confirm the diagnosis.
- Thus, proving the diagnosis "TBE" necessarily requires confirmation of TBEV-infection by detection of the virus or by demonstration of specific antibodies from serum and/or cerebrospinal fluid.
- During the phase of clinical symptoms from the CNS, the TBEV can only rarely be detected in the cerebrospinal fluid of patients.
- Most routinely used serological tests for diagnosing TBE (ELISA, HI, IFA) show cross reactions resulting either from infection with other flaviviruses or with other flavivirus vaccines

Clinical confirmation of suspected TBEV infection

Tick-borne encephalitis (TBE) manifests as a non-specific disease with symptoms of a febrile, influenza-like illness and, in some cases, an inflammatory infection of the central nervous system (CNS) that follows a few days later. Due to the lack of specific symptoms, a definitive confirmation of the diagnosis requires taking the history of the patient with regard to a possible tick bite or ingestion of unpasteurized milk in a known or suspected endemic area, plus a positive result from a classical virological test that confirms TBEV-infection either directly by the detection of virus or indirectly via detection of specific anti-virus antibodies.[1] Prior to the introduction of molecular detection technologies such as polymerase chain reaction (PCR), the only technique available to detect TBEV infection was virus isolation, but this is rarely used today.

The most common method of detecting TBEV infection nowadays is via serological assays, which have been developed from complement fixation or hemagglutination inhibition tests, through to modern immunoglobulin (Ig)-specific tests such as ELISAs and immunofluorescence (IF) assays.

Understanding of the pathogenesis and immunology of TBEV infection is essential for the selection and interpretation of appropriate diagnostic tests (Fig. 1). For example, the European subtype of TBEV often induces a biphasic clinical course, whereas a monophasic course may be more prominent in those infected with the Far Eastern subtype or Siberian subtype.[2] Following a bite from an infected tick, the virus is assumed to replicate locally within antigen-presenting cells and then subsequently within nearby lymph nodes. After replicating within the lymph nodes, the virus then spreads to the internal organs via the lymph and blood (causing viremia) and begins to replicate within the reticuloendothelial system.[3] It is during this phase of the disease that the infected individual will often show non-specific, influenza-like symptoms. These symptoms will then begin to improve for several days before a second phase appears in up to 30% of infected individuals, and which includes CNS involvement varying in severity from meningeal irritation to meningoencephalo-myelitis and even death. The choice of whether a specific patient should be tested using an assay that directly or indirectly detects TBEV infection therefore depends on the phase of the infection of a given patient.

Direct detection of TBEV infection

Virus isolation

The isolation of TBEV was the first diagnostic technique established for the confirmation of clinically suspicious CNS infections such as TBE. In the past, virus isolation from blood and brain samples was performed in newborn mice, with many of the 'old' TBEV strains (e.g., Scharl, Absettarov, Sofjin, KEM II, Alsace, Schaffhausen, etc.) isolated by intracerebral inoculation of patient material or tick suspensions. Cell culture was subsequently introduced and there are now a number of immortalized cell lines that can be used to isolate TBEV from patient material. The most frequently used cell lines are currently PS cells (porcine fetal kidney cells), Vero cells (green monkey fetal kidney cells), BHK-21 (baby hamster kidney cells), and A549 cells (human lung adenocarcinoma cells), although other lineages such as human neuroblastoma cells may also be used.

Figure 1: Natural course of TBE with clinical symptoms, virus replication, and evolution of specific anti-TBE antibodies

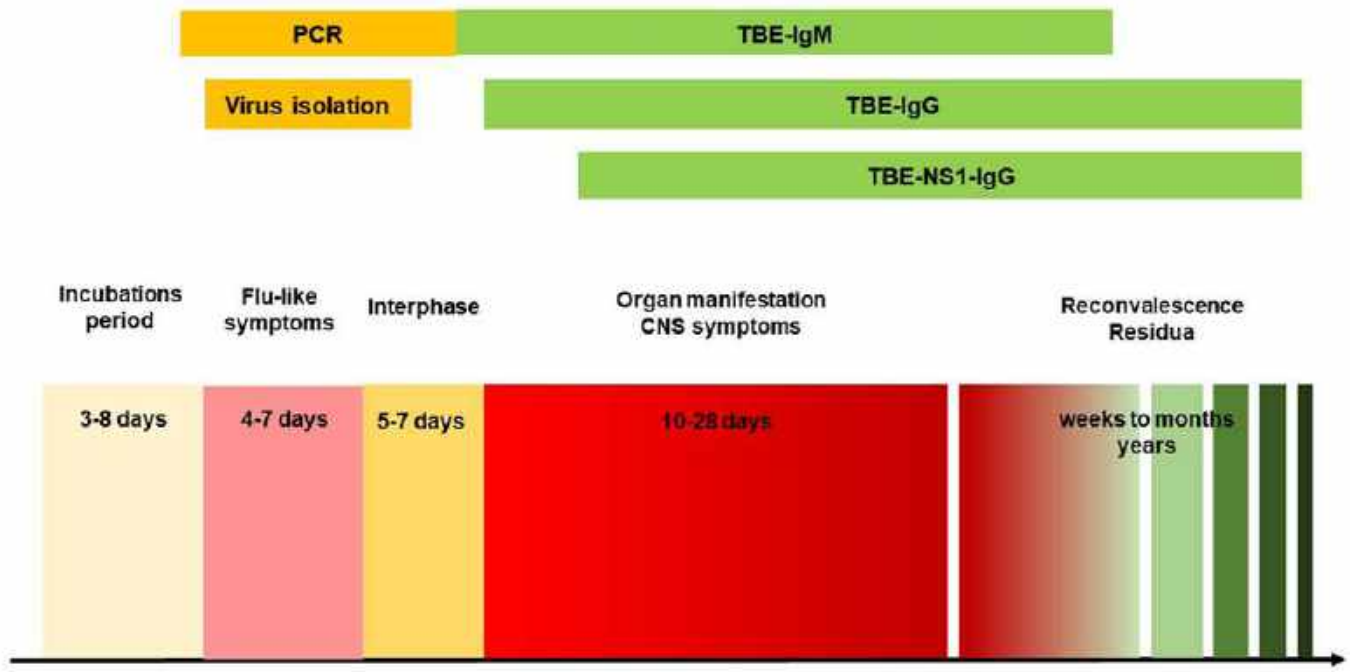


Table 1: Detection of TBEV by RT-PCR in patient samples according to stage of infection[4]

Antibody status	Serum	Blood	CSF	Brain tissue
IgM-/IgG-	30/30 (100%)	19/19 (100%)	1/10 (10%)	-
IgM+/IgG-	3/13 (23%)	3/5 (60%)	0/2 (0%)	-
IgM+/IgG+	1/34 (3%)	1/6 (16%)	0/19 (0%)	1/1 (100%)

Virus can be detected in an infected individual's blood during the first febrile phase of the disease and can be detected predominantly in brain tissue during the second phase involving neurologic symptoms.[4] The cerebrospinal fluid (CSF) does not usually contain viable virus and should therefore only be used for virus isolation under special circumstances. No systematic studies on the discharge of viable TBEV in the urine of patients infected with TBEV are available to date, but discharging in an immuno-compromised patient was observed to last for at least 56 days[5] and intermittent discharging in urine was observed for a period of more than 700 days in experimentally infected monkeys.[6]

Virus isolation is no longer routinely used for diagnosis of a TBE infection but is still needed to identify the subtype of TBEV present in brain tissue samples from fatal cases or in blood samples taken during the febrile phase of the disease. Virus isolation is also used to isolate TBEV strains from other biological material (e.g., ticks, rodents, etc.) for use in subsequent genetic and phenotypic characterization.

PCR

The current technology of choice for the detection of TBEV is PCR, and there are several formats available. The earliest PCR-based method for detecting TBEV infection was nested RT-PCR,[7–9] but a number of real-time RT-PCR assays for the detection of viral ribonucleic acid (RNA) in various clinical and biological samples have also been described.[10] PCR-based methods have no clear role in the diagnosis of TBEV infection during the phase involving CNS symptoms because viral RNA cannot usually be detected in blood or CSF samples during this phase of the disease.[4,8] However, TBEV can be detected in blood samples during the first febrile phase of TBE as well as in brain tissue (if available) during the phase involving CNS symptoms. The RT-PCR format is therefore a valuable diagnostic tool when there is a need to confirm an infection with TBEV as the cause of a febrile illness following a tick bite, or when confirmation of a TBEV infection is sought in fatal cases. A recent Swedish study reported that TBEV RNA could also be detected by RT-PCR

in urine samples from patients for up to 19 days after the start of neurologic symptoms.[11] Another application of RT-PCR in this setting is the diagnosis of potential TBEV infections in immunosuppressed patients unable to develop antibodies to the virus. In these cases, TBEV RNA may be detectable within blood and CSF samples over a longer period of time compared with immunocompetent patients. Detectable TBEV was reported to be shed over a period of at least 56 days in 1 immunocompromised patient.[5]

Indirect detection of TBEV infection

Purified antigenic components of the TBEV particle are essential in order to be able to detect antibodies produced by a potential host. The main immunodominant structure of a TBEV particle is the dimeric envelope (E) protein, which induces hemagglutinating, neutralizing, and protective antibodies following infection or immunization. The capsid protein and nonstructural protein 1 (NS1) are antigens against which the host generates complement-fixing antibodies. A more detailed description of the proteins encoded by the TBEV genome can be found in Chapter 2b.

Complement fixation assay

The complement fixation assay (CFA) is one of the oldest tests for detecting antibodies against TBEV and other flaviviruses,[12] and was used to detect anti-virus antibodies in the early phase of a potential infection. The CFA cannot differentiate between different antibody isotypes, however, because IgM and IgG (IgG1, IgG2, and IgG3 subclasses) can all bind complement. Early data showed that infected individuals display a marked increase in the generation of complement-fixing antibodies during the second phase of the infection involving CNS symptoms, about 10-14 days after being infected.[13] The titer of complement-fixing antibodies reaches a peak after 5-10 weeks and then decreases to a lower level or disappears completely following a period of up to 1 year. The detection of complement-fixing antibodies is therefore an indicator of an acute or recent TBEV infection. The test usually involves demonstrating a significant increase in antibody titer in 2 serum samples taken 10-14 days apart. During the acute phase of the disease, a 3- to 4-fold increase in titer may be expected. The CFA is cross-reactive with antibodies against other flaviviruses and can also give positive results for some time after a TBE vaccination. The CFA relies on the quality of the reagents used being excellent, especially the TBEV antigen (which was formerly mouse brain extract but extracts from infected cell cultures were subsequently used). The introduction of modern, standardized, less time-consuming assays and the lack of antigen of appropriate quality means that the CFA is now obsolete.

Hemagglutination inhibition test

The hemagglutination inhibition (HI) test exploits the ability of the E protein of TBEV and other flaviviruses to agglutinate erythrocytes isolated from male geese.[14] The agglutinating phenotype of the TBEV is lost in the presence of host antibodies against the E protein and only a small pellet of erythrocytes forms at the bottom of the test tube, whereas a larger layer of erythrocytes can be seen to form at the bottom of the tube in the absence of host anti-virus antibodies. The test can be standardized using a defined quantity/activity of antigen (usually 4 hemagglutination units), a defined concentration of erythrocytes, and serial dilutions of the serum being tested. The test can therefore be quantitated and the level of dilution at which the serum inhibits agglutination is referred to as the HI titer. It should be noted that serum contains many substances that inhibit hemagglutination and these must be removed by acetone extraction or kaolin absorption before the serum can be used in the HI test. Usually the viral antigen used in the test is isolated from infected mouse brain, although cell culture supernatant can also be used as a source of antigen when testing for other viruses.

The hemagglutination reaction detects both IgM and IgG antibody isotypes. Historically, the HI test was used to demonstrate a significant (usually 4-fold) increase in the end titer that would be indicative of an acute infection. The test was also used in seroprevalence studies because hemagglutinating antibodies usually persist for many years.

A further development in the HI test was the treatment of serum samples with 2-mercaptoethanol in order to reduce the disulfide bonds present in native IgM pentamers to leave inactive IgM monomers.[15] This additional treatment step will cause HI titers to decrease in the presence of IgM antibodies, with a significant (at least 4-fold) decrease in HI titer indicating acute TBEV infection.

One disadvantage of the HI test is that there is a broad cross-reactivity with all flaviviruses[14] and therefore samples from patients infected with more than 1 flavivirus, or from those recently vaccinated, may lead to non-specific cross-reaction and inaccurate determinations of titer. The HI test is still used in several countries and is recommended by the World Health Organization for distinguishing between primary and secondary flavivirus infection.

Immunofluorescence assay

The use of IF to detect antibodies against TBEV usually involves indirect assays that require cells infected with TBEV to be spotted, fixed, and permeabilized on slides.[16] A characteristic, fluorescent, cytoplasmic staining pattern can be seen and quantified using serial dilutions of the serum being tested; antibody isotypes can be distinguished using

fluorescent conjugates specific to IgM or IgG. For IgM testing, the higher-affinity IgG antibodies must be removed in order to avoid false-negative results. The sensitivity of IF assays appears to be like the HI test (the author's personal observation). IF assays that detect IgM antibodies against TBEV are moderately specific and occasionally show low levels of cross-reactivity to other anti-flavivirus antibodies following a recent infection or vaccination in the patient's history (the author's personal observation). According to our laboratory's experience, IF assays that detect IgG antibodies against TBEV perform specifically if there is only a TBEV infection or vaccination in the medical history. In contrast, diagnosis of patients with a history of infection or vaccination by a flavivirus other than TBEV can be difficult due to cross-reacting antibodies.

Low antibody titers that subsequently become undetectable occur following TBE vaccination and therefore IF assays are not recommended to test for immunity against TBE. After 2 flavivirus infections or vaccinations, a secondary response similar to the one seen in the HI test can often be detected as a high and broadly cross-reactive titer (the author's personal observation).

Neutralization test

The neutralization test (NT) exploits the capacity of antibodies to neutralize infectious viruses,[17] with several different formats available. One type of NT uses a standardized virus preparation and varying serum dilutions, while another format uses a standardized serum dilution and varying virus concentrations. Other examples are the plaque reduction NT (PRNT), which is used to evaluate the neutralization titer by analyzing the serum dilution at which the number of viral plaque-forming units is reduced by 50% or 90%, and the 'tissue culture infection dose 50% (TCID50) test. The TCID50 test involves a defined number of infectious or lethal doses undergoing neutralization by varying concentrations of the serum being tested. The dilution at which 50% of the original quantity of virus is neutralized is termed the TCID50 titer and is usually calculated using the formula of Reed and Muench.[18]

Neutralizing antibodies usually occur about 2 weeks after vaccination or infection. They are thought to be the most specific antibodies produced by the host, and with the lowest cross-reactivity to other flaviviruses. Therefore, one scenario that indicates the use of an NT is when it is necessary to distinguish between specific anti-TBEV antibodies and antibodies against other flavivirus types. A second scenario in which an NT is useful is when there needs to be a reliable demonstration of immunity: only the detection of neutralizing antibodies is thought to be a reliable surrogate marker for an existing immunity against TBE.

ELISA

The ELISA format is the most commonly used test for detecting antibodies against TBEV.[19,20] The ELISA is usually conducted in a standardized format and can be automated. The various formats of anti-TBEV ELISAs on the market use different antigens, such as European subtype strains (e.g., Hypr, K23, Neudoerfl, K 1074) or Far Eastern subtype strains (e.g., Moscow B-4). The antigens used in the assays are whole-cell lysates or purified extracts derived from whole-cell lysates.[21] The results obtained from different ELISAs are not comparable due to the different antigens and different amounts of antigen used. In general, ELISAs exhibit high levels of sensitivity but only moderate specificity due to cross-reactivity with other flaviviruses. Depending on local flavivirus circulation as well as on the individual vaccination history, positive ELISA tests should be confirmed by a TBEV-neutralization test, specifically if antibodies against dengue virus, yellow fever virus or any other flavivirus may be present in an individual patient or in a specific region (see also chapter 12 for more details).

The various formats of ELISA can distinguish between different antibody isotypes, although only IgM and IgG are usually relevant for a diagnosis of TBEV infection (IgA does not play any role in diagnosis but may be detectable in serum and CSF). IgM antibodies are usually already present at the onset of clinical CNS disease, or at least a few days after onset of neurologic symptoms, and can be detected for about 6 weeks after the onset of CNS symptoms. A μ-capture ELISA has the highest specificity for IgM testing. When using the 2-layer ELISA format, IgG has to be removed before testing in order to avoid false-negative results. Diagnostic tests for anti-TBEV IgM are usually more specific than IgG tests with regard to cross-reactivity with other flaviviruses (the author's personal observation).

Assays evaluating IgG antibodies are usually produced in a conventional 2-layer sandwich format. Anti-TBEV IgG is broadly cross-reactive with other anti-flavivirus IgG antibodies. ELISAs for detecting IgG anti-TBEV antibodies display a high sensitivity (up to 99%), but only moderate specificity (40–80%) if sera from patients or vaccinees exposed to other flaviviruses are tested.[21] The specificity can be up to 97%, however, when samples with no history of exposure to other flaviviruses are tested. IgG antibodies against TBEV are usually present at the onset of CNS symptoms, reach a maximum titer after about 6 weeks, and persist for years. The antibody titers present after natural infections are usually much higher than those that develop after vaccination.[22]

As with diagnostic tests for other flaviviruses, different types of antigen have been investigated in ELISAs in order to increase the sensitivity and specificity of testing. The use of NS1 protein as the antigen to be detected shows some

increase in specificity but a decrease in sensitivity. ELISAs based on NS1 do not detect anti-TBEV antibodies after vaccination, and therefore this format could be capable of distinguishing between an infection-induced and vaccination-induced immune response, which might be a relevant diagnostic question when CNS symptoms occur after vaccination. In a recent development, antibodies against the non-structural protein 1 (NS1) showed a high specificity. The detection of NS1 antibodies against TBE is also the proof for an active viral replication and therefore indicates past or recent TBE virus infection. Although it could be shown in a recent publication that traces of NS1 were detectable by mass spectrometry, it could be clearly shown that this test was able to differentiate between vaccine-induced and infection-induced antibodies.[23-25]

Secondary antibody response type

Pre-existing immunity due to previous infection or vaccination with other flaviviruses could modify the immune response to TBEV infection or TBE vaccination. In such cases, a low IgM and high IgG antibody response can usually be observed (the author's personal observation). In addition, reactivity against other flaviviruses (dengue virus, West Nile virus, yellow fever virus, Japanese encephalitis virus) can be observed independent of whether these infections, or vaccinations against these viruses, have occurred or not. Therefore, broad cross-reactivity against different flaviviruses or high IgG antibody titers should raise the suspicion of a secondary immune response (Fig. 2). Patients with TBE vaccination failure can often also display a serologic pattern consistent with a secondary immune response.

Avidity testing

The avidity of an antibody is an artificial index that indicates the binding activity of an antibody to a specific antigen. The avidity of an antibody usually increases with time after infection[26] and reaches its peak after weeks to months. The avidity index may therefore help to differentiate recent and past infections. The testing of avidity is performed by testing the sera in parallel ELISAs with and without washing with 8M urea. The avidity index is calculated as a percentage using the formula: (optical density [OD] of IgG with urea / OD of IgG without urea) ×100. Sera with an avidity index <40% are of low avidity and indicate a recent infection, whereas an avidity index >80% indicates an old infection. Avidity testing is used in suspected West Nile virus infections as there is sometimes a persistent IgM that can confound interpretation of whether an infection is recent or not. In TBEV infections, persistent IgM from a past

Figure 2: Schematic diagram of the course of specific anti-TBE antibodies in primary or secondary flavivirus infection

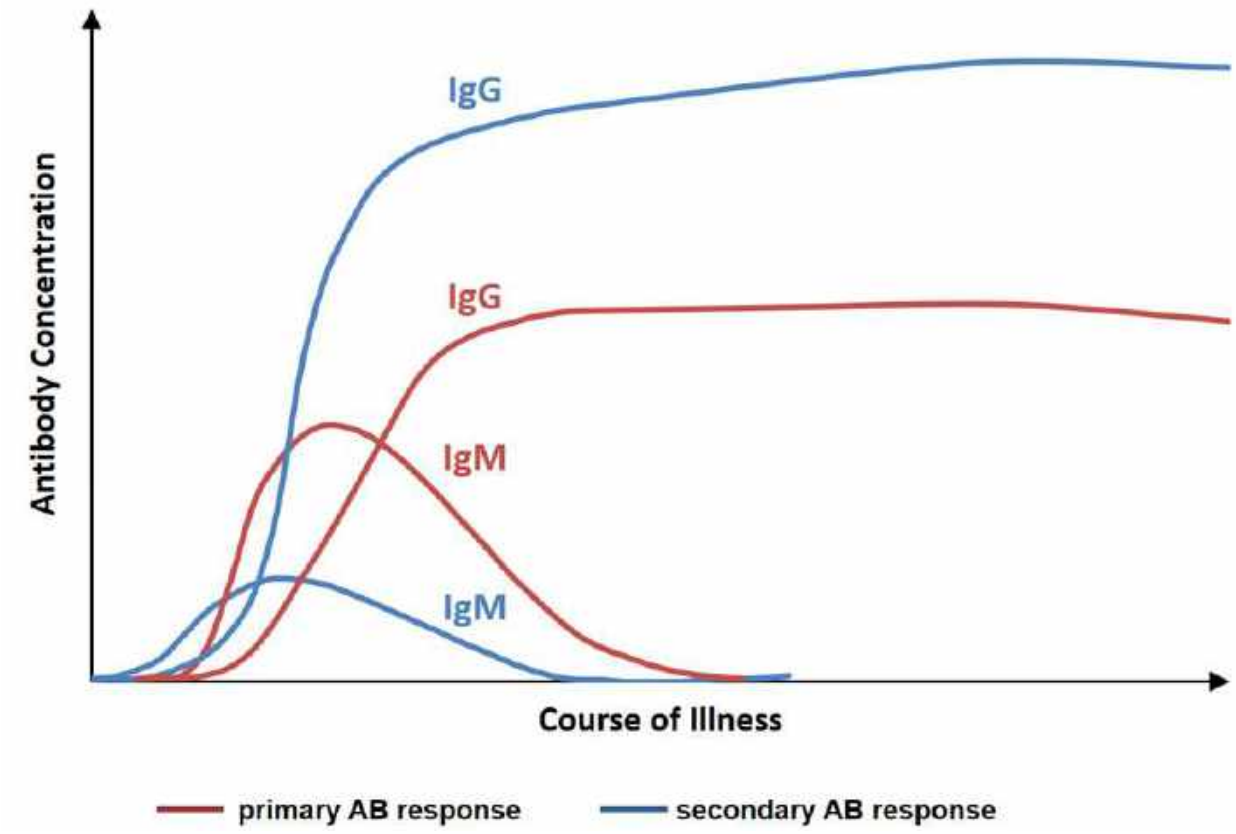


Table 2: Possible serologic constellations, their possible interpretation, and steps necessary for confirmation of TBE infection

Serologic constellation				Local CSF antibody production	Interpretation	Activity
IgM (serum)	IgG (serum)	IgM (CSF)	IgG (CSF)			
+	-	-	-	-	False-positive IgM; early phase of infection	Serologic control after 7 days; re-testing with other test format
+	+	-	-	-	Possible status after previous vaccination; very early in state of TBE infection	Serologic control after 7 days (increase in antibodies); cerebrospinal re-testing after 7 days
-	+	-	-	-	Past infection or vaccination; passive antibody transfer	Avidity testing in cases with neurologic symptoms
+	+	+	+	+	Acute or post-acute TBE infection	
-	-	-	+	Not calculable	Possibly incorrect result	Re-testing with other test format
-	-	+	-	Not calculable	Possibly incorrect positive result	Re-testing with other test format

infection is uncommon and therefore avidity testing is not routinely performed in cases of suspected TBEV infection.[19] In our laboratory, avidity testing is used to differentiate passively transferred IgG antibodies from infection-induced antibodies, e.g. to exclude Guillain-Barré syndrome in suspicious cases. Preliminary avidity testing of IgG in vaccinated persons shows that high avidity IgG is only produced after a complete basic vaccination (the author's personal observation).

Antibody testing of CSF

Both IgM and IgG anti-TBEV antibodies can be detectable in CSF at the onset of CNS symptoms, and their detection can be important in special circumstances or for supporting the diagnosis of a TBEV infection. IgM is produced locally within the CNS but is not passively transferred into the CSF to a great extent.

IgG is transferred passively, however, especially during inflammatory processes in the CNS that disturb the blood–brain barrier. The detection of IgG in the CSF is therefore not primarily indicative of an acute TBEV infection.

IgM can be detectable within the CSF during the first days of CNS symptoms in only 50% of patients and may only become detectable in the remainder during the next 10 days.[1] Therefore, the detection of IgM in serum samples is superior to the detection of IgM in CSF for the diagnosis of TBE. The detection of IgM in CSF may help to distinguish an acute TBEV infection from the antibody response induced by a recent vaccination; an 'IgM index' can be calculated for this purpose (Fig. 3).

Figure 3: Calculation of IgM index

$$\text{IgM index} = \frac{\text{Titer TBE-IgM (CSF)}}{\text{Titer TBE-IgM (SER)}} > \frac{\text{Total IgG (CSF)}}{\text{Total IgG (SER)}}$$

The production of IgG antibodies within the CSF must be demonstrated in order to prove that a patient has a neurologic TBEV infection,[27] and this can be evaluated by calculating the CSF serum index according to Reiber et al.[28]

There are different options for the calculation, with the most commonly used shown in Fig. 4.

Figure 4: Calculation of intrathecal antibody production

$$\text{IgG index} = \frac{\frac{\text{OD TBE-IgG (CSF)}}{\text{OD TBE-IgG (SER)}}}{\frac{\text{Total IgG (CSF)}}{\text{Total IgG (SER)}}} > 2$$

$$\text{IgG index} = \frac{\frac{\text{OD TBE-IgG (CSF)}}{\text{OD TBE-IgG (SER)}}}{\frac{\text{Albumin (CSF)}}{\text{Albumin (SER)}}} > 2$$

Serological cross reactions with other flaviviruses

Due to the close genetic relationship between the members of the genus Flavivirus within the family Flaviviridae some cross-reactions in the available serological tests might be expected. These serological cross-reactions are mainly directed against the E protein of the flaviviruses and known for most of the available serological tests and they may cause difficulties in the serological diagnosis of flavivirus infections.

Structural test formats like ELISA are especially prone to serological cross reactions; however, also hemagglutination inhibition and indirect immunofluorescence test systems show varying degrees of cross-reactions between flavivirus infections or flavivirus vaccinations. The test with the highest specificity against other flaviviruses is the neutralization test, which is believed to be highly specific for the respective flavivirus.

But beside the test systems, also the different immunoglobulin classes exhibit varying degrees of cross-reactivity. While different IgG-class antibodies show high cross-reactions among the members of the flaviviruses, antibodies of the IgM-class are highly specific and usually exhibit low or no cross-reactions.

The degree of cross-reactions between different flavivirus antibodies is also dependent on the serological status of the patient resp. vaccinee. In patients exhibiting a primary immune response due to the first contact of his immune system with a flavivirus a monospecific immune response can be mainly seen with only low and mainly short-lived cross-reactions against other flaviviruses. The titer difference, which can usually be found is significant, which means there is a significantly higher titer to the infecting resp. vaccinating flavivirus in comparison to other related, but non-applied flaviviruses.

If a patient or a vaccinee was already infected with or vaccinated with/against another flavivirus, a second flavivirus infection or vaccination may cause a serological response of the secondary type. Here high antibodies against a different number of flaviviruses can be seen. The titers are high against all flaviviruses and the infecting resp. vaccinated flavivirus can no longer be distinguished. Sometimes the second flavivirus induces a strong serological answer of the IgG antibodies against the flavivirus of the first infection or vaccination, which might cause disturbance and may lead to a false diagnosis.

These cross-reactions are also important for defining immunity. Cross-reacting antibodies are non-protective. If a vaccinee gets e.g. yellow fever vaccine and Japanese encephalitis vaccine, there may also be cross-reacting antibodies against TBEV. If only an ELISA test is conducted this test may become positive and lead to the suspicion of immunity, which is not the case in this situation. Therefore, the diagnosis and immunity testing of flaviviruses should always include an evaluation of immune responses against different flaviviruses like TBEV, yellow fever virus, Japanese encephalitis virus, dengue viruses and West Nile virus. Only the history of the patient or vaccinee together with the serological results against the most common flaviviruses and flavivirus vaccinations will give a realistic picture of the immune status and of a potential infection.

Contact: gerharddobler@bundeswehr.org

Citation: Dobler G. Diagnostic of TBEV-infections. Chapter 11. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_11-7

References

1. Holzmann H. Diagnosis of tick-borne encephalitis. *Vaccine*. 2003;21Suppl 1:S36-40.

2. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet*. 2008;371:1861-71.

3. Ruzek D, Dobler G, Donoso Mantke O. Tick-borne encephalitis: pathogenesis and clinical implications. *Travel Med Infect Dis.* 2010;8:223-32.

4. Saksida A, Duh D, Lotric-Furlan S, Strle F, Petrovec M, Avsic-Zupanc T. The importance of tick-borne encephalitis virus RNA detection for early differential diagnosis of tick-borne encephalitis. *J Clin Virol*. 2005;33:331-5.

5. Caracciolo I, Bassetti M, Paladini G, et al. Persistent viremia and urine shedding of tick-borne encephalitis virus in an infected immunosuppressed patient from a new epidemic cluster in North-Eastern Italy. *J Clin Virol*. 2015;69:48-51. doi:10.1016/j.jcv.2015.05.019

6. Pogodina VV, Frolova MP, Malenko GV, et al. Persistence of tick-borne encephalitis virus in monkeys. I. Features of experimental infection. *Acta Virol*. 1981;25:337-43.

7. Whitby JE, Ni H, Whitby HE, et al. Rapid detection of viruses of the tick-borne encephalitis virus complex by RT-PCR of viral RNA. *J Virol Methods*. 1993;45:103-14.

8. Puchhammer-Stockl E, Kunz C, Mandl CW, Heinz FX. Identification of tick-borne encephalitis virus ribonucleic acid in tick suspensions and in clinical specimens by a reverse transcription-nested polymerase chain reaction assay. *Clin Diagn Virol*. 1995;4:321-6.

9. Suess J, Beziat P, Ramelow C, Kahl O. Tick-borne encephalitis virus (TBEV)-specific RT-PCR for characterization of natural foci of TBE and for other applications. *Zentralbl Bakteriol*. 1997;286:125-38.

10. Schwaiger M, Cassinotti P. Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick-borne encephalitis virus (TBEV) RNA. *J Clin Virol*. 2003;27:136-45.

11. Veje M, Studahl M, Norberg P, et al. Detection of tick-borne encephalitis virus RNA in urine. *J Clin Microbiol*. 2014;52:4111-2.

12. Kunz C, Krausler J. Bildung und Überdauern der komplementbindenden Antikörper nach Infektion mit Frühsommer-Meningoenzephalitis (Tick-borne encephalitis virus). *Arch Virusforsch*. 1964;14:499-507.

13. Slonim D, Hloucal L. Bildung und Überdauern der komplementbildenden und neutralisierenden Antikörper bei der Zeckenenzephalitis. *Zentralbl Bakteriol*. 1959;175:55-9

14. Clarke D, Casals J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am J Trop Med Hyg*. 1958;7:561-73.

15. Kunz C, Hofmann H. Due Frühdiagnose der Früsommer-meningoenzephalitis (FSME) im Hämaggflutinationshemmungstest durch Behandlung des Serums mit 2-Mercaptoäthanol (Early diagnosis of tick-borne encephalitis in the hemagglutination-inhibition-test by treatment of serum with 2 mercaptoethanol). *Zentralbl Bakteriol Orig* A. 1971;218(3):273-279.

16. Sonnenberg K, Niedrig M, Steinhagen K, et al. State-of-the-art serological techniques for detection of antibodies against tick-borne encephalitis virus. *Int J Med Microbiol.* 2004;293 Suppl 37:148-51.

17. Heinz F, Herzig P, Asmera J, Benda R. [Comparison of the sensitivity of complement fixation tests, virus neutralization tests and the indirect immunofluorescence methods in the serologic diagnosis of tick-borne encephalitis]. *Cesk Epidemiol Mikrobiol Imunol*. 1969;18:193-8.

18. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. *Am J Hyg.* 1938;27:493-7.

19. Hofmann H, Heinz FX, Dippe H. ELISA for IgM and IgG antibodies against tick-borne encephalitis virus: quantification and standardization of results. *Zentralbl Bakteriol Mikrobiol Hyg A*. 1983;255:448-55.

20. Roggendorf M, Heinz F, Deinhardt F, Kunz C. Serological diagnosis of acute tick-borne encephalitis by demonstration of antibodies of the IgM class. *J Med Virol*. 1981;7:41-50.

21. Litzba N, Zelena H, Kreil T, et al. Evaluation of different diagnostic methods for tick-borne encephalitis virus: enzyme-linked immunosorbent, immunofluorescence, and neutralization assay. *Vector Borne Zoonotic Dis*. 2014;14:149-59.

22. Holzmann H, Kundi M, Stiasny K, et al. Correlation between ELISA, hemagglutination inhibition, and neutralization tests after vaccination against tick-borne encephalitis. *J Med Virol.* 1996;48:102-7.

23. Girl P, Bestehorn-Willmann M, Zange S, Borde JP, Dobler G, Buttlar HV. Tick-borne encephalitis virus (TBEV): non-structural protein (NS1) IgG ELISA differentiating infection vs. vaccination antibody responses. *J Clin Microbiol* 2020; 58(4):

24. Albinsson B, Vene S, Rombo L, Blomberg J, Lundkvist Å, Rönnberg B. Distinction between serological responses following tick-borne encephalitis virus (TBEV) infection vs vaccination, Sweden 2017. *EuroSurveill.* 2018, 23.

25. Salat J, Mikulasek K, Larralde O, Pokorna Formanova P, Chrdle A, Haviernik J, Elsterova J, Teislerova D, Palus M, Eyer L, Zdrahal Z, Petrik J, Ruzek D. Tick-borne encephalitis virus vaccines contain non-structural protein 1 antigen and may elicit NS1-specific antibody responses in vaccinated individuals. *Vaccines* (Basel). 2020 Feb 12;8(1). pii: E81.

26. Gassmann C, Bauer G. Avidity determination of IgG directed against tick-borne encephalitis virus improves detection of current infections. *J Med Virol. 1997;51:242-51.*

27. Günther G, Haglund M, Lindquist L, Sköldenberg B, Forsgren M. Intrathecal IgM, IgA and IgG antibody response in tick-borne encephalitis. Long-term follow-up related to clinical course and outcome. *Clin Diagn Virol. 1997;8:17-29.*

28. Reiber H, Lange P. Quantification of virus-specific antibodies in cerebrospinal fluid and serum: sensitive and specific detection of antibody synthesis in brain. *Clin Chem. 1991;37:1153-60.*

Chapter 12

Epidemiology of TBE

Wilhelm Erber, Michael Broeker, Gerhard Dobler,
Lidia Chitimia-Dobler, Heinz-Josef Schmitt

Key points

- TBE is a flavivirus infection of the central nervous system (CNS), transmitted by ticks and in some rare instances by ingestion of unpasteurized milk
- It is diagnosed in the Boreal and Temperate Forest Belt of Eurasia ranging from the UK, eastern France, The Netherlands and Norway down to Italy through central and Eastern Europe, Russia, Kazakhstan, and China to Japan.
- About 10,000 cases of TBE are reported annually, likely a significant underestimate as serological testing is more sporadic than complete and, in some countries, (like Japan) not even available.
- The European Centre for Disease Prevention and Control (ECDC) have put TBE on their list of notifiable diseases. Their case definition requires clinical symptoms of CNS infection plus virological or serological confirmation of the infection, usually by detection of specific immunoglobulins IgG and IgM.
- Vaccination against TBE is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system.
- Surveillance of TBE and the TBEV is incomplete. Reported incidences do not reflect actual risk since this fluctuates annually as a result of changes in exposure, vaccine uptake, intensity of case finding and reporting, climate factors, reservoir animals and ticks - just to mention the most relevant factors.
- For largely unknown reasons (including human behavior, improved diagnostics, or climate change) TBEV appears to be spreading north (e.g. northern Scandinavia), west (e.g. United Kingdom, even south (e.g. Tunisia) and to higher altitudes (e.g. in the Alps) to areas that were previously believed to be free of the virus.
- The vectors for TBE virus are ticks like *Ixodes ricinus* and *Ixodes persulcatus*. Reservoir animals for TBE virus are mainly small rodents.

Burden of disease and case definition

1. Burden of disease and case definition

Since the first description of the clinical symptoms of TBE and the detection of TBEV in Far Eastern Russia nearly a century ago[1], TBE has become the most important tick-borne viral disease across Eurasia. To date, tick-borne encephalitis virus (TBEV) foci have been identified in Europe, Russia, through to northern and eastern Asia up to Japan. Up to 12,000 human tick-borne encephalitis (TBE) cases are registered annually from countries where the disease is reportable. However, this number likely represents an underestimate due to under-diagnosis and/or underreporting. Case fatality rates between 0.2% to 20% are reported, depending on region and perhaps on viral subtype[2]. Severe long-term sequelae of TBE are well described both in children and in adults (see Chapters 8 and 9).

1.1 TBE case definition

Because TBEV is present in reservoir animals in nature, eliminating or eradicating the disease is impossible. Thus TBE is an important concern for the potentially exposed individual who becomes infected, but it is also of public health relevance, as acknowledged by the World Health Organization (WHO) in all position reports from 1983 to date (2011)[3-5]. Moreover, TBE vaccination against TBE is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a healthcare system[6]. In addition, in 2012 the European Center for Disease Prevention and Control (ECDC) decided to add TBE to the list of mandatory notifiable diseases and provided for the first time ever a uniform disease case definition[7] (Table 1).

As ECDC case definition and reporting have not been universally implemented around the globe or even throughout Europe, data on the burden of disease from

Table 1: TBE case definition by the ECDC4 "NA"= Not applicable

TICK-BORNE ENCEPHALITIS

1. Clinical criteria

Any person with symptoms of inflammation of the CNS (e.g. meningitis, meningoencephalitis, encephalomyelitis, encephaloradiculitis)

2. Laboratory criteria

Laboratory criteria for case confirmation:*

At least one of the following five:

- TBE specific IgM AND IgG antibodies in blood
- TBE specific IgM antibodies in CSF
- Sero-conversion or four-fold increase of TBE-specific antibodies in paired serum samples
- Detection of TBE viral nucleic acid in a clinical specimen,
- Isolation of TBE virus from clinical specimen

Laboratory criteria for a probable case:

- Detection of TBE-specific IgM-antibodies in a unique serum sample

3. Epidemiological criteria

Exposure to a common source (unpasteurized daily products)

Case classification

A. **Possible case** NA

B. **Probable case**

Any person meeting the clinical criteria and the laboratory criteria for a probable case

OR

Any person meeting the clinical criteria and with an epidemiological link

C. **Confirmed case**

Any person meeting the clinical and laboratory criteria for case confirmation

**Serological results should be interpreted according to the vaccination status and previous exposure to other flaviviral infections. Confirmed cases in such situations should be validated by serum neutralization assay or other equivalent assays.*

different countries are difficult to compare. Even if clear case definitions are provided and routinely implemented by local authorities, differences between countries exist regarding the classification of clinical diseases associated with TBEV infections. For example, Austria reports only "serologically proven hospitalized cases," whereas the Czech Republic reports any case with "clinical and laboratory signs of aseptic meningitis / meningo-encephalitis, not necessarily associated with hospitalization and Germany reports all diagnosed (serology, virus detection) human infections, irrespective of their clinical manifestation."[8]

In addition to the use of different case definitions and case classifications, there is a lack of implementation of routine diagnostics in any patients with CNS infection . This is exemplified by the Polish experience: between 2004 and 2008, only 39% of the country's hospitals had access to TBEV-serology. Therefore, a pilot project of enhanced surveillance for TBE was implemented in 2009. Routinely testing for TBE in patients with signs of meningitis or encephalitis in the entire country doubled case numbers in 2009 compared to previous years, moreover, and additional 38 endemic districts were identified. Seven of the „new" endemic districts were located far away from previously known endemic foci, most notably in the northwest of the country[9].

Finally, vaccine uptake substantially modifies the number of cases in a TBE risk area, as exemplified again by Austria, where in the last decade fewer than 100 cases are reported annually while this number, however, had been up to 700 cases annually before the introduction of a vaccination program. The 7-fold difference is easily explained by the about 84% vaccine uptake in Austria. Neighboring countries with lower vaccine uptake continue to have increasing TBE case numbers[10].

It should be noted, that there are many "fever only" TBE virus infections without ZNS symptoms not being captured by the ECDC definition[11].

1.2. Burden of disease. Incidence and trends

A characteristic feature of TBE is that the incidence of the disease in risk areas can vary significantly from year to year. However, in addition to short-term fluctuations, there are also longer-range undulations of incidence rates recognizable in many countries. (TBE cases by country and year see table 2).

In Estonia for example, a country with one of the highest overall TBE incidences in Europe case numbers in the years 2005 - 2017 fluctuated between 6.2 and 18.6 with a mean incidence between 5.2 and 52.8 (see Chapter 12b, Estonia).

These longer-range undulations are well recognized and in synchrony in a time interval of 12-15 years in countries like Germany, Czech Republic, Slovakia, Switzerland, Austria

(see figure 1a), and similarly in Poland and Slovenia. The long-term trend, however, shows an increase in Germany, Austria, Slovakia, Switzerland and Poland, a constant trend the last 22 years in the Czech Republic and decreasing in Slovenia.

Countries like Lithuania, Estonia, Latvia show a similar long-range undulation of about 12-15 years, time-wise incongruous to the central European countries. Trends in case numbers however are constant over time (Lithuania, Latvia) or even decreasing long term (Estonia) (see figure 1b).

Disease numbers in Sweden, Finland, Norway and even Italy have shown a substantial and continuous increase in the last couple of years (see figure 1c). In Sweden there is an increase reported from approximately 1.9/100,000 inhabitants in 2010 to 5.1/100,000 inhabitants in 2021[12]. However, those countries do not have the same long-range awareness and screening as the countries mentioned above. So this observed increase may at least in part be explained by an increasing awareness and surveillance in the respective country. (e.g. Sweden[13])

But also new countries, until recently regarded TBE-virus free, have been identified in the last decade as areas where TBEV circulates.

Since 2016, the Netherland[14,15], Belgium[16] and the United Kingdom[17,18] have reported autochthonous human cases. Recently, TBEV has even been detected in ticks collected in North Africa, in Tunisia (see country chapter). These findings illustrate that increased awareness and forced investigations to detect TBEV can lead to identification of new TBEV endemic areas and "artificially" increase cases numbers.

In recent years, new TBE foci have been reported from altitudes up to 2100 meters above sea level[19-22]. New endemic zones in previously unaffected alpine regions in western Austria[23] and in Switzerland were established, and a first report of TBEV being detected at locations in Norway up to more than 65°N latitude was published 2018[24].

Various factors may explain all these findings, at least in part: social factors (socio-political changes with changes in human behavior, duration, and type of leisure-time activities), ecological factors (e.g., effects of climate changes on the tick population and change in availability of tick host species, new flight routes of migrating birds which may lead to importation of TBE virus infected ticks into areas which have so far been free of TBE virus), and/or technological factors (advanced diagnostics, increased medical awareness).

There is increasing research interest in habitat suitability modeling to define universal environmental characteristics of TBEV foci, to predict suitable conditions where potentially human TBEV infections may occur[25-27].

Certainly, reporting of TBE cases has improved substantially over the years, and TBE is now a notifiable disease in the EU. In the end, all factors mentioned above play an "interactive role" resulting in complex interactions that may explain the observed changes in TBE epidemiology. But still, TBE surveillance in Europe is in many countries more sporadic than systematic, and TBE cases are likely underreported[2,28].

2. TBE risk areas

2.1. Risk area definition

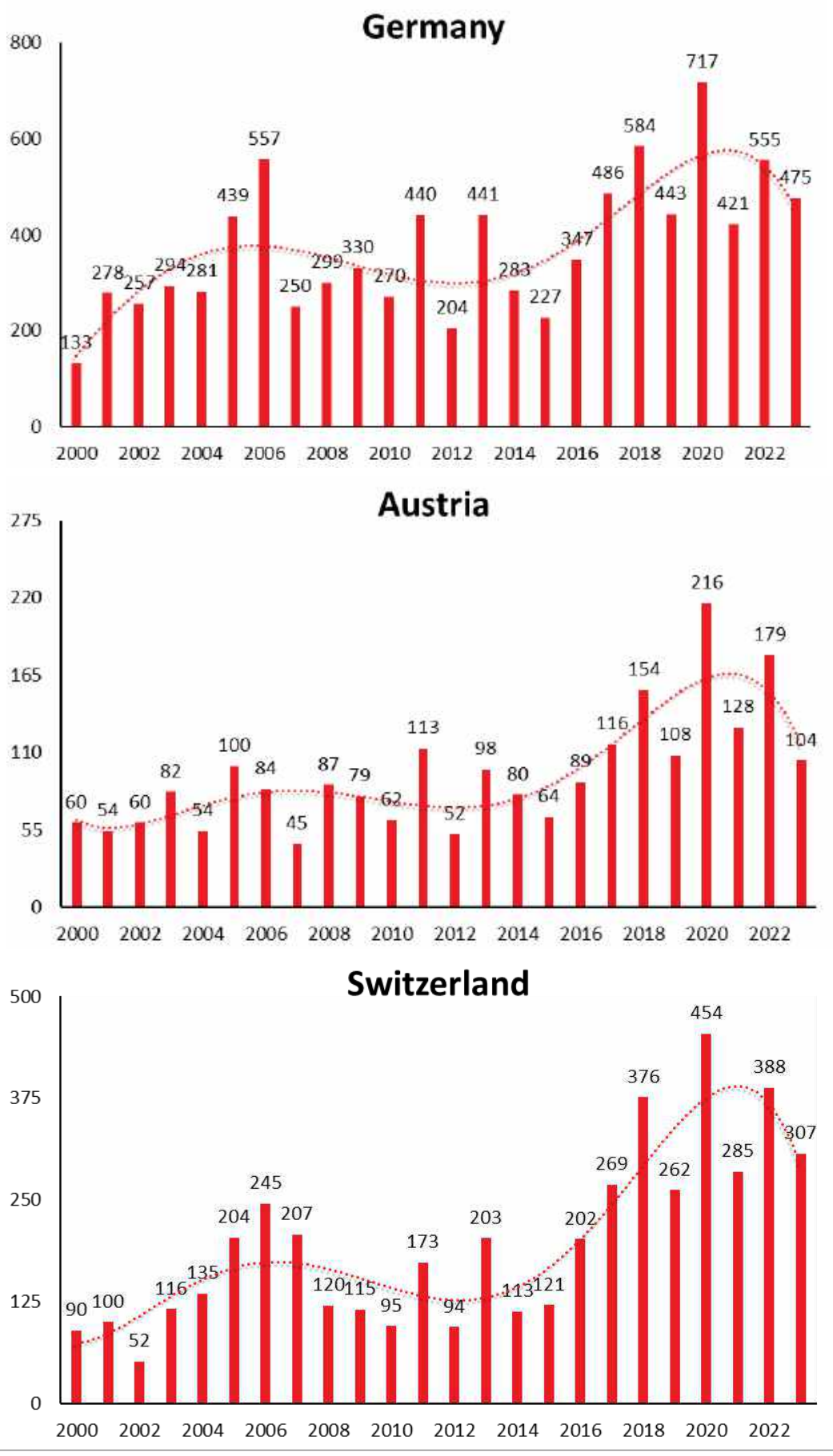
The TBE virus is restricted to specific endemic regions, and various procedures can be used to assess if and where TBE virus is circulating.

1. One of the most common methods used are antibody-prevalence studies in sera from humans or animals using ELISAs or indirect immunofluorescence tests which have the advantage that a high number of sera can be tested in relatively short time.
2. However, cross-reactivity with other flaviviruses can be misleading, and therefore, confirmation by neutralising tests are of upmost importance.
3. Another approach, but less often used, is the detection of TBEV-specific genomic sequences in ticks or in samples of milk from infected hosts like sheep, goats or cows.

For many countries in Eurasia, which are classified as TBE risk areas and are part of the TBE belt, this assessment is based on the sum of different documented evidence. Interpreting the results of such investigations and the definition of such risk areas is tricky and may be influenced by a number of factors:

- Very often the exact place of human TBE infection cannot be determined with certainty
- Epidemiology of TBE is the result of a complex interaction between reservoir animals, birds, ticks, vegetation, climate, weather.
- Human case numbers are to be interpreted with care. Behavior may change from time to time, and population density may be different in different regions of the world (see following country chapters for details). Finland for example is the eighth-largest country in Europe and the most sparsely populated country in the European Union (Population density is 18 inhabitants per square kilometer. This is the third-lowest population density of any European country). The majority of the population lives in the central and southern parts of the country. However, according to monitoring data for 2015–2019, the calculated incidence of tick-borne encephalitis in 2019 is as high as 53 per 100.000 inhabitants in the municipality of Pargas, 42 in Simo, 20 in Kustavi, and it is 30 on the island of Åland. Recommendations per municipality are

Figure 1: TBE disease cases (as of table 2) from 2000-2023, polynomial trendlines added



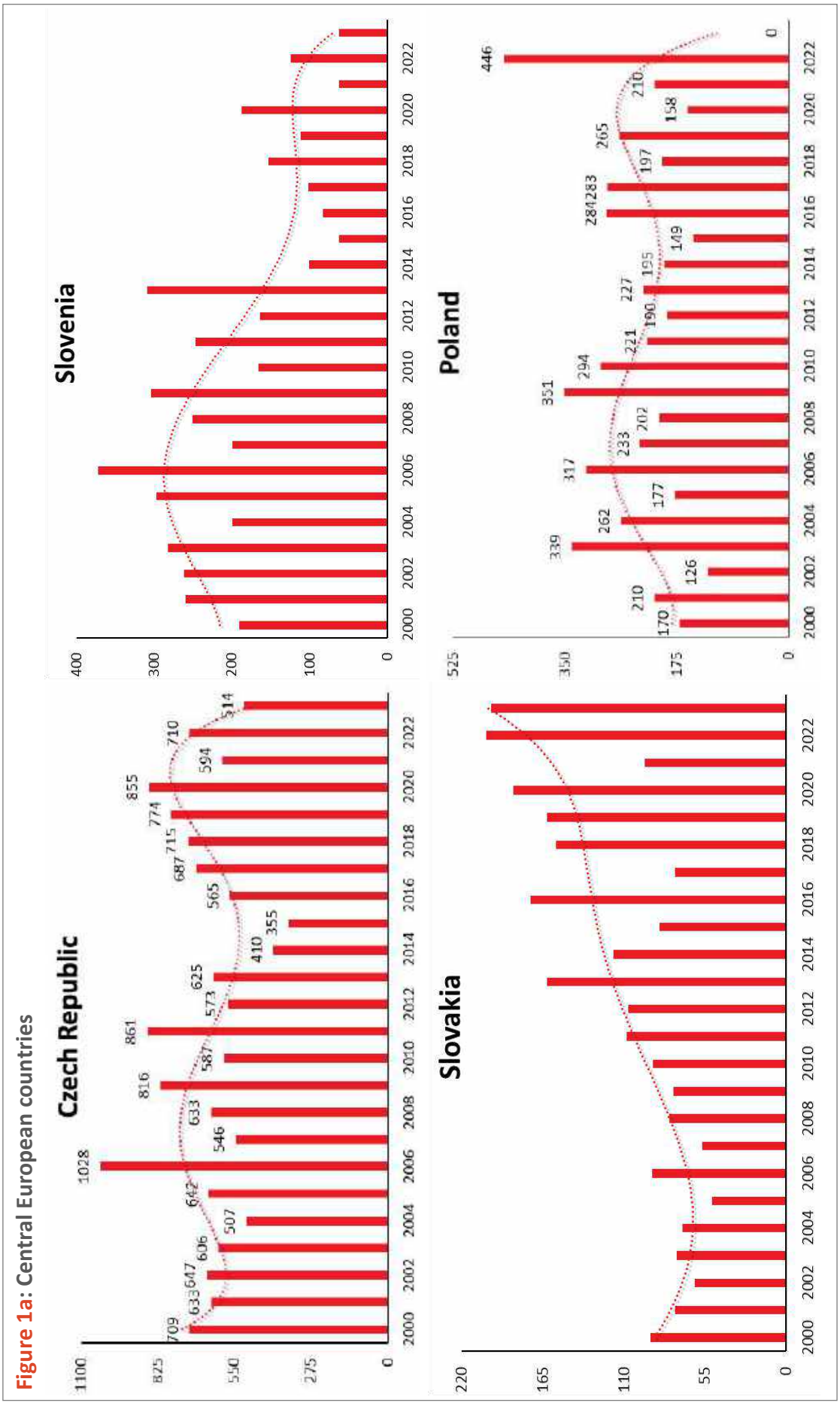
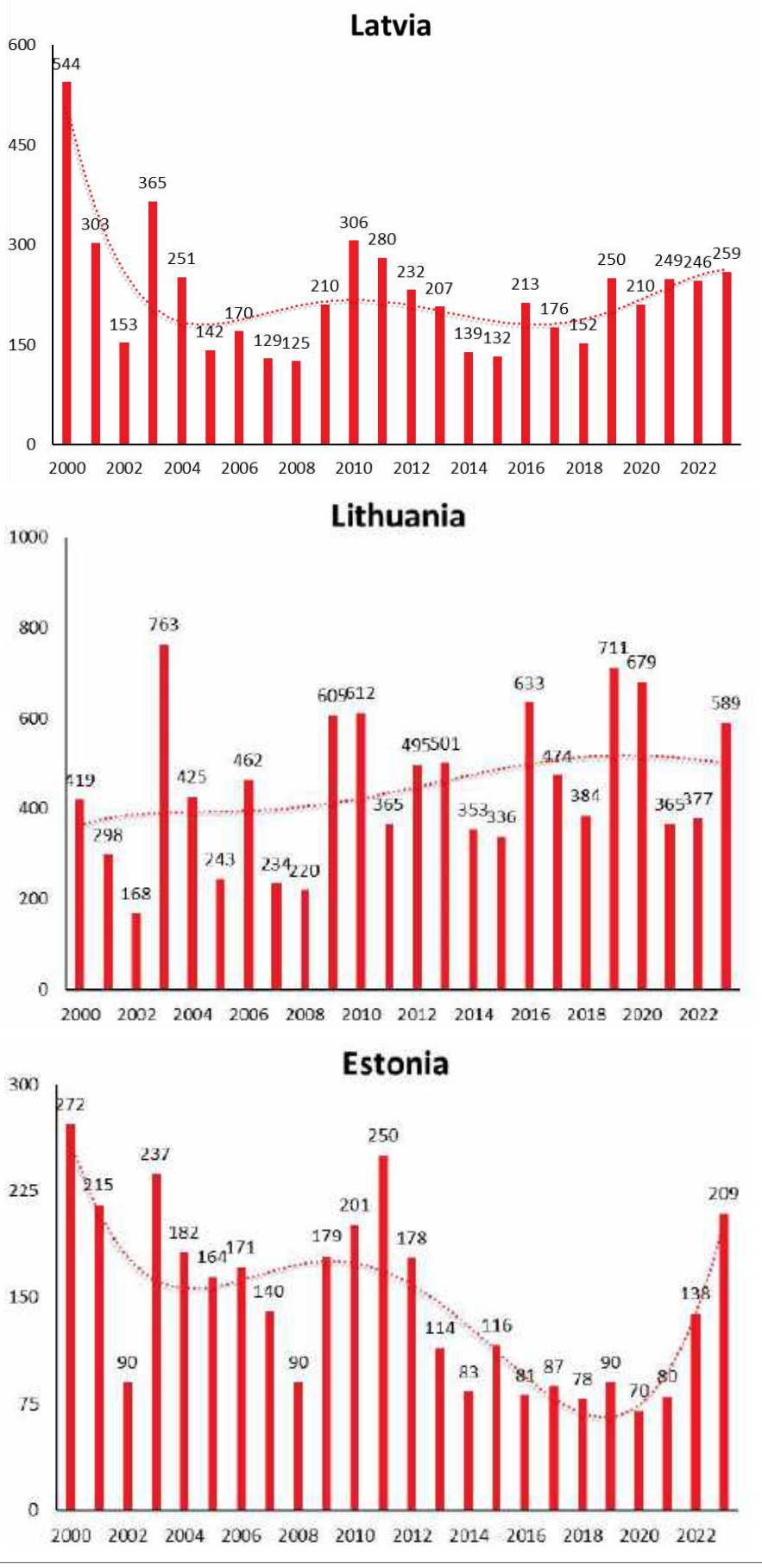


Figure 1a: Central European countries

Figure 1b: Baltic countries



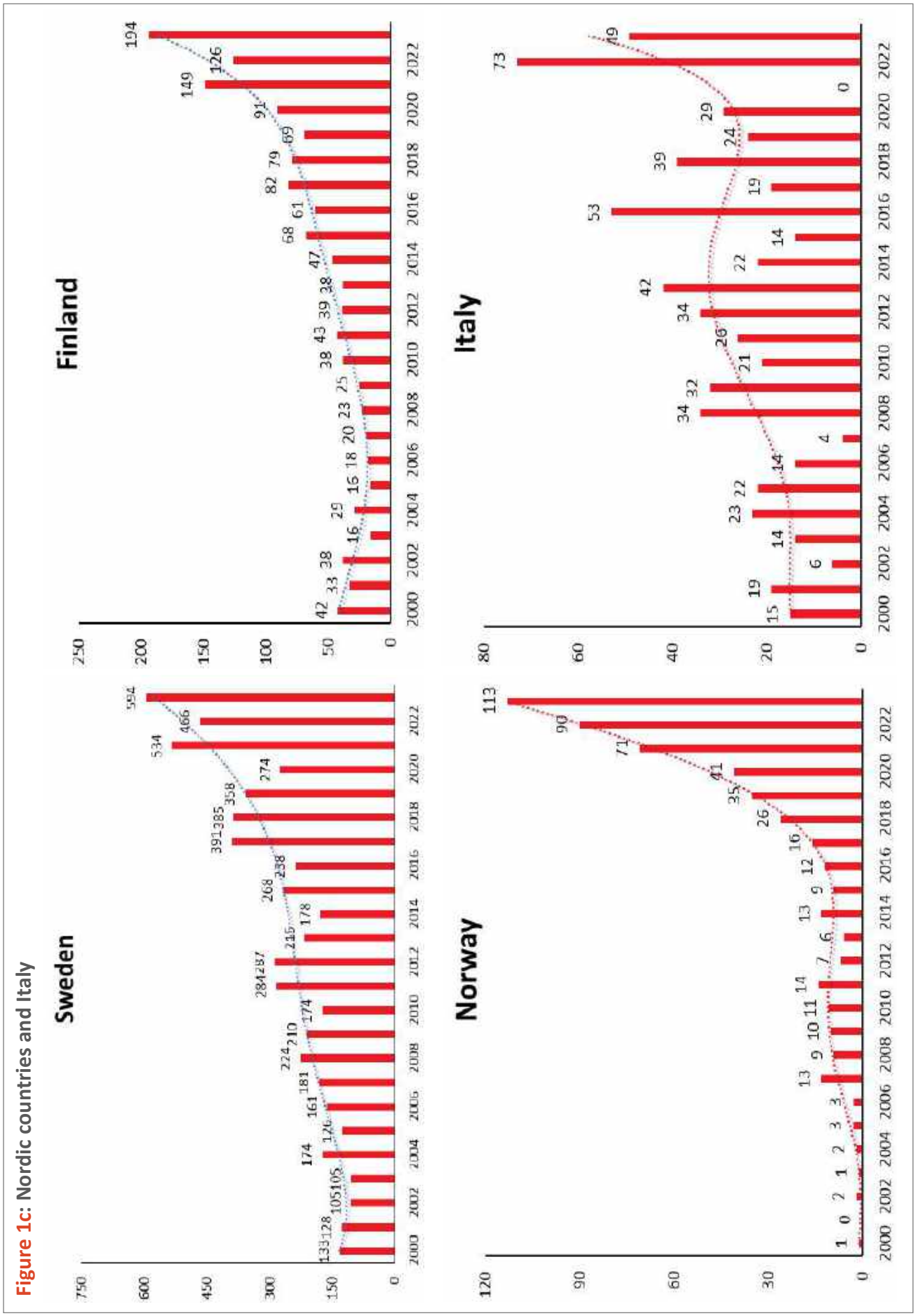


Figure 1c: Nordic countries and Italy

Table 2: TBE cases by year and country (source: data provided by the authors of Chapter 13, among others available upon request)

Country	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Austria	128	84	102	178	109	128	99	62	41	60	54	60	82	54	100	84	45	87	79	62	113	52	98	80	64	89	116	154	108	216	128	179	104
Belarus										23	61	18	53	44	46	108	82	66	88	91	108	122	109	119	77	141	142	135	171	108	102		n.a.
Belgium																							2	3	1	1	3	2	0	3	2	2	2
Bosnia and Herzegovina											1									2				5						0	n.a.	n.a.	n.a
Bulgaria																			2	0	0	1	0	0	2	0	1	0	1	2	1	0	0
China																															n.a.	n.a.	n.a.
Croatia	60	27	76	87	59	57	25	24	26	18	27	30	36	38	28	20	12	20	44	36	26	45	44	42	25	6	10	24	14	15	3	23	9
Czech Republic	356	337	618	619	727	571	412	422	490	709	633	647	606	507	642	1028	546	633	816	589	861	573	625	410	355	565	687	715	774	855	594	710	514
Denmark				2			2	3	4	3	3	1	4	8	2	2	1	2	2	4	1	1	3	1	1	1	0	4	5	5	7	5	12
Estonia	68	163	166	177	175	177	404	387	185	272	215	90	237	182	164	171	140	90	179	201	250	178	113	84	116	81	87	85	83	70	80	138	209
Finland		14	25	16	23	8	19	16	12	42	33	38	16	29	16	18	20	23	25	38	43	39	38	47	68	61	82	79	69	91	149	126	194
France	1	1	4	3	4	1	2	2	5	5	8	4	3	8	4	10	6	6	2	3	8	4	4	10	11	29	18	24		35	n.a		
Germany	44	142	118	306	226	114	211	148	115	133	255	239	277	274	432	544	239	289	313	260	424	195	420	264	221	353	485	582	443	717	421	555	475
Hungary	299	190	339	264	234	246	102	74	69	54	55	80	114	89	54	57	63	55	70	50	43	44	53	31	24	19	16	32	18	18	6	29	24
Italy	0	2	2	8	6	8	8	11	5	15	19	6	14	23	22	14	4	34	32	21	26	34	42	22	14	53	19	39	24	29	19	73	49
Japan			1																							1	2	1	0	0	0	0	0

**Autochthonous cases only. For travel-related cases, see country chapter.*

Table 2: TBE cases by year and country (source: data provided by the authors of Chapter 13, among others available upon request)

Country	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2002	2023
Kazakhstan	20	19	12	17	22	30	43	38	60	44	35	55	30	50	49	33	32	34	49	30	40	33	27	28	49	48	34	46	35	31	24	32	24
Kyrgyzstan																														n.a.	n.a.	n.a.	n.a.
Latvia	227	287	791	1366	1341	736	874	1029	350	544	303	153	365	251	142	170	129	125	210	306	280	232	207	139	132	213	176	152	250	210	249	246	259
Lithuania	14	17	198	284	427	310	645	548	171	419	298	168	763	425	243	462	234	220	605	612	365	495	501	353	336	633	474	384	711	679	365	377	589
Moldova																															n.a.		
Mongolia															5	6	52	12	8	9	13	6	15	7	40	52	62	32	19	20	5	8	34
Netherlands																	0	0	0	0	0	0	0	0	0	2	1	2	2	5	2	2	5
Norway								1	1	1	0	2	1	4	4	5	13	11	10	11	14	7	6	13	9	12	16	26	35	41	71	90	113
Poland	4	8	241	181	267	259	201	208	208	170	210	126	339	262	177	317	233	202	351	294	221	190	227	195	149	284	283	197	265	158	210	445	659
Romania																		8	4	3	3	3	1								n.a.		
Russia	5194	6239	7571	5640	5933	1037	6804	7531	1001	6010	6569	5231	4773	4178	4593	3433	3142	3140	3141	3094	3533	2716	2236	1978	2304	2035	1934	1727	1775	967	1015	1969	n.a
Serbia														1	6	1						4			4	1	5	13				1	1
Slovakia	24	16	51	60	89	82	76	54	63	92	75	62	74	70	50	91	57	79	76	90	108	107	162	117	88	174	75	156	161	185	96	203	200
Slovenia	118	80	197	531	157	406	274	137	150	196	260	262	282	199	297	372	199	251	304	166	247	164	309	100	62	83	102	153	111	187	62	124	62
South Korea																															n.a.	n.a.	n.a
Sweden	68	84	48	116	68	45	74	65	53	133	128	104	101	174	126	161	181	224	210	174	284	287	209	178	268	238	391	385	358	274	534	466	594
Switzerland	37	66	44	97	60	62	123	68	112	89	96	52	114	131	204	238	105	119	112	96	170	96	202	108	122	202	269	376	262	454	285	388	307
Tunisia																															0		
Ukraine												12	28	4	8	7	4	7	8	3	10	3	3	6	3	6	4	5	2	2	n.a.		
UK																													1	1	0	2	

**Autochthonous cases only. For travel-related cases, see country chapter.*

based on human incidence numbers exclusively and do not consider those many municipalities where there are only few people living.

- Environmental variables change annually resulting in great annual differences in case numbers, demonstrated in the country chapters as well as in Table 2. For instance, in some highly endemic areas, TBEV prevalence in ticks reaches 20– 40%, but in other areas it can be as low as 0.1–0.5%3 (see Chapter 11)
- A high local vaccine uptake may result in a low disease incidence, whereas the incidence in the unvaccinated (e.g., a traveler) may be much higher than the reported risk in the local population indicates. This is relevant information for travelers.
- TBEV-infected ticks are typically found in microfoci, i.e. the virus is often detectable in small areas only, whereas the surrounding areas are TBEV-free.

To date there is no commonly accepted definition to characterize "TBE risk areas". Most definitions and consequently vaccination recommendations (even from the WHO) so far are based on the human TBE incidence numbers in a given area.

A more holistic proposal was presented by the ECDC for assessing the risk for arbovirus infections in general[29].

- The key point from this is that "… any area where the chances of transmission of an arthropod-borne disease to humans are higher than nil is a risk area." This definition is compelling as it refrains from requiring any specific level of risk (which can be small or large), like incidence data, which vary from year to year even for the same region.
- The authors then graded risk areas as follows[29]:
    - A predisposed area is a risk area where existing conditions might enable the transmission of an arthropod-borne disease to humans, but the respective pathogen has not been detected. This may result from the fact that now surveillance for the TBEV had been accomplished to date.
    - An imperiled area is a risk area with no human cases detected, but where the pathogen has been detected in vectors, or transmission of the pathogen to animals or humans has been detected indirectly (by serology, e.g. if routine testing is not available).
    - An affected area is a risk area, where human TBE disease cases have occurred either sporadically or in a timewise restricted matter.
    - An endemic area is a risk area where recurrent transmission of TBE to humans is taking place over several seasonal cycles.

In order to assign an arbovirus-risk based on the ECDC definition[29] an area must be accurately determined geographically and by biological and epidemiological findings (surveillance of human and animal cases, field investigation etc.) in order to avoid misunderstandings and imprecision.

This however is NOT the case with TBE, as the quality of surveillance and reporting is significantly different among countries and data cannot be simply compared. Therefore, the ECDC classification is a way to grade available evidence by the time of evaluation. However, as noted above, the epidemiology of TBE is a "moving target", the process of unequivocal classification of a country as TBE risk area and the decision on vaccination recommendation is a stepwise process and can take many years. Countries with reasonable evidence for risk area assessment (see fig 2) are discussed in the respective country chapters (see below). For some countries, preliminary data are available regarding the prevalence of TBE virus which do not yet allow a risk area assessment. (see below paragraph 2.3.)

2.2. TBEV subtype and vector distribution

Three main TBEV subtypes have been described based on their distribution pattern and sequence similarity (see fig 3): the European subtype virus (previously CEE virus, Central European encephalitis virus; TBEV-EU), the Far Eastern subtype (previously RSSE virus; TBEV-FE), and the Siberian subtype (previously west Siberian virus; TBEV-Sib). In addition to the 3 primary TBEV subtypes, there is a fourth accepted subtype, designated as (Baikalian subtype (TBEV-BKL) with the prototype strain "886-84". Recently, two additional lineages have been described as possible TBEV subtypes, namely the "strain 178-79", and the Himalayan subtype (TBEV-HIM)[30] (details see chapter 11). So far, it is unclear whether the recently detected strain "Sallandse" from The Netherlands forms its own subtype or belongs to the European subtype.

TBEV-FE prevails in the regions of far-east Russia, in China, Mongolia and in Japan. TBEV-SIB prevails in eastern and western Siberia, in the Ural and European part of Russian territories. TBEV-EU is predominant in Eastern European countries including Ukraine and in central, western, and northern Europe. However, there is a big overlap in the distribution pattern of the three main subtypes as outlined in fig 3.

TBEV-BLK was found in East Siberia near Lake Baikal and in Northern Mongolia, and TBEV-HIM was recently isolated in wild rodents (Marmota himalayana) in the Qinghai-Tibet Plateau in China[30].

The principal vector as well as the reservoir for the TBEV-EU subtype is the tick *I. ricinus*, whereas TBEV-FE and TBEV-SIB subtypes are transmitted predominantly by *I. persulcatus*. The ranges of the 2 tick species as well as the TBEV subtypes overlap in Estonia, parts of Latvia, Finland, northern Sweden, and the European part of Russia. Interestingly in Finland *I. ricinus* infected with TBEV-Sib and I. persulcatus infected with TBEV-Eu have both been detected[31,32]

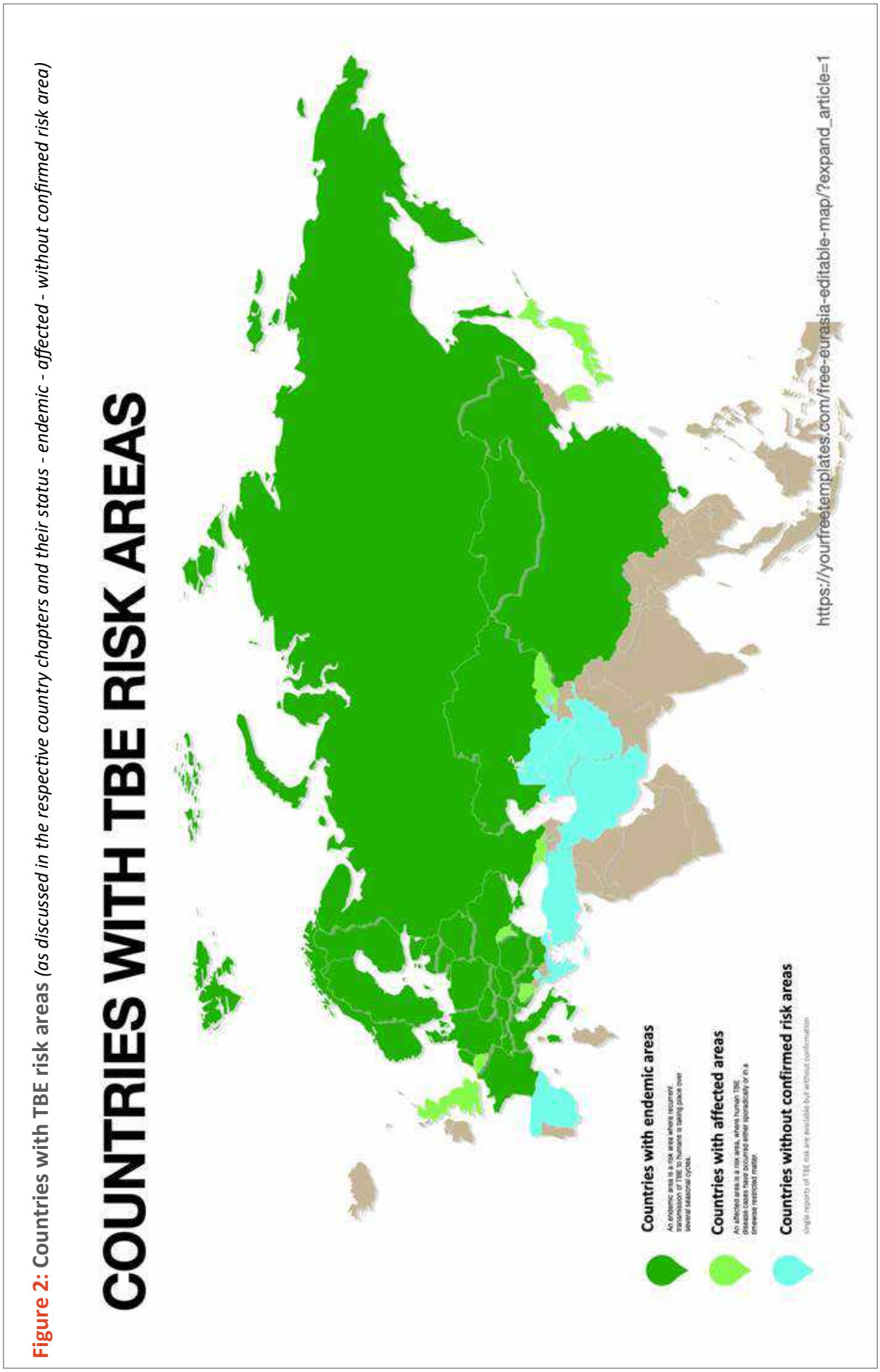


Figure 2: Countries with TBE risk areas *(as discussed in the respective country chapters and their status - endemic - affected - without confirmed risk area)*

Figure 3 : Distribution of TBEV subtypes by country

Distribution of TBEV subtypes:

TBEV-Eu	dotted blue line: prevails Europe, virus isolates in Siberia also, most eastern virus isolation Lake Baikal
TBEV-Sib	dotted red line: prevails Siberia and Ural region, most western virus isolation Baltics and Moldavia, most eastern virus isolation far eastern region of Russia
TBEV-Fe	dotted brown line: prevails far eastern region of Russia, most western virus isolation Baltics and Moldavia, most eastern virus isolation Hokkaido, Japan
	Islands of unusual TBEV subtype distribution are reported in South Korea (TBEV-EU)

All 3 main TBEV subtypes have been found in Estonia and Latvia[33,34]. From the limited virus isolates available from the Ukraine so far, there is evidence that all TBEV subtypes are present on the Crimean peninsula, too[35,36]. The TBEV-SIB has been detected in Bosnia as well[36].

TBEV-EU foci have been reported from South Korea, approximately 7000 km away from the European range of the TBEV-EU subtype circulation[37]. TBEV strains related to the TBEV-EU subtype were isolated in rodents and humans in eastern and western Siberia as well as in the Ural territory[36,38]. TBEV-FE foci have not only been reported from Crimea, about 3000 km away from the known TBEV-FE circulation area[39] but also from the Republic of Moldova between 2010 and 2011[40].

Geographical circulation of the TBEV subtypes, unusual TBEV subtype foci, and various carrier vectors are well described in more detail in Chapter 2.

2.3. Areas without confirmed TBE risk assessment

As mentioned TBE-virus risk area assessment is a stepwise process and one should consider some core assumptions.

i) Can tick species which are known as vectors for TBEV be found in the region to be analysed?

ii) Is the climate of the region and the landscape suitable for these tick vectors?

iii) How specific are the tests used to detect TBEV? What about cross-reactivity with other flaviviruses especially with those to be expected in the region?

A variety of flaviviruses genetically related to TBEV has been described (without being complete):

- Louping ill virus
- Spanish goat encephalitis virus
- Spanish sheep encephalitis virus
- Greek goat encephalitis virus
- Turkish sheep encephalitis virus
- Powassan virus
- Omsk haemorrhagic fever virus
- Alkhurma haemorrhagic fever virus
- Kyanasur forest disease virus
- Langat virus
- Negishi Virus
- West Nile fever virus
- Yellow fever virus
- Dengue virus
- Zika virus
- Japanese Encephalitis virus
- West Nile virus

Depending on the region where serological studies are carried out, at least one of these flaviviruses may interact with the test and may lead to cross-reactive false-positive results. The detection of a TBE positive serum (either in humans or animals and by ELISA or IFA) in an area so far not known as TBE endemic can only be a first sign and has to be followed by additional tests to confirm seropositivity. The golden standard for confirmation is the neutralisation test, and even this test has some minor cross reactivity.

When sera from animals are tested as sentinel, one has to take into consideration that samples from post mortem wild animals may lead to false-positive ELISA results and some samples may be toxic to cell cultures in the neutralization assay (e.g. from horses and foxes). When planning a seroprevalence study in animals, it should also be kept in mind that some animal species may be unsuitable as sentinels due to the fact that they do not seem to seroconvert, e.g. cats[41].

When animal or human sera have been found to definitely be TBE sero-positive in a given region, the next step to demonstrate that TBEV is circulating in this area is the detection of TBEV in ticks. While ticks may be found in a wide range of different landscapes and places in that region, TBE foci, that means ticks infected by TBEV, may be small, sometimes smaller than a soccer field, and the prevalence of infected ticks may be low (mostly less than 1%). Consequently, it may be very useful, to contact individuals who had TBE and/or are TBE antibody-positive and can remember where they had been bitten by a tick about one to three weeks before onset of disease. The localization of potential TBE foci can help to increase the chance to detect TBEV genome in ticks collected by flagging. This approach to identify TBE foci is much more effective than just collecting ticks in the landscape.

Investigations on TBEV or TBE in areas outside of the Eurasian continent have been successful during the last decade. TBE foci and/or TBE virus could be identified on the British Islands - now the most western part of the TBE belt - and in Japan – now the most eastern part of the TBE belt. Surprisingly, TBE virus could also be detected in Tunisia, which today is the most southern TBE virus endemic region and so far the only one on the African continent. It is assumed that migrating birds are responsible for the extension of the TBE belt by transporting ticks over wide distances. This assumption is supported by genomic sequence analyses of strains isolated from new foci and which show a close genomic relationship to strains from other regions of the TBE belt.

For the following countries preliminary data are available regarding the prevalence of TBE virus which do not yet allow a risk area assessment.

Countries close to the well-known TBE belt

Spain

The first systematic studies to investigate the probable occurrence of TBEV in Spain were carried out from 2006 to 2008. A total of more than 1800 *Ixodes ricinus* nymphs and 630 adult ticks collected in northern Spain were analysed by real-time reverse transcriptase PCR. All test results were negative, and the authors concluded that TBEV prevalence in northern Spain was either very low or absent in the investigated regions of northern Spain[42].

A sero-epidemiological study of West Nile virus, Usutu virus and TBEV in dogs has been carried out in Spain. Flavivirus antibodies were detected in 39/815 dogs using a commercial blocking ELISA. This test system detects antibodies targeting epitopes on domain III of the envelope protein common to antigenically related flaviviruses and thus, ELISA-positive results indicate the presence of antibodies against flaviviruses. TBE positivity was confirmed using a neutralisation test in 14 dog blood samples collected in southern (Andalusia) and southwestern (Extremadura) Spain[43].

A sero-epidemiological study in breeding and sport horses resident in nine autonomous communities across Spain was carried out between 2011 and 2016. A total of 14/458 (3.1%) sera were positive in a TBE serum neutralisation test[44]. The authors discussed that the neutralization test used would not enable differentiation between TBEV and LIV, both members of the TBE sero-complex.

In 2011/12, a sero-epidemiological study was carried out in horses in order to assess seropositivity for various flaviviruses (Usutu virus, West Nile virus, TBEV), and 291 blood samples were taken from 172 horses[45]. The IgG seroprevalence for TBE was 0.6%. Seroprevalence for WNV was 6.4% and for USV was 1.2%. The authors concluded that zoonotic arboviruses circulate in Mallorca.

Greece

In a sero-epidemiological study across Greece, 1.7% TBE positive samples were identified in apparently healthy persons by immunofluorescence testing. It is worth mentioning that the highest seroprevalence rate was found in a region where no *Ixodes ricinus* ticks have been shown to be prevalent[46].

In a sero-epidemiological study, 921 sera and cerebrospinal fluid from individuals with infections of the central nervous system and living in northern Greece were analysed for IgM and IgG TBE antibodies. In two percent of the general population, TBE antibodies were found (0%-5.8% in different prefectures), but TBE could not be confirmed by laboratory analyses, and the authors concluded that a flavivirus of the TBE sero-complex is circulating in the investigated region[47,48].

A dog with a history of tick infection and which displayed neurological symptoms was analysed for TBE by using a commercial IgM and IgG TBE ELISA. The dog was tested positive for both IgM and IgG, and the authors concluded that diagnosis of TBE infection was confirmed by combining the clinical symptoms with this seropositivity[49]. The authors stated that one limitation of the study was that no confirmation test by serum neutralisation assay was carried out.

In a review article about tick-borne pathogens and diseases in Greece[50], the authors concluded from the above cited publications that a flavivirus of the TBE sero-complex is circulating in Greece.

Turkey

In 2007, a seroprevalence study was carried out for WNV and TBE analyzing sera from 181 samples collected at two state medical hospitals in the southeastern part of Turkey. Using a commercial TBE IgG ELSA, 10.5% were positive and 23% of the IgG positive sera were also positive in a TBE IgM ELISA. In an immunofluorescence test, 16% of the sera were positive for WNV, of which four sera were also positive for TBE. The authors concluded the possible presence of TBEV in southeastern Turkey[51].

Some years later, a total of 2450 sera from healthy blood donors in central and northern Anatolia were analyzed by a commercial TBE IgG ELISA, and 47 donor samples (1.9%) were tested positive. One sample from the Black Sea region was positive in a plaque reduction neutralisation test. The authors discussed that the blood donors have had exposure to TBE virus or an antigenically similar tick-borne flavivirus[52].

When 110 sera from Turkish children with fever and/or arthritis were analyzed by TBE IgM, five samples were tested positive. No sample was TBE IgG positive. Two samples were positive for WNV IgM and six sera were tested positive for WNV IgG. The authors concluded that children in Turkey were exposed to TBEV and WNV[53].

In the Samsun province, a total of 419 human sera from healthy individuals were analyzed by TBE IgM and IgG ELISA. Four samples were positive for IgG and one sample tested positive for IgM. However, none of these sera were confirmed positive in a neutralization assay[54].

A TBE seroprevalence study has been carried out among domestic animals in northern Turkey, and ticks were collected from animals (cattle, goat, sheep) and were analyzed for TBEV. No TBEV-specific genomic sequences were detected in a total of 2625 ticks. Screening of serum samples by a commercial TBE IgG ELISA revealed positive results in cattle (61/198, 30.8%), in goats (7/115, 6.1%) and sheep (15/147, 10.2%). The authors concluded that their study supports previous findings which indicates that TBEV is distributed in Turkey[55].

Albania and Bosnia and Hercegovina

Sero-epidemiological studies in humans and animals were carried out in the 1990s to analyse the distribution of

arboviruses in Albania, and TBE positive sera were detected[56]. However, the tests used in these investigations were based on indirect immunofluorescence techniques, and results may have been false-positive due to cross-reactivity with other flaviviruses. During the 2nd International Symposium on Tick-Borne Encephalitis in June 1991, Eltari reported about TBE cases in Albania[57]. Later, Sherifi et al. (2018) admitted that no accurate data were available on TBE in Albania, and their attempts to detect TBEV-specific genome in ticks collected by flagging had been negative[58].

There is only one report from Bosnia mentioning human TBE cases (Burger, 2017).

However, Zlobin et al. (2006) isolated three Siberian TBEV subtype strains - Bosnian lineage, two strains from one male and one female Dermacentor marginatus and 1 strain isolated from Ixodes ricinus nymph[59,60]

In total, the southwestern Balkans (Albania, Bosnia and Herzegovina, Macedonia, Montenegro) have only a few or no studies about TBE and TBE related reports.

North Macedonia

In a study to assess the prevalence of antibodies to *Borrelia burgdorferi* and TBEV in North Macedonia and Serbia, one serum sample from a female in North Macedonia was positive for neutralising TBE antibodies. This result suggests the potential existence of TBE foci in North Macedonia, however, there is still the alternative explanation that this person was exposed to TBEV during a short stay in Austria[61].

Afghanistan

In a serological study dealing with the seroprevalence of various flaviviruses, a commercial IgG and IgM ELISA was used to assess seropositivity among individuals in Afghanistan. A total of 30.8% of the sera were IgG-positive for TBE, and 20.6% were co-reactive in a WNV-ELISA. 2.2% of the sera were TBE-IgM positive. The authors concluded that TBEV may circulate in Afghanistan[62]. However, these high prevalence rates may be due to another circulating flavivirus of the tick-borne mammalian group, Royal Farm virus, which was isolated in Afghanistan in 1968 from soft ticks. With no NT testing available the situation remains unclear.

Georgia

In Georgia, 7% of acute febrile patients showed TBEV seropositivity[63]. Non-published data show that TBEV-EU may circulate in Georgia. The interpretation of the data is unclear.

Iran

Raw milk samples collected from local dairy markets around Qazvin, a city in northern Iran, have been analysed by using nested and multiplex PCR methods for the presence for various foodborne and zoonotic viruses. TBEV genomic sequences were detected in 42/492 (18.91%) of the analysed samples[64]. The authors concluded that the presence of TBEV in raw milk may pose an immediate health risk for milk and dairy consumers, even without any reported TBE cases in the Qazvin area.

In a conference report[65], data on the presence of TBEV in raw milk samples from sheep (4.4%), goat (4.4%) and cows (0%) in northwest Iran were presented, and TBE antibodies evaluated by ELISA were found in the milk of sheep (4.4%), goats (2.2%) and cows (1.1%). However, we did not find these data anywhere in a peer-reviewed journal.

A cross-sectional sero-epidemiological study has been carried out in rural areas in northern Iran in order to analyse the prevalence of TBE antibodies among the general population using a commercial TBEV ELISA IgG kit. A total of 16/448 serum samples tested positive. The authors discuss that there are uncertainties about the accuracy of positive results on serological tests, such a ELISA, owing to the antigenic cross-reactivity among flaviviruses, and they concluded that confirmation is needed by neutralisation test and that the results should be interpreted with caution[66].

Central Asian countries

Within the Central Asian countries there are reports of TBE in Kazakhstan and Kirgizstan (see country chapters), the only other single report without any further details is from Turkmenistan[67].

Countries remote from the TBE belt

Comores

A cross-sectional survey of arboviral infections in humans was conducted on three islands of the Union of Comores in 2011. Using a commercial TBE IgG ELISA, 3/400 sera were positive in the TBE ELISA, but no neutralisation/ confirmation tests were carried out[68].

Kenya

A seroprevalence study was carried out in Kenya in 2000 to 2004 to evaluate the prevalence of arboviral infections. A high seroprevalence of TBE IgG (16% in older persons, 6% in children) was found using a commercial indirect immunofluorescent test, and the authors concluded that this was a result of cross-reactions amongst related flaviviruses[69].

Djibouti

In a sero-epidemiological study carried out in Djibouti to assess the burden of a variety of arboviral diseases, antibodies to Dengue were the most frequent (21.8).In 2/1045 sera, TBE antibodies were detected using a commercial ELISA. While the first serum sample was negative in a TBE specific neutralisation assay and negative also for Alkhurma virus, the second serum was slightly positive for both viruses. The authors discussed that these two TBE seropositive individuals may have been migrants with a specific exposure to tick bites in a rural environment[70].

Vietnam

TBE sero-epidemiological analyses using an indirect immunofluorescence antibody test (IFAT) of sera from humans and rats gave positivity rates of 47.3% and 5.4%, respectively. The authors concluded that the TBE reactivity in both humans and rodents detected by the IFAT most likely reflected cross-reactivity with other flaviviruses, especially with Dengue virus and Japanese encephalitis virus[71].

Malaysia

Among farms workers, a TBE seropositivity of 36.5% was found using a commercial TBE IgG ELISA. However, when testing these sera against three antigenically related flaviviruses (DEN, WN, JEV), only 4.2% of the sera did not show cross-reactivity. The authors discussed that the remaining TBE seropositivity may be due to cross-reactivity to Langat virus and they concluded that even a virus neutralisation test could still lead to false TBE seropositivity results[72].

Summary

In this book, we did all possible to identify predisposed, imperiled, affected and endemic areas. For the countries mentioned in the end, surveillance data using TBEV-NT would be most simple to confirm TBEV circulation – which would be relevant for travelers. In endemic countries reporting should be enhanced and commercial tests for TBE should be easily accessible. Clearly, the country-specific information on TBE – epidemiology is still scarce and results in relevant underdiagnosis.

The following country reports in Chapter 13 provide standardized information, as available on:

- The risk area assessment based on the ECDC definition (see above)
- The history of TBE in the respective country as well as various specific aspects
- Virus, vector, transmission of TBE
- TBE-reporting and prevention by vaccination
- TBE case numbers over time
- Local demographics of TBE
- TBEV-isolation and TBE cases – risk area distribution

The risk map in chapter 13 shows the extent of TBEV based on documented TBE cases, TBEV infection, as well as on the detection of TBEV-circulation in nature (i.e., imperiled, affected and endemic areas). The map does not reflect the incidence of the disease or the universal prevalence of the virus in a given area. As the quality, intensity and completeness of epidemiological surveillance varies between different countries, the map presented here must be incomplete, and very likely TBEV infections and thus TBE may occur in additional ("new") areas.

The risk map distribution is based on the second and third level of the Nomenclature of territorial units for statistics (NUTS) used for subnational analysis, depending on availability (Eurostat, the statistical office of the European Union. Nomenclature of Territorial Units for Statistics. Luxembourg: Eurostat. [Accessed: 7 Mar 2023]. Available from: http://ec.europa.eu/eurostat/web/nuts/overview)

Acknowledgement: We thank all authors and the co-editors of THE TBE BOOK, who left no stone unturned in their efforts to find current country-specific information on as many countries as possible and we would like to thank again all authors of Chapter 13 for providing their timely reports.

Contact: Wilhelm.Erber@gmail.com

Citation:

Erber W, Broeker M, Dobler G, Chitimia-Dobler L, Schmitt HJ. Epidemiology of TBE. Chapter 12. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt, HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi: 10.33442/26613980_12-7

References

1. Mazanik A. Arbovirology and Cold War Collaborations: A Transnational History of the Tick-borne Encephalitis Vaccine, 1930-1980. *J Hist Med Allied Sci.* Published online September 8, 2023. doi:10.1093/jhmas/jrad054
2. Kollaritsch H, Krasilnikov V, Holzmann H, et al. Background document on vaccines and vaccination against tick–borne encephalitis. Geneva, WHO Strategic Advisory Group of Experts on Immunization. Accessed 29 May, 2012. https://www.who.int/immunization/sage/6_TBE_backgr_18_Mar_net_apr_2011.pdf
3. Tick-borne encephalitis and haemorrhagic fever with renal syndrome in Europe. Report on a WHO meeting. *EURO Rep Stud*. 1986;(104):1-79.
4. Vaccines against tick-borne encephalitis: WHO position paper. *Wkly Epidemiol Rec*. 2011;86(24):241-256.

5. World Health Organization. World Health Organization model list of essential medicines: 21st list 2019, W.H.O. Geneva, *Editor.* Accessed 27 April, 2024. *https://www.who.int/medicines/publications/essentialmedicines/en/.*

6. European Commission. Commission Decision 2002/253/EC of 19 March 2002 laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. *EC.* 2012.

7. Donoso Mantke O, Escadafal C, Niedrig M, Pfeffer M, Working Group For Tick-Borne Encephalitis Virus C. Tick-borne encephalitis in Europe, 2007 to 2009. *Euro Surveill*. 2011;16(39):19976. Published 2011 Sep 29. doi:10.2807/ese.16.39.19976-en

8. Stefanoff P, Zielicka-Hardy A, Hlebowicz M, et al. New endemic foci of tick-borne encephalitis (TBE) identified in districts where testing for TBE was not available before 2009 in Poland. *Parasit Vectors*. 2013;6:180. Published 2013 Jun 18. doi:10.1186/1756-3305-6-180

9. Heinz FX, Stiasny K, Holzmann H, et al. Vaccination and tick-borne encephalitis, central Europe. *Emerg Infect Dis*. 2013;19 (1):69-76. doi:10.3201/eid1901.120458

10. Bogovič P, Kastrin A, Lotrič-Furlan S, et al. Clinical and Laboratory Characteristics and Outcome of Illness Caused by Tick-Borne Encephalitis Virus without Central Nervous System Involvement. *Emerg Infect Dis*. 2022;28(2):291-301. doi:10.3201/eid2802.211661

11. Kjær LJ, Johansson M, Lindgren PE, et al. Potential drivers of human tick-borne encephalitis in the Örebro region of Sweden, 2010-2021. *Sci Rep*. 2023;13(1):7685. Published 2023 May 11. doi:10.1038/s41598-023-34675-x

12. Albinsson B, Hoffman T, Kolstad L, et al. Seroprevalence of tick-borne encephalitis virus and vaccination coverage of tick-borne encephalitis, Sweden, 2018 to 2019. *Euro Surveill*. 2024;29(2):2300221. doi:10.2807/1560-7917.ES.2024.29.2.2300221

13. Weststrate AC, Knapen D, Laverman GD, et al. Increasing evidence of tick-borne encephalitis (TBE) virus transmission, the Netherlands, June 2016. *Euro Surveill*. 2017;22 (11):30482. doi:10.2807/1560-7917.ES.2017.22.11.30482

14. Dekker M, Laverman GD, de Vries A, Reimerink J, Geeraedts F. Emergence of tick-borne encephalitis (TBE) in the Netherlands. *Ticks Tick Borne Dis*. 2019;10(1):176-179. doi:10.1016/j.ttbdis.2018.10.008

15. Superior Health Council. Vaccination against Tick-Borne Encephalitis. 2019;9435

16. Holding M, Dowall SD, Medlock JM, et al. Detection of new endemic focus of tick-borne encephalitis virus (TBEV), Hampshire/Dorset border, England, September 2019. *Euro Surveill*. 2019;24(47):1900658. doi:10.2807/1560-7917.ES.2019.24.47.1900658

17. Holding M, Dowall SD, Medlock JM, et al. Tick-Borne Encephalitis Virus, United Kingdom. *Emerg Infect Dis*. 2020;26(1):90-96. doi:10.3201/eid2601.191085

18. Daniel M, Danielová V, Kríz B, Jirsa A, Nozicka J. Shift of the tick *Ixodes ricinus* and tick-borne encephalitis to higher altitudes in central Europe. *Eur J Clin Microbiol Infect Dis*. 2003;22(5):327-328. doi:10.1007/s10096-003-0918-2

19. Holzmann H, Aberle SW, Stiasny K, et al. Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. *Emerg Infect Dis*. 2009;15(10):1671-1673. doi:10.3201/eid1510.090743

20. Lukan M, Bullova E, Petko B. Climate warming and tick-borne encephalitis, Slovakia. *Emerg Infect Dis*. 2010;16(3):524-526. doi:10.3201/eid1603.081364

21. Briggs BJ, Atkinson B, Czechowski DM, et al. Tick-borne encephalitis virus, Kyrgyzstan. *Emerg Infect Dis*. 2011;17 (5):876-879. doi:10.3201/eid1705.101183

22. Heinz FX, Stiasny K, Holzmann H, et al. Emergence of tick-borne encephalitis in new endemic areas in Austria: 42 years of surveillance. *Euro Surveill*. 2015;20(13):9-16. Published 2015 Apr 2. doi:10.2807/1560-7917.es2015.20.13.21077

23. Soleng A, Edgar KS, Paulsen KM, et al. Distribution of *Ixodes ricinus* ticks and prevalence of tick-borne encephalitis virus among questing ticks in the Arctic Circle region of northern Norway. *Ticks Tick Borne Dis*. 2018;9(1):97-103. doi:10.1016/j.ttbdis.2017.10.002

24. Gray JS, Dautel H, Estrada-Peña A, Kahl O, Lindgren E. Effects of climate change on ticks and tick-borne diseases in europe. *Interdiscip Perspect Infect Dis.* 2009;2009:593232. doi:10.1155/2009/593232

25. WHO. World health report of an international consultation: methodology for risk mapping of the international spread of vector-borne diseases via air travel. 2018: Geneva, Switzerland.

26. Borde JP, Glaser R, Braun K, et al. Decoding the Geography of Natural TBEV Microfoci in Germany: A Geostatistical Approach Based on Land-Use Patterns and Climatological Conditions. *Int J Environ Res Public Health.* 2022;19 (18):11830. Published 2022 Sep 19. doi:10.3390/ijerph191811830

27. Suess J. Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asia-an overview. *Ticks Tick Borne Dis*. 2011;2(1):2-15. doi:10.1016/j.ttbdis.2010.10.007

28. Domanovic D, Giesecke J. How to define an area where transmission of arthropod-borne disease is occurring? *Euro Surveill*. 2012;17(20).

29. Dai X, Shang G, Lu S, Yang J, Xu J. A new subtype of eastern tick-borne encephalitis virus discovered in Qinghai-Tibet Plateau, China. *Emerg Microbes Infect*. 2018;7(1):74. doi:10.1038/s41426-018-0081-6

30. Jääskeläinen A, Tonteri E, Pieninkeroinen I, et al. Siberian subtype tick-borne encephalitis virus in *Ixodes ricinus* in a newly emerged focus, Finland. *Ticks Tick Borne Dis*. 2016;7 (1):216-223. doi:10.1016/j.ttbdis.2015.10.013

31. Jääskeläinen AE, Tonteri E, Sironen T, Pakarinen L, Vaheri A, Vapalahti O. European subtype tick-borne encephalitis virus in Ixodes persulcatus ticks. *Emerg Infect Dis*. 2011;17(2):323-325. doi:10.3201/eid1702.101487

32. Lundkvist k, Vene S, Golovljova I, et al. Characterization of tick-borne encephalitis virus from Latvia: evidence for co-

circulation of three distinct subtypes. *J Med Virol.* 2001;65 (4):730-735. doi:10.1002/jmv.2097

33. Golovljova I, Vene S, Sjölander KB, Vasilenko V, Plyusnin A, Lundkvist A. Characterization of tick-borne encephalitis virus from Estonia. *J Med Virol*. 2004;74(4):580-588. doi:10.1002/jmv.20224

34. Yurchenko OO, Dubina DO, Vynograd NO, Gonzalez JP. Partial Characterization of Tick-Borne Encephalitis Virus Isolates from Ticks of Southern Ukraine. *Vector Borne Zoonotic Dis*. 2017;17(8):550-557. doi:10.1089/vbz.2016.2094

35. Tkachev SE, Babkin IV, Chicherina GS, et al. Genetic diversity and geographical distribution of the Siberian subtype of the tick-borne encephalitis virus. *Ticks Tick Borne Dis*. 2020;11 (2):101327. doi:10.1016/j.ttbdis.2019.101327

36. Demina TV, Dzhioev YP, Verkhozina MM, et al. Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. *J Med Virol*. 2010;82(6):965-976. doi:10.1002/jmv.21765

37. Adelshin RV, Melnikova OV, Karan LS, Andaev EI, Balakhonov SV. Complete Genome Sequences of Four European Subtype Strains of Tick-Borne Encephalitis Virus from Eastern Siberia, Russia. *Genome Announc*. 2015;3(3):e00609-15. Published 2015 Jun 18. doi:10.1128/genomeA.00609-15

38. Evstaf'ev IL. Itogi 20-letnego izucheniia kleshchevogo éntsefalita v Krymu [Results of the 20-year study of tick-borne encephalitis in Crimea]. *Zh Mikrobiol Epidemiol Immunobiol*. 2001;(2):111-114.

39. Ponomareva EP, Mikryukova TP, Gori AV, et al. Detection of Far-Eastern subtype of tick-borne encephalitis viral RNA in ticks collected in the Republic of Moldova. *J Vector Borne Dis*. 2015;52(4):334-336.

40. Topp AK, Springer A, Mischke R, et al. Seroprevalence of tick-borne encephalitis virus in wild and domestic animals in northern Germany. *Ticks Tick Borne Dis*. 2023;14(6):102220. doi:10.1016/j.ttbdis.2023.102220

41. Barandika JF, Hurtado A, Juste RA, García-Pérez AL. Seasonal dynamics of Ixodes ricinus in a 3-year period in northern Spain: first survey on the presence of tick-borne encephalitis virus. *Vector Borne Zoonotic Dis.* 2010;10(10):1027-1035. doi:10.1089/vbz.2009.0148

42. García-Bocanegra I, Jurado-Tarifa E, Cano-Terriza D, Martínez R, Pérez-Marín JE, Lecollinet S. Exposure to West Nile virus and tick-borne encephalitis virus in dogs in Spain. *Transbound Emerg Dis*. 2018;65(3):765-772. doi:10.1111/tbed.12801

43. Camino E, Schmid S, Weber F, et al. Detection of antibodies against tick-borne encephalitis flaviviruses in breeding and sport horses from Spain. *Ticks Tick Borne Dis*. 2020;11 (5):101487. doi:10.1016/j.ttbdis.2020.101487

44. Vanhomwegen J, Beck C, Desprès P, et al. Circulation of Zoonotic Arboviruses in Equine Populations of Mallorca Island (Spain). *Vector Borne Zoonotic Dis*. 2017;17(5):340-346. doi:10.1089/vbz.2016.2042

45. Antoniadis A, Alexiou-Daniel S, Malissiovas N, et al. Seroepidemiological survey for antibodies to arboviruses in Greece. In: Calisher CH, ed. Hemorrhagic Fever with Renal Syndrome, Tick- and Mosquito-Borne Viruses. *Springer Vienna*; 1990:277-285. doi:10.1007/978-3-7091-9091-3_31

46. Pavlidou V, Geroy S, Diza E, Antoniadis A, Papa A. Epidemiological study of tick-borne encephalitis virus in northern Greece. *Vector Borne Zoonotic Dis*. 2007;7(4):611-615. doi:10.1089/vbz.2007.0107

47. Pavlidou V, Gerou S, Diza E, Antoniadis A, Papa A. Genetic study of the distribution of Greek goat encephalitis virus in Greece. *Vector Borne Zoonotic Dis*. 2008;8(3):351-354. doi:10.1089/vbz.2007.0215

48. Sioutas G, Tsakou K, Top C, Jongejan F, Papadopoulos E. First clinical case of tick-borne encephalitis (TBE) in a dog in Greece. *Ticks Tick Borne Dis*. 2023;14(6):102226. doi:10.1016/j.ttbdis.2023.102226

49. Efstratiou A, Karanis G, Karanis P. Tick-Borne Pathogens and Diseases in Greece. *Microorganisms*. 2021;9(8):1732. Published 2021 Aug 14. doi:10.3390/microorganisms9081732

50. Ergunay K, Ozer N, Us D, et al. Seroprevalence of West Nile virus and tick-borne encephalitis virus in southeastern Turkey: first evidence for tick-borne encephalitis virus infections. *Vector Borne Zoonotic Dis*. 2007;7(2):157-161. doi:10.1089/vbz.2006.0574

51. Ergünay K, Saygan MB, Aydoğan S, et al. Confirmed exposure to tick-borne encephalitis virus and probable human cases of tick-borne encephalitis in Central/Northern Anatolia, Turkey. *Zoonoses Public Health*. 2011;58(3):220-227. doi:10.1111/j.1863-2378.2010.01342.x

52. Yilmaz H, Barut K, Karakullukcu A, et al. Serological Evidence of Tick-Borne Encephalitis and West Nile Virus Infections Among Children with Arthritis in Turkey. *Vector Borne Zoonotic Dis*. 2019;19(6):446-449. doi:10.1089/vbz.2018.2349

53. Aslan Başbulut E, Gözalan A, Sönmez C, et al. Samsun Kırsalında Borrelia burgdorferi ve Kene Ensefaliti Virusu Seroprevalansının Araştırılması [Seroprevalence of Borrelia burgdorferi and tick-borne encephalitis virus in a rural area of Samsun, Turkey]. *Mikrobiyol Bul.* 2012;46(2):247-256.

54. Asal G, Tamer C, Albayrak H. Serological survey and molecular investigation of tick-borne encephalitis virus in Northern Turkey. *Etlik Veteriner Mikrobiyoloji Dergisi*. 2022;33(1):34-39. doi:10.35864/evmd.1064554

55. Eltari E. Epidemiology of tick-borne encephalitis in Albania. *Ellipse*. 1991;29:449–450.

56. Eltari E, et al. Some data on arboviruses, especially tick-borne encephalitis, in Albania. *Giornale di Malattie Infettive e Parassitarie*. 1993;45:404.

57. Sherifi K, Rexhepi A, Berxholi K, et al. Crimean-Congo Hemorrhagic Fever Virus and Borrelia burgdorferi sensu lato in Ticks from Kosovo and Albania. Front Vet Sci. 2018;5:38. Published 2018 Mar 6. doi:10.3389/fvets.2018.00038

58. Zlobin VI, Verkhozina MM, Demina TV, et al. *Vopr Virusol*. 2007;52(6):4-13.

59. Omeragic J. TICKS AND TICK-BORNE PATHOGENS IN BOSNIA AND ERZEGOVINA, in Specialty training course for young scientists of West Balkan countries “New methods in Parasitology: Tick-borne disease research and targeted selective anthelmintic treatment”. 2013: Munich, Germany.

60. Jakimovski D, Mateska S, Dimitrova E, et al. Tick-Borne Encephalitis Virus and Borrelia burgdorferi Seroprevalence in Balkan Tick-Infested Individuals: A Two-Centre Study. *Pathogens*. 2023;12(7):922. Published 2023 Jul 9. doi:10.3390/pathogens12070922

61. Elyan DS, Moustafa L, Noormal B, et al. Serological evidence of flaviviruses infection among acute febrile illness patients in Afghanistan. *J Infect Dev Ctries*. 2014;8(9):1176-1180. Published 2014 Sep 12. doi:10.3855/jidc.4183

62. Kuchuloria T, Imnadze P, Mamuchishvili N, et al. Hospital-Based Surveillance for Infectious Etiologies Among Patients with Acute Febrile Illness in Georgia, 2008-2011. *Am J Trop Med Hyg*. 2016;94(1):236-242. doi:10.4269/ajtmh.15-0400

63. Pakbin B, Rossen JWA, Brück WM, et al. Prevalence of foodborne and zoonotic viral pathogens in raw cow milk samples. *FEMS Microbiol Lett*. 2022;369(1):fnac108. doi:10.1093/femsle/fnac108

64. Parsadanians A, Mirshahabi H, Yavarmanesh M. Frequency of Tick-Borne Encephalitis Virus (TBEV) in Raw Milk Samples in Zanjan, Northwest of Iran. 2016.

65. Salehi-Vaziri M, Pouriayevali MH, Azad-Manjiri S, Vasmehjani AA, Baniasadi V, Fazlalipour M. The seroprevalence of tick-borne encephalitis in rural population of mazandaran province, northern iran(2018 - 2019). *Arch Clin Infect Dis*. 2020;15(1). doi:10.5812/archcid.98867

66. Atkinson B, Hewson R. Emerging arboviruses of clinical importance in Central Asia. J Gen Virol. 2018;99(9):1172-1184. doi:10.1099/jgv.0.001125

67. Dellagi K, Salez N, Maquart M, et al. Serological Evidence of Contrasted Exposure to Arboviral Infections between Islands of the Union of Comoros (Indian Ocean). *PLoS Negl Trop Dis*. 2016;10(12):e0004840. Published 2016 Dec 15. doi:10.1371/journal.pntd.0004840

68. Sutherland LJ, Cash AA, Huang YJ, et al. Serologic evidence of arboviral infections among humans in Kenya. Am J Trop Med Hyg. 2011;85(1):158-161. doi:10.4269/ajtmh.2011.10-0203

69. Andayi F, Charrel RN, Kieffer A, et al. A sero-epidemiological study of arboviral fevers in Djibouti, Horn of Africa. *PLoS Negl Trop Dis*. 2014;8(12):e3299. Published 2014 Dec 11. doi:10.1371/journal.pntd.0003299

70. Van Cuong N, Carrique-Mas J, Vo Be H, et al. Rodents and risk in the Mekong Delta of Vietnam: seroprevalence of selected zoonotic viruses in rodents and humans. *Vector Borne Zoonotic Dis*. 2015;15(1):65-72. doi:10.1089/vbz.2014.1603

71. Mohd Shukri M, Ling Kho K, Ghane Kisomi M, et al. Seroprevalence report on tick-borne encephalitis virus and Crimean-Congo hemorrhagic fever virus among Malaysian's farm workers. *BMC Public Health*. 2015;15:704. Published 2015 Jul 24. doi:10.1186/s12889-015-1901-4

Chapter 13

TBE in Austria

Karin Stiasny, Simon Raffl, Stephan W. Aberle and Judith H. Aberle

E-CDC risk status: endemic *(last edited: date 29.02.2024, data from 2023)*

History and current situation

Since 1972, the documentation of human cases of tick-borne encephalitis (TBE) in Austria has been performed by the Center for Virology, Medical University of Vienna, which acts as the National Reference Laboratory for TBE and other flavivirus infections. Only hospitalized patients with a recent tick-borne encephalitis virus (TBEV) infection confirmed by laboratory diagnosis are counted as cases. Confirmation is usually based on immunoglobulin (Ig) serology (namely enzyme-linked immunosorbent assay [ELISA] for IgM and IgG). However, this confirmation may be supplemented by virus neutralization and polymerase chain reaction (PCR) analyses if needed.

In 2012, TBE became a notifiable disease in Austria as in other countries of the European Union.[1] The annual incidence rates of TBE in Austria have declined substantially since the 1980s.[2] This decline was associated with an increasing rate of vaccination and was not observed in some neighboring countries, for example, Czech Republic and Slovenia, where vaccination coverage is much lower than in Austria.[2]

Incidences of TBE in the total and unvaccinated population in Austria from 2010 to 2023 are shown in Figure 1. Strong annual fluctuations are a characteristic feature of the epidemiology of TBE in Austria, indicating a complex interplay of factors that control viral transmission dynamics in natural hosts and human risk exposure. The age distribution of TBE incidences in Austria is strongly shifted towards older people[2] and reveals a peak in the population 41 to 80 years of age (Figure 2). In addition to virus transmission by tick bites, alimentary infections through the consumption of infected goat cheese have been documented.[3,4] TBE viruses isolated in Austria from ticks and humans were shown through molecular analyses to be members of the European subtype of TBEV (TBEV-Eu)[5] (and Gerhard Dobler, personal communication; Stephan W. Aberle and Jeremy V. Camp, unpublished results).

Mapping of the most likely sites of human infections has been performed by the National Reference Laboratory since 1972 through the use of questionnaires sent to hospitalized TBE patients with confirmed laboratory diagnosis.[6] These data are shown in Figure 3. Although many of the most affected regions remained constant throughout the observation period, new endemic zones – especially in previously unaffected alpine regions in western Austria –

Overview of TBE in Austria

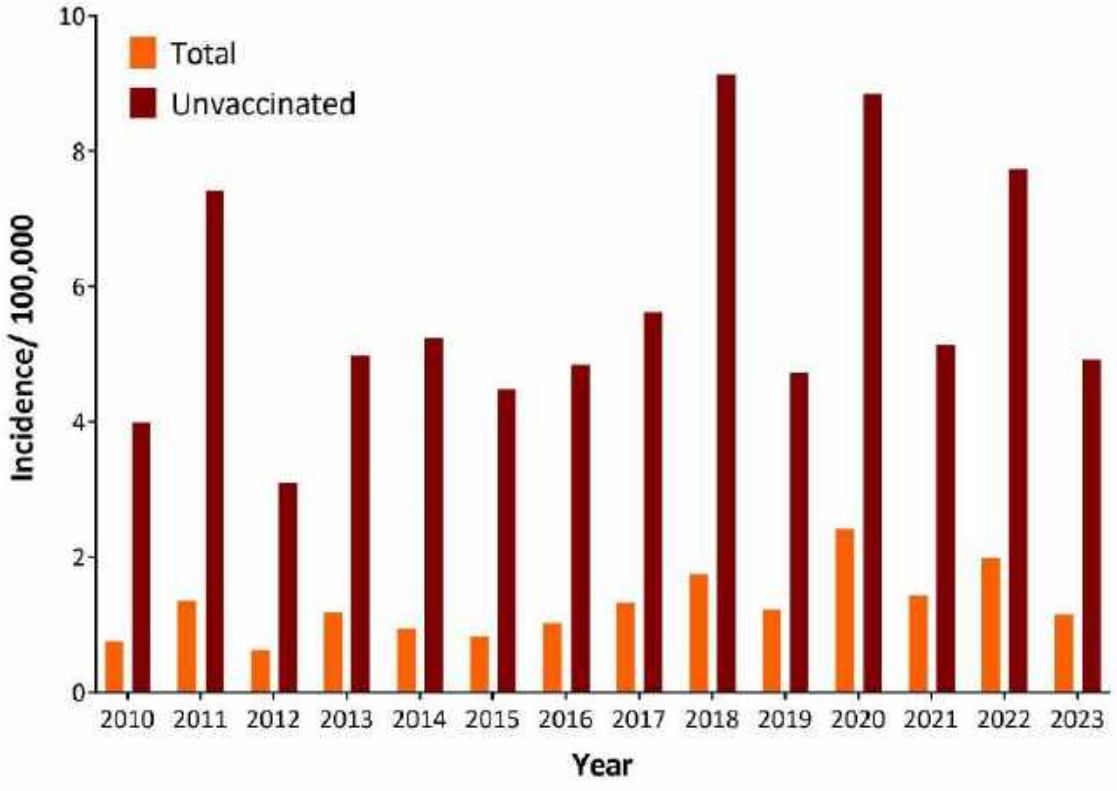
Table 1: TBE in Austria

Viral subtypes, distribution	European TBEV subtypes[5] (and Gerhard Dobler, personal communication; Stephan W. Aberle and Jeremy V. Camp, unpublished results.)
Reservoir animals	No information available
Percentage infected ticks	No information available
Dairy product transmission	Small outbreaks[3,4]
Case definition used by authorities	ECDC
Completeness of case detection and reporting	No information available on the % of undetected cases
Type of reporting	Mandatory for clinically and serologically verified viral meningoencephalitis[8]
Other TBE surveillance	No information available
Special clinical features	Mild clinical course (febrile illness, meningitis): 36.5%. Severe clinical course (meningoencephalitis, encephalomyelitis, radiculitis): 63.5%. Data of the National reference center for 2023.
Licensed vaccines	Encepur Erwachsene, Encepur Kinder (Bavarian Nordic) FSME-IMMUN Erwachsene, FSME-IMMUN Kinder (Pfizer)
Vaccination recommendations	General recommendation https://www.sozialministerium.at/Themen/Gesundheit/Impfen/Impfplan-%C3%96sterreich.html
Vaccine uptake	~80%[9]
National Reference center for TBE	National reference center for human arbovirus infections Center for Virology, Medical University of Vienna Kinderspitalgasse 15, 1090 Vienna, Austria virologie@meduniwien.ac.at

have become established.[6] The first TBE case in the federal province of Tyrol was documented in 1984 and in Vorarlberg in 2000. In the subsequent years, certain valleys in both states became sites of infection for a substantial number of human TBE cases.[6] In parallel, the incidences in the northeastern part of the country (comprising regions with relatively low altitudes) declined,[6] suggesting a change to less favorable conditions for virus circulation in this area. In the traditional core TBE zones of Austria, no evidence has been seen for a shift of infection sites to higher altitudes.[6]

The causes for establishment of new endemic regions in Austria as well as the decline of TBE in other parts of the country are unknown. Surprisingly, these changes are not paralleled by similar alterations in the incidence of borreliosis, which is transmitted by the same ticks as TBEV but remained relatively constant over time in all parts of Austria.[7] These data rule out that the substantial geographical shifts of TBE incidence are only caused by changes in tick abundance or human behavior affecting the risk of tick exposure. The discordant epidemiology of TBE and borreliosis in some parts of Austria rather suggests the existence of yet undefined virus-specific factors that control the circulation of TBEV in its animal reservoir and is independent of general factors controlling the proliferation of ticks.

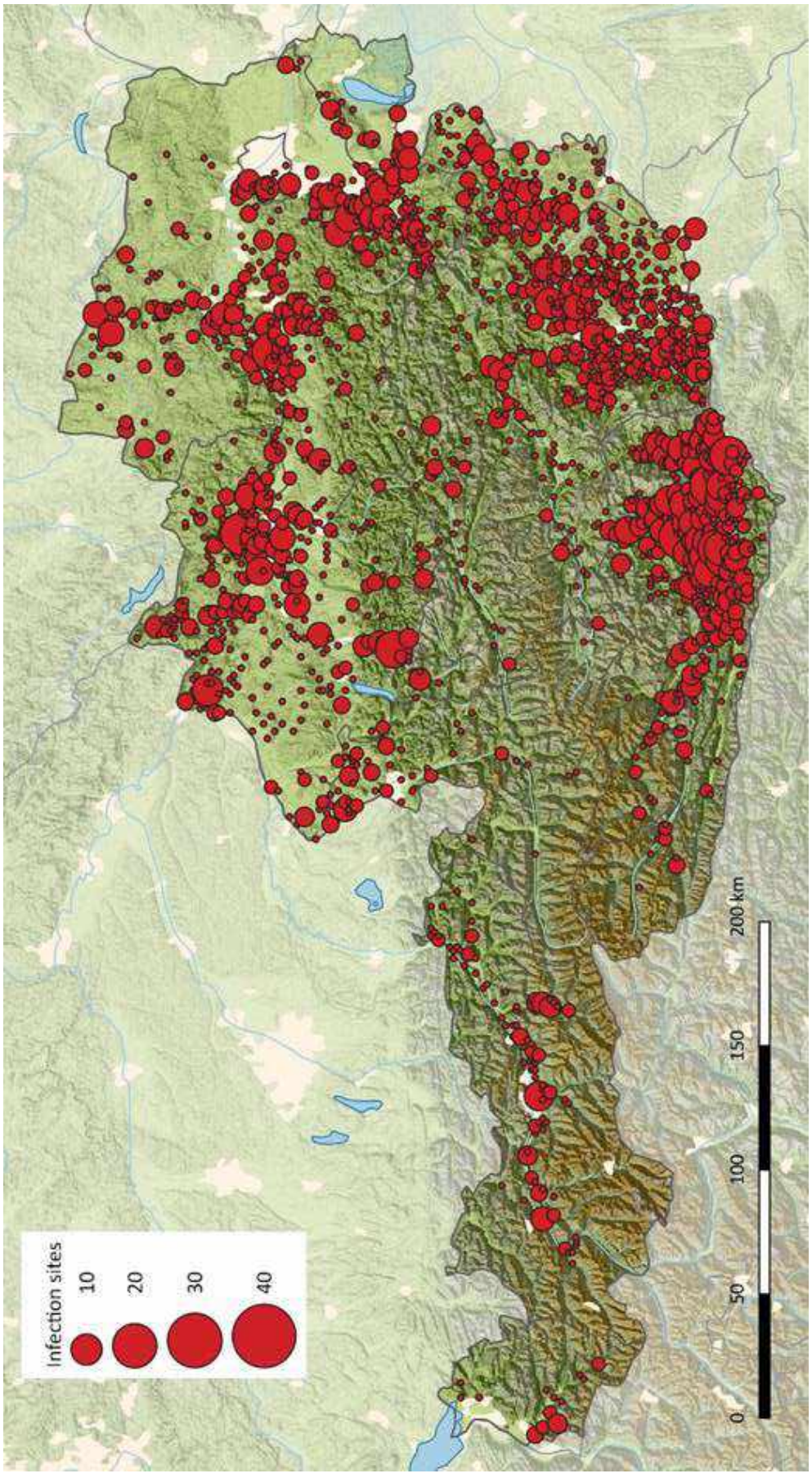
Figure 1: Incidence of TBE in Austria in total and unvaccinated population, 2010–2023



Orange columns: TBE incidence in the total population
***Magenta** columns: TBE incidence in the unvaccinated population (based only on patients with a documented status of 'no vaccination'). Population data were obtained from the Austrian Statistical Office ("Statistik Austria", https://www.statistik.at/) and vaccine-coverage data from reference[10]).*

Source Data: Appendix—Figure 1

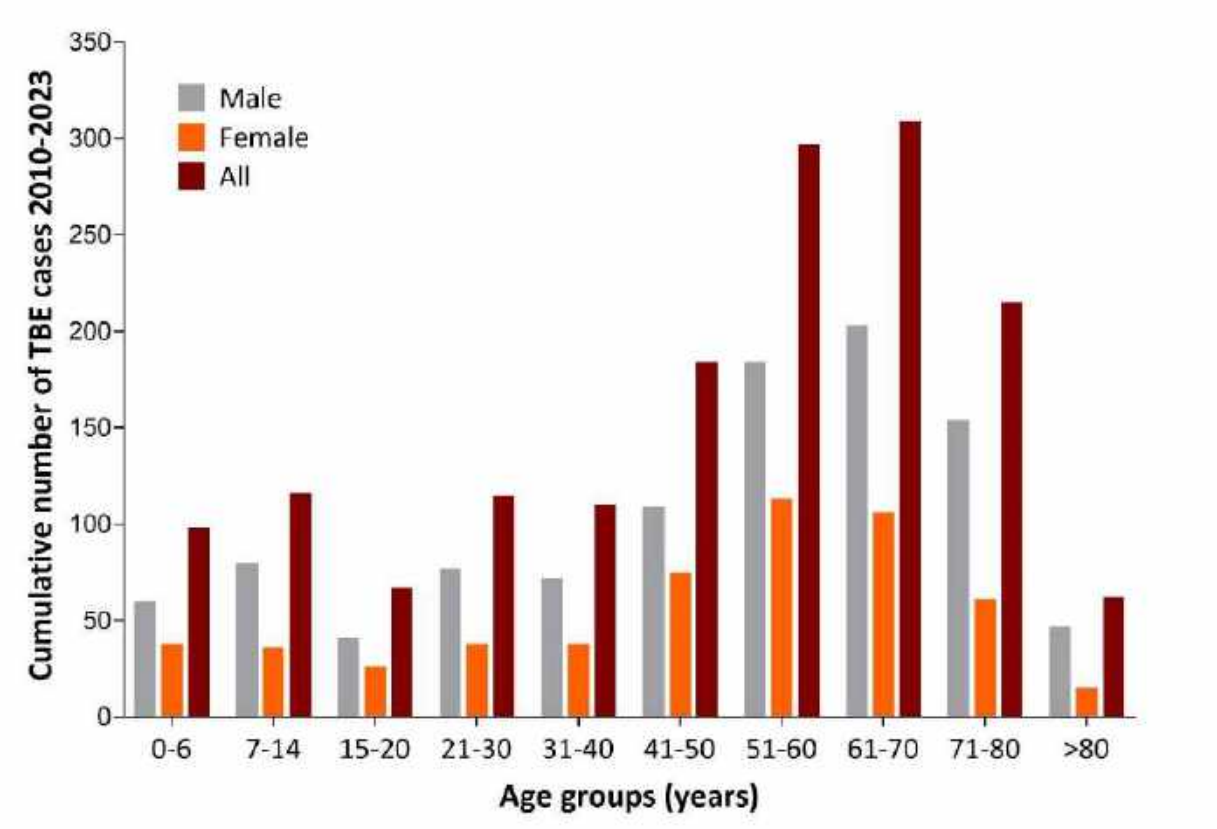
Figure 3: Sites of TBEV infection in Austria, 1972–2023



Red circles: Cumulative infection sites of TBE patients for the period from 1972 to 2023

Infection sites were geocoded and processed for spatial mapping by QGIS (https://www.qgis.org/). Spatially close sites were aggregated using a 1.5 km raster for Austria, and centroids were calculated for each square. These centroids formed the center of the red circles with diameters proportional to the number of documented infection sites within this area. The base map was built with Natural Earth Data [borders, rivers, lakes, cities; http://www.naturalearthdata.com/] and Global Multi-Resolution Topography (GMRT) synthesis data of the Marine Geoscience Data System (MGDS) [topography [11], http://www.marine-geo.org/tools/GMRTMapTool/].

Figure 2: Age distribution and gender of TBE in Austria, 2010–2023



Source Data: Appendix—Figure 2

Appendix

Source data: Figure 1

Incidence/100,000

Year	Total	Unvaccinated
2010	0.75	3.99
2011	1.35	7.41
2012	0.62	3.09
2013	1.17	4.98
2014	0.94	5.23
2015	0.82	4.48
2016	1.02	4.85
2017	1.32	5.62
2018	1.74	9.13
2019	1.22	4.72
2020	2.42	8.85
2021	1.43	5.14
2022	1.98	7.73
2023	1.15	4.92

Source data: Figure 2

Cumulative number of cases by age and gender

Age group (years)	Males	Females	All
0-6	60	38	98
7-14	80	36	116
15-20	41	26	67
21-30	77	38	115
31-40	72	38	110
41-50	109	75	184
51-60	184	113	297
61-70	203	106	309
71-80	154	61	215
>80	47	15	62

Authors and affiliations

Karin Stiasny, Simon Raffl, Stephan W. Aberle, Judith H. Aberle
Center for Virology, Medical University of Vienna, 1090 Vienna, Austria

Contact: karin.stiasny@meduniwien.ac.at

Citation:
Stiasny K, Raffl S, Aberle SW, Aberle JH. TBE in Austria. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-1-7

References

1. Amato-Gauci A, Zeller H. Tick-borne encephalitis joins the diseases under surveillance in the European Union. *Euro Surveill*. 2012;17(42):20299.
2. Heinz FX, Stiasny K, Holzmann H, et al. Vaccination and tick-borne encephalitis, central Europe. *Emerg Infect Dis*. 2013;19 (1):69-76. doi:10.3201/eid1901.120458.
3. Holzmann H, Aberle SW, Stiasny K, et al. Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. *Emerg Infect Dis*. 2009;15(10):1671-3. doi:10.3201/eid1510.090743.
4. Mylonaki E, Seiberl M, Jones N, et al. Tick-borne encephalitis virus RNA found in frozen goat's milk in a family outbreak. *Int J Mol Sci*. 2022;23(19). doi:10.3390/ijms231911632.
5. Ecker M, Allison SL, Meixner T, Heinz FX. Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia. *J Gen Virol*. 1999;80:179-85. doi:10.1099/0022-1317-80-1-179.
6. Heinz FX, Stiasny K, Holzmann H, et al. Emergence of tick-borne encephalitis in new endemic areas in Austria: 42 years of surveillance. *Euro Surveill*. 2015;20(13):9-16. doi:10.2807/1560-7917.es2015.20.13.21077.
7. Stiasny K, Santonja I, Holzmann H, et al. The regional decline and rise of tick-borne encephalitis incidence do not correlate with Lyme borreliosis, Austria, 2005 to 2018. *Euro Surveill*. 2021;26(35):2002108. doi:10.2807/1560-7917.ES.2021.26.35.2002108.
8. Bundesministerium für Soziales G, Pflege und Konsumentenschutz. Anzeigenpflichtige Krankheiten in Österreich (gem. Epidemiegesetz, BGBl. Nr. 186/1950 idgF, Tuberkulosegesetz BGBl. Nr. 127/1968, AIDS-Gesetz, BGBl. Nr. 728/1993 idgF, Geschlechtskrankheitengesetz, StGBl. Nr. 152/1945 idgF). In. *Anzeigenpflichtige Krankheiten in Österreich*. Vol 12023:8.
9. Pilz A, Erber W, Schmitt HJ. Vaccine uptake in 20 countries in Europe 2020: Focus on tick-borne encephalitis (TBE). *Ticks Tick Borne Dis*. 2023;14(1):102059. doi:10.1016/j.ttbdis.2022.102059.
10. Kunze M, Erber W, Haditsch M. TBE as a matter of public health. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book* (6th edition). Global Health Press, Singapore, 2023:365-372. doi:10.33442/26613980_13-4
11. Ryan WBF, Carbotte SM, Coplan JO, et al. Global Multi-Resolution Topography synthesis. *Geochem Geophys*. 2009;10(3):2008GC002332. doi:10.1029/2008GC002332

Chapter 13

TBE in Belarus

Oksana Hanenko, Natalia Kolomiets, Alla Dashkevich,
Vlada Zapolskaya, Igor Stoma

E-CDC risk status: endemic *(last edited: date 01.04.2024)*

History and current situation

Tick-borne encephalitis is endemic in Belarus. The Tick-Borne Encephalitis Virus (TBEV) was first isolated in the country from *Ixodes ricinus* ticks in 1939 and from humans in 1954.[1,2] According to the multi-year follow-up data (2014-2023), a rise in TBE incidence among the national population has been recorded since 2022 (2.8 cases per 100,000 population) and reached its current peak with 4.1 cases per 100,000 population in 2023 (Figure 1).

From 2020 to 2023, a total of 844 TBE cases were registered in Belarus. During this period, there was a tendency for an incidence increase in all administrative territories of the country, except for the Gomel region. TBE incidence rates in the Grodno and Brest regions were the highest and exceeded the national average in all the years of observation (Figures 2 and 3). The age structure of patients was dominated by people over 18 years old (802 out of 844 [95.0%]). Gender structure was dominated by males (526 out of 844 [62.3%]). In the vast majority of cases transmission mode was by the bite of infected ticks (766 out of 844 [90.8%]), whereas for 33 (3.9%) of patients it was by consumption of infected raw goat milk. For 45 patients (5.3%) the transmission route was not identified. With regard to seasonality, the share of those who fell ill in July and August accounted for 24.5% (207/844) and 22.9% (193/844), respectively.

Overview of TBE in Belarus

Table 1: TBE in Belarus	
Virus subtypes isolated	Regional circulation of the European (TBEV-Eu) virus subtype has been established; single natural isolates have been identified as the Far Eastern (TBEV-FE) subtype[3,4].
Reservoir animals	Epidemiologically significant *Ixodidae* ticks is presented by two mass species: *Ixodes ricinus* and *Dermacentor reticulatus*. Their parasitization has been observed on more than 65 species of vertebrates living in forests, as well as on cattle and domestic animals[2]. Some few isolates from natural reservoirs have been characterized as *Ixodes persulcatus*[2,5].
Percentage of infected ticks	The detection of TBEV in *Ixodes ricinus* and *Dermacentor reticulatus* ticks was 0,27% in 2022 and 0,37% in 2021 out of the total number of specimens examined in those years, 3978 and 3741, respectively[6].
Dairy product transmission	Documented for 3.9% of cases
Case definition used by authorities	None specified
Completeness of case detection and reporting	Unknown
Type of reporting	Mandatory
Other TBE surveillance	None
Special clinical features	Out of 844 patients, 79 patients (9,4%) had a severe clinical form of the disease; 3 cases had a fatal outcome (case fatality rate: 0,4%). Fatal cases were registered in highly endemic areas of the country (Grodno and Brest regions).
Licensed vaccines	TBE vaccines registered in Belarus[7]: TICOVAC, TICOVAC JUNIOR, Tick-E-Vak, Encevir
Vaccine Recommendations	Risk groups: employees of forest managing organizations working in the territories of: the National Park "Belovezhskaya Pushcha"; the Berezinski Biosphere Reserve; other enzootic areas[8]. Vaccination is also recommended for all the people travelling to or living in endemic areas[9]
Vaccine Uptake	Unknown
National reference center for TBE	None

Figure 1: Yearly notification rate of tick-borne encephalitis cases per 100 000 population, Belarus, 2014–2023

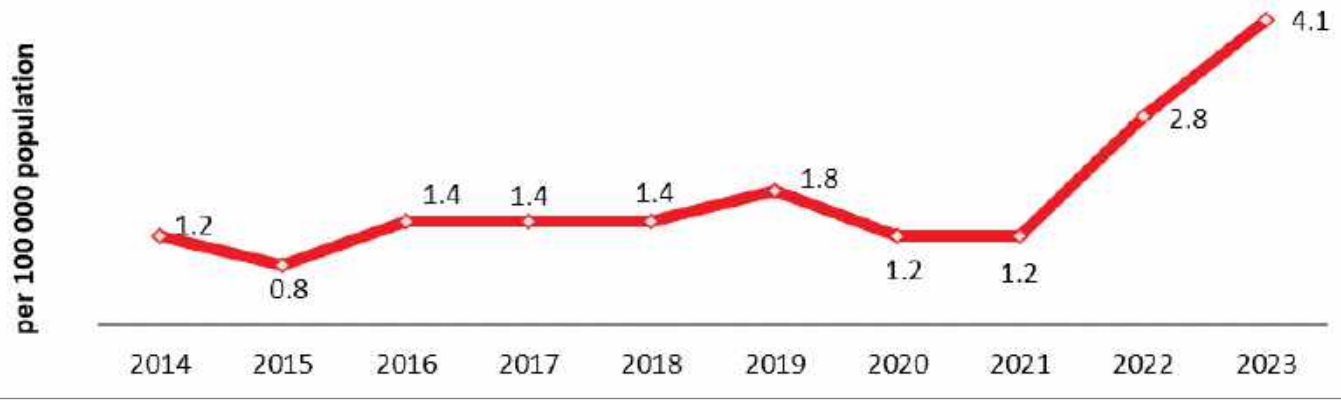
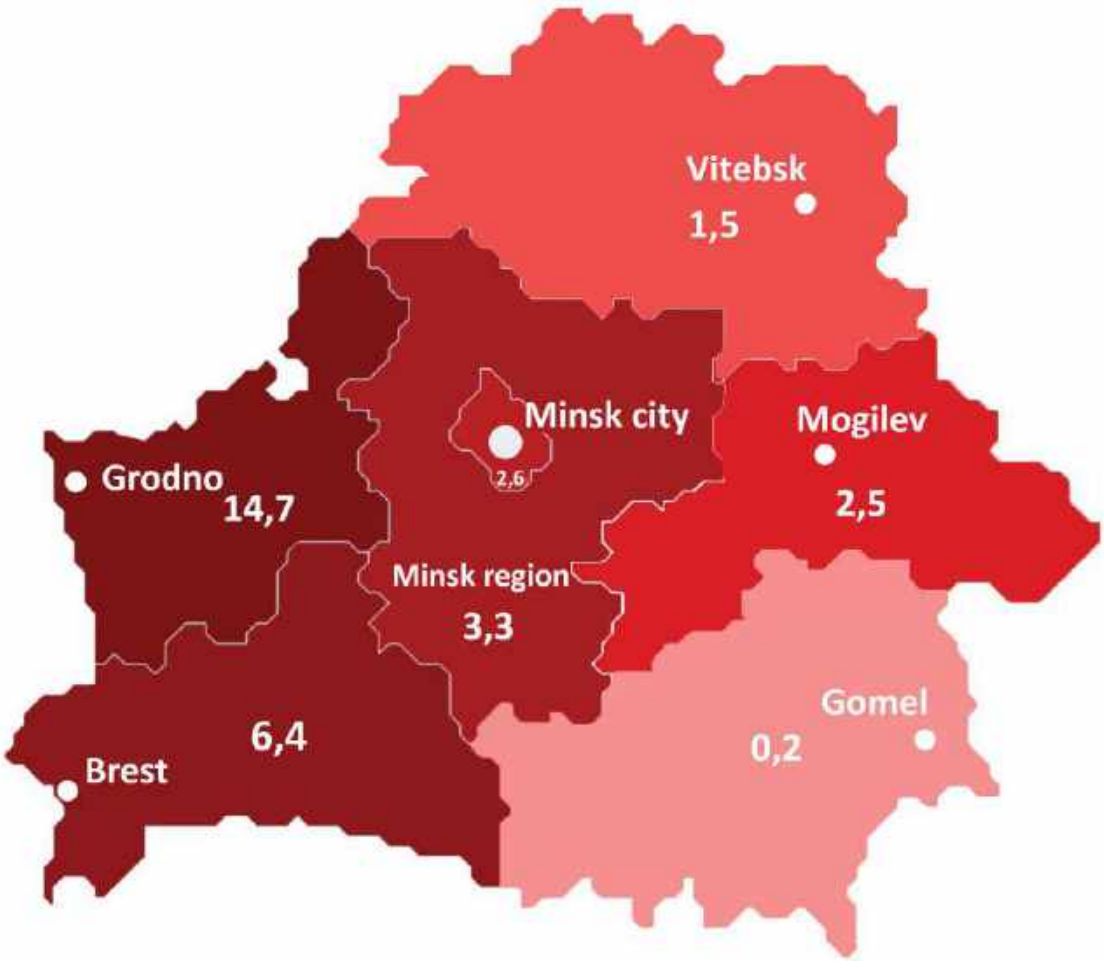


Figure 3: Map of TBE cases per 100 000 population in the administrative territories of Belarus in 2023



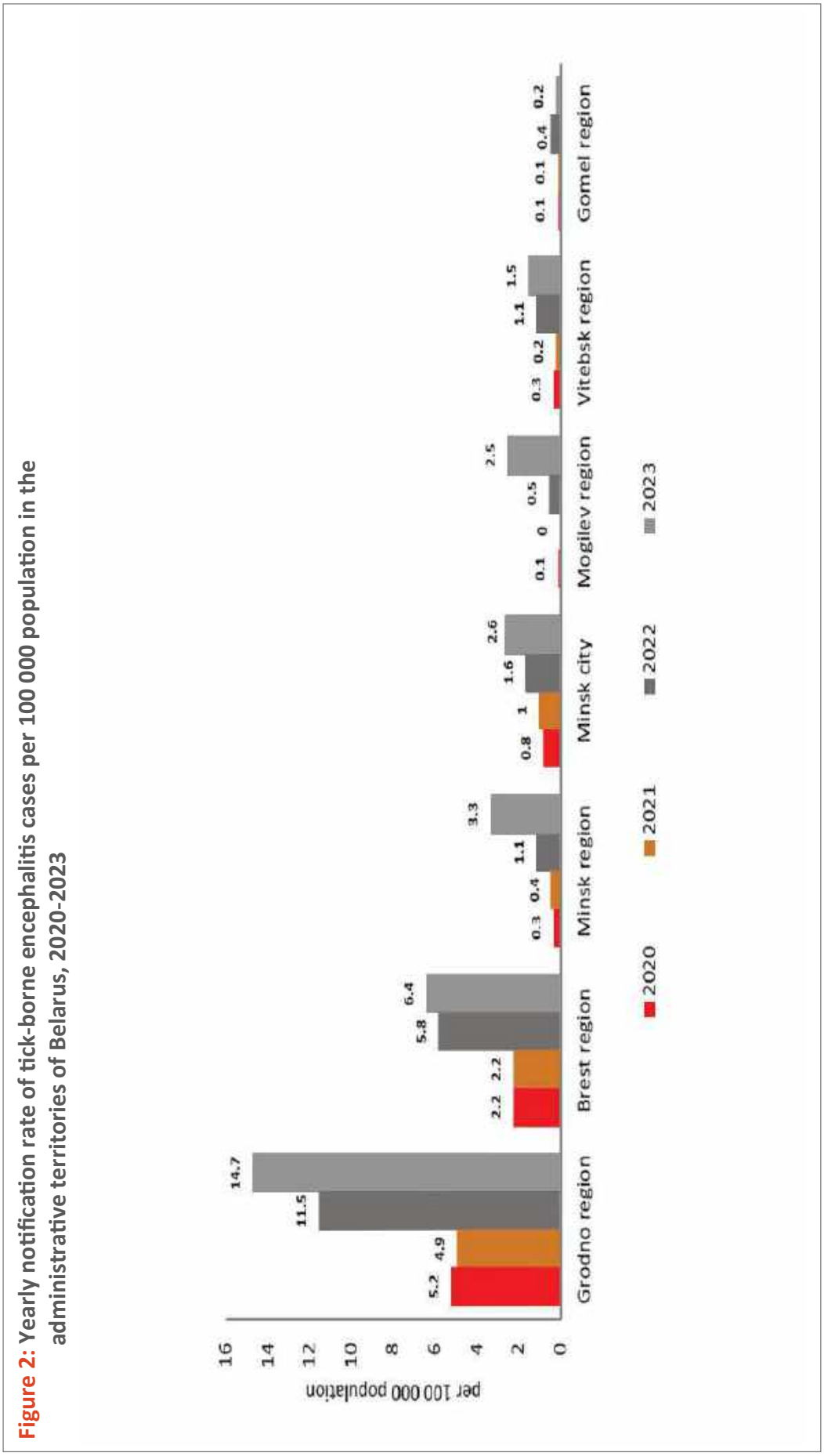


Figure 2: Yearly notification rate of tick-borne encephalitis cases per 100 000 population in the administrative territories of Belarus, 2020-2023

Appendix

Source data: Figure 1

Year	Cases
2014	113
2015	75
2016	133
2017	136
2018	134
2019	168
2020	108
2021	108
2022	260
2023	368

Authors and affiliations

Oksana Hanenko[1], Natalia Kolomiets[1], Alla Dashkevich[2], Vlada Zapolskaya[2], Igor Stoma[3]

[1]Institute of Advanced Training and Retraining of Healthcare Personnel of the Belarusian State Medical University, Minsk, Belarus

[2]State Institution «Republican Center for Hygiene, Epidemiology and Public Health», Minsk, Belarus

[3]Educational Institution «Gomel State Medical University», Gomel, Belarus

Contact: igor.stoma@gmail.com

Citation:
Hanenko O, Kolomiets N, Dashkevich A, Zapolskaya V, Stoma I. TBE in Belarus. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-2-7

References

1. Votyakov VI, Protas II, Zhdanov VM. Western tick-borne encephalitis. 1978.
2. Bychkova EI, Fedorova IA, Yakovich MM. [Ixodid ticks (Ixodidae) in Belarus]. 2015.
3. Samoilova TI, Votyakov VI, Mikhailova AA, et al. Genotyping of tick-borne encephalitis viruses isolated in Belarus using nucleotide sequence sequencing. 2007. (in Russian). Accessed April 9, 2024. https://med.by/dmn/book.php?book=07-7_3.
4. Zlobin VI, Verkhozina MM, Demina TV, et al. [Molecular epidemiology of tick-borne encephalitis]. *Vopr Virusol*. 2007;52 (6):4-13.
5. Bespyatova LA, Bychkova EI, Yakovich MM, Bugmyrin SV. Manifestation peculiarities of natural focuses of tickborne infections on the territory of Karelia and Belarus. Natural resources. 2018;(1):86-91. (in Russian).
6. Ministry of Health of Belarus. On the sanitary and epidemiological situation in Belarus in 2022: report.
7. State register of medicines of the Republic of Belarus. Accessed April 9, 2024. https://www.rceth.by/Refbank/reestr_lekarstvennih_sredstv/results.
8. On preventive vaccinations: by resolution of the Ministry of Health of Belarus 2018;42. Accessed April 9, 2024. https://minzdrav.gov.by/ru/dlya-belorusskikh-grazhdan/vaktsinatsiya/natsionalnyy-kalendar-privivok.php
9. On approval of the Instructions on the tactics of carrying out preventive vaccinations among the population in the Republic of Belarus: Order of the Ministry of Health of Belarus № 191; 2014 (in Russ.). Available at: https://minzdrav.gov.by/ru/dlya-spetsialistov/normativno-pravovaya-baza/baza-npa.php?ELEMENT_ID=331869

Chapter 13

TBE in Belgium

Marjan Van Esbroeck, Tinne Lernout and Steven Van Gucht

ECDC risk status: affected (*last edited: date 14.2.2024, data from 2023*)

History and current situation

Until 2018, only imported cases of TBE were detected in Belgium, mainly infected in other parts of Europe such as Estonia, Germany[1], Austria, Scandinavia, Slovenia[2] and the Czech Republic, but also Kyrgyzstan, Russia and the USA. In the summer of 2020, the first three confirmed autochthonous cases were diagnosed at the National Reference Centre of arboviruses (the Institute of Tropical Medicine, Antwerp, Belgium)[3]. Already in 2018, two cases possibly/probably infected in Belgium were reported, but patients had also traveled during the incubation period. No autochthonous cases have been detected since 2020 (Figure 1). The distribution of reported cases by age and gender is comparable to what is observed in other European countries, with a higher number of cases in males, and more cases in the older age groups (45+).

Based on the current epidemiological findings, Belgium is classified as an affected country for TBE, with possible presence of the virus spread over the territory (Figure 2).

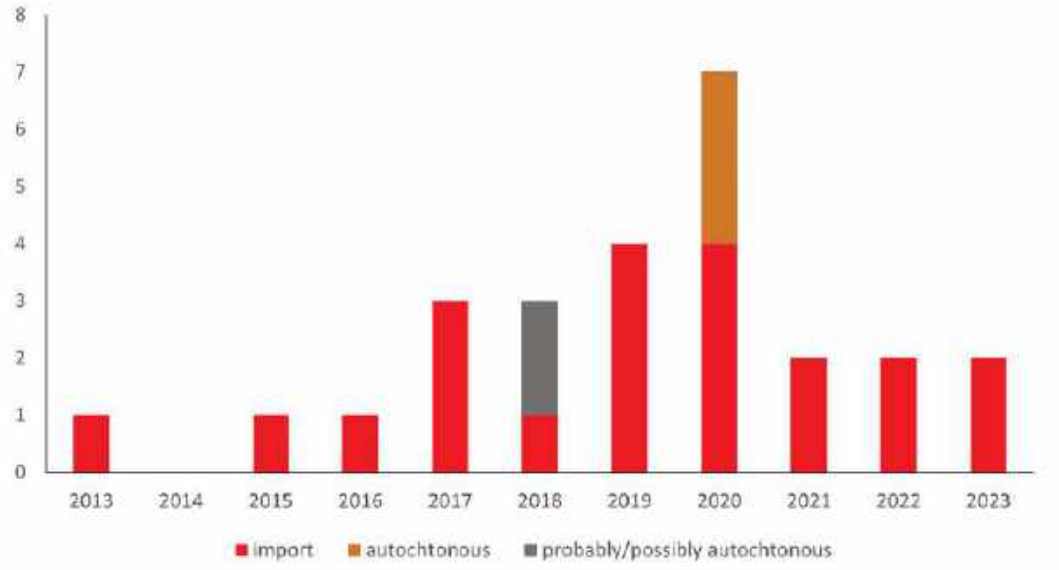
The finding of autochthonous cases was not surprising as several (sero)prevalence studies in sentinel animals suggested that the virus had been circulating at a low level for at least several years. Depending on the animal species, prevalence rates ranging from 0.11% in dogs in 2009 (Belgium) to 9.27% in wild boars in 2019/2020 (Flanders) have been reported.[4-8] The results of the study on wild boars in 2020 suggest an increase in TBEV prevalence over the last decade.[8]

Two out of the three patients with an autochthonous infection, diagnosed in 2020, had been exposed in a geographical location adjacent to an area with known TBEV seropositivity in animals.[3]

Several screening programs set up to detect TBEV in ticks, have been undertaken since 2017. Screening for TBEV by PCR in 1,307 ticks collected through flagging in the surrounding nature of the autochthonous cases in 2018, 2019, 2022 and 2023 did not reveal the presence of TBEV (Van Esbroeck, personal communication). Using a citizen science approach based on an existing notification tool for tick bites, 1,599 and 928 ticks removed from humans, 99% of which belonged to Ixodes ricinus, were collected across Belgium in 2017 and 2021 respectively. None of the ticks tested positive for TBEV by PCR.[8-10]

In 2019, a seroprevalence study in Flanders among 195 forestry workers exposed to tick bites during professional activities, of which 85% with more than 10 years of exposure and 42% reporting at least one tick bite/month during the tick season, revealed that none had antibodies showing evidence of infection.[11]

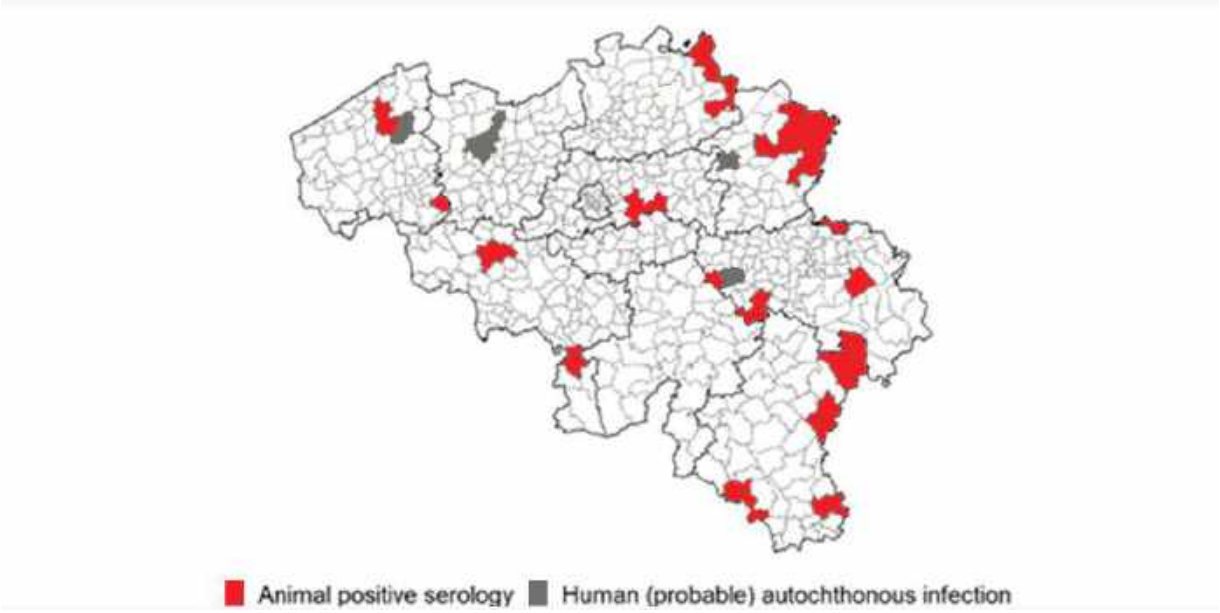
Figure 1: TBE case numbers over time, vaccination status unknown



Overview of TBE in Belgium

Table 1: TBE in Belgium	
Viral subtypes, distribution	No information available in humans. No virus-positive animals or ticks have been reported to date.
Reservoir animals	Seropositive cattle and sheep at national level and roe deer and wild boar in Flanders have been identified[4-8]
Percentage infected ticks	No positive ticks have been detected[8-10] (Van Esbroeck personal communication)
Dairy product transmission	No information available
Case definition used by authorities	ECDC case definitions
Completeness of case detection and reporting	No information available
Type of reporting	Annual reporting to the ECDC
Other TBE surveillance	1. A national reference center (NRC) for TBE performs laboratory confirmation in suspected human cases 2. Ad hoc seroprevalence monitoring in animals[4-8] 3. PCR testing of ticks collected from humans, from animals and by flagging[8-10] (Van Esbroeck personal communication)
Special clinical features	No
Licensed vaccines	FSME-IMMUN (Pfizer)
Vaccine recommendations	In the current epidemiological setting, vaccination is only recommended for travelers to endemic regions doing outdoor activities in forested areas during the tick season and for people handling TBEV in a laboratory setting[12]
Vaccine uptake	No data available
National Reference center for TBE	Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium, +32 3 247 64 45. www.itg.be

Figure 2: Cumulative sites of TBEV-infection in Belgium, 2018-2023



Acknowledgments

The National Reference Center for TBE is partially supported by the Belgian Ministry of Social Affairs through a fund within the Health Insurance System.

The Belgian Institute for Health, Sciensano is supported by the Belgian regional authorities (Department of Care and Agency for Nature and Forests (Flanders), and Agency for a qualitative life (Wallonia)).

Authors and affiliations

Marjan Van Esbroeck, Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp

Tinne Lernout, Belgian institute for health Sciensano, Brussels

Steven Van Gucht, Belgian institute for health Sciensano, Brussels

Contact: mvesbroeck@itg.be

Citation:
Van Esbroeck M, Lernout T, Van Gucht S. TBE in Belgium. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-3-7

References

1. Gils S, Frans J, Ho E, et al. Case report: tick-borne encephalitis (TBE) in a Belgian traveller returning from Germany. *J Travel Med*. 2018;25(1):10.1093/jtm/tay078. doi:10.1093/jtm/tay078
2. Du Four S, Mertens R, Wiels W, De Keyser J, Bissay V, Flamez A. Meningoencephaloradiculitis following infection with tick borne encephalitis virus: case report and review of the literature. *Acta Neurol Belg*. 2018;118(1):93-96. doi:10.1007/s13760-017-0873-9
3. Stoefs A, Heyndrickx L, De Winter J, et al. Autochthonous Cases of Tick-Borne Encephalitis, Belgium, 2020. *Emerg Infect Dis*. 2021;27(8):2179-2182. doi:10.3201/eid2708.211175
4. Roelandt S, Heyman P, De Filette M, et al. Tick-borne encephalitis virus seropositive dog detected in Belgium: screening of the canine population as sentinels for public health. *Vector Borne Zoonotic Dis*. 2011;11(10):1371-1376. doi:10.1089/vbz.2011.0647
5. Roelandt S*, Suin V*, Riocreux F, et al. Autochthonous tick-borne encephalitis virus-seropositive cattle in Belgium: a risk-based targeted serological survey. *Vector Borne Zoonotic Dis*. 2014;14:640-7. doi:10.1089/vbz.2014.1576. *Equal contribution.
6. Tavernier P, Sys SU, De Clercq K, et al. Serologic screening for 13 infectious agents in roe deer (Capreolus capreolus) in Flanders. *Infect Ecol Epidemiol*. 2015;5:29862. Published 2015 Nov 24. doi:10.3402/iee.v5.29862
7. Roelandt S, Suin V, Van der Stede Y, et al. First TBEV serological screening in Flemish wild boar. *Infect Ecol Epidemiol*. 2016;6:31099. Published 2016 Apr 15. doi:10.3402/iee.v6.31099
8. Adjadj NR, Vervaeke M, Sohier C, Cargnel M, De Regge N. Tick-Borne Encephalitis Virus Prevalence in Sheep, Wild Boar and Ticks in Belgium. *Viruses*. 2022;14(11):2362. Published 2022 Oct 26. doi:10.3390/v14112362
9. Lernout T, De Regge N, Tersago K, Fonville M, Suin V, Sprong H. Prevalence of pathogens in ticks collected from humans through citizen science in Belgium. *Parasit Vectors*. 2019;12(1):550. Published 2019 Nov 21. doi:10.1186/s13071-019-3806-z
10. Geebelen L, Philippe C, Hermy M, Mori M, Lernout T. Recherche de pathogènes chez les tiques, 2021. Accessed March 27, 2023. https://www.sciensano.be/sites/default/files/pathogenes_chez_les_tiques_2021_final.pdf
11. Lernout T, Roelandt A, Vandervelden J, et al. Epidemiological situation of tick-borne encephalitis in Belgium, an overview. Poster presented at the International Symposium on Tick-Borne Pathogens and Disease ITPD; October 22-25, 2023; Vienna, Austria.
12. Superior Health Council. Vaccination against Tick-Borne Encephalitis (TBE). Brussels. Report nr 9435. Accessed February 29, 2019. https://www.health.belgium.be/sites/default/files/uploads/fields/fpshealth_theme_file/shc_9435_tbe.pdf

Chapter 13

TBE in Bosnia and Herzegovina

Wilhelm Erber and Tamara Vuković-Janković

E-CDC risk status: affected (*last edited: date 25.06.24, data as of end 2022*)

History and current situation

Very limited information is available for Bosnia showing the occurrence of TBE.[7]

Even though there have been some elder case reports in the northern parts of the country, including alimentary infections, details have not been published.[3]

In early 1996, United States military forces were deployed to Bosnia as part of Operation Joint Endeavor. Only 4 (0.42%) unvaccinated individuals, all males, demonstrated a 4-fold seroconversion. All 4 seemingly were infected with TBE virus (or a closely-related variant) during their 6–9-month deployment period in Bosnia, but did not report with symptoms to any health care provider.[2,4,5]

The only official TBE case report data so far are from the Centralized Information System for Infectious Diseases ([CISID] – WHO: incidence of tick-borne encephalitis) where 1 case was reported in 2001, and 2 cases were reported in 2010, and additionally 5 cases of alimentary outbreak were reported in 2014 by the Institute of Public Health in Serbia (Institute of Public Health FBIH https://www.zzjzfbih.ba/biblioteka/) [Accessed October 2016].

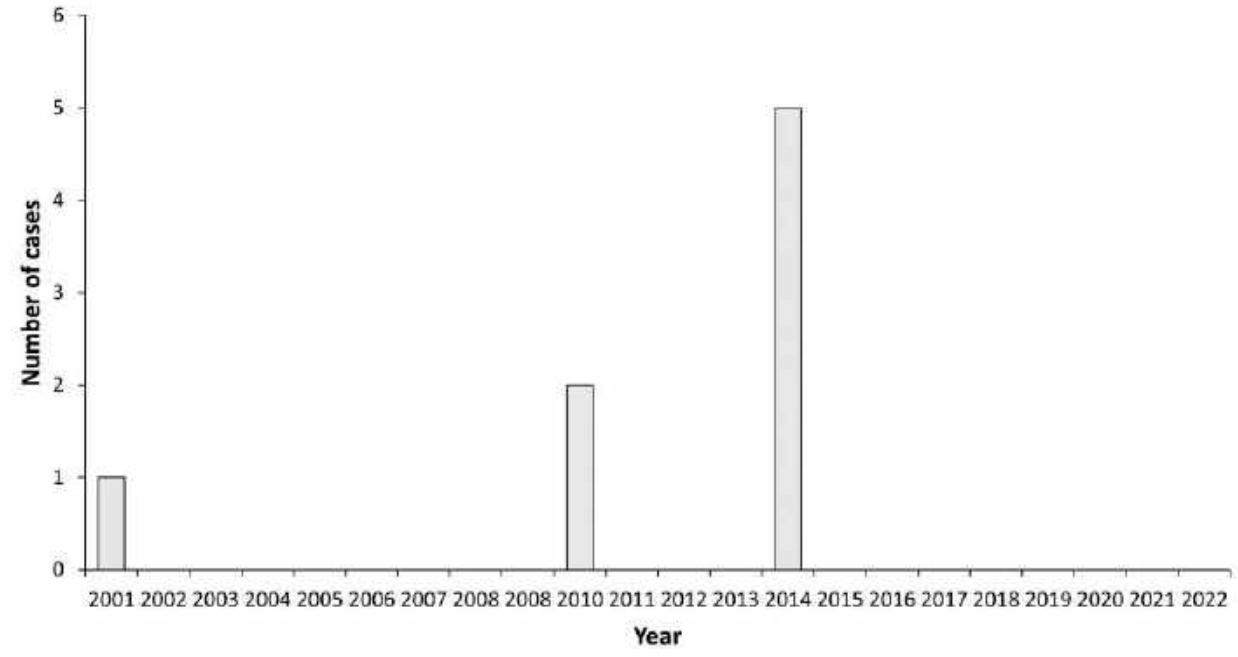
Overview of TBE in Bosnia and Herzegovina

Table 1: Virus, vector, transmission of TBE in Bosnia and Herzegovina

Viral subtypes, distribution	TBEV-SIB[1,2], TBEV-EU?
Reservoir animals	There is a lack of data on TBEV-seroprevalence among wild animals[8]
Infected tick species (%)	*I. ricinus*[1,2]
Dairy product transmission	Has been reported[3]

However, the proven record about the spread of the TBE virus in Bosnia and Herzegovina is the isolation of five strains of the TBEV-Sib genotype 3 in *Ixodes ricinus*.[1,2] Siberian TBEV strains from Bosnia, the Crimean Peninsula, Kyrgyzstan and Kazakhstan are clustered into a newly described Bosnia lineage.[3]

Figure 1: Burden of TBE in Bosnia and Herzegovina over time[2,4,5,7]



Source Data: Appendix—Figure 1

Appendix

Source data: Figure 1

Year	Number of cases
2001	1
2002	
2003	
2004	
2005	
2006	
2007	
2008	
2008	
2010	2
2011	
2012	
2013	
2014	5
2015	
2016	
2017	
2018	
2019	
2020	
2021	
2022	

Contact: wilhelm.erber@gmail.com

Citation:
Erber W, Janković TV. TBE in Bosnia and Herzegovina. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-4-7

References

1. Demina TV, Dzhioev YP, Verkhozina MM, et al. Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. *J Med Virol*. 2010;82(6):965-976.
2. Tkachev S, et.al. Genetic diversity and geographical distribution of the Siberian subtype of the tick-borne encephalitis virus. Medical Biodefense Conference 2018; Munich.
3. Tkachev SE, Babkin IV, Chicherina GS, et al. Genetic diversity and geographical distribution of the Siberian subtype of the tick-borne encephalitis virus. *Ticks Tick Borne Dis.* 2019;11(2):101327.
4. Suess J. Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine*. 2003;21 Suppl 1:S19-35.
5. Craig SC, Pittman PR, Lewis TE, et al. An accelerated schedule for tick-borne encephalitis vaccine: the American Military experience in Bosnia. *Am J Trop Med Hyg*. 1999;61(6):874-878.
6. Sanchez JL Jr, Craig SC, Kohlhase K, Polyak C, Ludwig SL, Rumm PD. Health assessment of U.S. military personnel deployed to Bosnia-Herzegovina for operation joint endeavor. *Mil Med*. 2001;166(6):470-4.
7. Amicizia D, Domnich A, Panatto D, et al. Epidemiology of tick-borne encephalitis (TBE) in Europe and its prevention by available vaccines. *Hum Vaccin Immunother*. 2013;9(5):1163-71.
8. Hukić M, et al. Surveillance of wildlife zoonotic diseases in the Balkans Region. *Med Glas Ljek komore Zenicko-doboj kantona.* 2010;7(2):96-105.

Chapter 13

TBE in Bulgaria

Iva Christova

E-CDC risk status: endemic (lack of consistent testing and reporting, data as of end 2023)

History and current situation

First cases of probable tick-borne encephalitis (TBE) were reported in 1961 by Andonov et al in eastern regions of Bulgaria.[1] Possible TBE cases with the typical two-wave fever, originating from consumption of raw goat milk, were described back in 1953 by Vaptzarov et al in southern Bulgaria.[2] Investigations in the 1960s were able to isolate 3 tick-borne encephalitis virus (TBEV) strains from *Haemaphysalis punctata* and 1 from *Dermacentor marginatus* ticks from goats and sheep in the district of Plovdiv.[3] The antigenic properties of these 4 virus strains were identical to the highly virulent strain "Hypr" of the European subtype of TBEV (TBEV-EU).[3]

Laboratory diagnosis of TBE, based on serology using complement fixation assay, was introduced in Bulgaria in the 1970s. Since then single case reports of presumed TBE have been reported, but these lack reliable microbiological confirmation.[4-5] However, investigations of ticks between 1974 and 2002 detected TBEV in ticks in Bulgaria. A total of 6849 ticks were investigated, and 8 TBEV strains were isolated.[6]

Beginning in 2009, the National Reference Laboratory of Vector-Borne Pathogens introduced reliable laboratory diagnosis methods for TBE, based on polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), and identified the first 3 confirmed TBE cases in Bulgaria: 2 cases in 2009 and 1 case in 2012.[7] Two more TBE cases were identified in 2015, one case was reported in 2017, one case in 2019, two cases in 2020 and one in 2021 (Fig. 1). Most of the cases reported in the last few years originate from a focus in Western Bulgaria close to the capital city (Fig.3).

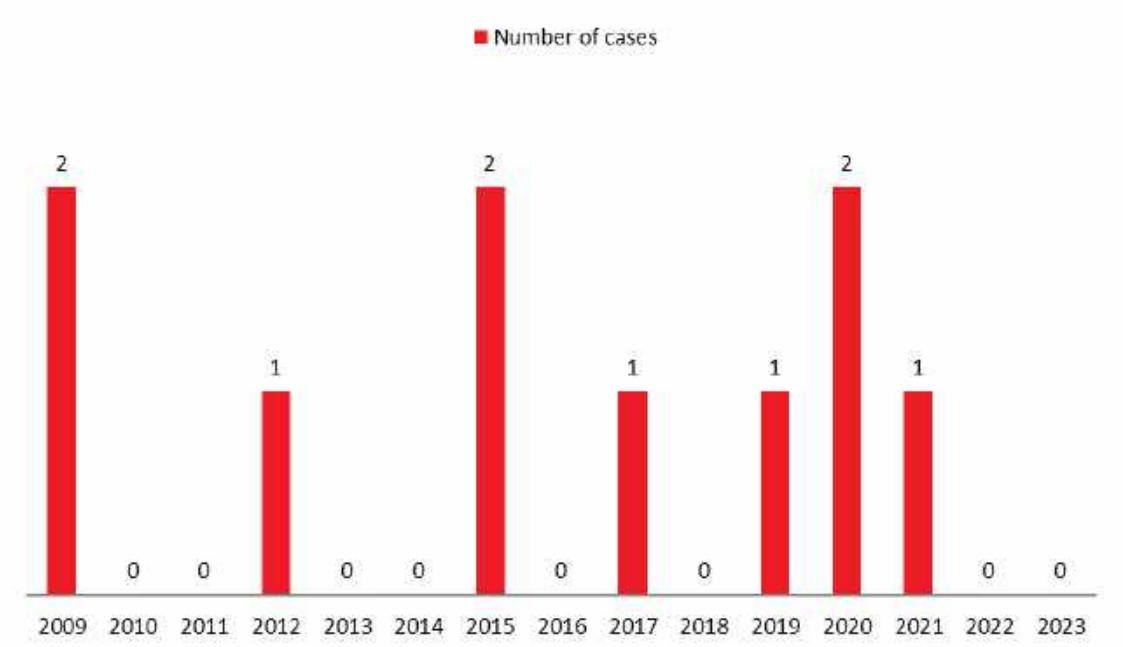
Nationwide seroprevalence survey on circulation of TBE virus in Bulgaria found an overall seroprevalence of 0.6% (Fig. 4). However, district analysis showed TBEV seroprevalence to be up to 4.0%-4.8%, indicating that the TBEV infection seems to be more widespread in the country than previously described.[8-10]

Though TBE cases are reported sporadically, TBEV circulates in Bulgaria, causing human cases, associated with either tick bites or consumption of unpasteurized milk.

Overview of TBE in Bulgaria

Table 1: TBE in Bulgaria	
Viral subtypes, distribution	European subtype of TBEV (TBEV-EU)[3]
Reservoir animals	Not known
Infected tick species (%)	*Dermacentor marginatus, Haemaphysalis punctata*
Dairy product transmission	Yes
Case definition used by authorities	ECDC case definition for confirmed, probable, and possible TBE case
Type of reporting	Mandatory since 2014. Both physicians and laboratories must report cases.
Other TBE surveillance	No
Special clinical features	Biphasic disease
Licensed vaccines	None commercially available
Vaccination recommendations	No
Vaccine uptake	No
Name, address/website of TBE NRC	National reference laboratory of vector-borne pathogens at the National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria *www.ncipd.org*

Figure 1: Burden of TBE in Bulgaria over time (confirmed cases only)



Source Data: Appendix—Figure 1

Age and gender distribution of TBE in Bulgaria

No table can be provided, the number of cases is too low to give any meaningful interpretation.

Figure 2: Sites of TBEV infection in Bulgaria, 1953-2023

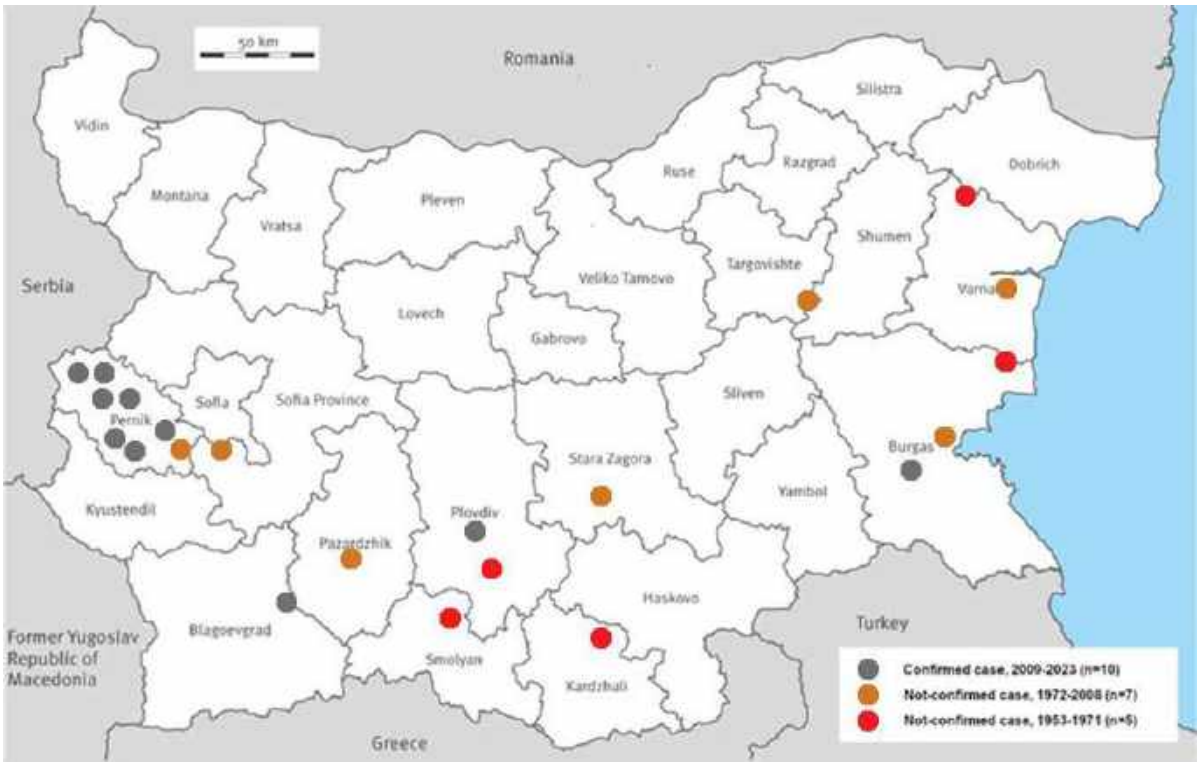
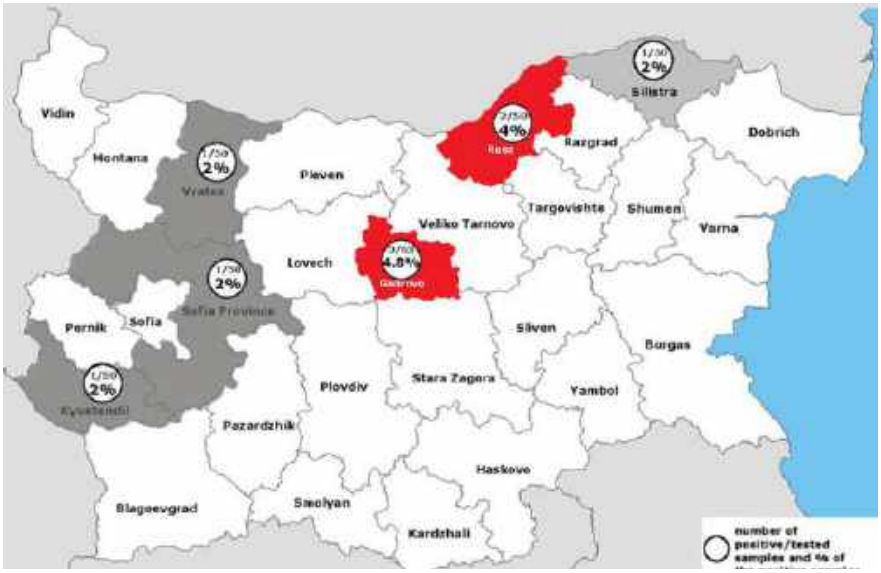


Figure 3: Seroprevalence in Bulgaria, in 2015



Appendix

Source data: Figure 1

Burden of TBE in Bulgaria over time

Year	Number of cases	Incidence / 10^5
2009	2	n.c.
2010	0	n.c.
2011	0	n.c.
2012	1	n.c.
2013	0	n.c.
2014	0	n.c.
2015	2	n.c.
2016	0	n.c.
2017	1	n.c.
2018	0	n.c.
2019	1	n.c.
2020	2	n.c.
2021	1	n.c.
2022	0	n.c.
2023	0	n.c.

Author and affiliation

Iva Christova

National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

Contact: iva_christova@ncipd.org

Citation:

Christova I. TBE in Bulgaria. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-5-7

References

1. Andonov P, Rusakiev M, Hristova T. Natural outbreak of TBE in Eastern Balkan mountain. *Hygiena* 1961;6:23-26.
2. Vaptzarov I, Turpomanov A, Spasov Z, Nikov D, Dragiev M. Recurrent viral meningitis in South Bulgaria. *Suvr Med* (Sofia) 1954;5(2):86-103.
3. Pavlov P., Daniel M, Georgriev B, Kolman JM, Rashev Kh, Arnaudov D, Ignatov D. The natural focus of tick-borne encephalitis of sheep and man in the Rhodope mountains (Bulgaria). *Folia Parasitol* (Praha) 1972;19(1):33-40.
4. Georgiev B, Rosický B, Pavlov P, Daniel M, Arnaudov D. The ticks of the natural focus of tick-borne encephalitis of sheep and man in the Rhodope Mountains (Bulgaria). *Folia Parasitol* (Praha) 1971;18(3):267-73.
5. Kaneva Z. Infectious diseases. Sofia: *Znanie* 2006, pp. 261-2.
6. Dikov I, Gacheva N, Kamarinchev B. Tick-borne encephalitis.In: Serbezov V, Kalvatchev Z, editors. Arboviral infections. Sofia: *Iztok-Zapad* Ltd; 2005, pp. 92-106.
7. Mohareb E, Christova I, Soliman R, Younan R, Kantardjiev T. Tick-borne encephalitis in Bulgaria, 2009 to 2012. *Euro Surveill* 2013;18(46). pii:20635.
8. Christova I, Panayotova E, Tchakarova S, Taseva E, Trifonova I, Gladnishka T. A nationwide seroprevalence screening for West Nile virus and Tick-borne encephalitis virus in the population of Bulgaria. *J Med Virol*. 2017;89(10):1875-1878.doi:10.1002/jmv.24855
9. Taseva E, Christova I, Panayotova E, Ilieva D, Pavlova V. Is there an outbreak of tick-borne encephalitis in Pernik district, Bulgaria? Four cases registered for a period of four years – clinical manifestations and epidemiological relations. *Probl Infect Parasit Dis*. 2021;49(1): 19-25. doi: 10.58395/pipd.v49i1.57
10. Panayotova E, Christova I, Tchakarova S, Taseva E, Trifonova I, Gladnishka T. Seroprevalence of Tick-borne encephalitis virus in domestic animals in Bulgaria. Probl *Infect Parasit Dis*. 2017;45(1):28-30.

Chapter 13

TBE in China

Junfeng Yang and Heinz-Josef Schmitt

E-CDC risk status: endemic in Northern China (last edited March 2024)

History and current situation

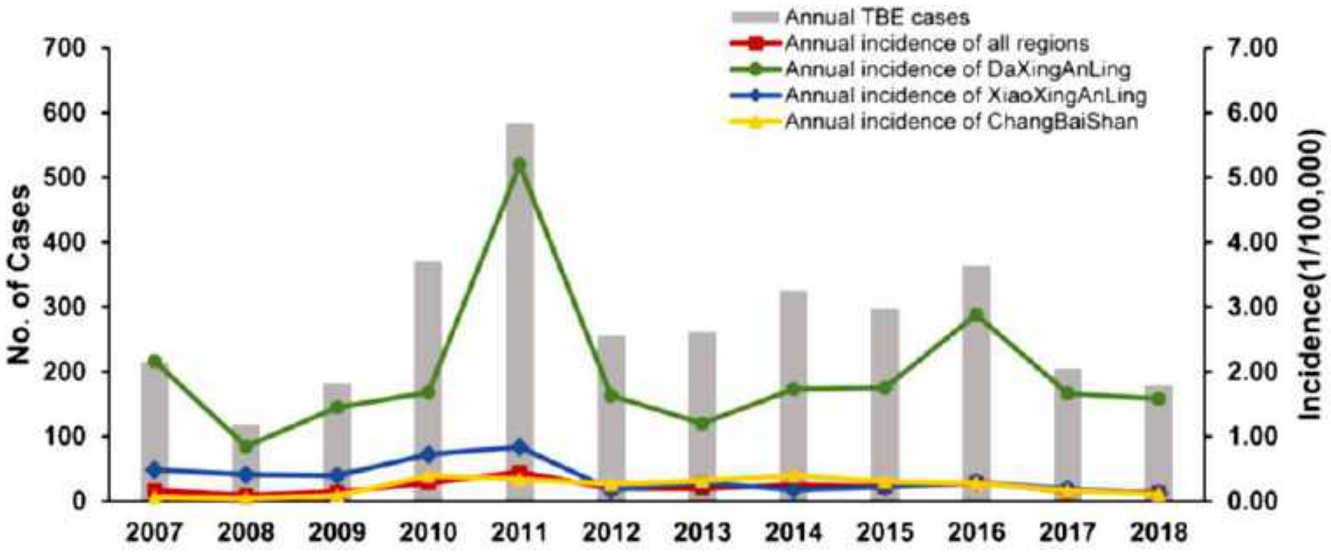
Tick-borne encephalitis (TBE) is an endemic disease in some regions of northern China. The first TBE patients were reported in 1943 and TBE virus (TBEV) was isolated from brain tissues of two patients in 1944 by Japanese military scientists[1] as well as from patients and ticks (*I. persulcatus* and *Haemaphysalis concinna*) in 1952 by Chinese researchers[2].

In China, the Far Eastern (TBEV-FE) subtype is the endemic subtype which has been isolated from the 3 major endemic regions (northeastern China, western China and south-western China). It is mainly transmitted by *Ixodes persulcatus*[3]. No European (TBEV-Eu), and Siberian (TBEV-Sib) subtypes were isolated to date according to our knowledge. Recently, Himalayan (Him-TBEV) subtype has been identified in wild rodents in Qinghai-Tibet Plateao in China[4].

TBE patients are mainly reported from the epicenter: northeastern China, including Inner Mongolia Autonomous Region (Daxing'an Mountains), Heilongjiang Province (Xiaoxing'an Mountains) and Jilin Province (Changbai Mountains). Patients are also reported from another important epidemic area, the Tianshan Mountains and the Altai Mountains of the Xinjiang Autonomous Region[5] as well as from other areas which were not considered to be endemic in the past (see map, Figure 3). Cases may be missed as TBE is not a notifiable disease in China, especially in regions with lower TBE incidences, due to a lack of awareness among both physicians and the population and also due to a local lack of availability of serological testing.

The incidence of TBE decreased in China during the 1980s. However, it has been rising since 2008, as noted by disease control and prevention sectors and local hospitals[5]. Case numbers remained stable in recent years[6]. TBE patients before the 1980s were mainly forest workers, however, it has been reported that changes in the occupation / type of "exposure risk" occurred among TBE patients ever since and in particular since the late 1990s with 70%-95% of the most recent patients being non-forest working farmers, housewives, domestic workers, students, or anyone with any occupation who entered the endemic forest areas[7]. Cases among tourists may be underreported, considering that the Chinese "TBE-epicenters" are also tourist resorts, and probably fewer protection measures are applied by tourists.

Figure 1: TBE case numbers and incidence in China, 2007 to 2018.[6] As opportunities for TBE-diagnostics (serology) are limited, and as there is no mandatory reporting of TBE in China, the approximate 300 - 400 documented cases in China each year since 2007 are probably just the tip of the iceberg.



Overview of TBE in China

Table 1: TBE in Northern China	
Viral subtypes, distribution	Far Eastern TBEV subtype[1]
Reservoir animals	Mice and insectivorous animals; migratory birds; lagomorphs, goats[8]
Percentage infected ticks	*I. persulcatus*, however TBEV has also been isolated from *H. concinna*, *H. japonica*, *Dermacentor silvarum*, and *I. ovatus*[7]
Dairy product transmission	Not known
Case definition used by authorities	Clinical case: symptoms (such as acute fever, headache, vomiting and/or typical central nervous system symptoms) + exposure in forests during spring or summer, or a tick bite history; Laboratory-confirmed case: clinical case + confirmed by laboratory serological tests (increased anti-TBEV IgG and IgM or ≥4-fold increase in specific antibody to TBEV between acute and convalescent serum samples) or PCR test positive for TBEV RNA if necessary[9]
Completeness of case detection and reporting	NA
Type of reporting	Mandatory in Heilongjiang Province. Clinical TBE cases have been reported to the Chinese Information System for Diseases Control and Prevention (CISDCP) by the majority of provinces since 2002, such as Heilongjiang, Inner Mongolia Autonomous Region, Jilin, and Liaoning. No data publicly available[5]
Other TBE Surveillance	Detection of TBE virus in ticks have been conducted in endemic areas sporadically[10,11]
Special clinical features	Biphasic disease not reported from China. Different symptoms among patients with different disease severities; in the early 1950s, CFR of TBE in the northeastern forest areas was over 25%, but since the 1980s it has decreased to around 8%. Long-lasting sequelae of TBE are common, almost one-third of the patients in the 1952 outbreak had paralysis in the neck muscles or the shoulder muscles. Recently the complications of TBE over a ten-year period was reported to be 16.6% (90/542)[12-15]
Licensed vaccines	TaiSenBao produced in China with Sen-Zhang strain as seed strain in PHK cell (Changchun Institute of Bio-product)[16]
Vaccine recommendations	Residence in endemic areas, travelers to endemic areas, with no reimbursement[17]
Vaccine uptake	NA
National Reference center for TBE	Chinese Center for Disease Prevention and Control http://ivdc.chinacdc.cn/
Additional relevant information	Seropositivity in the population: 19.7% in southwestern China; 35.4% in northwestern China; 0-10.9%, 0-9.8% and 7.6% in northeastern China[8]

Figure 2: Age and gender distribution of TBE in China, 2007-2018[6]

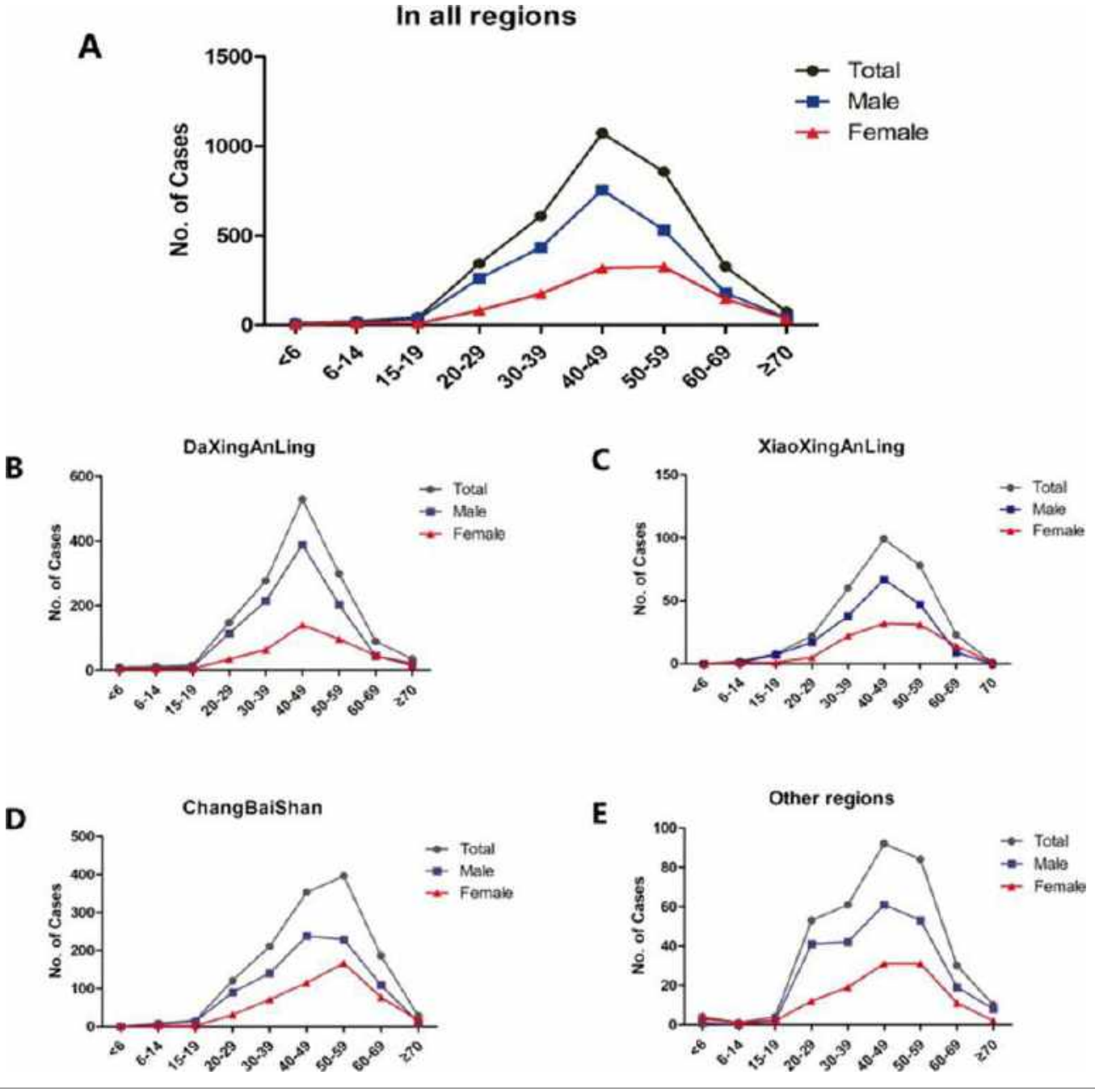
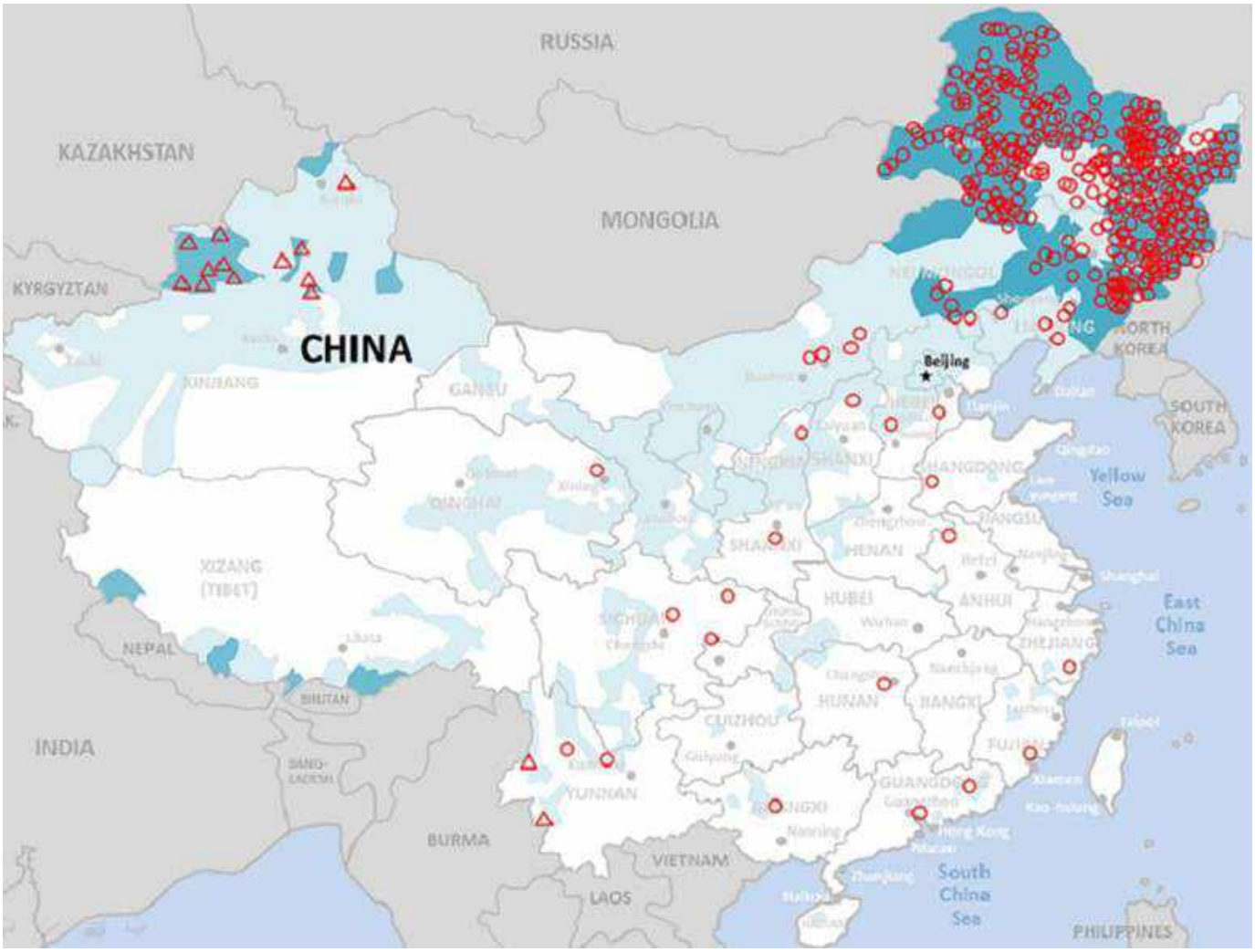


Figure 3. Sites of confirmed and predisposed TBEV infection in China since 2006[5,6]



○ *Reported TBE cases 2006–2013*
△ *Confirmed TBEV foci in Xinjiang and Yunnan*

***Intensity of blue color**: Reflects the probability of an area to be endemic for TBEV, dark blue = 100%, light blue, lower probabilities based on various environmental and climate criteria as published by Sun et al. 2017*

Authors and affiliations

Junfeng Yang, Medical Affairs, Pfizer Investment Co., Ltd., Beijing, China

Heinz-Josef Schmitt, Editor-in-Chief, GHP

Contact: Junfeng.yang@pfizer.com

Citation:
Yang J, Schmitt HJ. TBE in China. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-6-7

References

1. Liu YJ, Jiang YT, Guo CS, Guan BP. Epidemiological characteristics of tick borne encephalitis natural foci in Jilin. Zhongguo Ren Min Jie Fang Jun Jun Shi Yi Xue Ke Xue Yuan Yuan Kan (Bulletin of the Academy of Military Medical Sciences) [in Chinese]. 1979;3:109-20.

2. Yin DM, Liu RZ. Review on the control of forest encephalitis in the forest areas of north-east China. Zhonghua Liu Xing Bing Xue Za Zhi (Chinese Journal of Epidemiology) [in Chinese]. 2000;21:387-9.

3. Bi WM, Deng HP, Bu XY. Regionalisation of natural foci of forest encephalitis. Shou Du Shi Fan Da Xue Xue Bao (Journal

of Capital Normal University: Natural Science Edition) [in Chinese]. 1997;18:100-7.

4. Dai X, Shang G, Lu S, Yang J, Xu J. A new subtype of eastern tick -borne encephalitis virus discovered in Qinghai-Tibet Plateau, China. *Emerg Microbes Infect*. 2018;7(1):74. doi: 10.1038/ s41426-018-0081-6. PMID: 29691370; PMCID: PMC5915441.

5. Sun RX, Lai SJ, Yang Y, et al. Mapping the distribution of tick-borne encephalitis in mainland China. *Ticks Tick Borne Dis*. 2017;8(4):631-639. doi:10.1016/j.ttbdis.2017.04.009

6. Chen X, Li F, Yin Q, et al. Epidemiology of tick-borne encephalitis in China, 2007- 2018. *PLoS One*. 2019;14 (12):e0226712. Published 2019 Dec 26. doi:10.1371/ journal.pone.0226712

7. Xing Y, Schmitt HJ, Arguedas A, Yang J. Tick-borne encephalitis in China: A review of epidemiology and vaccines. *Vaccine*. 2017;35(9):1227-1237. doi:10.1016/j.vaccine.2017.01.015

8. Huang WL, Hou ZL, Zi DY, Gong ZD, Lei YM, Mi ZQ, et al. Investigation of the Russian Spring-Summer Encephalitis virus in Yunnan province. Zhongguo Yu Fang Shou Yi Xue Bao (Chinese Journal of Preventive Veterinary Medicine) [in Chinese]. 2001;23:231-3.

9. The Ministry of Health ofPeople's Republic of China. Diagnostic Criteria of Occupational Forest Encephalitis (2002) (in Chinese). Available at: http://www.nhc.gov.cn/wjw/ pyl/201212/34564.shtml (accessable on 29 Feb. 2024)

10. Wang D, Ji HW, Wang ZD, et al. Isolation and Identification of Forest Encephalitis Virus Carried by Ticks in Part of Northeast China [J]. Chin. J. Prev. Vet [in Chinese]. 2021.DOI:10.16303/ j.cnki.1005-4545.2021.03.10.

11. Wang XY, Sun ZD, Suo JN,et al. Survey on the Distribution of Ticks and the Carriage Situation of Tick Borne Encephalitis Virus in Heilongjiang Province [J]. China Public Health Management [in Chinese]. 2020,36(5):750-752.DOI:10.19568/ j.cnki.23-1318.2020.05.039.

12. Liu YJ, Jiang YT, Guo CS, Guan BP. Epidemiological characteristics of tick borne encephalitis natural foci in Jilin. Zhongguo Ren Min Jie Fang Jun Jun Shi Yi Xue Ke Xue Yuan Yuan Kan (Bulletin of the Academy of Military Medical Sciences) [in Chinese]. 1979;3:109-20.

13. Yin DM, Liu RZ. Review on the control of forest encephalitis in the forest areas of north-east China. Zhonghua Liu Xing Bing Xue Za Zhi (Chinese Journal of Epidemiology) [in Chinese]. 2000;21:387-9.

14. Li H. Analysis of 90 cases of disability as the sequela of forest encephalitis. Zhongguo Lin Chuang Kang Fu (Chinese Journal of Clinical Rehabilitation) [in Chinese]. 2002;6:374.

15. Zhang DH, Zhang ZX, Wang YM, Wang DH. Trends of forest encephalitis endemic in Heilongjiang. Ji Bing Jian Ce [Disease Surveillance] [in Chinese]. 2000;15:57-8.

16. Changchun Institute of Bio-product (Chinese). https:// www.ccbio.net/publicity/show-45.html (accessable on 29 Feb. 2024)

17. Label of Tick borne encephalitis vaccine (Chinese). https:// wenku.baidu.com/ view/22151e19b8d528ea81c758f5f61fb7360b4c2bad.html?_wkts_=1709193206519&bdQuery=%E6%A3%AE%E6%9E%97%E8%84%91%E7%82%8E%E7%96%AB%E8%8B%97+%E8%AF%B4%E6%98%8E%E4%B9%A6&needWelcomeRecommand=1 (accessable on 29 Feb. 2024)

18. Zhang GL, Liu R, Sun X, Zheng Y, Liu XM, Zhao Y, et al. Investigation on the endemic foci of tick-borne encephalitis virus in Xiaerxili Natural Reserve, Xinjiang. Zhonghua Liu Xing Bing Xue Za Zhi (Chinese Journal of Epidemiology) [in Chinese]. 2013;34:438-42.

19. Cai ZL, Lu ZX, Hu LM, Zhao ZL, Jin XT, He YY. Epidemiology survey of Russian Spring Summer encephalitis in the northeast area, China. Wei Sheng Wu Xue Za Zhi (Journal of Microbiology) [in Chinese]. 1996;16:19-22.

20. Zhang ZQ, Wu YM, Feng L, Wang HJ, Wang LQ, Liu GP, et al. A serological survey of forest encephalitis in northeast of China. Shenyang Bu Dui Yi Yao (Shenyang Army Medical Journal) [in Chinese]. 2006;19:112-3.

21. Guo Y. Serological survey of forest encephalitis in Milin, Tibet. Zhong Wai Jian Kang Wen Zhai (World Health Digest) [in Chinese]. 2010;7:9-10.

Chapter 13

TBE in Croatia

Tatjana Vilibić-Čavlek, Maja Bogdanić, Vladimir Savić, Ljubo Barbić, Vladimir Stevanović and Bernard Kaić

E-CDC risk status: endemic (last edited: April 2024, data from 2023)

History and current situation

In Croatia, TBE was reported for the first time in 1953 near Križevci (Stara Ves, northwestern region).[1] In addition to this first focus, several continental foci (Bjelovar, Pakrac, Koprivnica, Karlovac, Varaždin) have been recorded since 1961. Moreover, TBEV antibodies were detected in residents of the Croatian littoral near the islands of Zadar, Pula, and Brac.[2] In 1991, TBEV emerged in the mountainous area of Gorski Kotar.[3] The disease is also endemic in northwestern and eastern regions between the Sava and Drava rivers. Endemicity is highest in northwestern counties, with average incidence rates ranging from 3.61 to 6.78 per 100,000 inhabitants.[4,5] In 2015 and 2019, two TBE clusters after consumption of raw goat milk were observed.[6,7]

TBE in Croatia shows a bimodal seasonality with a larger peak during the spring and summer months (April–August) and a smaller one in October–November. A recent study showed that the majority of TBE patients are in the age group of 40–69 years (58.3%) with a male predominance (70.2%). Males predominate in all age groups with male-to-female ratios ranging from 1.3:1 (for those under 20 years) to 5:1 (for those between 50 and 59 years). Meningitis (54.8%) and encephalitis (30.9%) are the main clinical presentations in hospitalized patients with TBE. The abortive form („febrile headache“) was reported in 13.1% of patients, and meningoencephalomyelitis in 1.2% of patients.[8]

In addition to human cases, 2.1% of TBEV asymptomatic seropositive individuals were detected in the same study (2017–2023). In contrast to acute cases, there is only a comparatively small difference in the seroprevalence between males (2.6%) and females (3.6%) as well as between age groups (2.5–3.7%). Recent serosurveys showed the presence of TBEV antibodies in animals as well. Seropositive horses were detected in continental Croatian counties in the period from 2017 to 2020. The overall seroprevalence rate was 12.1%, ranging from 7.3% to 17.1%. In 2022, 9.7% of sheep from the easternmost Vukovar-Srijem county tested positive for TBEV IgG antibodies.[8]

Ixodes ricinus ticks are the main vector of TBEV in Croatia. From 2017 to 2023, hard ticks were sampled using the dragging–flagging method and hand-picked from both dead wild and live domestic animals. Ticks were collected in the Medvednica and Papuk mountain areas, and in the area between the Drava, Sava, and Danube Rivers. In the Alpine biogeographic region, ticks were mostly collected in the Gorski Kotar area. The seasonal tick dynamic was similar to the reported human cases.[8]

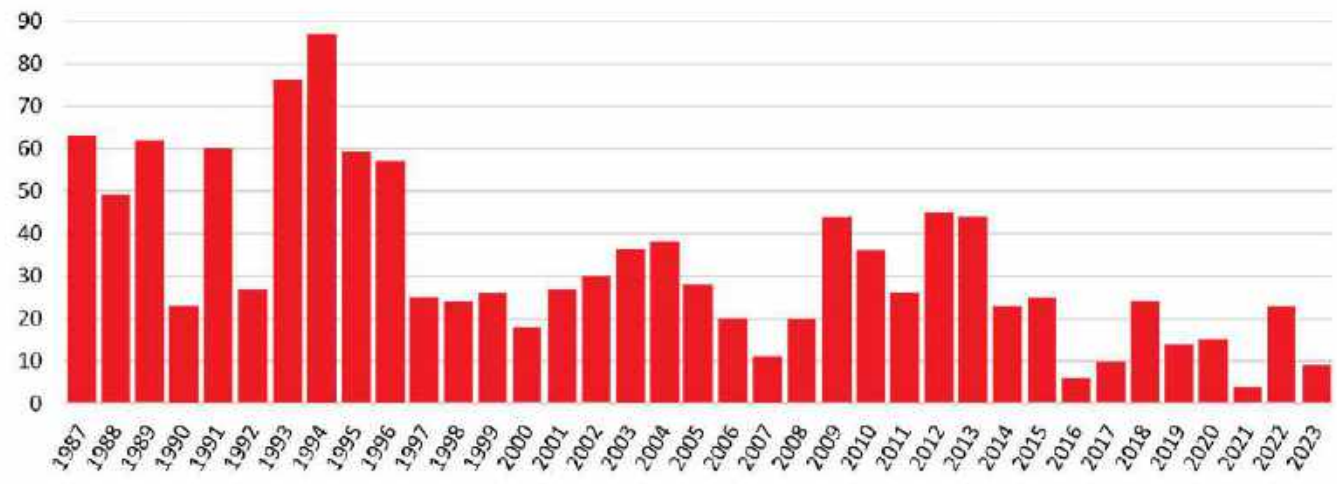
A study on the TBEV detection in ticks removed from red fox (*Vulpes vulpes*) carcasses hunted in endemic areas in northern Croatia was performed during two hunting seasons (2010-2011 and 2011-2012). TBEV was detected in adult *Ixodes ricinus* and *Ixodes hexagonus* ticks showing a prevalence of 1.6%. Furthermore, two spleen samples (1.1%) from 182 red deer (*Cervus elaphus*) were found positive for TBEV.[9]

Phylogenetic analysis of one TBEV strain detected in a urine sample from a patient with severe meningoencephalitis (2017) and strains from ticks and deer spleen showed that all clustered the TBEV European subtype.[4,9]

Overview of TBE in Croatia

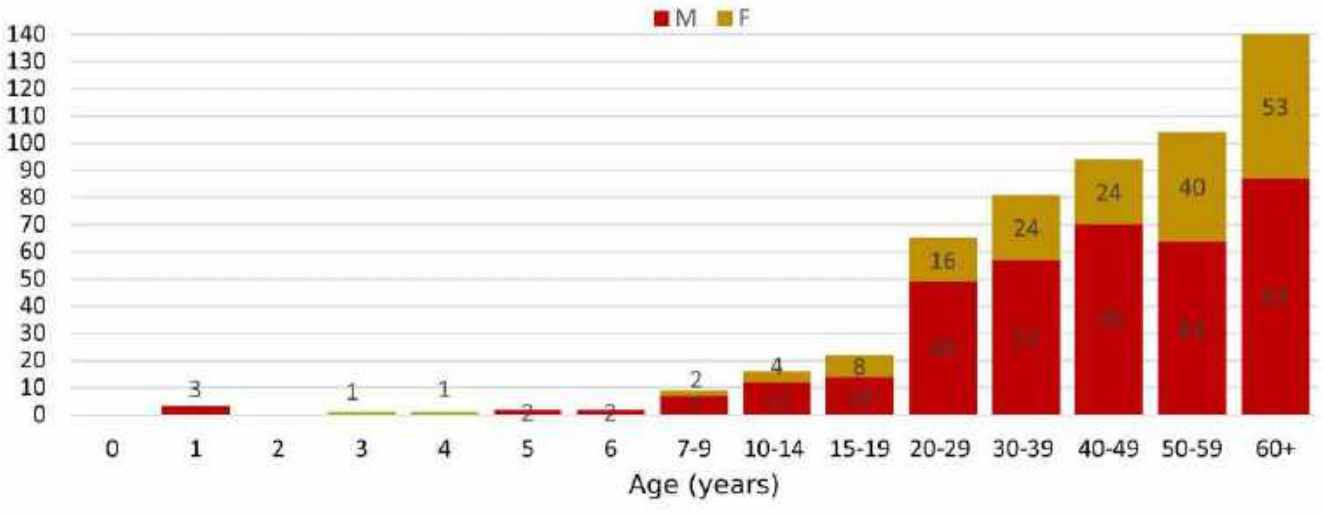
Table 1: TBE in Croatia	
Virus subtypes isolated	TBEV European subtype[4,8]
Reservoir animals	Rodents
Infected tick species (%)	1.6% in one study
Dairy product transmission	2015 – 7 cases of TBEV (Bjelovar region) after consuming fresh goat milk and cheese[6] 2019 – 5 cases of TBEV (Gorski Kotar region) after consuming raw goat milk from the same farm[7]
Case definition used by authorities	ECDC case definition[10]
Completeness of case detection and reporting	No data
Type of reporting	Mandatory[11]
Other TBE surveillance	Occasional serosurveys[8]
Special clinical features	The majority of cases are in the age group 40–69 years. Meningitis (54.8%) and encephalitis (30.9%) are the most common clinical presentations in hospitalized patients. An abortive form "febrile headache" was detected in 13.1% of patients.[8]
Licensed vaccines	FSME-IMMUN
Vaccine recommendations	Risk groups (forestry workers, hunters, people who reside in endemic areas/visit endemic areas)
Vaccine uptake	No data
National Reference Center for TBE	National Reference Laboratory for Arboviruses, Reference Center for Diagnosis and Surveillance of Viral Zoonoses of the Croatian Ministry of Health, Department of Virology, Croatian Institute of Public Health

Figure 1: TBE cases notified over time, 1987–2023



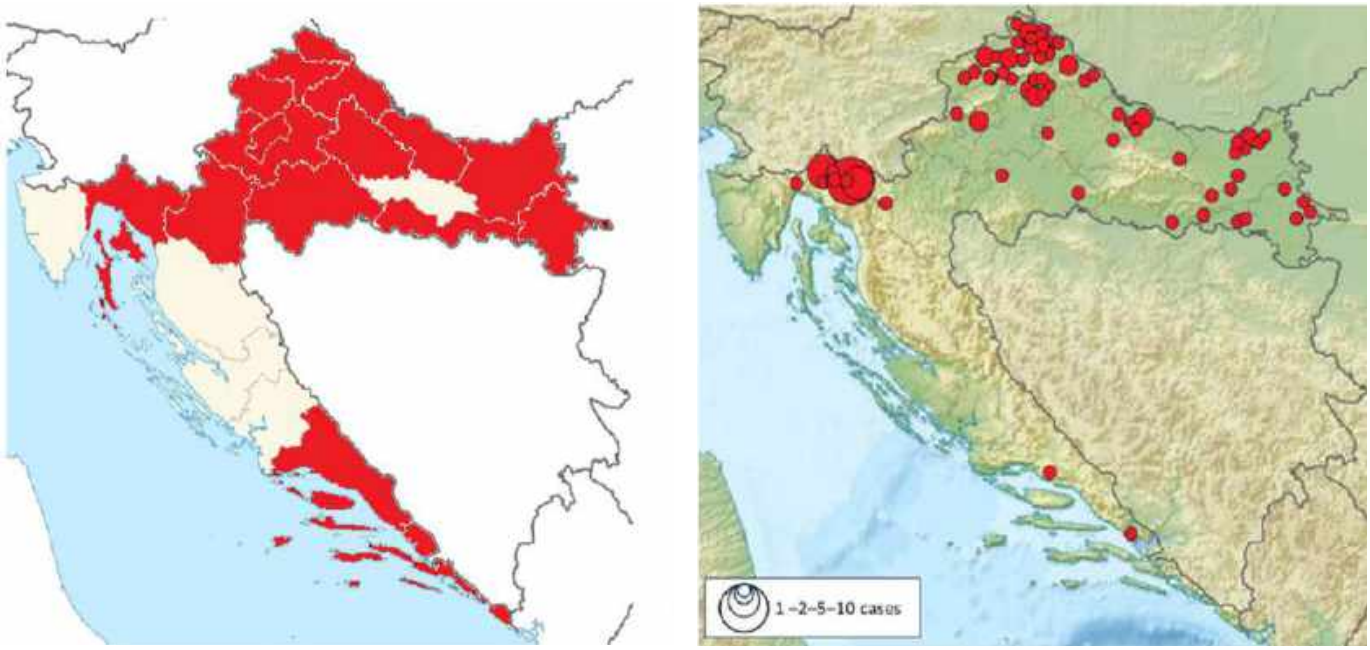
Preliminary data for 2023, reporting is still ongoing until April 2024; source: Reference Center for Epidemiology Croatian Ministry of Health; Croatian Institute of Public Health

Figure 2: Age and gender distribution of notified TBE cases in Croatia, 2000–2020



Source: Reference Center for Epidemiology Croatian Ministry of Health; Croatian Institute of Public Health

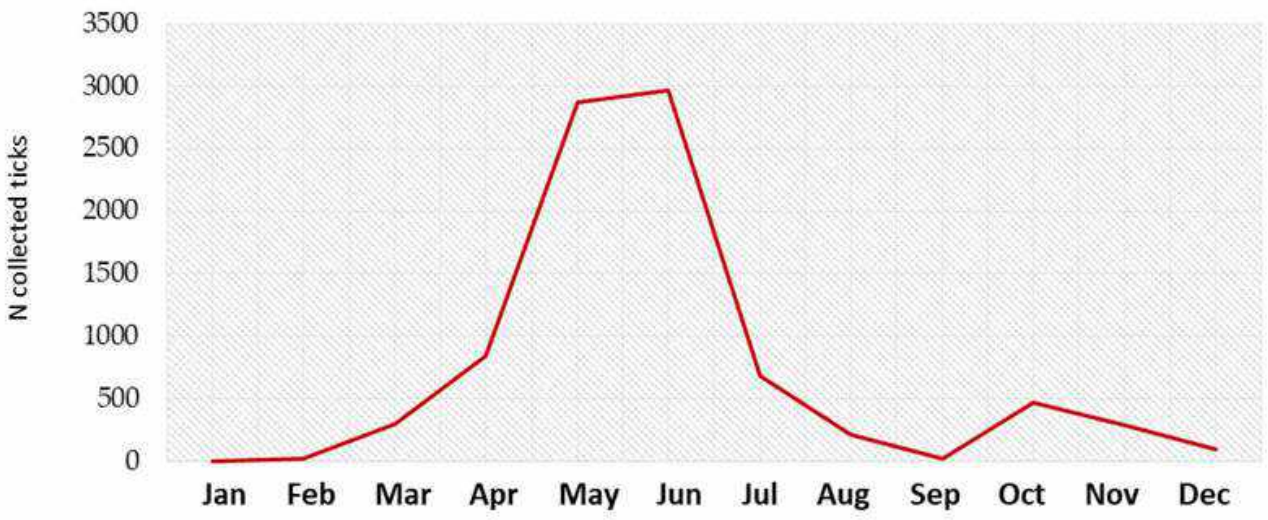
Figure 3: Sites of TBEV detection in Croatia, 2016–2023



Red shadowed areas: counties with reported cases; Red circles: Cumulative infection sites of TBE patients for the period from 2016 to 2023

Source: Vilibic-Cavlek T, et al. Microorganisms 2024; 12(2):386.

Figure 4: Seasonal dynamic of *Ixodes ricinus* ticks in Croatia, 2016–2023



Source: Vilibic-Cavlek T, et al. Microorganisms 2024; 12(2):386.

Acknowledgments

TBE surveillance was supported by the projects: Croatian Science Foundation, project IP 2016-06-7456: Prevalence and molecular epidemiology of emerging and re-emerging neuroinvasive arboviral infections in Croatia; CRONEUROARBO (2017-2021), and the Next Generation EU project supported by Ministry of Science and Education of the Republic of Croatia, grant number NPOO 1 of the Croatian Veterinary Institute: Epidemiology of avian flaviviruses in the "One Health" context, FLAVIR.

Authors and affiliations

Tatjana Vilibić-Čavlek, Maja Bogdanić
National Reference Laboratory for Arboviruses, Reference Center for Diagnosis and Surveillance of Viral Zoonoses of the Croatian Ministry of Health, Department of Virology, Croatian Institute of Public Health, 10000 Zagreb, Croatia

Vladimir Savić
Poultry Center, Croatian Veterinary Institute, 10000 Zagreb, Croatia

Ljubo Barbić, Vladimir Stevanović
Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine University of Zagreb, 10000 Zagreb, Croatia

Bernard Kaić
Reference Center for Epidemiology of the Croatian Ministry of Health, Department of Communicable Disease Epidemiology, Croatian Institute of Public Health, 10000 Zagreb, Croatia

Contact: tatjana.vilibic-cavlek@hzjz.hr

Citation:
Vilibić-Čavlek T, Bogdanić M, Savić V, Barbić L, Stevanović V and Kaić B. TBE in Croatia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book.* 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-7-7

References

1. Vesenjak-Hirjan J. Tick-Borne Encephalitis in Croatia. Rad JAZU, Book 372; Croatian Publisher RAD JAZU: Zagreb, Croatia, 1976; pp. 1–10.
2. Vesenjak-Hirjan J, Galinović-Weisglass M, Brudnjak Z. Infections with Tick-Borne Encephalitis Virus in Pannonian Focus Stara Vas. Serological Studies in 1972; Rad JAZU, Book 372; Croatian Publisher RAD JAZU: Zagreb, Croatia, 1976; pp. 29–36.
3. Borčić B, Kaić B, Gardasević-Morić L. Krpeljni meningoencefalitis u Gorskom kotaru--nove spoznaje [Tick-borne meningoencephalitis in Gorski Kotar--new findings]. *Lijec Vjesn*. 2001; 123(7-8):163-4.
4. Vilibic-Cavlek T, Barbic L, Mrzljak A, Brnic D, Klobucar A, Ilic M, et al. Emerging and Neglected Viruses of Zoonotic Importance in Croatia. *Pathogens* 2021; 10:73. doi:10.3390/pathogens10010073
5. Vilibic-Cavlek T, Janev-Holcer N, Bogdanic M, Ferenc T, Vujica Ferenc M, Krcmar S, Savic, et al. Current status of vector-borne diseases in Croatia: Challenges and Future Prospects. *Life.* 2023;13(9):1856. doi:10.3390/life13091856.
6. Markovinović L, Kosanović Ličina ML, Tešić V, Vojvodić D, Vladušić Lucić I, Kniewald T, Vukas T, Kutleša M, Krajinović LC. An outbreak of tick-borne encephalitis associated with raw goat milk and cheese consumption, Croatia, 2015. *Infection*

2016; 44(5):661-5. doi:10.1007/s15010-016-0917-8

7. Ilic M, Barbic L, Bogdanic M, Tabain I, Savic V, Kosanovic Licina ML, et al. Tick-borne encephalitis outbreak following raw goat milk consumption in a new micro-location, Croatia, June 2019. *Ticks Tick Borne Dis.* 2020; 11(6):101513. doi:10.1016/j.ttbdis.2020.101513
8. Vilibic-Cavlek T, Krcmar S, Bogdanic M, Tomljenovic M, Barbic L, Roncevic D, et al. An Overview of Tick-Borne Encephalitis Epidemiology in Endemic Regions of Continental Croatia, 2017-2023. *Microorganisms.* 2024; 12(2):386. doi:10.3390/microorganisms12020386
9. Jemeršić L, Dežđek D, Brnić D, Prpić J, Janicki Z, Keros T, Roić B, Slavica A, Terzić S, Konjević D, Beck R. Detection and genetic characterization of tick-borne encephalitis virus (TBEV) derived from ticks removed from red foxes (*Vulpes vulpes*) and isolated from spleen samples of red deer (*Cervus elaphus*) in Croatia. *Ticks Tick Borne Dis*. 2014; 5(1):7-13. doi:10.1016/j.ttbdis.2012.11.016
10. Croatian Institute of Public Health. [Definitions of infectious diseases that are mandatory reported]. Accessed 16 April, 2024. https://www.hzjz.hr/wp-content/uploads/2023/04/definicije-24_01_2024_promjenaCOVID19.pdf (In Croatian)
11. Ministry of Health of the Republic of Croatia. [List of infectious diseases which control and prevention are of interest to the Republic of Croatia]. Accessed 16 April, 2024. https://narodne-novine.nn.hr/clanci/sluzbeni/2014_05_60_1119.html

Chapter 13

TBE in the Czech Republic

Petr Pazdiora

E-CDC risk status: endemic (last edited: date 28.03.2024, data from 2023)

History and current situation

The TBE virus (TBEV) was first isolated in the Czech Republic by a Czech scientist in 1948-1949 from both a patient and also from *Ixodes ricinus* ticks[1]. However, even before 1948, etiologically unclear summer cases of viral meningoencephalitis had been reported, and likely, at least in part, they are attributable to the TBEV. These cases were reported mostly from patients in the districts of Beroun (Central Bohemia), Hradec Králové (East Bohemia), Vyškov (South Moravia), and occasionally from the neighborhood of Prague. The official reports of these probable cases of "tick-borne encephalitis" were registered in the database of the National Institute of Public Health in Prague since 1945.

The first TBEV isolation was accomplished from blood and cerebrospinal fluid of a patient with meningoencephalitis. Other successful isolations were from subjects with a history of a tick bite. The first successful attempt of isolation of the TBEV from different developmental stages of *I. ricinus* ticks collected in forests of the district Beroun was in 1949. The analysis of an outbreak of meningoencephalitis in Rožňava in south-eastern Slovakia in 1951 from Czech and Slovak specialists ended with the discovery of the alimentary transmission of the TBEV.

The definition of TBE for reporting changed in the following decades. Following a ministerial decree from 1970, only clinically-manifested, laboratory-confirmed cases of TBE were to be reported to the central surveillance center. The number of case characteristics collected from TBE patients has gradually increased ever since 1982. Since 1993, the national reporting system (EPIDAT) has been computerized. TBE surveillance was established by Regulation No. 275/2010, Annex No. 28.

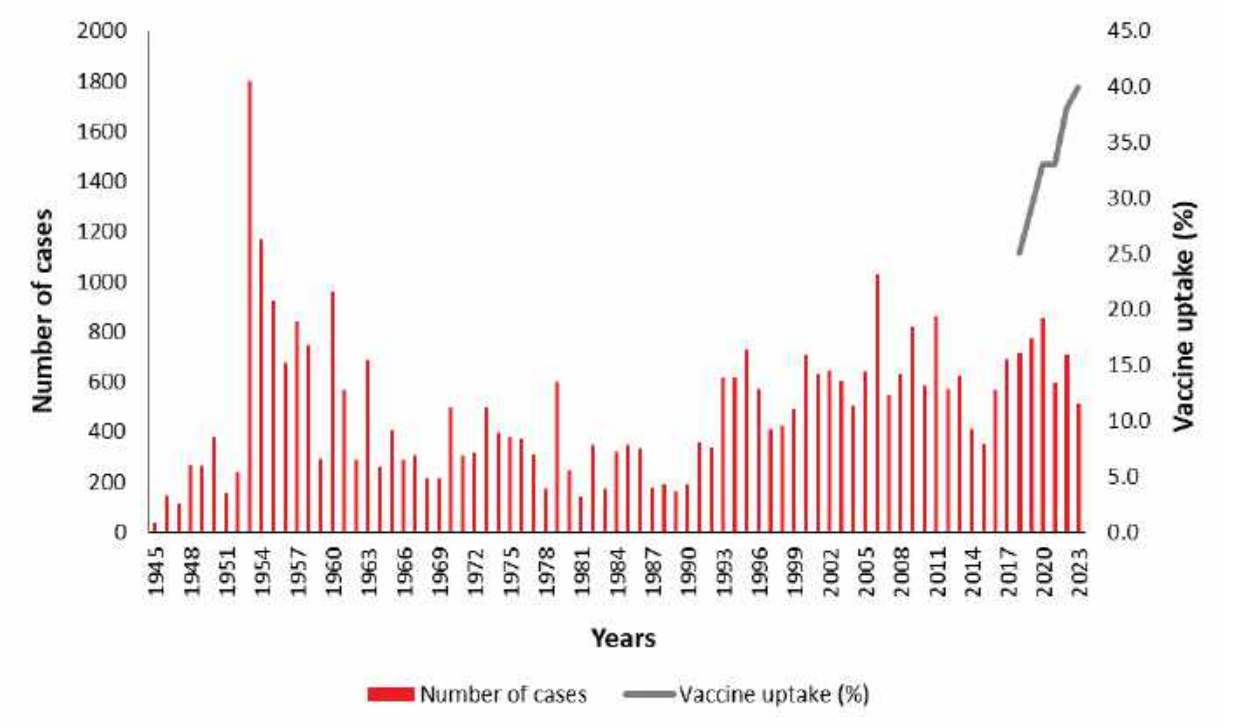
The Czech Republic is a highly TBE endemic country. Many cases are associated with outdoor activities (camping, living in secondary residences in the countryside, hiking, hunting, fishing, mushrooming), while the incidence of possible occupational transmission has decreased over the last years (in 2007-2023 289 cases, i.e. 2.7% among foresters, and farmers mostly). Numbers of imported cases from abroad are very low with only 5 cases (0.7%) in 2022, and 12 cases (2.3%) in 2023. The geographical distribution of TBE is changing. The gradual spread of TBE into formerly unaffected districts, namely into the border districts of the country at higher altitudes is highlighted. Long-term observations confirm a shift of age-specific incidence rates to older age groups. The period of the transmission of TBE is changing, too. The "TBE-season" with detection of cases is longer than 30-50 years ago and lasts from March to December. These changes of basic epidemiological characteristics may be due to climatic changes, changes of environmental and/or other factors. These factors are affecting the different interactions between TBEV, its vectors and vertebrate hosts, too.

Vaccine uptake is very low, the highest rate is reached in the age group of 18-24 year-olds, the lowest among children younger than 4 years, however there is no central vaccination registry. Data from 8 international telephone surveys in 2009, 2013, 2015, 2018, 2019, 2020, 2021, 2022, and 2023 which covered the whole Czech population and defined a "vaccinated person" as someone having received ≥ 1 dose vaccine uptake, was estimated to be 16, 23, 24, 25, 29, 33, 33, 38 and 40%, respectively. Substantial regional differences in uptake were observed in the Czech Republic (Prague Region 51%, Pardubice Region 32%). Similar differences in uptake were observed in individual age-groups (18-24 years 64.7%, 0-3 years 18.6%). Unpublished data from some Czech regions indicate that vaccine uptake with ≥ 3 dose is even lower.

Overview of TBE in Czech Republic

Table 1: TBE in Czech Republic	
Virus subtypes isolated	European subtype - no other information available
Reservoir animals	*Apodemus sylvaticus*, *Apodemus flavicollis*, *Myodes glareolus*, *Microtus agrestis*, *Sciurus vulgaris*, *Erinaceus roumanicus*, *Sorex araneus*, *Talpa europaea*[15]
Percentage infected ticks	1970–2023: 157/128,005 (0.123%)[18]
Dairy product transmission	Rare: 1997-2008: 0.9%13; 1993-2019: 3.4%20; 2007-2023: 0.5%[16] Children and adolescents (1993-2019): 6.8%[19]
Case definition used by authorities	Based on ECDC
Completeness of case detection and reporting	There is not enough valid data to estimate the % of undetected cases
Type of reporting	Mandatory, only confirmed cases on the basis of clinical and lab criteria are reported1
Other TBE surveillance	Detection in ticks (National Reference Laboratory for arboviruses)
Special clinical features	Biphasic disease: 1994-1997: 80%[17] Children and adolescents (1993-2012): 58%[12] Risk groups: No information available % with sequelae: children and adolescents (1993-2012): 3%[12] Mortality: case fatality rate (1960-2019): 0.79%[19]; (1970-2008): 0.55%[14]; (2018-2023): 0.5%[16] Children and adolescents (1960-2019): 0.2%[19]
Licensed vaccines	FSME-IMMUN since 1990, Encepur since 1996
Vaccination recommendations	General, first recommendation 1990, last recommendation February 8, 2016 Partial reimbursement from health insurances started in 1993, different strategies of different health insurances in individual years Total reimbursement from health insurances for people 50 years old and over started in 2022
Vaccine uptake	Vaccine uptake in the general population of 16, 23, 24, 25, 29, 33, 33, and 38% (years 2009, 2013, 2015, 2018, 2019, 2020, 2021, 2022 and 2023[3,4,5,6,7,8,9,10,11]
National Reference center for TBE	National Reference Laboratory for arboviruses, Public Health Institute of Ostrava, Partyzánské nám. 7, 702 00 Ostrava https://www.zuova.cz/Home/Page/NRL-arboviry[18]

Figure 1: TBE case numbers over time according to vaccination status



Source Data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE in the Czech Republic (2023)

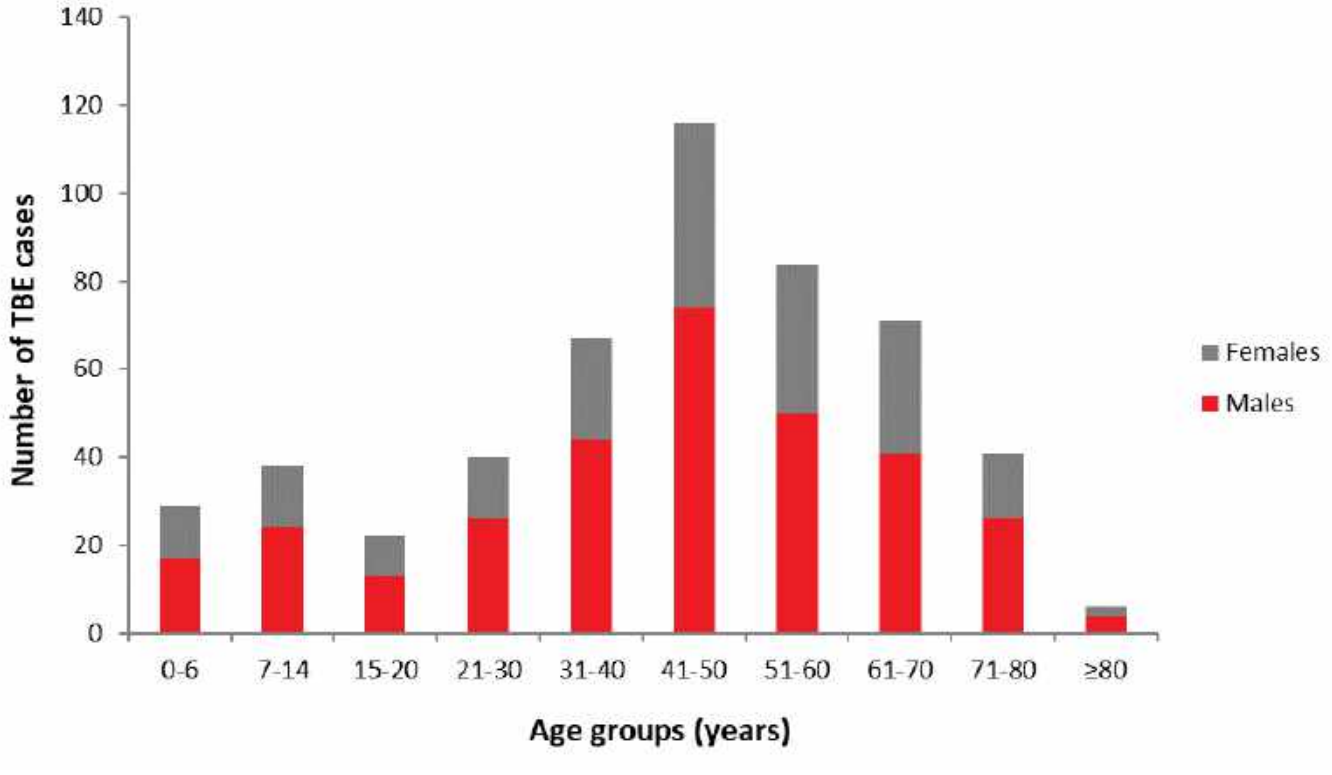
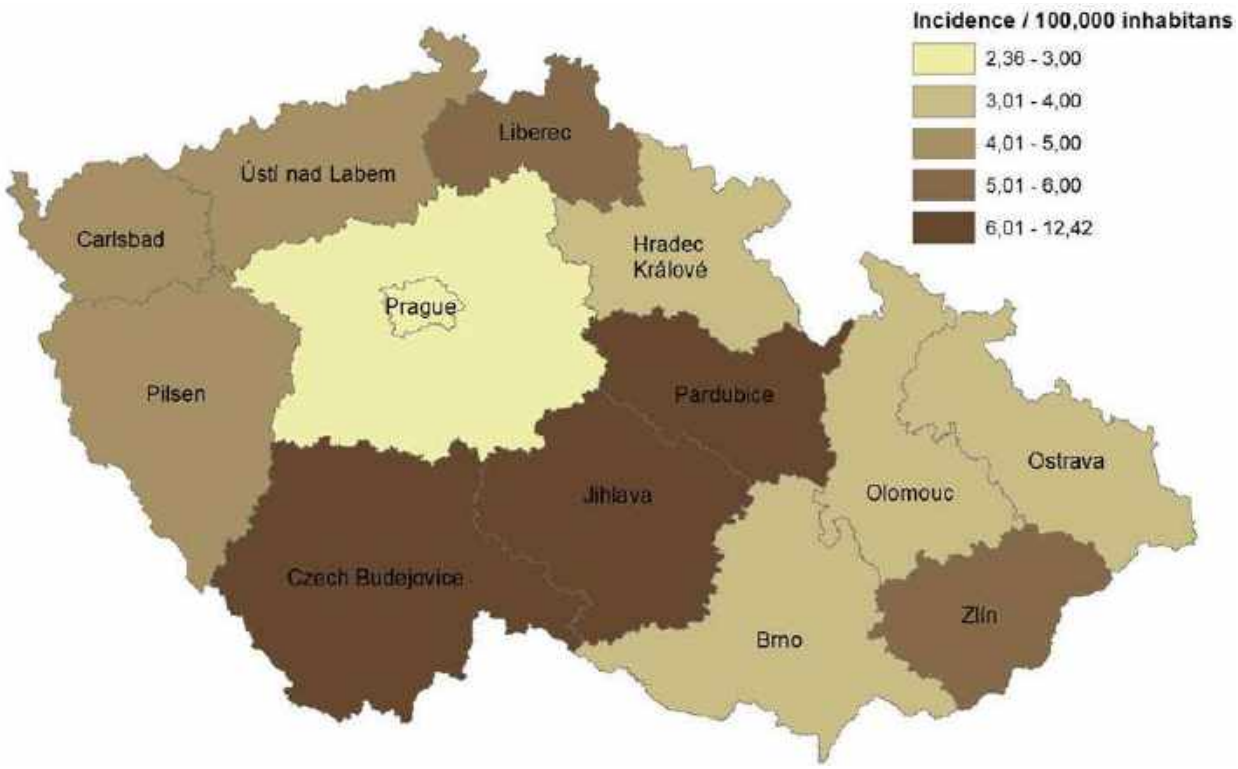


Figure 3: Incidence of TBE in individual regions in the Czech Republic, 2023



Authors and affiliations

Petr Pazdiora
Department of Epidemiology, Medical Faculty of Charles University Pilsen, Czech Republic

Contact: pazdiora@fnplzen.cz

Citation:

Pazdiora P. TBE in Czech Republic. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJS, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-8-7

Appendix

Source data: Figure 1

Year	Number of cases	Incidence/ 10^5
1945	35	0.33
1946	146	1.53
1947	112	1.28
1948	267	3
1949	265	2.98
1950	375	4.2
1951	155	1.72
1952	240	2.65
1953	1800	19.69
1954	1167	12.68
1955	927	10
1956	675	7.23
1957	839	8.93
1958	744	7.89
1959	294	3.11

Year	Number of cases	Incidence/ 10^5
1960	958	9.92
1961	564	5.88
1962	285	2.96
1963	685	7.08
1964	258	2.65
1965	407	4.16
1966	289	2.94
1967	308	3.13
1968	216	2.19
1969	217	2.19
1970	502	5.12
1971	305	3.1
1972	316	3.2
1973	502	5.06
1974	397	3.97

Year	Number of cases	Incidence/ 10^5
1975	378	3.76
1976	374	3.69
1977	309	3.03
1978	175	1.71
1979	598	5.81
1980	246	2.38
1981	139	1.35
1982	348	3.37
1983	172	1.63
1984	320	3.16
1985	350	3.44
1986	333	3.22
1987	178	4.81
1988	191	1.84
1989	166	1.6

Year	Number of cases	Incidence/ 10^5
1990	193	1.86
1991	356	3.45
1992	337	3.28
1993	618	6.09
1994	619	5.99
1995	727	7.19
1996	571	5.54
1997	412	4.03
1998	422	4.1
1999	490	4.77
2000	709	7
2001	633	6.19

Year	Number of cases	Incidence/ 10^5
2002	647	6.34
2003	606	5.94
2004	507	4.97
2005	642	6.28
2006	1028	10.02
2007	546	5.29
2008	631	6.05
2009	816	7.78
2010	589	5.6
2011	861	8.2
2012	573	5.45
2013	625	5.94

Year	Number of cases	Incidence/ 10^5
2014	410	3.9
2015	355	3.4
2016	565	5.3
2017	687	6.5
2018	715	6.7
2019	774	7.3
2020	855	8
2021	594	5.6
2022	710	6.8
2023		

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	43	25	68
10-19	34	28	62
20-29	37	22	59
30-39	52	39	91
40-49	74	57	131
50-59	55	43	98
60-69	64	48	112
≥70	53	36	89
Total	412	298	710

References

1. Daniel M, Benes C, Danielová V, Kríz B. Sixty years of research of tick-borne encephalitis--a basis of the current knowledge of the epidemiological situation in Central Europe. *Epidemiol Mikrobiol Imunol*. 2011;60(4):135-155.
2. Daniel M, Brabec M, Malý M, Danielová V, Vráblík T. The influence of meteorological factors on the risk of tick-borne encephalitis infection. Vliv meteorologických faktorů na riziko infekce klíšťovou encefalitidou. *Epidemiol Mikrobiol Imunol*. 2023;72(2):67-77.
3. Fessel – GfK. Tick-borne encephalitis, a study sponsored by Baxter. 2009.
4. Fessel – GfK. Tick-borne encephalitis, a study sponsored by Baxter. 2013.
5. Fessel – GfK. Tick-borne encephalitis, a study sponsored by Pfizer. 2016.
6. Fessel – GfK. Tick-borne encephalitis, a study sponsored by Pfizer. 2018.
7. Ipsos: TBE Awareness Coverage and Compliance Research 2019.
8. Ipsos: TBE Awareness Coverage and Compliance Research 2020.
9. Ipsos: TBE Awareness Coverage and Compliance Research 2021.
10. Ipsos: TBE Awareness Coverage and Compliance Research 2022.
11. Ipsos:TBE Awareness Coverage and Compliance Research 2023.
12. Krbkova L, Stroblova H., Bednarova J. Clinical course and sequalae for tick-borne encephalitis among children in South Moravia (Czech Republic). *Eur J Pediatr*. 2015; 174(4):449-458.
13. Kriz B, Benes C, Daniel M. Alimentary transmission of tick-borne encephalitis in the Czech Republic (1997-2008). *Epidemiol Mikrobiol Imunol*. 2009; 58(2):98-103.
14. Kriz B, Maly M, Benes C, Daniel M. Epidemiology of tick-borne encephalitis in the Czech Republic 1970-2008. *Vector Borne Zoonotic Dis*. 2012; 12(11):994-999. doi:10.1089/vbz.2011.0900
15. Pazdiora P, Struncova V, Svecova M. Tick-borne encephalitis in children and adolescents in the Czech Republic between 1960 and 2007. *World J Pediatr*. 2012; 8(4):363-366. doi:10.1007/s12519-012-0383-z
16. Pazdiora P, Gasparek M, Sebestova H, Lenz P, Vlckova I. The analysis of the register of compulsorily notifiable diseases (EPIDAT 1993-2017; ISIN 2018-2023) – data published on local, regional seminars, conferences, congresses.
17. Ruzek D, Danielova V, Daniel M, Chmelik V, Chrdle A, Pazdiora P, et al. Klíšťová encefalitida. 1st ed. Praha; 2015.
18. Zelena H. Průkaz viru KE v klíšťatech, Česká republika 1970-2022; 2023. Accessed 29 March, 2024. https://www.zuova.cz/Home/Page/NRL-arboviry.
19. Pazdiora P, Prokopova M, Svecova M, Tomaskova H. Tick-borne Encephalitis in Children and Adolescents in the West Bohemian Region (Czech Republic) between 1960 and 2019. The 29th Meeting of the Czech Society for Epidemiology and Microbiology, PED 2020, in Pilsen, the Czech Republic, from 15 -17 September, 2020.
20. Pazdiora P, Prokopova M, Kudova J, Tomaskova H. Tick-borne Encephalitis in the West Bohemian Region (Czech Republic) between 1960 and 2021. The Meeting of the Czech Society for Epidemiology and Microbiology, Prague, 1st March 2022.

Chapter 13

TBE in Denmark

Anders Fomsgaard

E-CDC risk status: endemic (data as of end 2023)

History and current situation

Since the 1950s tick-borne encephalitis (TBE) has been endemic in Denmark but only on the island of Bornholm. Bornholm is situated east of mainland Denmark, south of Sweden (Figure 3) and has a different fauna and flora from the rest of Denmark. Bornholm has about 45,000 inhabitants, but about 500,000 tourists visiting every year.

Freundt et al carried out a sero-survey during 1958- 1962[1] and found TBE antibodies in 1.4% of blood donors and 30% of woodworkers on Bornholm but no antibodies in subjects living in mainland Denmark. In 1963, Freundt found that 8 of 12 patients admitted to the hospital with acute meningoencephalitis of unknown etiology during 1951–1960 had antibodies to tick-borne encephalitis (TBEV).[2] In 2000 TBE was re-discovered on Bornholm, where a retrospective study covering the period 1994–2002 (7 years) identified 14 TBE cases; 2 cases were tourists and 12 were inhabitants of Bornholm, giving an incidence of 3.81 per 100,000 inhabitants.[3] At least 5 patients (37.7%) developed permanent sequelae. In addition, 32 forest workers on Bornholm were tested in 2000, and 20% had IgG antibodies but no symptoms. This is similar to the finding of Freundt in 1960. It was concluded that the data did not provide evidence of an increase in incidence of TBE. Ticks (*Ixodes ricinus*) from Bornholm were investigated for TBEV in 2000 and 2% were found to be infected.[4] Since 2001 an average of 2.5 (range 1–8) TBE cases per year have been reported in Bornholm (Figure 1).

In 2009 we identified a TBEV microfocus (size app. 1000 m2) outside Bornholm in a forested area, Tokkekøb Hegn on Zealand just north of Copenhagen, which had two severe TBE cases reported, a forest worker in 2009 and a wood kindergarten teacher in 2008.[5] Both subjects had a typical biphasic disease and TBE was diagnosed. Both experienced persistent neurological sequelae. TBEV European (Western) sub-type (TBEV-E) was identified in 2009 in *I. ricinus* tick adults and nymphs collected from this focus.[5] In July and Sept. 2011 TBEV-Eu was again identified in adults and nymphs at the same Tokkekøb microfocus, and TBEV isolated (isolates T2 and T3)[6], but in 2016 the Tokkekøb TBEV microfocus disappeared. The Tokkekøb TBEV WGS-sequence grouped with isolates from Sweden-Norway probably carried by infected ticks on migrating birds from Norway.

In contrast, one Bornholm TBEV from 2012 grouped into a different subclade from South and Central Bohemia.[6] And an additional (2018) TBEV isolate from Bornholm (lake Rubinsøen) grouped with TBEV from Switzerland and Finland.[7] TBEV was not identified in 58 tick pools collected 2010–2011 in North Zealand, Fuen, and Jutland by flagging or from roe deer. In addition, 78 patients in North Zealand with 'summer flu' after tick bites (July–Sept. 2010) and 96 hospitalized encephalitis patients after tick bites (2007–2009) who were negative for Borrelia all tested negative for TBE antibodies.[6] This supports a limited TBEV introduction into the new temporary (2008-2016) microfocus in Tokkekøb.

In the hot summer in 2018 two sporadic and independent cases of TBE occurred outside Bornholm: probably somewhere in Jutland (north of Esbjerg) and on Fuen (near Faaborg), respectively.[8]

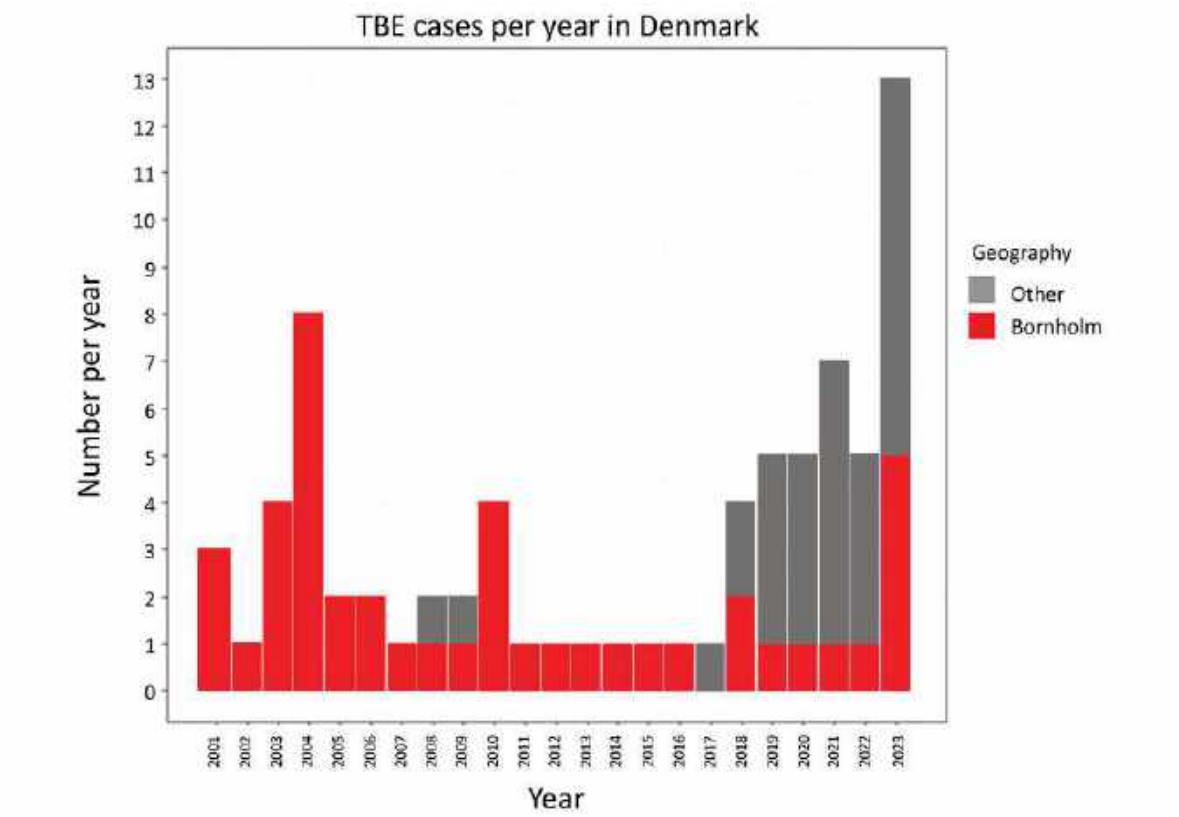
During June-July 2019 four independent TBE cases were suddenly hospitalized, infected in the same wood area Tisvilde Hegn in Northern Zealand, at the same specific wood playground (Figure 3). By flagging we identified a new TBEV micro-focus (1000 m2 in size) with a very high TBEV prevalence of 8% among the ticks (only in nymphs). Whole genome sequencing showed clustering with a TBEV from Norway probably from migrating birds.[9] Later in 2019 three more clinical TBE cases appeared infected in the same wood Tisvilde Hegn but not at the playground microfocus. Since 2019 there have been 4-6 TBE cases yearly spreading from Tisvilde Hegn to more wood areas in Zealand including new areas in Tokkekøb Hegn, so far culminating in 2023 with 8 TBE cases (plus 5 cases on Bornholm). All cases reported here are autochthonous as confirmed by individual patient history. Another 13 Danish TBE patients were infected in our neighboring country Sweden during 2023.

Serological testing of roe deer 'sentinels' in 2002-2003 and again in 2013-2014 have suggested an increasing TBEV appearance in the whole of Denmark apparently with a delay in the appearance of also clinical TBE cases.[10] Since ELISA antibodies to TBEV may cross-react to Louping ill virus (LIV) in roe deer, the presence of LIV outside of Bornholm and/or TBEV needs to be confirmed. Either TBEV and/or LIV are now widespread in Denmark.

Overview of TBE in Denmark

Table 1: TBE in Denmark	
Viral subtypes, distribution	TBEV European subtype [5,6,7,9]
Reservoir animals	Roe deer[10]
Percentage infected ticks	2% - 8% in hotspot [4,7,9]
Dairy product transmission	No
Case definition used by authorities	Based on ECDC
Type of reporting	TBE has been a notifiable disease in Denmark (DK) since 2023 and SSI reports to ECDC (TESSy)
Other TBE surveillance	Detection in ticks, seroprevalence in roe deer.[10] Flagging from locations with more than one TBE case.
Special clinical features	Biphasic. Encephalitis, meningitis, meningo-radiculoneuritis[3,5,8,9]
Licensed vaccines	TicoVac (Pfizer) and Encepur (Bavarian Nordic)
Vaccine recommendations	Regular movement in wood areas with TBE cases
Vaccine uptake	Unknown. In 2023 51,709 adult- and 16,713 pediatric TBE vaccine doses were sold in DK for an unknown number of persons.
National Reference Center for TBE	Laboratory: Dept. Virus & Microbiological Special Diagnostic, Statens Serum Institut, 5 Artillerivej, DK2300 Copenhagen, Denmark. (www.ssi.dk)

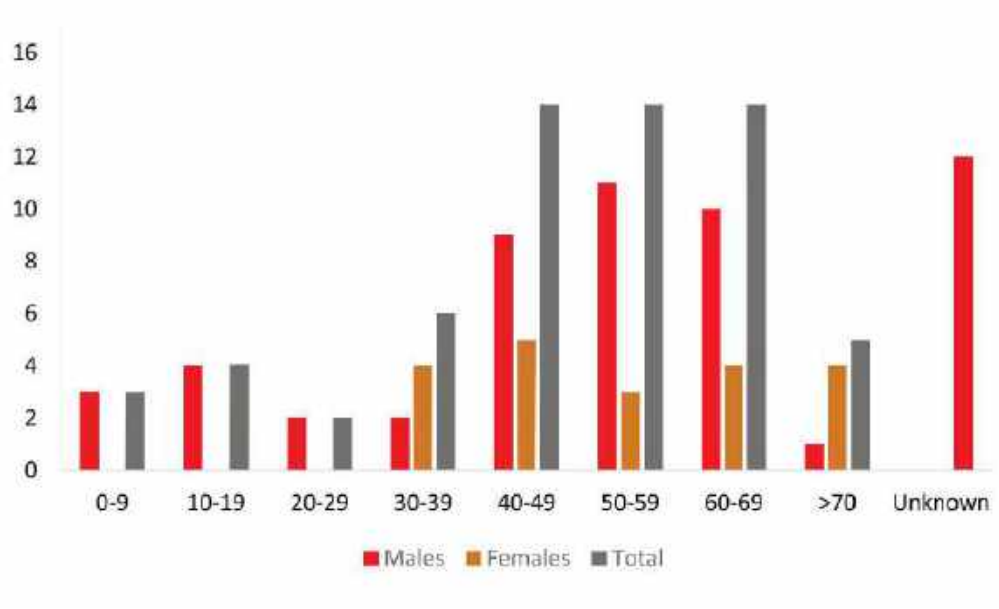
Figure 1: TBE case numbers over time



Note: Travel-related cases are excluded by individual patient history, all cases reported here are confirmed as autochthonous.

Source Data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE in Denmark, 2001 – 2023



Source Data: Appendix—Figure 2

Figure 3: Sites of TBEV infection in Denmark, 2001-2023[5,6,7,9,10]

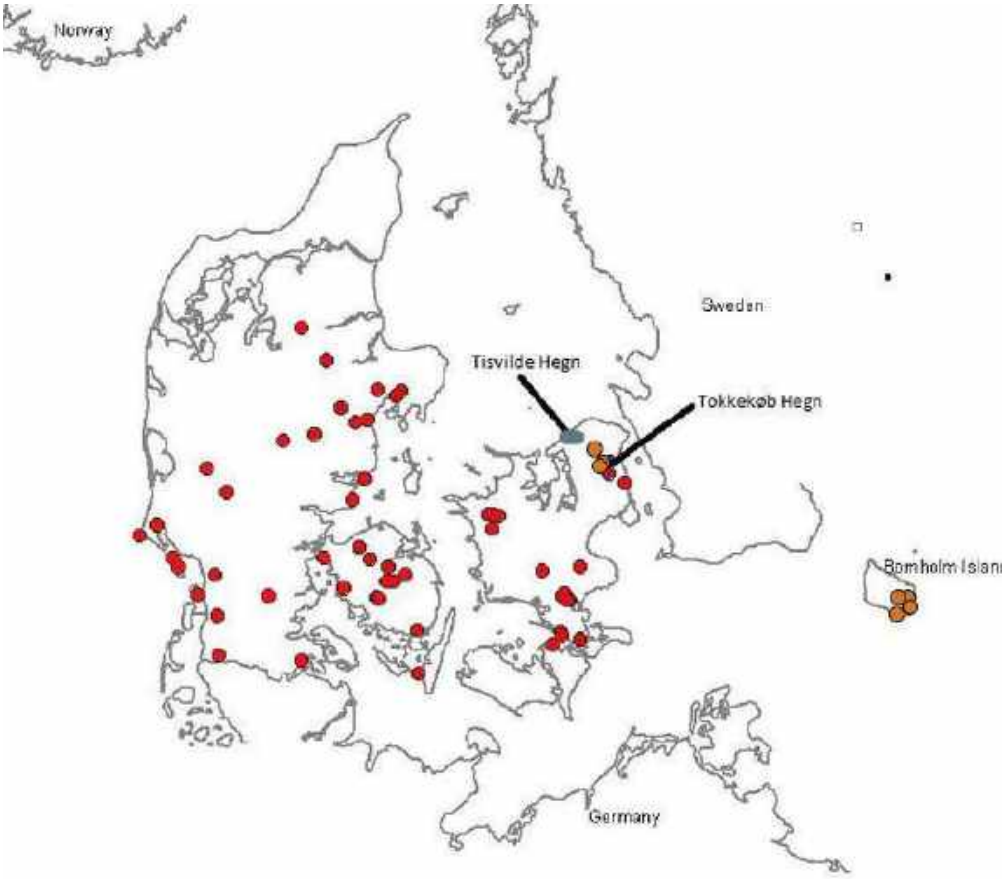


Figure of Denmark showing endemic Bornholm and the TBEV microfoci in Tokkekøb Hegn, North Zealand (TBEV isolate T2, 2011)[6] and Tisvilde Hegn 2019[9]; red dots indicate tick sampling from roe deer[10], blue dots indicate flagging.[6]

Appendix

Source data: Figure 1

Year	Number of cases	Incidence / 10^5
1951-1960	8	
		…
1994	2	
1995	?	
1996	?	
1997	2	
1998	3	
1999	4	
2000	3	3.81
2001	3	
2002	1	
2003	4	
2004	8	
2005	2	
2006	2	
2007	1	
2008	2	
2009	2	
2010	4	
2011	1	
2012	1	
2013	3	
2014	1	
2015	1	
2016	1	
2017	0	
2018	4	
2019	5	
2020	5	
2021	7	
2022	5	
2023	13	

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	3	0	3
10-19	4	0	4
20-29	2	0	2
30-39	2	4	6
40-49	9	5	14
50-59	11	3	14
60-69	11	3	14
>70	1	4	5
Unknown			12

Authors and affiliation

Anders Fomsgaard. Department of Virus & Microbiological Special Diagnosis, Statens Serum Institut, 5 Artillerivej, 2300 Copenhagen, Denmark.

Contact: afo@ssi.dk

Citation:
Fomsgaard A. TBE in Denmark. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-9-7

References

1. Freundt EA. The incidence of antibodies to the Russian Spring -Summer encephalitis complex and viruses in man and animals on Bornholm. *Acta Pathol Microbiol Scand*. 1962;Suppl.154:334-6.
2. Freundt EA. Endemisk forekomst på Bornholm af Centraleuropæisk virus. Meningoencephalitis, overført af skovflåter. *Ugeskrift for Læger*. 1963;125:1098-104.
3. Laursen K, Knudsen JD. Tick-borne encephalitis: a retrospective study of clinical cases in Bornholm, Denmark. *Scand J Infect Dis*. 2003;35:354-7. doi:10.1080/00365540310005305
4. Kristiansen K, Rønne T, Bro-Jørgensen K. Tick-borne encephalitis på Bornholm. Copenhagen: Statens Serum Institut. *EpiNyt*. 2001; no. 17. ISSN 1396-8599.
5. Fomsgaard A, Christiansen C, Bodker R. First identification of tick-borne encephalitis in Denmark outside of Bornholm, August 2009. *Euro Surveill*. 2009;14:19325.
6. Fomsgaard A, Fertner ME, Essbauer S, et al. Tick-borne encephalitis virus, Zealand, Denmark, 2011. *Emerg Infect Dis*. 2013;19:1171-3. doi:10.3201/eid1907.130092
7. Andersen NS, Bestehorn M, Lidia C-D, et al. Phylogenetic characterization of tick-borne encephalitis virus from Bornholm, Denmark. *Ticks Tick Borne Dis*. 2019;10(3):533-9. doi:10.1016/j.ttbdis.2018.12.008
8. Laugesen NG, Stenør C. Tick-borne encephalitis-associated meningoradiculoneuritis acquired in the south-western part of Denmark. *Ugeskr Laeger*. 2019;181(27):V03190197.
9. Agergaard CN, Rosenstierne MW, Bødker R, Rasmussen M, Andersen PHS, Fomsgaard A. New tick-borne encephalitis virus hot spot in Northern Zealand, Denmark, October 2019. *Euro Surveill*. 2019;24(43):1900639. doi:10.2807/1560-7917.ES.2019.24.43.1900639
10. Andersen NS, Larsen SL, Olesec CR, Stiasny K, Kolmos HJ, Jensen PM, Skarphédinsson S. Continued expansion of tick-borne pathogens: Tick-borne encephalitis virus complex and Anaplasma phagocytophilum in Denmark. *Ticks Tick Borne Dis.* 2019;10:115–23. doi:10.1016/j.ttbdis.2018.09.007

Chapter 13

TBE in Estonia

Kuulo Kutsar

E-CDC risk status: endemic (data as of end 2023)

History and current situation

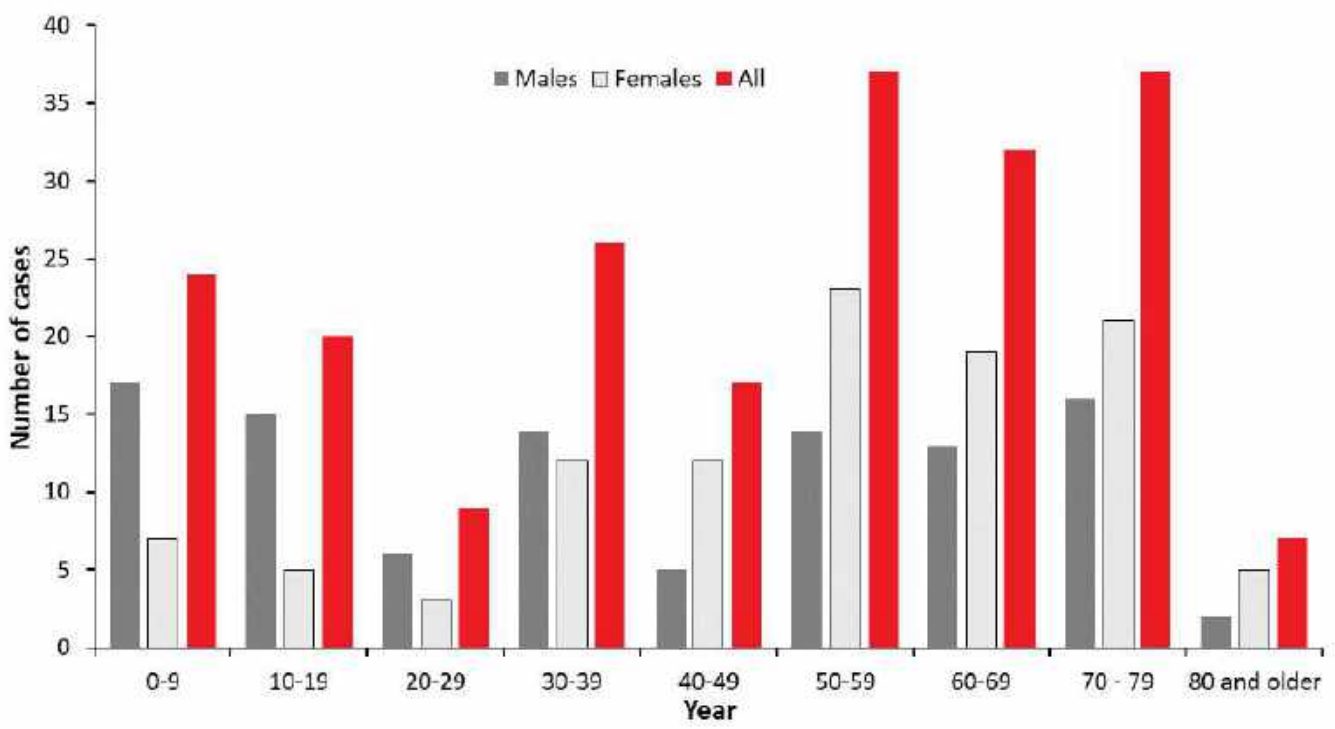
The first cases of tick-borne encephalitis (TBE) in Estonia were identified in 1949. Today, Estonia is a TBE-endemic country. A TBE-endemic area in Estonia is defined as an area with circulation of the TBEV between ticks and vertebrate hosts as determined by detection of the TBEV or the demonstration of autochthonous infections in humans or animals within the last 20 years.

Euro-Asian genotypes of TBEV – the Western or European (TBEV-EU), Siberian (TBEV-Sib), and Far-Eastern (TBEV-FE) subtypes are co-circulating in Estonia. Vectors of TBEV, the tick species *Ixodes ricinus* and *Ixodes persulcatus*, are distributed throughout the country.

The high-risk season for infection coincides with the period of biological activity of ticks and lasts for 7 months from April to November, peaking in June to August.

Most TBE cases are diagnosed in persons ≥60 years of age and the incidence among the rural population is 1.8 times higher than among the urban population.

Figure 1: Age and gender distribution of TBE cases in Estonia, 2023



TBE seasonality: case numbers, Estonia 2023

January – 1, February – 1, March – 0, April – 0 , May – 3 , June – 20 , July – 19, August – 49, September – 45, October – 56, November – 11, December – 4 cases

TBE total cases 209 and incidence 15.6 per 100 000 population in Estonia 2023

Source Data: Appendix—Figure 1

Overview of TBE in Estonia

Table 1: TBE in Estonia

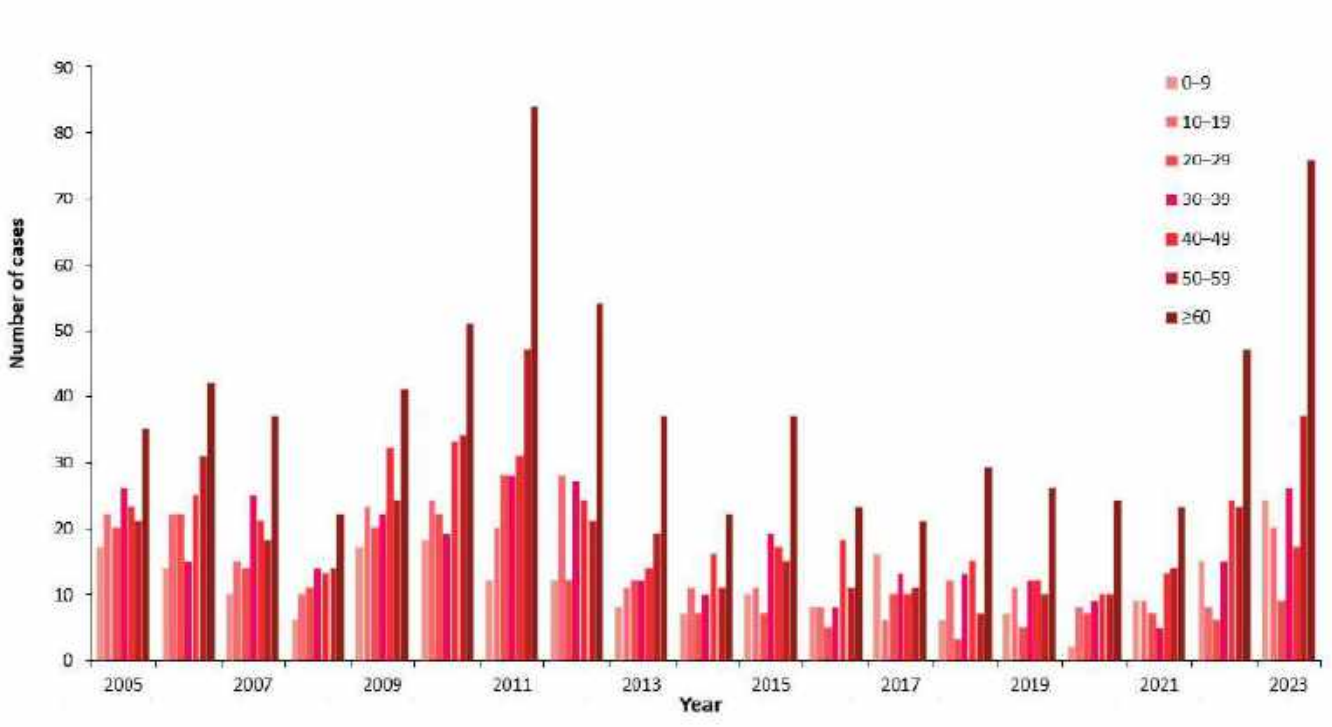
Viral subtypes, distribution	Co-circulation of European (TBEV-EU), Far-Eastern (TBEV-FE), and Siberian (TBEV-Sib) subtypes
Reservoir animals	Rodents, ruminants, game
Infected tick species (%)	2011: *I. persulcatus* 8%, *I. ricinus* on mainland 0.6% – 0.8% and Saaremaa 3.0%. 2013: Estonia: I. persulcatus 4.23%, I. ricinus 0.42%. 2018: Tallinn 0.44% - 2.7%. 2023: Estonia 1.1% - 8.3%: Valga county 6.1% and Viljandi county 8.3%.
Dairy product transmission	Documented but rare
Mandatory TBE reporting	**Reporting**: neurologists, infectious disease specialist **Case definition** Clinical criteria: a person with symptoms of the central nervous system (meningitis, meningoencephalitis, encephalomyelitis, encephaloradiculitis) **Laboratory criteria for case confirmation**: At least 1 of the following: • TBE-specific IgM and IgG antibodies in blood • TBE-specific IgM antibodies in CSF • Seroconversion of 4-fold increase of TBE-specific antibodies in paired serum samples • Detection of TBE viral nucleic acid in a clinical specimen • Isolation of TBEV from clinical specimens. Probable case: detection of TBE-specific IgM antibodies in a unique serum sample **Epidemiological criteria** Exposure to a common source (unpasteurized dairy product). Case classification: • Possible case: not applicable • Probable case: a person meeting the clinical criteria and the laboratory criteria for a probable case OR a person meeting the clinical criteria and with an epidemiological link • Confirmed case: a person meeting the clinical and laboratory criteria for case confirmation
Other TBE surveillance	Laboratory and epidemiological surveillance
Special clinical features	Biphasic disease: meningitis, meningoencephalitis, or meningoencephalomyelitis. Risk groups: people who often spend time outdoors (in nature)
Available vaccines	ENCEPUR CHILDREN, ENCEPUR ADULTS, TICOVAC CHILDREN, TICOVAC ADULTS

TBE vaccination by age in Estonia, 2022

Age	Vaccination (3 doses)	Revaccination (dose 4 or more)
1 - 14	6513	6544
15 - 17	418	1261
Adults	14475	25800
General population of Estonia 2022: 1,331,796		

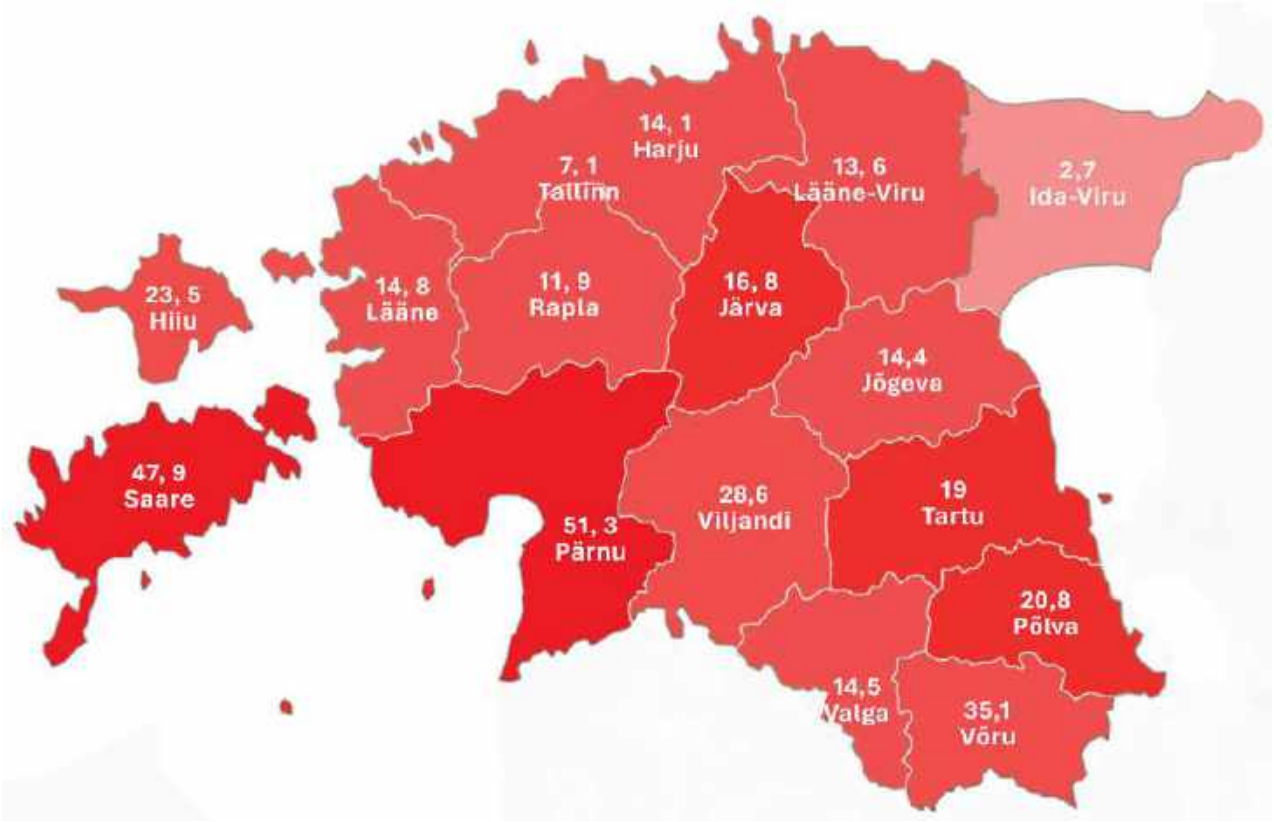
Vaccination recommendations and reimbursement	Vaccination recommendations 1998. No reimbursement; self-paid
Vaccine uptake by age group/risk group/ general population	Vaccine uptake by general population (vaccinated and revaccinated): 2018 – 3.1%; 2019 – 3.7%; 2020 – 3.4%; 2021 – 2.6%, 2022 – 4.1%, 2023 – 5.8%.
Name, address/website of TBE National Reference Center	Health Board, Tallinn Paldiski St 81; https://www.terviseamet.ee

Figure 2: Age distribution of TBE in Estonia, 2005–2023



Source Data: Appendix—Figure 2

Figure 3: Sites of TBEV-infection in Estonia, 2023



Contact: kkutsar@hotmail.com

Citation:
Kutsar K. TBE in Estonia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-10-7

Appendix

Source data: Figure 1

Year	Males	Females	All
0 - 9	17	7	24
10 - 19	15	5	20
20 - 29	6	3	9
30 - 39	14	12	26
40 - 49	5	12	17
50 - 59	14	23	37
60 - 69	13	19	32
70 - 79	16	21	37
80 and older	2	5	7
Total	102	107	209

Source data: Figure 2

Year	Vanusrühmad (aastates) / Age groups (years)						
	0-9	**10-19**	**20-29**	**30-39**	**40-49**	**50-59**	**60≤**
2005	17	22	20	26	23	21	35
2006	14	22	22	15	25	31	42
2007	10	15	14	25	21	18	37
2008	6	10	11	14	13	14	22
2009	17	23	20	22	32	24	41
2010	18	24	22	19	33	34	51
2011	12	20	28	28	31	47	84
2012	12	28	12	27	24	21	54
2013	8	11	12	12	14	19	37
2014	7	11	7	10	16	11	22
2015	10	11	7	19	17	15	37
2016	8	8	5	8	18	11	23
2017	16	6	10	13	10	11	21
2018	6	12	3	13	15	7	29
2019	7	11	5	12	12	10	26
2020	2	8	7	9	10	10	24
2021	9	9	7	5	13	14	23
2022	15	8	6	15	24	23	47
2023	24	20	9	26	17	37	76

Source data: Figure 3

Counties	Cases
Tallinn (capital)	31
Harjumaa	25
Hiiumaa	2
Ida-Virumaa	3
Järvamaa	5
Jõgevamaa	4
Läänemaa	3
Lääne-Virumaa	8
Pärnumaa	45
Põlvamaa	5
Raplamaa	4
Saaremaa	15
Tartumaa	30
Valgamaa	4
Viljandimaa	13
Võrumaa	12
Total	209

References

1. Health Board of Estonia. [Nakkushaiguste esinemine ja immunoprofülaktika]. 2022. Accessed 9 April, 2024. https://www.terviseamet.ee/sites/default/files/Nakkushaigused/Haigestumine/epid_ulevaade_2022_0.pdf

2. Katargina O, Russakova S, Geller J et al. Detection and characterization of tick-borne encephalitis virus in Baltic countries and Eastern Poland. *PLoS One*. 2013;8(5):e61375

3. Geller J, Vikentjeva M. [Ticks as disease carriers in the green areas of Tallinn and the surrounding area]. 2020. Accessed 30 March, 2024. https://www.tai.ee/sites/default/files/2021-03/159852954118_Pealinna%20rohealade%20puugid%20ja%20puugihaigused.pdf

4. Vikentjeva M, Geller J. Linnapuugid 2023 – puukide levimus ja puugihaiguste oht Eesti linnade avalikel haljasaladel. Tervise Arengu Instituut. 2023. Accessed 30 March 2024. https://tai.ee/sites/default/files/2023-09/Linnapuugid_2023.pdf

Chapter 13

TBE in Finland

Anu Jääskeläinen and Heidi Åhman

E-CDC risk status: endemic (data as of beginning of 2023)

History and current situation

Finland is at the northernmost edge of the TBE endemic area in Europe. Here TBE is focally endemic. An aseptic encephalitis disease has been known in Kumlinge Island in Åland Islands since the 1940s.[1] TBE is also known in Finland by the name Kumlinge disease.

According to a legend, tick-borne encephalitis–like disease was known in the Åland Islands already in the 18th century. However, this is apparently a misunderstanding due to a doctoral thesis of archipelago fever in the Turku region published 1781, which describes malaria, not TBE.[2]

TBEV foci were determined in the 1960s by screening TBEV antibodies in cattle from all over the country.[3] The endemic areas remained the same throughout decades until the 1990s, when Isosaari Island at the archipelago of Helsinki was found to be TBE endemic.[5] Since then, sporadic human cases have appeared in new areas, like in Närpiö on the western coast and in eastern Finland in Varkaus, in the Kuopio region and in the Kotka archipelago.[6] 2008 human cases were traced to Simo, the world's northernmost TBE endemic foci in Finnish Lapland,[7] which is nowadays a high endemic focus where residents are vaccinated against TBE in national immunization program.

Tick distribution in the country was studied in 1950s[8] and 2015 using crowdsourcing.[9] Compared with the nationwide distribution map drawn in 1960s, the distribution of ticks has extended up to 200 km northwards.[9]

The northernmost tick samples were from latitudes of 67°, but it is unclear whether ticks there are from stable populations or are stragglers transported there with animals. However, populations have established in new locations, i.e., the Bothnian Bay coast and the eastern part of central Finland. In addition, TBEV RNA has been detected or TBEV isolated from ticks in areas formerly unknown to be TBE endemic and areas where only sporadic TBE cases have been reported.[9]

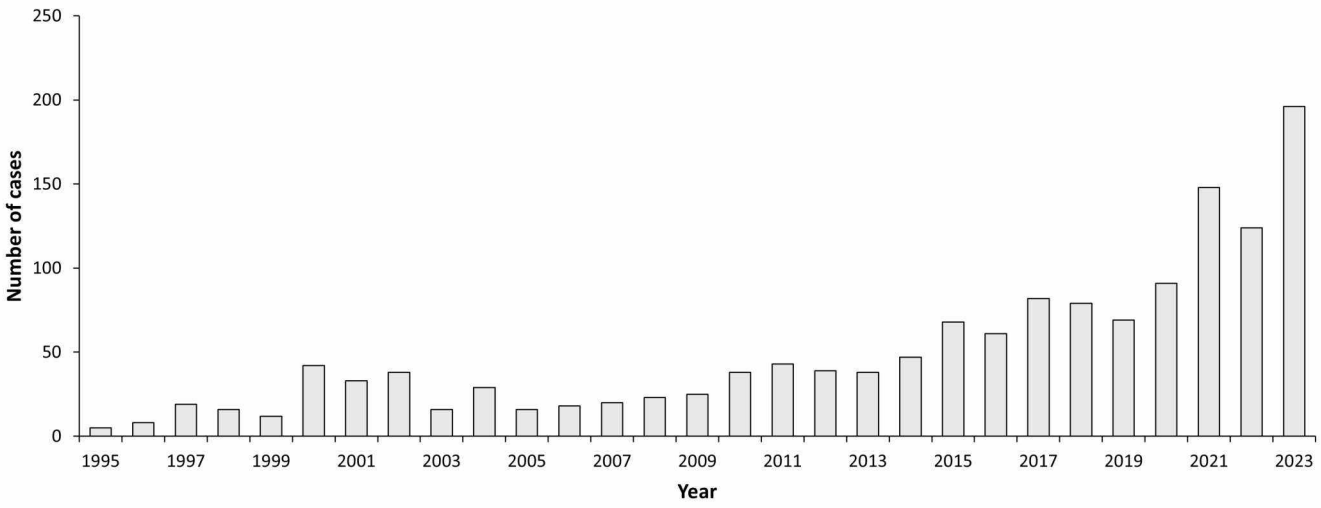
Both TBEV vector tick species, *Ixodes ricinus* and *Ixodes persulcatus,* are distributed in Finland.[4,10] *I. persulcatus* is more abundant than *I.* ricinus in certain areas, such as in northern Finland where it is the dominant tick species. Both species have been shown to transmit TBEV-Eur and TBEV-Sib in Finland.[6,7]

The overall prevalence of TBEV in ticks in Finland is reported to be 1.6%.[9] TBEV prevalence was higher in *I. persulcatus* (3.0%) than in *I. ricinus* (0.2%) in 2015 based on ticks sampled by crowdsourcing[9] but varies greatly within Finland.

Overview of TBE in Finland

Table 1: TBE in Finland	
Viral subtypes, distribution	European and Siberian subtypes[4,9]
Reservoir animals	*Microtus agrestis, Myodes glareolus*[10]
Infected tick species (%)	*I. ricinus, I. persulcatus.* In average 1.6%; *I. ricinus* 0.2%, *I. persulcatus* 3.0%[9] In (suspected) endemic foci, TBEV RNA prevalence in field-collected ticks has been reported to be about 0.1%–3.0%[4,10,11]
Mandatory TBE reporting	All patients with TBEV IgM antibodies are reported to National Infectious Diseases Register at National Institute for Health and Welfare; a group of experts interviews the patients and/or reviews the reports to confirm the place of acquisition and that the cases are true TBE cases by definition
Other TBE surveillance	Sentinel animals not systematically screened
Special clinical features	Biphasic disease reported in about 30%[12]
Available vaccines	Encepur, Encepur Lapset (Bavarian Nordic), TicoVac and TicoVac Junior (Pfizer)
Vaccination recommendations and reimbursement[13]	Eligible for the TBE vaccines as part of the national program are persons aged 3 years and over who are domiciled in Finland and who live permanently in the following regions: • Åland • The southern districts of Kemi • Simo • Kotka archipelago • Sammonlahti district of Lappeenranta • Off the coast of Raahe on the island of Preiskari • Parainen • Lohjanjärvi archipelago and the postal code areas of Ojamo (08200), Kirkniemi (08800), Lylyinen/Hormajärvi (08450) and Vohloinen/Virkkala (08700) • Kustavi • Kirkkonummi in the postal code areas of Luoma (02440) and Masala (02430) • Parts of the Sipoo archipelago Persons staying for long periods of time in holiday homes in these risk areas are also entitled to free vaccination. The vaccine is necessary only for persons who are active in nature for at least 4 weeks during the snow-free season. A previously unvaccinated person will receive three free doses of the vaccine. A person who has not completed the basic series will also receive remaining doses of primary series free of charge as part of the vaccination program. Booster vaccinations for those who have received a three-dose vaccination series are currently not included in the vaccination program. TBE vaccination recommendations for other risk areas are based on incidence and case-by-case consideration. The vaccine is paid for by the vaccinee. In some situations, the employer is responsible for protecting the worker, in which case the need for vaccination is assessed by the occupational health service.
Vaccine uptake by age group/risk group/general population	21%[14]
Name, address/website of TBE NRC	National Institute for Health and Welfare, THL, Mannerheimintie 166, 00300 Helsinki https://www.thl.fi

Figure 1: Burden of TBE in Finland 2013–2022 (Reference: National Registry of Infectious Diseases)[14]



Please note that TBE is not evenly distributed throughout Finland.
The local incidence rates vary from 0 to >15/100,000.

Source Data: Appendix—Figure 1

Figure 2: Number of TBE cases during 2017–2021[16]

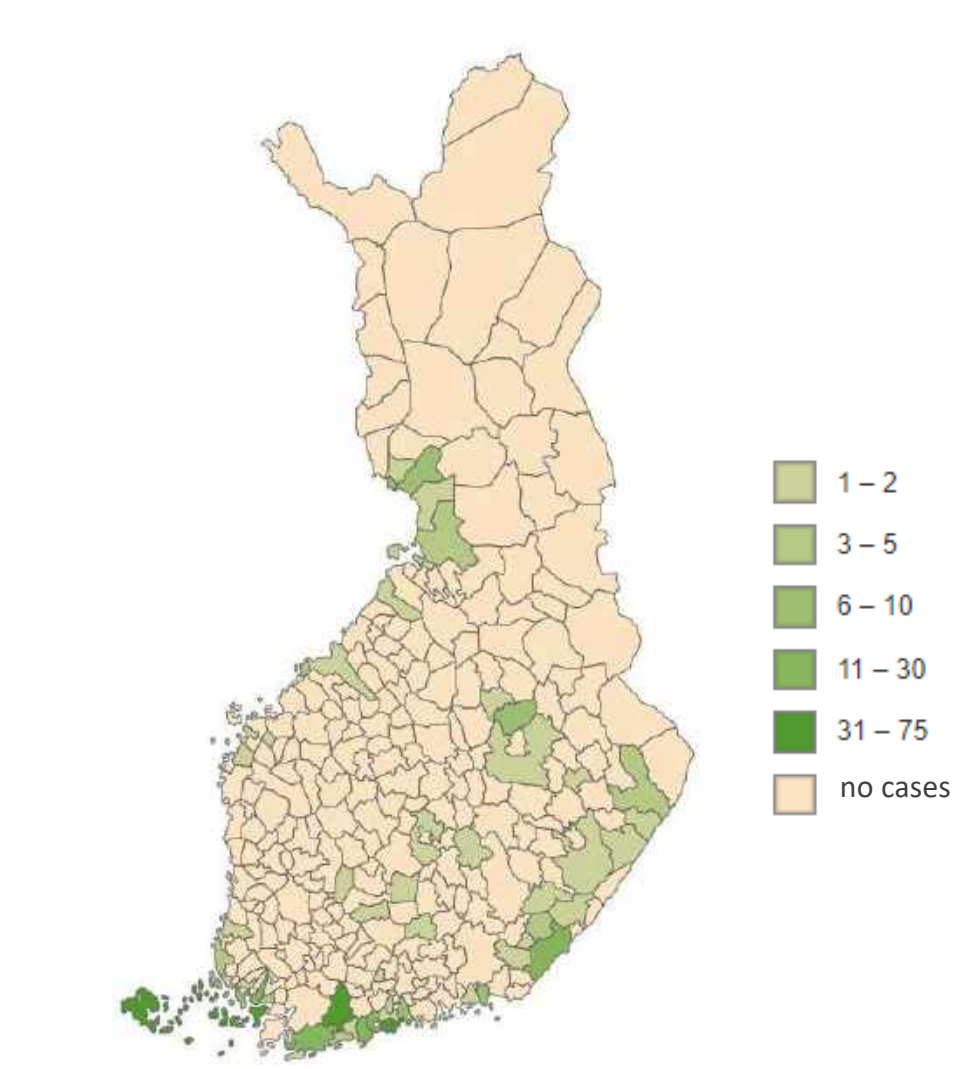
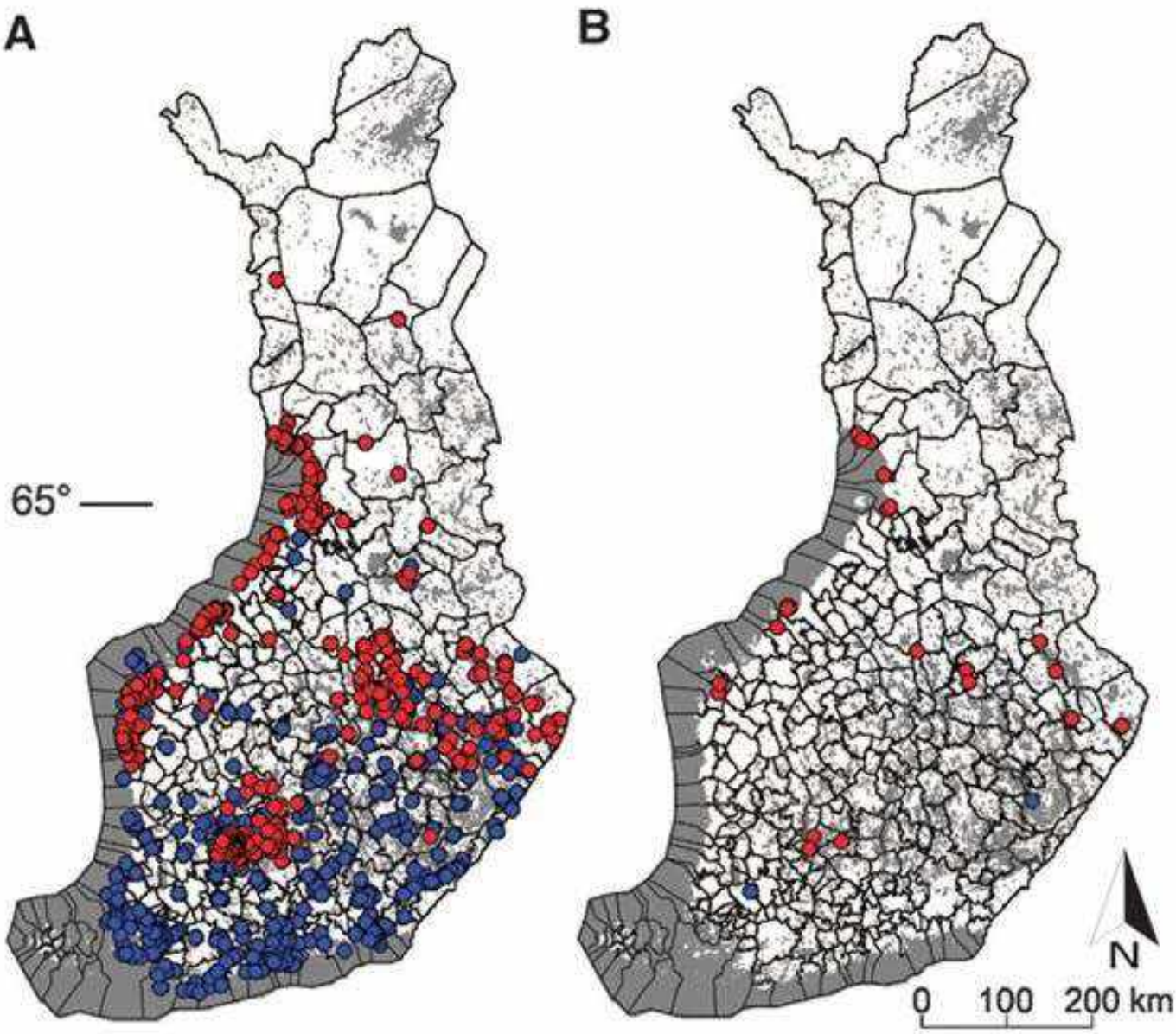


Figure 3:



(A) Distribution of samples (n=2038) screened for pathogens. Blue dots indicate collection points for *I. ricinus* samples (n=1044) and red dots indicate collection points for *I. persulcatus*.

(B) Distribution of the samples that were positive for TBEV (n=32). Adapted from Laaksonen M, et al. 2007.[10]

Contact: heidi.ahman@pfizer.com

Citation:
Jääskeläinen A, Åhman H. TBE in Finland. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-11-7

Appendix

Source data: Figure 1

Year	Number of cases	Incidence / 10^5
1995	5	0.0
1996	8	0.16
1997	19	0.38
1998	16	0.31
1999	12	0.23
2000	42	0.81
2001	33	0.64
2002	38	0.73
2003	16	0.31
2004	29	0.56
2005	16	0.31
2006	18	0.34
2007	20	0.38
2008	23	0.43
2009	25	0.47
2010	38	0.71
2011	43	0.80
2012	39	0.72
2013	38	0.71
2014	47	0.86
2015	68	1.25
2016	61	1.11
2017	82	1.49
2018	79	1.43
2019	69	1.25
2020	91	1.64
2021	148	2.67
2022	124	2.23
2023	196	3.5

References

1. Oker-Blom N. Kumlinge disease; a meningoencephalitis occurring in the Aaland Islands. *Ann Med Exp Fenn*. 1956;34:309-18.
2. Brummer-Korvenkontio M. Virusten ja prionien luonnonhistoria. Helsinki University Press 2007. In Finnish
3. Tuomi J, Brummer-Korvenkontio M. Antibodies against viruses of the tick-borne encephalitis group in cattle sera in Finland. *Ann Med Exp Biol Fenn*. 1965;43(3):149-54
4. Jääskeläinen AE, Tikkakoski T, Uzcátegui NY, Alekseev AN, Vaheri A, Vapalahti O. Siberian subtype tickborne encephalitis virus, Finland. *Emerg Infect Dis*. 2006;12(10):1568-71.
5. Han X, Aho M, Vene S, et al. Prevalence of tick-borne encephalitis virus in *I. ricinus* ticks in Finland. *J Med Virol.* 2001;64(1):21-8
6. Jääskeläinen A, Tonteri E, Pieninkeroinen I, et al. Siberian subtype tick-borne encephalitis virus in *I. ricinus* in a newly emerged focus, Finland. *Ticks Tick-Borne Dis*. 2016;7(1):216-23.
7. Jääskeläinen AE, Tonteri E, Sironen T, Pakarinen L, Vaheri A, Vapalahti O. European subtype tick-borne encephalitis virus in *I. persulcatus* ticks. *Emerg Infect Dis*. 2011;17(2):323-5
8. Öhman C. The geographical and topographical distribution of *I. ricinus* in Finland. *Acta Soc. Pro Fauna et Flora Fenn*. 1961;76 (4):1-25
9. Laaksonen M, Sajanti E, Sormunen J, et al. Crowdsourcing-based nationwide tick collection reveals the distribution of *I. ricinus* and *I. persulcatus* and associated pathogens in Finland. *Emerg Microbes Infect*. 2017;6(5):e31. doi:10.1038/emi.2017.17.
10. Tonteri E, Jääskeläinen A, Tikkakoski T, et al. Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008-2009. *Emerg Infect Dis*. 2011;17(1):72-5. doi:10.3201/eid1701.100051
11. Jääskeläinen AE, Sironen T, Murueva GB, et al. Tick-borne encephalitis virus in ticks in Finland, Russian Karelia and Buryatia. *J Gen Virol.* 2010;91(Pt 11):2706-12.
12. Metsi J, Vuorla M, Kantele A, Kuusi M and Oksi J. Puutiaisaivokuume Suomessa 2010-2012. *Duodecim.* 2015;131:1367-75.
13. National Institute for Health and Welfare 2022. https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/rokotteet-a-o/tbe-rokote-eli-punkkirokote. Updated March 1, 2022 (Accessed February 6, 2023).
14. TBE Compliance and Coverage 2019 – Finland. Ipsos, November 2019.
15. National Registry of Infectious Diseases, National Institute for Health and Welfare THL. https://www.thl.fi/ttr/gen/rpt/tilastot.html (Accessed May 10, 2023).
16. National Institute for Health and Welfare https://www.thl.fi/ttr/gen/atlas/html/atlas.html?show=tbe_riskienarviointi (Accessed February 6, 2023).

Chapter 13

TBE in France

Yves Hansmann and Aurélie Velay

E-CDC risk status: endemic (data as of end 2022, updated May 2023)

History and current situation

The first human case of tick-borne encephalitis virus (TBEV) infection in France was reported in 1968 in Alsace, an eastern region next to the German border: a gamekeeper working in a forest near Strasbourg.[1] Between 1970 and 1974, an extensive research survey confirmed the presence of TBEV in ticks and rodents in this French region. Eight percent of adult tick batches collected were infected (*I. ricinus*) by the TBEV. Tick collection occurred in a forest near Strasbourg, the main city in the region. Nymphs were more rarely infected (1.6% of the collected lots).[1] These data were confirmed in 2011 in Alsace in Guebwiller's Valley, a middle altitude forest, with identification of western (European) subtype TBEV (TBEV-EU). The infection rate still remains low: TBEV was detected only in the *I. ricinus* nymphs (2.48%) that were collected during May; however, not in those collected during the other spring or summer months. In a more recent study, Bestehorn et al., collected ticks (953 male, 856 female adult ticks and 2,255 nymphs) in endemic foci in the upper Rhine region in France and Germany between 2016, 2017 and 2018 by flagging.[2] The minimal infection rate (MIR) of the collected ticks in the Foret de la Robertsau (France) was estimated to 0,11% (1 nymph/944 ticks). The isolated and sequenced TBEV strain from Foret de la Robertsau (F) is related to circulating TBEV isolates from eastern Bavaria and the Czech Republic. In the French department Alsace, there are today at least two independent TBEV strains circulating: the historical Alsace strain isolated in 1971 and the newly identified strain from Foret de la Robertsau. Other wooded regions (Ardennes) were explored for TBEV in ticks, but without evidence of virus infection.[3]

Between 1968 and 2018, more than 200 human tick-borne encephalitis (TBE) cases have been described in France.[4,5] The majority of cases (more than 90%) were diagnosed in Alsace. Twenty-two cases were imported, including eight imported cases in 2017.[6] Among them, 14 cases came from Germany (after staying in the Black Forest, a mountainous area bordering eastern France). The 8 other imported cases were acquired in Austria, Finland, Poland, Romania, Russia, Slovakia, Sweden, and Switzerland.

Among the autochthonous cases, the majority of the patients were infected in Northeastern France, especially in Alsace (more than 70% of the autochthonous cases during the five last years). Although Alsace remains the area with the highest prevalence of TBE in France, a secondary hotspot was identified in the Alpine region, in a Swiss neighboring area (Savoie and Haute Savoie) during the last ten years with 8 patients presented with TBE. In 2006, 1 patient was infected close to Bordeaux (not a known endemic area). In 2017 and 2018, 3 patients were infected in Haute Loire (in the surrounding countryside of Saint Etienne), making this region a new possible emerging area of TBE, and new foci have been identified in the Auvergne-Rhone region.[7] In Alsace, some small areas with higher TBEV endemicity have been identified, especially in the southern Vosges valley, a middle-altitude mountain, and some forests around Strasbourg.[4]

There are currently 3 medical laboratories that test for TBEV in France: the national reference center, the virology laboratory of Strasbourg University Hospital in eastern France, and 1 private laboratory. All 3 of these laboratories participate in the collection of data for any patients diagnosed with TBE as confirmed by the presence of specific TBE immunoglobulin M (IgM) and IgG in serum samples. However, in France, patients with encephalitis are tested for TBE only if they have risk factors (especially travelling to high-endemic regions). Considering Alsace as an endemic region, only patients living in this region are regularly tested for TBE. Only patients with clinical signs compatible with TBE meningoencephalitis are kept for further analyses that are presented here.

Until 2016, in humans, the annual number of cases in France each year ranged from 1 to 12. In 2016, we noticed a recrudescence of infection with 29 cases of TBEV infection.[5] In 2017 and 2018, 18 and 24 cases were reported, respectively, by the 3 laboratories involved in TBE testing. Except for the year 2017, in 2016 and 2018 more than 80% of the cases were autochthonous. From 2013 to 2018, the transmission period for TBEV is from April to October, with a peak in June and July in half of all cases.

From 2013 to 2017, 60% of the patients presented with meningoencephalitis.[6] All patients were hospitalized. The female-to-male ratio was 0.4; mean age was 53 years. Also, 63% of the patients remembered a tick bite during the weeks before the beginning of symptoms that led to TBE diagnosis. Consuming raw milk cheese before the onset of symptoms was recorded for 1 patient, but without any proof that this was the source of the TBEV infection.

Between April and May 2020, a TBE outbreak due to alimentary transmission (non-pasteurized goat milk and milk products) was reported by Santé Publique France in the Auvergne-Rhônes Alpes Region (département de l'ain); data in French available on the web site (www.santepubliquefrance.fr/les-actualites/2020/foyer-de-cas-d-encephalite-a-tiques-lies-a-la-consommation-de-fromage-de-chevre-au-lait-cru-dans-l-ain.-point-au-19-juin-2020). A total of 33 TBE cases were confirmed by the National reference center of arboviruses (Marseille) and 11 are still under investigation. Including these 33 cases results in an estimated total of 68 TBE cases in France in 2020, pending final confirmation. Among the remaining 35 patients, all diagnosed by the laboratory of Virology of Strasbourg University Hospital, the median age was 53.2 years (range: 11–78), 19 of them were male. Transmission occurred by tick bite in 17 (48.6%), it was the alimentary route in 6 (17.14%) and it remained unknown in 12 cases. The 6 additional cases identified as alimentary transmission were all linked to the outbreak previously mentioned above. Only one case was imported (due to COVID-19 lockdown). The two main endemic areas in France are still the Alsace and the Alpine regions.

In 60% of cases, an initial disease stage with fever and flu-like symptoms occurred prior to the onset of meningitis or encephalitis symptoms. Among those cases, 37% had meningitis without any other neurological symptoms and 54.3% had neurological signs associated with meningitis. For 2 patients, a clinical diagnosis of meningo-radiculitis was established.

Between May 2021 and December 2022, 62 cases were notified (31 cases in each year): M/F ratio= 1.6; median age 50 years [IIQ 27–60]; 2 cases were children. 57 cases presented neurological signs: 30 encephalitis or meningoencephalitis, 23 meningitis, 3 encephalomyelitis, and 1 myelitis.

34 cases out of 62 (55%) reported a tick bite before the onset of signs. 52 cases (84%) had acquired their infection in France. Among them, 8 cases (15%) had a job exposing them to tick bites or dairy products made from raw milk from animals at risk. For 6 cases (12%), food contamination in the Auvergne-Rhône-Alpes (ARA) region was suspected:

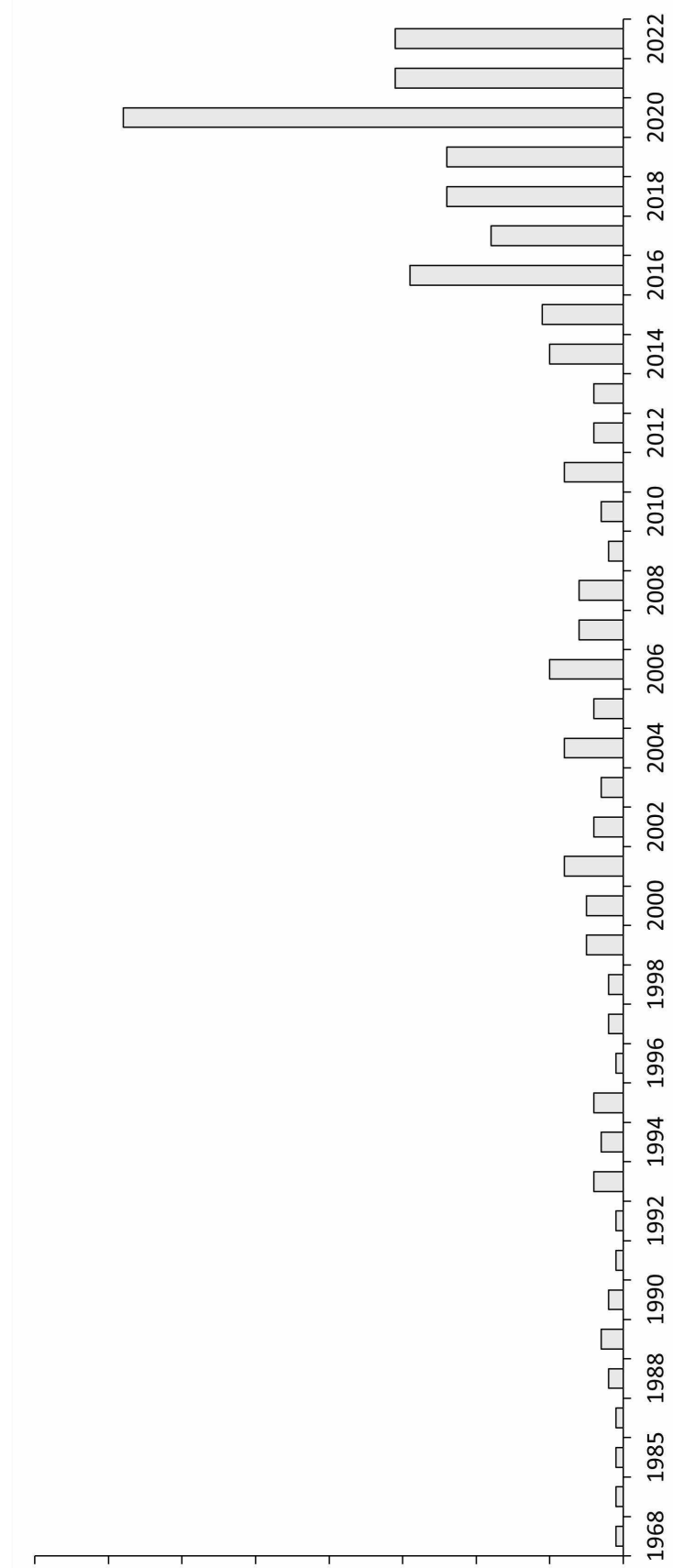
- Two cases had consumed cheese from the same farm.
- One case worked on a goat farm and reported another case among the employees.
- One case lived on a farm that could not be investigated.
- One case occurred in a breeder whose herd and products were also contaminated.

Two clusters were highlighted in the ARA region in an area not previously known to be at risk.

Overall of TBE in France

Table 1: TBE in France	
Viral subtypes, distribution	Western subtype
Reservoir animals[1]	Red-backed voles *(Clethryonomis glareolus)* and field mice (*Apodemus sylvaticus* and *A. flavicollis*)
Infected tick species (%)[1]	• Infected *I. ricinus* adults: 0.6–0.79% according to the site and the year of collection • Infected *I. ricinus* nymphs: 0.04–0.12% much more rarely isolated virus (numerous negative lots) • No infected *I. ricinus* larvae
Dairy product transmission	Documented since 2020; see text above
Mandatory TBE reporting	Mandatory reporting planned — expected to be effective in 2022
Other TBE surveillance	Mainly three laboratories establish the diagnosis for TBE in France: • The National reference center of arboviruses (Marseille) • The laboratory of Virology of Strasbourg University Hospital (Strasbourg) • Cerba (a private laboratory) The 2020 data above and in the table/graph are those reported by us, the laboratory of Virology of Strasbourg University Hospital, and they are not exhaustive. TBE notification became mandatory since May 2021. Case definition: Positive findings with at least one of the following methods: • Direct detection of virus • Nucleic acid detection (e.g. PCR) • IgM and IgG antibody detection in blood • IgM antibody detection in CSF • Four-fold rising of antibody titer or seroconversion in two successive samples Probable case definition: the same clinical definition as confirmed cases but with isolated IgM antibody in blood.
Special clinical features	Approximately 50% of biphasic disease 1% mortality
Available vaccines	Ticovac and Encepur
Vaccination recommendations and reimbursement	Recommendations only for travelers going to endemic areas No reimbursement
Vaccine uptake by age group/risk group/general population	No information available
Name, address/website of TBE NRC	Arbovirus Reference Center, Institut de Recherche Biomedicale des Armées (Irba), Hôpital d'Instruction des Armées Laveran – Service de Biologie BP 60149 13384 MARSEILLE CEDEX 13 Laboratoire de Virologie, Hôpitaux Universitaires de Strasbourg, 3, rue Koeberlé, 67000 Strasbourg

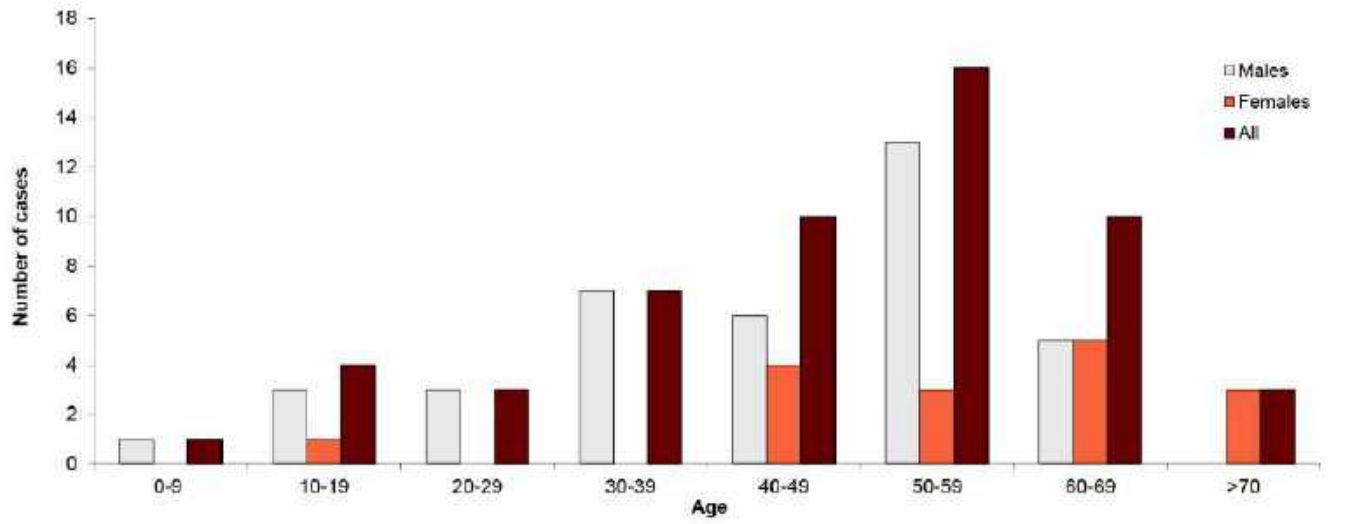
Figure 1: Burden of TBE in France over time; (Hansmann, Velay 2018; updated May 2023)



Note: Vaccine became available in 2005

Source data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE in France in (2013–2016)[5]



Source data: Appendix Figure 2

Figure 3: TBEV-isolation and TBE cases in France

TBE endemic area

Regular sporadic TBE cases area

Alimentary outbreak between April and May 2020 (département de l'Ain)

Appendix

Source data: Figure 1

Year	Number of cases	Incidence/10^5
1968	1	
1970	1	
1985	1	
1986	1	
1988	2	
1989	3	
1990	2	
1991	1	
1992	1	
1993	4	
1994	3	
1995	4	
1996	1	
1997	2	
1998	2	
1999	5	
2000	5	
2001	8	
2002	4	
2003	3	
2004	8	
2005	4	Vaccine available
2006	10	
2007	6	
2008	6	
2009	2	
2010	3	
2011	8	
2012	4	
2013	4	
2014	10	
2015	11	
2016	29	
2017	18	
2018	24	
2019	24	
2020	68	
2021	31	
2022	31	

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	1	0	1
10-19	3	1	4
20-29	3	0	3
30-39	7	0	7
40-49	6	4	10
50-59	13	3	16
60-69	5	5	10
>70	0	3	3

Contact: yves.hansmann@chru-strasbourg.fr

Citation:
Hansmann Y, Velay A. TBE in France. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-12-7

References

1. Perez Eid C, Hannoun C, Rodhain F. The Alsatian tick-borne encephalitis focus: presence of the virus among ticks and small mammals. *Eur J Epidemiol.* 1992;8:178-86.
2. Bestehorn M, Weigold S, Kern WV, et al. Phylogenetics of tick-borne encephalitis virus in endemic foci in the upper Rhine region in France and Germany. *PLoS One*. 2018;13(10):e0204790.
3. Moutailler S, Valiente Moro C, Vaumourin E, et al. Coinfection of ticks: the rule rather the exception. *PLoS Negl Trop Dis*. 2016;10:e0004539
4. Hansmann Y, Pierre Gut J, Remy V, et al. Tick-borne encephalitis in Eastern France. *Scand J Infect Dis*. 2006;38:520-6.
5. Velay A, Solis M, Kack-Kack W, et al. A new hot spot for tick-borne encephalitis (TBE): A marked increase of TBE cases in France in 2016. *Ticks Tick Borne Dis*. 2018;9(1):120-125.
6. Velay A, Paz M, Cesbron M, et al. *Epidémiologie et prévention de l'encéphalite à tique en.* 2017. 19èmes JNI ; 13-15 juin 2018, Nantes
7. Botelho-Nevers E, Gagneux-Brunon A, Velay A, et al. Tick-Borne Encephalitis in Auvergne-Rhône-Alpes Region, France, 2017–2018. *Emerg Infect Dis*. 2019;25(10):1944–8.

Chapter 13

TBE in Germany

Gerhard Dobler

E-CDC risk status: endemic (data as of end 2023)

History and current situation

The beginning of research on TBE in Germany was influenced and inspired by the results and developments of TBE research in the former Czechoslovakia. There, TBE virus was detected in the Czechoslovak Republic in 1948. In Germany, the first evidence of the presence of TBE virus was found by Sinnecker and his group in the former German Democratic Republic (GDR).[1] The first virus strains were isolated also by Sinnecker's group in the early 1960s.[2] In the former Federal Republic of Germany (FRG), TBE research started with research on TBE virus in the region of Franconia by Scheid and Ackermann.[3,4] In the region of Lower Franconia, a virus was isolated which was called "Zimmern Virus'' after the location of the isolation.[5] Unfortunately, all these virus strains were lost but it can be assumed that they all belonged to the Western (European) subtype of TBE virus.

In the 1970s, a strong decrease of reported human TBE cases occurred in the former endemic areas of the German Democratic Republic.[6] In Western Germany, only few studies were conducted on the geographic appearance of human TBE cases, mainly led by the company IMMUNO, the first producer of a TBE vaccine in Western Europe. No systematic epidemiological studies are available from this time. TBE was not reportable during this time.

In 2001, TBE became a reportable disease by the new Infection Control Act. From this time on, reliable data on the prevalence of TBE in Germany are available. In the era of molecular detection studies in different areas of Germany on the prevalence of TBE virus in ticks were conducted. In non-engorged ticks the prevalence rates vary depending on the tick stage from 0.1% to 0.5% (nymphs) up to 5% (adult stages).[7,8] The molecular characterization of a number of virus strains isolated from ticks in Germany shows that so far all known strains belong to the European subtype of TBE virus.[8] *Ixodes ricinus*, the sheep tick, is the most important vector of TBE virus in Germany. In 2016, TBE virus was detected for the first time in Dermacentor reticulatus in the Federal State of Saxony. In 2016 and 2017, also for the first time in about 50 years, two goat milk-borne outbreaks of TBE were registered in Germany (districts of Reutlingen, Tübingen, Baden- Württemberg).

In Germany, TBE is found mainly in the southern part, with the federal states of Bavaria and Baden-Württemberg comprising 80% to 90% of all reported human cases in Germany. There is an increasing number of districts in Saxony, Thuringia and for the first time in 2019 in Lower Saxony and Brandenburg which are classified as risk districts by the RKI. The annual reported human cases range from 200 to >550 (RKI, SurvStat). Seroprevalence rates before vaccination programs started in endemic areas in the human population ranged between 3% to 8%, with high clustering in some human populations, indicating a highly focal geographic distribution within the endemic areas. Calculating the incidence of the overall German population is generally low (<0.1/100,000), but these figures may give a strongly underestimated risk for some districts in Southern Germany, where the highest incidence rates in Germany can reach >10/100,000 in particular districts (e.g., Amberg, Bavaria and Ortenaukreis, Baden-Württemberg). Actual studies in the district of Ortenaukreis show that the prevalence of antibodies indicating infection (NS1 IgG) is 5.6% in a population of blood donors and subtracting the vaccinated (and therefore protected) portion, the prevalence of antibodies indicating infection was 12.8%[17].

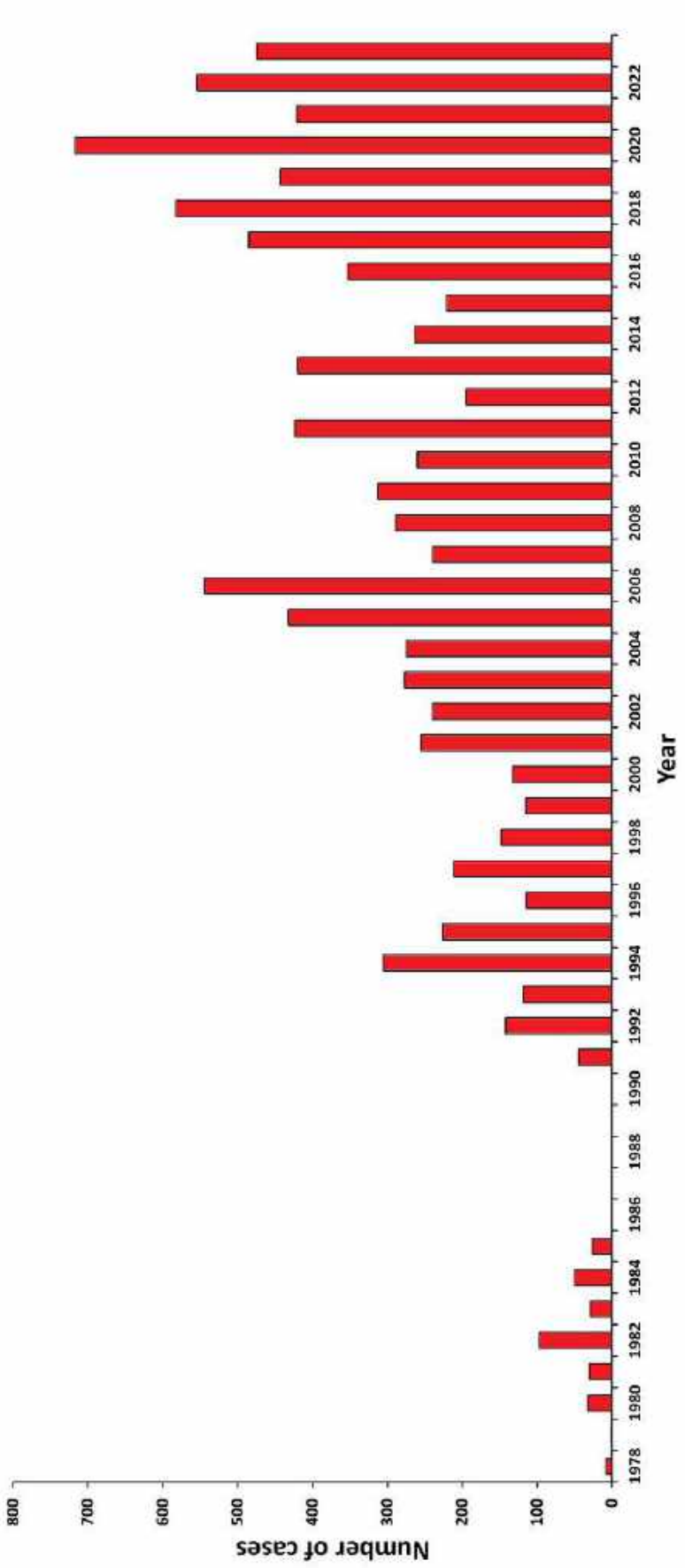
Overview of TBE in Germany

Table 1: Virus, vector, transmission of TBE in Germany

Viral subtypes, distribution	European TBEV subtype[7,8,13,14]
Reservoir animals	Main vertebrate reservoir animals assumed – *Myodes glareolus, Apodemus flavicollis, Apodemus agrarius, Apodemus sylvaticus, Microtus agrestis and Microtus arvalis, and Myodes glareolus*; detailed information and studies missing.[10]
Infected tick species (%)	*I. ricinus* (0.1%–5%); *D. reticulatus* (0.5%). (Chitimia-Dobler et al.[16]; Dobler, personal communication)
Dairy product transmission[14]	2016 first outbreak by goat milk and goat cheese for >50 years in Germany; 2 patients 2017 outbreak in school with 8 patients[18]

Figure 1: Burden of TBE in Germany over time

[The Center for Communicable Diseases and AIDS (2014). Available at: http://www.ulac.lt/ataskaitos]



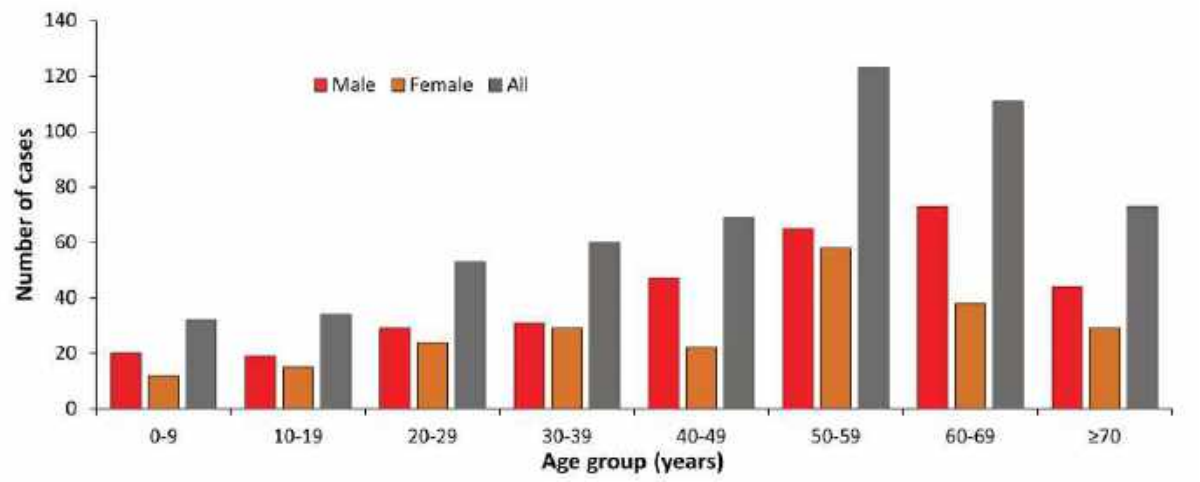
Source Data: Appendix—Figure 1

Please note that TBE is not evenly distributed throughout Germany; in some specific areas of the country, TBE incidence may be >10/100,000 (see text for details).

[Robert Koch-Institute, SurvStat. Available at: http://survstat.rki.de/Content/Query/Create]

Table 2: TBE reporting and vaccine prevention in Germany	
Mandatory TBE reporting	All patients with confirmed TBE by serological methods (TBEV IgM ± IgG) or by virus detection are reported to the State Public Health Authorities and to the Federal State Public Health Authority (Robert Koch-Institute: www.rki.de)
Other TBE surveillance	n/a
Special clinical features	Biphasic disease in about 50% Risk groups: permanent inhabitants and visitors of highly endemic areas; mainly acquired during leisure activities 40% of patients meningoencephalitis, 10% meningoencephalomyelitis; no reliable data available on neurological sequelae; in a large study 40%–50% of patients with long-term sequelae; mortality rate 1%–2%[9]
Available vaccines	Encepur Erwachsene, Encepur Kinder (Bavarian Nordic), FSME-IMMUN Erwachsene, FSME-IMMUN Kinder (Pfizer)
Vaccination recommendations and reimbursement	All inhabitants and visitors of known endemic areas with a risk of tick contact; (STIKO recommendation [www.rki.de])
Vaccine uptake by age group/ risk group/ general population	Vaccination rates in endemic areas 25% to 75%, depending on the district (Survey of the German Society of Consumption Research and personal seroprevalence studies).
Name, address/website of TBE National Reference Center	Robert Koch-Institute (Federal Authority of Public Health), Nordufer 20, 13353 Berlin, Germany (www.rki.de) Bundeswehr Institute of Microbiology, Neuherbergstrasse 11, 80937 München, Germany (gerharddobler@bundeswehr.org)

Figure 2: Age and gender distribution of TBE in Germany



[Robert Koch-Institute, SurvStat. Available at: http:// survstat.rki.de/Content/Query/Create.] *Source Data: Appendix—Figure 2*

TBEV-isolation and TBE cases in Germany			
Year of isolation	**Strain name**	**Source of isolation**	**Location of isolation**
1975[11]	K23	Tick	Karlsruhe, Baden-Württemberg
2006[8]	AS33	Tick	Amberg, Bavaria
2007[12]	Salem	Monkey brain	Salem, Baden-Württemberg
2009*	HM strains	Tick	Amberg, Bavaria
2011[13]	HB171/11	Tick	Heselbach, Bavaria
2014**	Bottnang	Tick	Stuttgart, Baden-Württemberg
2016*	HM-M1	Bank vole brain	Amberg, Bavaria
2016*,***	tbd	Goat milk cheese	Zwiefalten, Baden-Württemberg
2016[15]	tbd	Tick	Aubachstrasse, Baden-Württemberg
2017[15]	tbd	Tick	Schiltach, Baden-Württemberg
2017[16]		Tick (*D. reticulatus*)	Battaune, Saxony

**Dobler, personal communication; **Oehme, personal communication; ***Chitimia-Dobler et al.[16]; tbd, to be determined*

Appendix

Source data: Figure 1

Year	Number of cases	Incidence / 10^5
1978	8	
1979	1	<0.1
1980	32	<0.1
1981	30	<0.1
1982	97	0.17
1983	29	<0.1
1984	50	<0.1
1985	26	<0.1
1986	n.a.	
1987	n.a.	
1988	n.a.	
1989	n.a.	
1990	n.a.	
1991	44	<0.1
1992	142	0.18
1993	118	0.15
1994	306	0.38
1995	226	0.28
1996	114	0.14
1997	211	0.26
1998	148	0.18
1999	115	0.14
2000	133	0.16
2001	255	0.31
2002	239	0.29
2003	277	0.34
2004	274	0.33
2005	432	0.52
2006	544	0.66
2007	239	0.29
2008	289	0.35
2009	313	0.38
2010	260	0.32
2011	424	0.52
2012	195	0.24
2013	420	0.52
2014	264	0.33
2015	221	0.27
2016	353	0.43
2017	485	0.59
2018	582	0.70
2019	443	0.53
2020	717	0.86
2021	421	0.51
2022	555	0.66
2023	474	0.58

Source data: Figure 2 (2023, with data for 2010–2022 also shown):

Year	Gender	Age group (years)							
		0–9	10–19	20–29	30–39	40–49	50–59	60–69	≥70
2010	Male	3	12	13	18	39	26	26	23
	Female	6	4	7	16	28	24	8	7
	All	**9**	**16**	**20**	**34**	**67**	**50**	**34**	**30**
2011	Male	18	19	18	15	76	62	34	27
	Female	7	13	8	23	42	25	18	18
	Unknown		1						
	All	**25**	**33**	**26**	**38**	**118**	**87**	**52**	**45**
2012	Male	3	5	10	14	34	27	13	17
	Female	3	3	9	7	15	19	7	9
	All	**6**	**8**	**19**	**21**	**49**	**46**	**20**	**26**
2013	Male	17	22	25	26	47	53	33	38
	Female	5	5	15	24	36	35	17	21
	Unknown				1				
	All	**22**	**27**	**40**	**51**	**83**	**88**	**50**	**59**
2014	Male	5	5	11	17	39	39	25	27
	Female	4	3	8	14	24	20	10	13
	All	**9**	**8**	**19**	**31**	**63**	**59**	**35**	**40**
2015	Male	5	11	11	11	17	30	27	18
	Female	4	5	6	6	23	21	12	14
	All	**9**	**16**	**17**	**17**	**40**	**51**	**39**	**32**
2016	Male	14	16	18	18	25	35	48	28
	Female	6	8	11	14	32	50	19	11
	All	**20**	**24**	**29**	**32**	**57**	**85**	**67**	**39**
2017	Male	13	14	22	36	43	81	52	50
	Female	7	14	13	16	27	52	25	19
	Unknown						1		
	All	**20**	**28**	**35**	**52**	**70**	**134**	**77**	**69**
2018	Male	25	16	34	30	57	74	68	66
	Female	15	11	15	27	42	48	28	25
	Unknown						1		
	All	**40**	**27**	**49**	**57**	**99**	**123**	**96**	**91**
2019	Male	16	19	23	26	39	58	47	43
	Female	4	6	14	15	29	48	37	20
	All	**20**	**25**	**37**	**41**	**68**	**106**	**84**	**63**
2020	Male	28	31	38	41	50	102	76	75
	Female	13	20	18	28	33	80	51	28
	Unknown							1	
	All	**41**	**51**	**56**	**69**	**83**	**182**	**128**	**103**
2021	Male	16	21	19	30	31	59	48	38
	Female	6	3	10	19	17	49	24	27
	Unknown			1					
	All	**22**	**24**	**30**	**49**	**48**	**108**	**72**	**63**
2022	Male	20	19	29	31	47	65	73	44
	Female	12	15	24	29	22	58	38	29
	All	**32**	**34**	**53**	**60**	**69**	**123**	**111**	**73**
2023	Male	10	16	24	31	32	62	64	23
	Female	5	9	8	26	25	47	37	18
	All	**15**	**25**	**32**	**57**	**57**	**109**	**101**	**41**

Contact: gerharddobler@bundeswehr.org

Citation:
Dobler G. TBE in Germany. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-13-7

References

1. Sinnecker H. Zeckenenzephalitis in Deutschland. *Zentralbl Bakteriol Orig*. 1960;180:12-18.
2. Apitzsch L, Sinnecker H, Wigand R, Berndt D. Zeckenencephalitis-Virusisolierungen in der DDR 1965-66 und einige Stammdifferenzierungen [Tick-borne encephalitis virus isolation in the German Democratic Republic in 1965-66 and some strain-specific differences]. *Zentralbl Bakteriol Orig*. 1968;207(4):429-434.
3. Queisser H. Beobachtungen über verschiedene Fälle von Zeckenenzephalitis in Deutschland. *Münch Med Wochenschr*. 1962;47:2288.
4. Scheid W, Ackermann R, Bloedhorn H, Löser R, Liedtke G, Skrtic N. Untersuchungen über das Vorkommen der Zentraleuropäischen Enzephalitis in Süddeutschland. *Dtsch Med Wochenschr*. 1964;89:2313-7. doi:10.1055/s-0028-1113279
5. Ackermann R, Scheid W, Küpper B. Infektionen mit dem Virus der Zentraleuropäischen Enzephalitis in Südwest-Deutschland. *Dtsch Med Wochenschr*. 1966;91(25):1141-3. doi:10.1055/s-0028-1110717
6. Süss J, Sinnecker H, Sinnecker R, Berndt D, Zilske E, Dedek G, Apitzsch L: Epidemiology and ecology of tick-borne encephalitis in the eastern part of Germany between 1960 and 1990 and studies on the dynamics of a natural focus of tick-borne encephalitis. *Zentralbl Bakteriol*. 1992;277(2):224-35. doi:10.1016/s0934-8840(11)80617-1
7. Süss J, Beziat P, Rohr HP, Treib J, Haass A. Detection of the tick-borne encephalitis virus (TBEV) in ticks in several federal "Länder" of Germany by means of the polymerase chain reaction (PCR) – characterization of the virus. *Infection.* 1996;24:403-4. doi:10.1007/BF01716096
8. Kupča AM, Essbauer S, Zoeller G, et al. Isolation and molecular characterization of a tick- borne encephalitis virus strain from a new tick-borne encephalitis focus with severe cases in Bavaria, Germany. *Ticks Tick Borne Dis*. 2010;1(1):44-51. doi:10.1016/j.ttbdis.2009.11.002
9. Kaiser R. Tick-borne encephalitis: Clinical findings and prognosis in adults. *Wien Med Wochenschr*. 2012;162 (11-12):239-43. doi:10.1007/s10354-012-0105-0
10. Achazi K, Růžek D, Donoso-Mantke O, et al. Rodents as sentinels for the prevalence of tick-borne encephalitis virus. *Vector Borne Zoonotic Dis*. 2011;11(6):641-7. doi:10.1089/vbz.2010.0236
11. Heinz FX, Tuma W, Kunz C. Antigenic and immunogenic properties of defined physical forms of tick-borne encephalitis virus structural proteins. *Infect Immun*. 1981;33(1):250-7. doi:10.1128/iai.33.1.250-257.1981
12. Süss J, Dobler G, Zöller G, et al. Genetic characterization of a tick-borne encephalitis virus isolated from the brain of a naturally exposed monkey (Macaca sylvanus). *Int J Med Microbiol*. 2008;298(S1):295-300.
13. Dobler G, Bestehorn M, Antwerpen M, Överby-Wernstedt A. Complete genome sequence of a low-virulence tick-borne encephalitis virus strain. *Genome Announc*. 2016;4(5):e01145-16.
14. Brockmann SO, Oehme R, Buckenmaier T, et al. A cluster of two human cases of tick-borne encephalitis (TBE) transmitted by unpasteurized goat milk and cheese in Germany, May 2016. *Euro Surveill*. 2018;23(15):17-00336. doi:10.2807/1560-7917.ES.2018.23.15.17-00336
15. Bestehorn M, Weigold S, Kern WV, et al. Phylogenetics of tick-borne encephalitis virus in endemic foci in the upper Rhine region in France and Germany. *PLoS One*. 2018;13 (10):e0204790. doi:10.1371/journal.pone.0204790
16. Chitimia-Dobler L, Lemhöfer G, Krol N, Bestehorn M, Dobler G, Pfeffer M. Repeated isolation of tick-borne encephalitis virus from Dermacentor reticulatus ticks in an endemic area in Germany. *Parasit Vectors*. 2019;12(1):90. doi:10.1186/s13071-019-3346-6
17. Euringer K, Girl P, Kaier K, et al. Tick-borne encephalitis IgG antibody surveillance: vaccination- and infection-induced seroprevalences, south western Germany, 2021. *Euro Surveill*. 2023;28(12):2200408. doi:10.2807/1560-7917.ES.2023.28.12.2200408
18. Chitimia-Dobler L, Lindau A, Oehme R, et al. Tick-Borne Encephalitis Vaccination Protects from Alimentary TBE Infection: Results from an Alimentary Outbreak. *Microorganisms*. 2021;9(5):889. Published 2021 Apr 21. doi:10.3390/microorganisms9050889

Chapter 13

TBE in Hungary

Anna Nagy, Ferenc Schneider, Eszter Mezei, András Lakos

E-CDC risk status: endemic (data as of end 2023)

History and current situation

Hungarian scientists were among the pioneers in Europe as the tick-borne encephalitis virus (TBEV) was isolated in 1952, 30 years after the TBEV had been described in Russia (see chapters 3).[1] However, most of their observations were published in the Hungarian language, and therefore did not become widely distributed. Between 1981 and 1997, the average annual number of TBE cases reported to authorities was around 300, and as of that year, it decreased to fewer than 20 patients per year (Figures 1, 2). It has been speculated that the decrease is a result of underreporting of TBE, following a change in the reimbursement system for payments related to serologic TBE diagnosis.[2-4] However, two main arguments contradict the 'underreporting hypothesis':

During the 5 years before 1997, a total of 1,800,000 FSME vaccine doses were sold by pharmacies (Figure 1), and this convincingly explains the observed reduction of TBE cases. Furthermore, after 1997, lethal TBE cases decreased in parallel with decreased incidence. If lower incidences had resulted from underreporting, then lethal cases would not have changed since the etiology of a lethal case is regularly determined by mandatory autopsy and other diagnostic tests.

The incidence data from the Hungarian military are similar to that of the civilian population: no case has been reported since 2003. 'Underreporting'[5] in this context would be practically impossible. The reporting system for TBE has not changed, and a reduction of cases (most probably due to vaccination) sufficiently explains why the use of TBE serology was subsequently reduced.

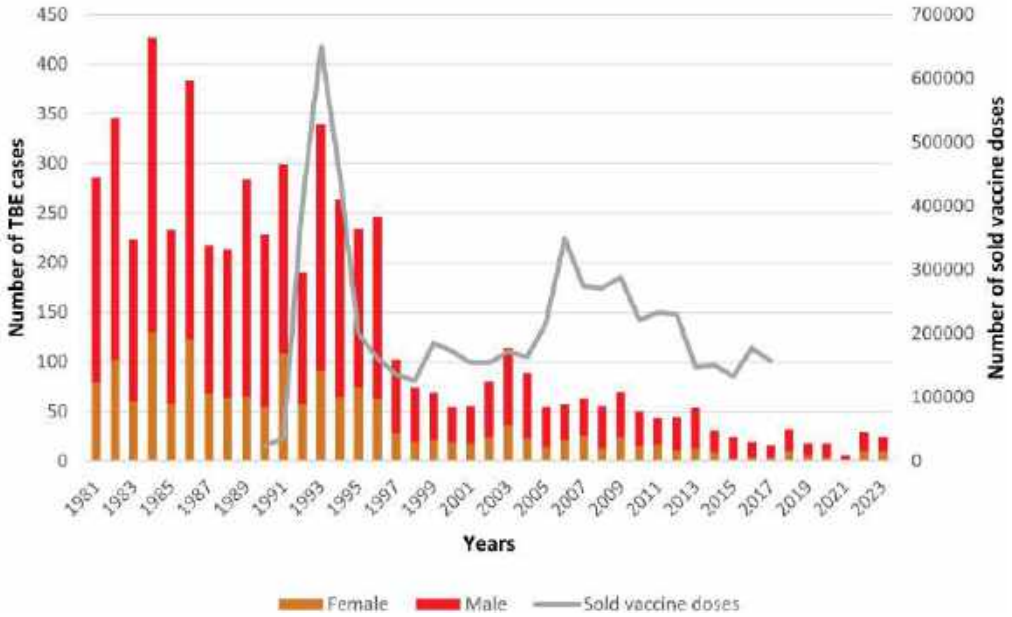
Overview of TBE in Hungary

Table 1: Virus, vector, transmission of TBE in Hungary

Viral subtypes, distribution	TBEV-EU[6]
Reservoir animals	*Apodemus agrarius, Apodemus flavicollis, Microtus arvalis, Myodes glareolus*[6] *Apodemus flavicollis, Apodemus agrarius, Myodes glareolus, Microtus subterraneus*[7]
Infected tick (Figure 3)	2/2485 = 0.08%[1] 6/8310 ≈ 0.07%[8] 40/51,746 ≈ 0.08%; the highest figure was 22/6738 ≈ 0.3% in this study[9] 1/17,500 ≈ 0.006%[10] 5/2196 ≈ 0.23%, only with PCR[11] 3/9616 ≈ 0.03%[7]
Dairy product transmission	Out of the 81 food-borne TBE cases registered between 1992 and 2011, 55.1% were male. Also, 4.4% of the total number of TBE cases were milk-borne. On average, 24.5% of people who drank infected goat milk suffered from clinical symptoms of neurologic infection. Historically, only 2 TBE epidemics in Hungary were caused by cow milk.[12] The largest epidemic came from a single goat (of the 75 tested animals) with 25 cases amongst 154 subjects who had consumed contaminated milk.[13] In that year (2007), almost half of the total number (30/63) of registered TBE cases were of alimentary origin.

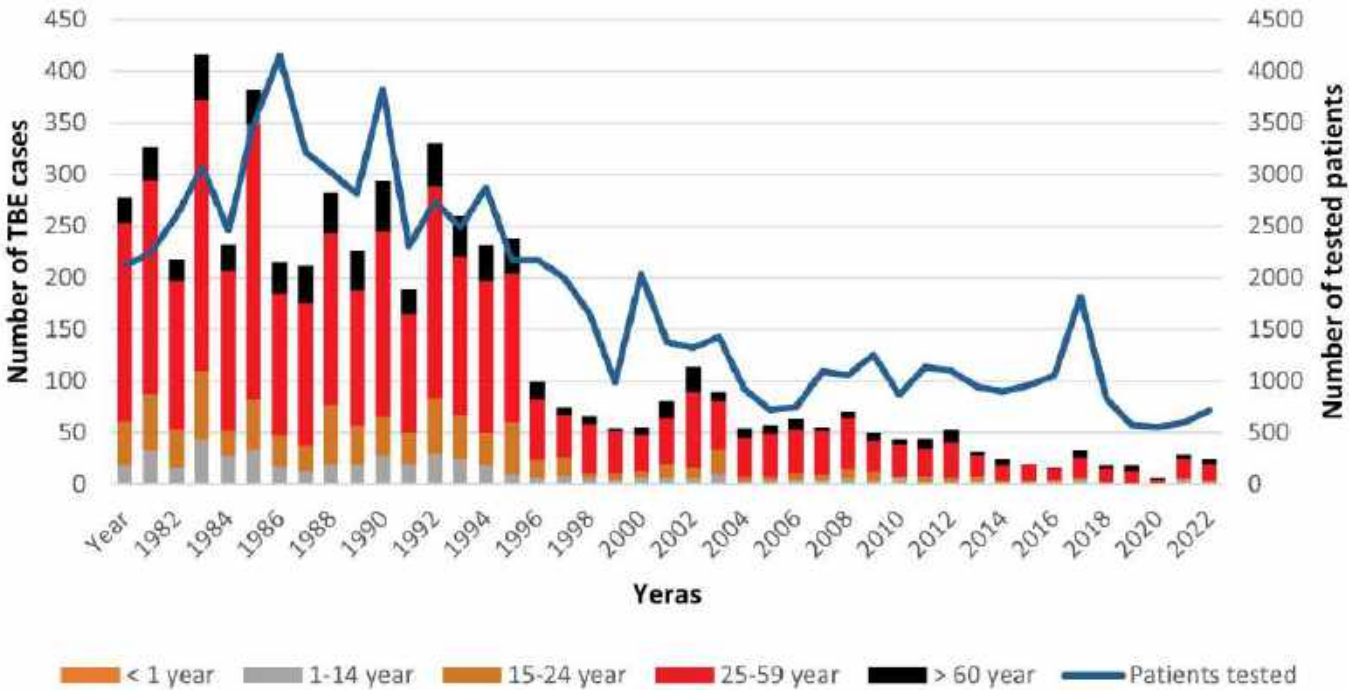
Table 2: TBE reporting and vaccine prevention in Hungary	
Mandatory TBE reporting	Every physician who establishes a diagnosis of TBE must report it. Practically, these are hospital-based specialists for infectious diseases, pediatricians, internists, and neurologists. Case definition: clinical symptoms of central nervous infection + presence of TBE immunoglobulin M (IgM) antibodies in serum and cerebrospinal fluid (CSF) OR TBEV-specific IgM in CSF OR isolation of infectious virus from clinical samples OR detection of TBEV RNA in clinical samples OR seroconversion and/or 4-fold specific IgG increase in a sample pair.[14]
Other TBE surveillance	No
Special clinical features	• In one study, 21% of retrospectively collected patient cases were agrarian, 16% forestry workers.[8] • Other work has shown 12% to 16% of patients with TBE were forestry workers.[9,10] • Similarly, another report found 10.4% of 5196 cases were forestry, 11% other agrarian workers.[15] • Also, 2% of the 1,670 forestry workers screened for Lyme borreliosis went through TBE (Lakos, unpublished data). • 65% of hospitalized patients could recall a biphasic course of their TBE.[16]
	In the same department of the Central Hospital for Infectious Diseases, during the years 1976–1980 (n=100), 27 patients showed paresis, 2 died. In 1987–1991 (n=93), only 5 patients had paresis, none of them died.[17] From 1985 to 2008, the death rate from TBE in Hungary was 29/3987 (0.73%).[18] However, in an earlier period from 1977 to 1996, the fatality rate was higher – 43/5196 (0.83%). Most of the fatal cases were male (85%), while the proportion of male patients in the total TBE population was 70%.[15]
Available vaccines	FSME IMMUN Inject vaccine has been available for public use since 1992; another vaccine, Encepur, was launched in 1995. Previously, between 1977 and 1990, some 150,000 doses were distributed for the at-risk population. (Note: during 1979 to 1983, the FSME IMMUN Inject vaccine was considered to be ineffective both clinically and serologically.[19] It has to be mentioned that TBE vaccination in Austria at the same time showed a field effectiveness 79.4%–100% after the second dose and 97.3%–100% after the third dose.[26]) From 1990 to 2017, 6 million doses were sold. (The Hungarian population is 10 million.)
Vaccination recommendations and reimbursement	When FSME IMMUN Inject was first available in Hungary in the early 1990s, the reimbursement rate was 95%; the pharmacy price was 59 HUF (≈20 euro cents). After a gradual decrease, the reimbursement was cancelled for the FSME IMMUN Inject and Encepur vaccines in 2008 and 2012, respectively. The present price is around 13,000 HUF (40 euros). For occupationally exposed workers, vaccination has been mandatory at the employers' expense since 1999.[20]
Vaccine uptake by age group/ risk group/general population	Not available.
Name, address/website of TBE National Reference Center	National Public Health Center, National Reference Laboratory for Viral Zoonoses, Budapest, Hungary [https://www.nnk.gov.hu/].

Figure 1: Gender distribution of TBE cases and the sold number of doses of TBE vaccines



The data of TBE cases in this graph originated from the National Reference Laboratory for Viral Zoonoses and from the Department of Epidemiological and Vaccination Surveillance of the National Public Health Center. The data for 1998 is missing, an estimation is plotted in the graph. No reliable information on the number of vaccine doses sold in 1995 could be found; estimated information was used. (The number of vaccine doses sold is not available from 2018.)

Figure 2: Burden of TBE in Hungary from 1981 to 2023.[24-25] Age distribution and the requested number of tested patients

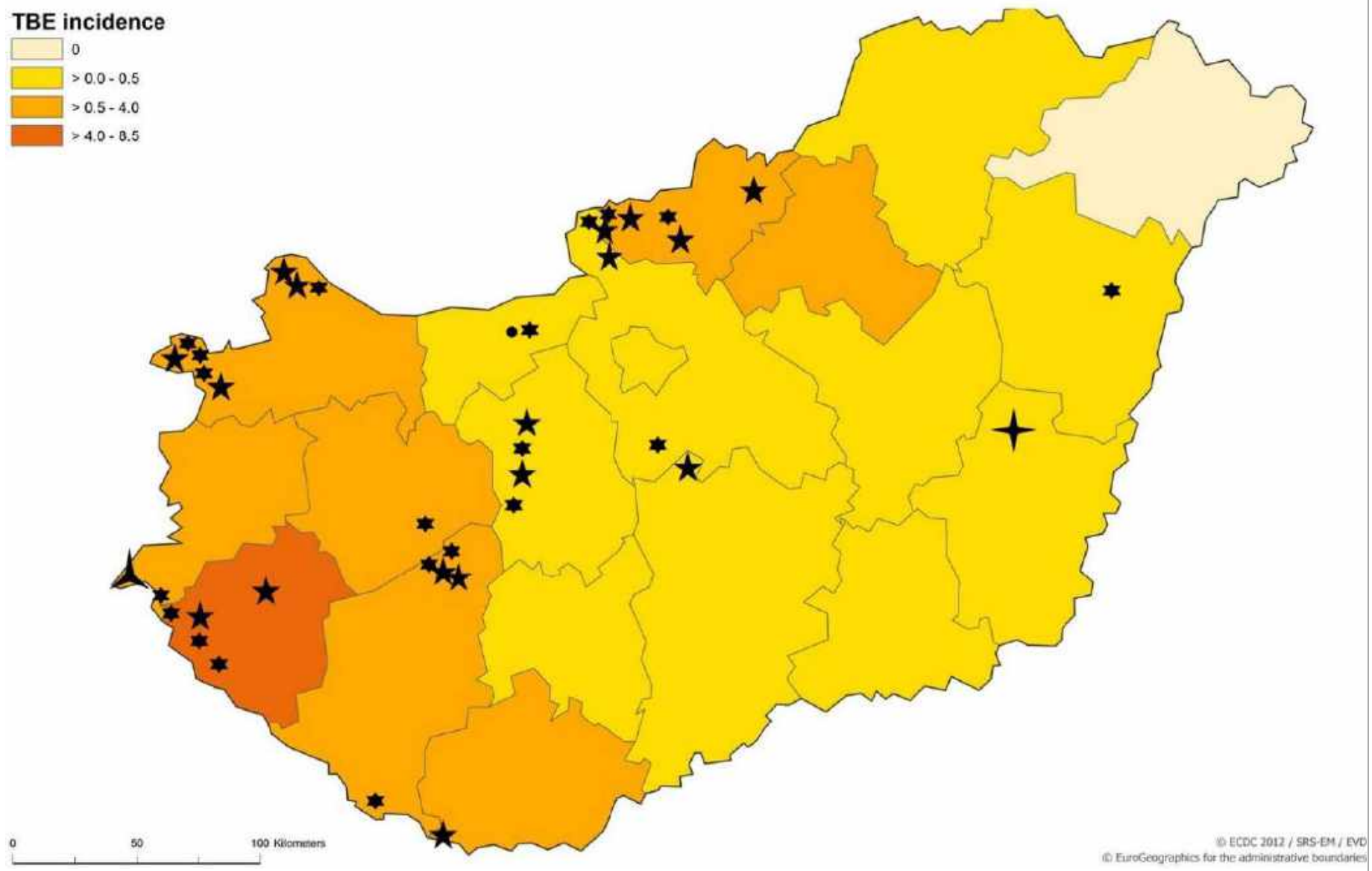


Source Data: Appendix-Figure 2

The data of TBE cases in this graph originated from the National Reference Laboratory for Viral Zoonoses and from the Department of Epidemiological and Vaccination Surveillance of the National Public Health Center.

The number of TBE cases decreased dramatically after a mass vaccination campaign from 1992 to 1995. The Hungarian population is approximately 10 million, so the incidence for 100 cases is 1/100,000. A West Nile virus epidemic resulted in 225 infections in 2018 (https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2019.24.28.1900038). That was the reason for the striking elevation of the requested TBE serological tests. The elevated number of tests coincided with the elevated number of verified TBE cases.

Figure 3: TBEV-isolation and TBE cases in Hungary



Map of Hungary showing human TBE incidence (ECDC, 2017).[22] TBEV isolation sites are marked by circles (Fornosi, 1954),[1] six-pointed stars (Molnár, 1979),[23] five-pointed stars (Gerzsenyi, 1980 and 1985),[9,10] four-pointed star (Pintér, 2013),[11] and three-pointed star (Zöldi, 2015).[7]
A map with more detailed incidence data[21] can be downloaded from http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20128.

Appendix

Source data: Figure 2

	Female	Male	<1 year	1–14 years	15–24 years	25–59 years	>60 years	Unknown age	Total TBE cases	Sold vaccine doses	Samples tested (IgG)
1981	79	207	0	18	43	192	25	8	286	N/A	2113
1982	102	244	0	32	55	207	32	20	346	N/A	2241
1983	60	163	0	16	37	144	21	5	223	N/A	2595
1984	130	297	0	43	67	262	44	11	427	N/A	3074
1985	58	175	0	28	24	155	25	1	233	N/A	2456
1986	123	260	0	33	49	267	33	1	383	N/A	3486
1987	68	149	0	17	30	138	30	2	217	N/A	4157
1988	64	149	0	13	24	139	35	2	213	N/A	3215
1989	65	219	0	19	58	166	39	2	284	N/A	3016
1990	54	174	0	19	37	132	38	2	228	23251	2809
1991	109	190	0	28	37	180	48	6	299	36,720	3823
1992	57	133	0	19	31	115	24	1	190	400,000	2301
1993	91	248	0	30	53	205	42	9	339	650,000	2737
1994	65	199	0	24	43	153	40	4	264	450,000	2488
1995	74	160	0	18	32	147	34	3	234	200,000	2875
1996	63	183	0	10	50	144	34	8	246	161,717	2168
1997	28	74	0	6	17	59	17	3	102	136,394	2168
1998	19	55	0	8	18	41	7	0	74	125,843	2000
1999	21	48	0	6	5	47	8	3	69	184,555	1649
2000	19	35	0	4	7	40	3	0	54	172,615	988
2001	18	37	0	6	7	35	7	0	55	153,941	2036
2002	24	56	0	6	13	45	16	0	80	154,165	1379
2003	36	78	0	5	11	73	25	0	114	171,151	1315
2004	23	66	0	10	23	47	9	0	89	163,347	1428
2005	14	40	0	2	5	38	9	0	54	215,238	927
2006	21	36	0	3	4	42	8	0	57	349,206	467
2007	26	37	0	4	7	42	10	0	63	274,396	750
2008	13	42	0	4	5	43	3	0	55	271,092	1636
2009	24	46	0	5	9	50	6	0	70	288,629	1527
2010	15	35	0	3	9	30	8	0	50	221,095	1154
2011	17	26	0	5	3	30	5	0	43	233,579	1003
2012	11	33	0	1	7	26	10	0	44	229,794	1095
2013	13	40	0	2	4	35	12	0	53	146,518	1099
2014	9	22	0	3	5	20	3	0	31	150,507	840
2015	3	21	0	1	2	15	6	0	24	132,878	855
2016	4	15	0	1	2	16	0	0	19	177,064	958
2017	4	12	0	1	3	11	1	0	16	157,687	1050
2018	10	22	0	4	2	19	7	0	32	N/A	1814
2019	6	12	0	0	1	14	3	0	18	N/A	830
2020	4	14	0	0	0	13	5	0	18	N/A	578
2021	2	4	0	0	1	3	2	0	6	N/A	553
2022	10	19	0	5	0	19	5	0	29	N/A	597
2023	10	14	0	1	2	16	5	0	24	N/A	719

N/A: data not available

Contact: nagy.anna@nnk.gov.hu

Citation:
Nagy A, Schneider F, Mezei E, Lakos A. TBE in Hungary. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-14-7

References

1. Fornosi F, Molnár E. [Isolation of encephalomyelitis virus from ticks. I. Isolation of the virus and its properties.] *Orv Hetil*. 1954;95:144-9.
2. Randolph SE. on behalf of the EDEN-TBD sub-project team. Human activities predominate in determining changing incidence of tick-borne encephalitis in Europe. *Euro Surveill*. 2010;15:24-31.
3. Petri E, Gniel D, Zent O. Tick-borne encephalitis (TBE) trends in epidemiology and current and future management. *Travel Med Infect Dis*. 2010;8:233-45.
4. WHO, 2004: The vector-borne human infections in Europe. Their distribution and burden on public health. www.euro.who.int/document/e82481.pdf. Accessed January 16, 2011.
5. Lakos A, Rókusz L. [Epidemiology of tick-borne encephalitis in Hungary – significance of the vaccination.] *Háziorvos Továbbképző Szemle*. 2011;16:38-40.
6. Pintér R, Madai M, Horváth G, et al. Molecular detection and phylogenetic analysis of tick-borne encephalitis virus in rodents captured in the Transdanubian region of Hungary. *Vector Borne Zoonotic Dis*. 2014;14:621-4.
7. Zöldi V, Papp T, Rigó K, Farkas J, Egyed L. A 4-year study of a natural tick-borne encephalitis virus focus in Hungary, 2010–2013. *Ecohealth*. 2015;12:174-82.
8. Kubinyi L, Molnár E, Szertich A, Pethő I. [Tick encephalitis in Zala and Győr-Sopron counties.] *Orv Hetil.* 1971;112:2931-5.
9. Gerzsenyi K. Tick-borne meningoencephalitis investigations in the Transdanubian and the Budapest regions and the prevention (in Hungarian). Forest Research Institute. 1980 p 23.
10. Gerzsenyi K. Natural foci of tick-borne encephalitis virus in Central and Eastern Hungary (in Hungarian). Forest Research Institute, 1985 p 25.
11. Pintér R, Madai M, Vadkerti E, et al. Identification of tick-borne encephalitis virus in ticks collected in Southeastern Hungary. *Ticks Tick Borne Dis*. 2013;4:427-31.
12. Zöldi V, Ferenczi E, Egyed L. [Milk-transmitted tick-borne encephalitis epidemics in Hungary.] *Magyar Állatorvosok Lapja*. 2013;135:48-56.
13. Balogh Z, Ferenczi E, Szeles K, et al. Tick-borne encephalitis outbreak in Hungary due to consumption of raw goat milk. *J Virol Methods*. 2010;163:481-5.
14. Kollaritsch H, Chmelík V, Dontsenko I, et al. The current perspective on tick-borne encephalitis awareness and prevention in six Central and Eastern European countries: report from a meeting of experts convened to discuss TBE in their region. *Vaccine*. 2011;29:4556-64.
15. Lontai I, Straub I. Tick-borne encephalitis and its prevention in Hungary. *Med Pregl*. 1998;51 Suppl 1:21-3.
16. Schneider F, Pergel R. [Tick-borne encephalitis in Vas county between 1988-1998.] *Vasi Szemle*. 2000;54:827-38.
17. Lakos A, Ferenczi E, Ferencz A, Tóth E. Tick-borne encephalitis. *Parasit Hung*. 1997;30:5-16.
18. Zöldi V, Erdős Gy, Szlobodnyik J. 2nd Guideline on tick control and prevention (in Hungarian). *EPINFO*. 2009;16:Suppl 3 p 61.
19. Ferenczi E, Molnár E. Tick-borne encephalitis in the last ten years. *Ellipse*. 1991;29:458-9.
20. Lakos A. [Tick-borne encephalitis, benefit of the vaccine.] *Háziorvosi Szemle*. 2012;17:36-7.
21. Caini S, Szomor K, Ferenczi E, et al. Tick-borne encephalitis transmitted by unpasteurised cow milk in western Hungary, September to October 2011. *Euro Surveill.* 2012;17. pii: 20128. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20128. Accessed January 8, 2017.
22. ECDC. Epidemiological situation of tick-borne encephalitis in the European Union and European Free Trade Association countries. http://ecdc.europa.eu/en/publications/Publications/TBE-in-EU-EFTA.pdf. Accessed January 5, 2017.
23. Molnár E. Location of the tick-borne encephalitis and other arboviruses and their burden in Hungary. DSc thesis.1979, p 193. Cited in: Zöldi V, Erdős Gy, Szlobodnyik J. 2nd Guideline on tick control and prevention (in Hungarian). *EPINFO*. 2009;16:Suppl 3 p 61.
24. OEK (National Centre for Epidemiology), 2016 data on file.
25. IMSHealth Hungary, 2010. Data on file
26. Kunz, C. Tick-borne encephalitis in Europe. *Acta Leiden.* 1992;60(2):1-14

Chapter 13

TBE in Italy

Valentina Tagliapietra, Martina Del Manso, Flavia Riccardo and Anna Teresa Palamara

E-CDC risk status: endemic (last edited: date 19/03/2024, data from 2023)

History and current situation

Italy is considered a low-incidence country for tick-borne encephalitis (TBE) in Europe[1]. Areas at higher risk for TBE within Italy are geographically clustered in the forested and mountainous regions and provinces of the northeastern part of the country, as suggested by TBE case series published over the last decade[2–4]. A national enhanced surveillance system for TBE has been established since 2017[5]. Before this, information on the occurrence of TBE cases at the national level in Italy was lacking. Both incidence rates and the geographical distribution of the disease were mostly inferred from endemic areas where surveillance was already in place, and from ad hoc studies and international literature. TBE has been recorded in Italy since 1967, with foci of infections in the northeast (Trento, Belluno and Gorizia) and central (Florence and Latina) provinces[6–9]. TBE presence in central Italy has not been confirmed by further studies on ticks and serosurveys conducted afterwards[10,11], nor by human cases, posing concerns about possible misdiagnosis.

Serological investigations of people at risk, such as forestry rangers, hunters, hikers and forest products collectors, have been performed in order to get information on human exposure to TBE virus (TBEV). Circulation in the pre-alpine and alpine regions reported partially NT-confirmed seroprevalence values of 0.6%, 1.07% and 3.2% in Friuli-Venezia Giulia[12], Trento province[13] and Turin province[14], respectively. Interestingly, Turin province has never reported TBE human cases, so far. A retrospective study conducted in 2015 in the northeast regions using the ECDC case definition of TBE[3], allowed the identification of 367 cases (0.38 per 100,000 inhabitants) during the period from 2000 to 2013[3]. TBE cases were mainly males (70%) and the majority of them were between 30 and 70 years of age (see also Figure 2). A significant increase in the annual incidence rate (IR) was observed during the study period, from 0.18 per 100,000 in the year 2000 up to 0.59 per 100,000 in 2013 (95% confidence interval [CI]: 1.02–1.08, P>0.01)[3]. The majority of TBE cases occurred between April and October, consistent with the seasonal activity of ticks. According to this study, the risk of TBE is associated with altitude, with the highest values found for municipalities between 400 and 600 m a.s.l., and the IR falling along with municipality altitude decrease or increase[3]. In 2022, TBE showed a record in the number of cases and mortality rates, with 72 cases, mainly from four northeastern Italian regions and provinces[15]: Trento (18 cases), Friuli-Venezia Giulia (12 cases) and Veneto (37 cases), and sporadically from other locations i.e. Emilia Romagna (2 cases), Liguria (2 cases) and Lazio (1 case) (Fig. 3) and 3 fatal events, resulting in an exceptionally high mortality rate of 4.17%.

In its natural enzootic cycle, TBEV transmission involves ixodid ticks, mainly belonging to the genus *Ixodes*, and the small mammal hosts (rodents and insectivores) which support both ticks population and TBEV circulation. The link between tree masting, rodent population dynamics, density of nymphal ticks and eventually the incidence of tick-borne diseases in humans, has been investigated in several studies highlighting the expected two-year lag between a masting event and the increase in (infected) nymphs[16,17]. In this context, a long-term study conducted in the Province of Trento positively correlated pollen data and TBE incidence in humans[18], therefore offering to public health agencies a potential early warning tool that might be used to plan preventive measures two years in advance. Of note is the fact that a huge mast event involving two important forest species (*Fagus sylvatica* and *Picea abies*) was recorded in 2020 and that the peak in the number of TBE cases happened in 2022.

In particular, the province of Trento showed a sharp increase in TBE incidence since 2012, despite vaccination efforts. To assess the current risk of infection in the provincial territory, an integrated One-Health research approach was applied, combining the analysis of the distribution of human cases, the study of seroprevalence in sentinel hosts (goats) and the direct screening of questing ticks[19]. A total of 1.56% of goats resulted positive for specific antibodies for TBEV. Sampling of ticks was concentrated in areas where TBEV circulation was observed both in seropositive goats or in humans, resulting in a prevalence of 0.17%. In particular these results revealed an increased prevalence of TBEV in ticks and the emergence of new active TBE foci which are located northward and at higher altitude (1.109 m a.s.l.) compared to previous investigations. None of the areas with seropositive goats was confirmed by TBEV detection in ticks and recent human

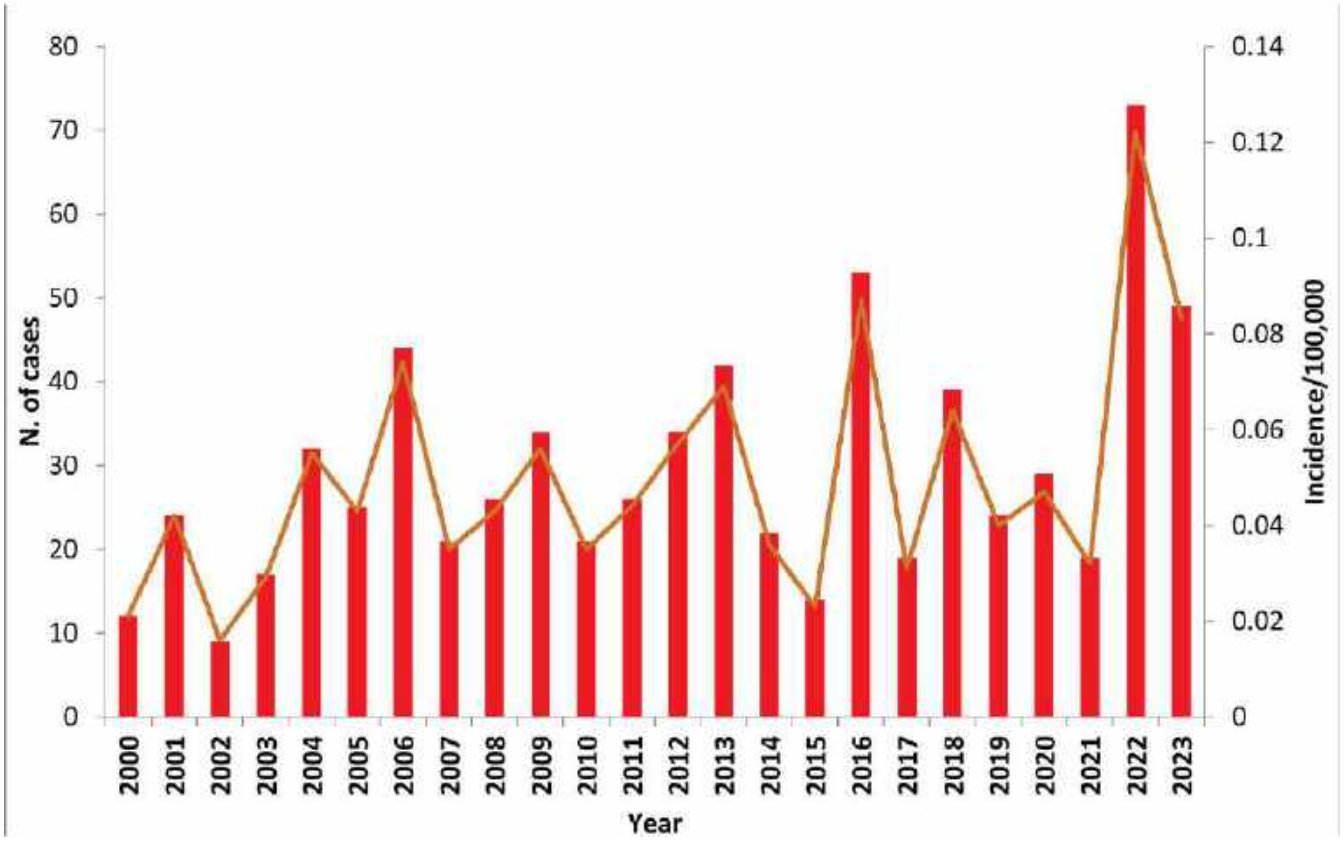
cases, but this aspect needs further confirmation.

Since the 1990s, rising cervid population numbers and changes in forest structure in the northeastern regions and provinces of Italy were observed in conjunction with an increase in TBE incidence[20], but this relationship is not always positive and at a threshold density level of ungulates, TBEV prevalence decreases[21]. Transmission of TBEV from infected nymphs to co-feeding uninfected ticks on rodents is considered the most efficient route for the amplification of this virus, therefore, studies regarding the ecological and abiotic conditions affecting tick feeding dynamics are important. Recently a long-term longitudinal field study highlighted that the autumnal cooling rate and the presence of roe deer and mice are crucial ecological drivers for co-feeding transmission which in turn is reflected in the maintenance of a TBEV hotspot[22]. The animal community composition and abundance are known to affect transmission of tick-borne diseases, suggesting that in highly diverse habitats TBE risk decreases. Using habitat richness as a proxy for vertebrate host diversity, high TBE risk corresponded to areas with intermediate richness. In endemic areas, such as those located in northeast Italy, TBE risk is higher probably because it features habitat types that are generally suitable for both ticks and hosts presence[23].

Vaccination for TBE is currently recommended in Italy among residents and occupationally exposed groups, living in rural endemic areas, but its impact on disease occurrence in the affected communities is not yet evaluated[24]. In the Friuli-Venezia Giulia region since 2013 and in the Autonomous Provinces of Trento and Bolzano since 2018, TBE vaccine is offered free of charge to the resident population.

In conclusion, the incidence of TBE in Italy is relatively low and the risk appears to be geographically restricted to the pre-alpine and alpine regions of the country. However, recent increase and spread in the number of cases (see Figure 3), pose concerns regarding the importance of disentangling the complex factors that are involved in the spread and maintenance of TBEV in an endemic focus and the early-warning predictors that should be identified.

Figure 1: Reported human cases and incidence of TBE, Italy, 2000-2023. Data on vaccine uptake not available.

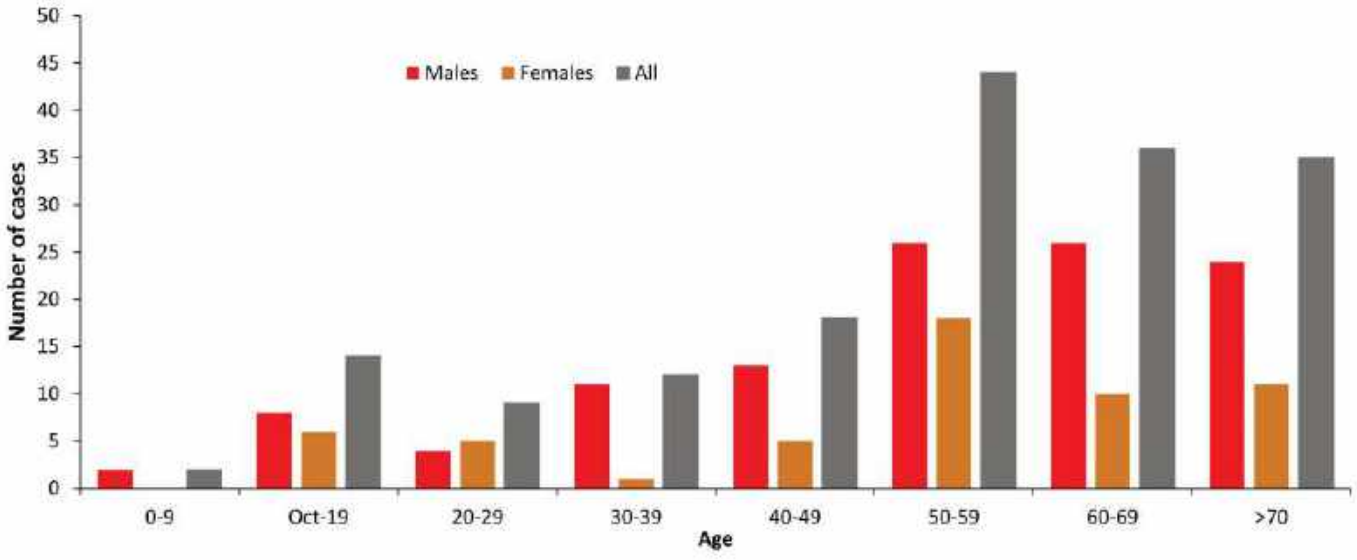


Data on vaccination rate : Appendix—Figure 1

Overview of TBE in Italy

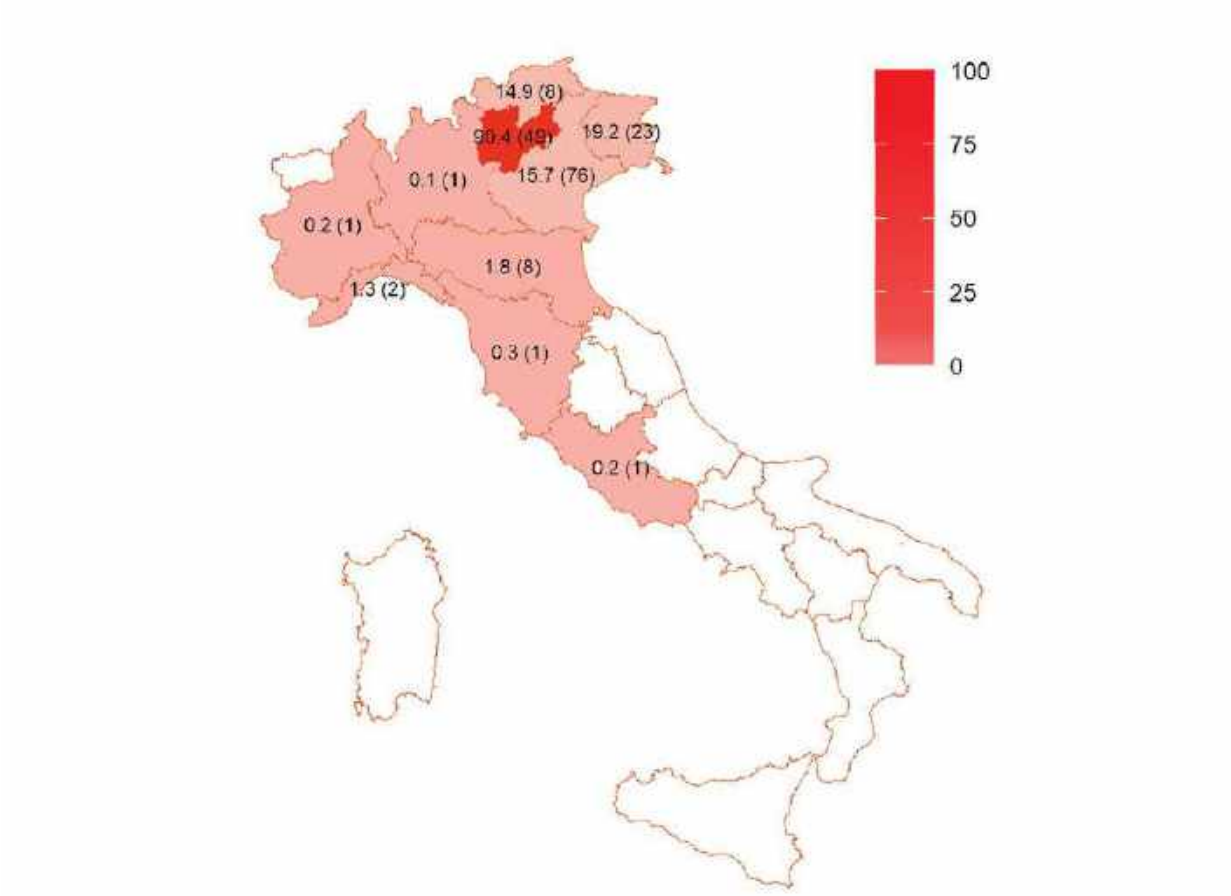
Table 1: TBE in Italy	
Viral subtypes, distribution	European TBEV subtype[19]
Reservoir animals	Rodents, ticks
Percentage infected ticks	0.17% (Trento Province,[19]); 2.1% (Belluno province,[25]).
Dairy product transmission	N/A
Case definition used by authorities	Case definition: Clinical criteria are any symptoms of inflammation of the CNS (for example, meningitis, meningo-encephalitis, encephalomyelitis, encephaloradiculitis). A TBE case is confirmed by at least one of the following five laboratory criteria: TBE specific IgM AND IgG antibodies in blood; TBE specific IgM antibodies in CSF; seroconversion or four-fold increase of TBE-specific antibodies in paired serum samples; detection of TBE viral nucleic acid in a clinical specimen; isolation of TBE virus from clinical specimen.
Type of reporting	Reported by Department of Infectious Diseases, National Institute of Health, Italy in collaboration with all the Infectious Diseases Units and Public Health Districts. Surveillance has been enhanced at the national level since 2017 and web-based from 2020. Presumed place of exposure and date of tick bite are recorded.
Other TBE surveillance	Ticks, rodents and sentinel animals screening.
Special clinical features	Bi-phasic disease is not reported.
Licensed vaccines	TICOVAC 0.5 mL and 0.25 mL (for pediatric use) (Pfizer Srl).
Vaccine recommendations	Vaccine is free of charge for residents in the Friuli-Venezia Giulia and Trentino-Alto Adige regions.
Vaccine uptake	Recommended for those who live, frequent or work in the woods or in rural areas i.e. hikers/trekkers, foragers, agricultural, forest or lumber workers.
National Reference center for TBE	Prof.ssa Anna Teresa Palamara Dipartimento Malattie Infettive Istituto Superiore di Sanità Viale Regina Elena, 299 00161 Roma, Italia https://www.iss.it

Figure 2: Age and gender distribution of reported human cases of neuro-invasive laboratory confirmed TBEV infections, Italy, 2020-2023.



Source Data: Appendix—Figure 2

Figure 3: Distribution (4-year incidence/100,000 and number of cases in 4 years (2020-2023)) of neuro-invasive laboratory confirmed TBE per region/autonomous province (incidence based on each region / province population size) of Italy



Acknowledgments

The authors thank Elisa Di Maggio for her support in data collection and analysis.

Authors and affiliations

Valentina Tagliapietra
Fondazione Edmund Mach, Centro Ricerca e Innovazione, 38010 San Michele all'Adige (TN), Italia

Martina Del Manso, Flavia Riccardo, Anna Teresa Palamara
Istituto Superiore di Sanità, 00161 Rome, Italy

Contact: valentina.tagliapietra@fmach.it

Citation:

Tagliapietra V, Riccardo F, Del Manso M, Palamara A.T. TBE in Italy. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book.* 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-15-7

Appendix

Source data: Figure 1

Year	Number of cases	Incidence/ 10^5	Vaccination rate (%)
2000	12	0.021	
2001	24	0.042	
2002	9	0.016	
2003	17	0.029	
2004	32	0.055	
2005	25	0.043	
2006	44	0.074	0.11
2007	21	0.035	0.11
2008	26	0.043	0.11
2009	34	0.056	0.14
2010	21	0.035	0.13
2011	26	0.044	0.16
2012	34	0.057	0.10
2013	42	0.069	0.18
2014	22	0.036	0.15
2015	14	0.023	
2016	53	0.087	
2017*	24	0.04	
2018*	39	0.065	
2019*	24	0.040	
2020*	21	0.047	
2021*	18	0.032	
2022*	73	0.122	
2023*	49	0.083	

* Neuroinvasive laboratory confirmed TBEV infections

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	2	0	2
10-19	8	6	14
20-29	4	5	9
30-39	11	1	12
40-49	13	5	18
50-59	26	18	44
60-69	26	10	36
>70	24	11	35

References

1. Dagostin F, Tagliapietra V, Marini G, et al. Ecological and environmental factors affecting the risk of tick-borne encephalitis in Europe, 2017 to 2021. *Euro Surveill*. 2023;28 (42). doi:10.2807/1560-7917.ES.2023.28.42.2300121
2. Beltrame A, Ruscio M, Cruciatti B, et al. Tickborne encephalitis virus, northeastern Italy. *Emerg Infect Dis*. 2006;12(10):1617-9. doi:10.3201/eid1210.060395
3. Rezza G, Farchi F, Pezzotti P, et al. Tick-borne encephalitis in north-east Italy: a 14-year retrospective study, January 2000 to December 2013. *Euro Surveill*. 2015;20(40):1560-7917. doi: 10.2807/1560-7917.ES.2015.20.40.30034
4. Regione Autonoma Friuli Venezia Giulia. L'encefalite da zecca (TBE) in Friuli Venezia Giulia. Accessed March 1, 2024. https://www.regione.fvg.it/rafvg/export/sites/default/RAFVG/salute-sociale/zecche/allegati/TBE_FVG.pdf.
5. Ministero della salute. Piano nazionale di sorveglianza e risposta all'encefalite virale da zecche e altre arbovirosi. Accessed March 1, 2024. https://www.salute.gov.it/portale/news/p3_2_1_1_1.jsp?lingua=italiano&menu=notizie&p=dalministero&id=3399.
6. Amaducci L, Arnetoli G, Inzitari D, Balducci M, Verani P, Lopes M. Tick-borne encephalitis (TBE) in Italy: report of the first clinical case. *Riv Patol Nerv Ment*. 1976;2(97):77-80.
7. Paci P, Leoncini F, Mazzotta F, et al. Meningoencefaliti da zecche (TBE) in Italia. *Ann Sclavo*. 1980;22(3):404-16.
8. Ciufolini M, Verani P, Nicoletti L, et al. Recent advances in the eco-epidemiology of tick borne encephalitis in Italy. *Alpe Adria Microbiology Journal*. 1999;8:81-83.
9. Verani P, Ciufolini MG, Nicoletti L. Arbovirus surveillance in Italy. *Parassitologia*. 1995;37:2-3.
10. Tomao P, Ciceroni L, D'Ovidio MC, et al. Prevalence and incidence of antibodies to Borrelia burgdorferi and to tick-borne encephalitis virus in agricultural and forestry workers from Tuscany, Italy. *Eur J Clin Microbiol Infect Dis*. 2005;24 (7):457-463. doi:10.1007/s10096-005-1348-0
11. Di Renzi S, Martini A, Binazzi A, et al. Risk of acquiring tick-borne infections in forestry workers from Lazio, Italy. *Eur J Clin Microbiol Infect Dis*. 2010;29(12):1579-1581. doi:10.1007/s10096-010-1028-6
12. Cinco M, Barbone F, Ciufolini MG, et al. Seroprevalence of tick-borne infections in forestry rangers from northeastern Italy. *Clin Microbiol Infect*. 2004;10(12):1056-1061. doi:10.1111/j.1469-0691.2004.01026.x
13. Cristofolini A, Bassetti D, Schallenberg G. Zoonoses transmitted by ticks in forest workers (tick-borne encephalitis and Lyme borreliosis): preliminary results. *Med Lav*. 1993;84(5):394-402.
14. Tomassone L, Berriatua E, De Sousa R, et al. Neglected vector-borne zoonoses in Europe: Into the wild. *Vet Parasitol*. 2018;15(251):17-26. doi:10.1016/j.vetpar.2017.12.018
15. Del MM, Di ME, Perego G, et al. Arbovirosi in Italia-2022. Istituto Superiore di Sanità. Accessed Mar 1, 2024. https://www.epicentro.iss.it/arbovirosi/dashboard
16. Brugger K, Walter M, Chitimia-Dobler L, Dobler G, Rubel F. Forecasting next season's Ixodes ricinus nymphal density: the example of southern Germany 2018. *Exp Appl Acarol*. 2018;75:281-288. doi:10.1007/s10493-018-0267-6
17. Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing F. Climate, deer, rodents and acorns as determinants of variation in lyme-disease risk. *PLoS Biol*. 2006;4(6):e145. doi:10.1371/journal.pbio.0040145
18. Marini G, Tagliapietra V, Cristofolini F, et al. Correlation between airborne pollen data and the risk of tick-borne encephalitis in northern Italy. *Sci Rep*. 2023;13(1):1-8. Published 2023 May 22. doi:10.1038/s41598-023-35478-w
19. Alfano N, Tagliapietra V, Rosso F, Ziegler U, Rizzoli A. Tick-borne encephalitis foci in northeast Italy revealed by combined virus detection in ticks, serosurvey on goats and human cases. *Emerg Microbes Infect*. 2020;9(1):474-84. doi:10.1080/22221751.2020.1730246
20. Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosà R. Forest structure and roe deer abundance predict Tick-borne encephalitis risk in Italy. *PLoS One*. 2009;4(2). doi:10.1371/journal.pone.0004336
21. Cagnacci F, Bolzoni L, Rosà R, et al. Effects of deer density on tick infestation of rodents and the hazard of tick-borne encephalitis. I: Empirical assessment. *Int J Parasitol*. 2012;42 (4). doi:10.1016/j.ijpara.2012.02.012
22. Rosà R, Tagliapietra V, Manica M, et al. Changes in host densities and co-feeding pattern efficiently predict tick-borne encephalitis hazard in an endemic focus in northern Italy. *Int J Parasitol*. 2019;49(10):779-787. doi:10.1016/j.ijpara.2019.05.006
23. Dagostin F, Tagliapietra V, Marini G, et al. High habitat richness reduces the risk of tick-borne encephalitis in Europe: A multi-scale study. *One Heal.* 2023;18:100669. Published 2023 Dec 30. doi:10.1016/j.onehlt.2023.100669
24. Piano Nazionale di Prevenzione Vaccinale 2017-2019. Accessed March 1, 2019. https://www.salute.gov.it/imgs/C_17_pubblicazioni_2571_allegato.pdf.
25. Capelli G, Ravagnan S, Montarsi F, et al. Occurrence and identification of risk areas of Ixodes ricinus-borne pathogens: a cost-effectiveness analysis in north-eastern Italy. *Parasit Vectors*. 2012;5(1):61. Published 2012 Mar 27. doi:10.1186/1756-3305-5-61

Chapter 13

TBE in Japan

Kentaro Yoshii

E-CDC risk status: affected, possibly endemic

(this information will also go to the TBE global map down to county level, last edited: date 02.02.2024, data from 2023)

History and current situation

In Japan the Japanese encephalitis virus (JEV), one of mosquito-borne flaviviruses, has been widely endemic on the main and on the southern islands with more than 1,000 Japanese encephalitis (JE) cases reported annually in the late 1960s.[1] In contrast, until 1993, no TBE case had ever been reported and it was considered that there was no endemic focus of TBEV.

In 1993, a case of viral encephalitis in Hokuto city, in the southern part of Hokkaido, was diagnosed as TBE.[2] The patient had suffered from fever, headache, and neurological symptoms such as seizures. Hemagglutination inhibition (HI) test against JEV showed significant increase in HI antibodies. However, 2-mercaptoethanol-sensitive HI antibodies were not detected, and it was unlikely that JEV infection occurred in Hokkaido, where JEV was not endemic. Furthermore, blood-sucking vector mosquitoes were not active at the end of autumn in the area. Further serological analysis was conducted against other flaviviruses. IgM-ELISA and neutralization tests revealed very low antibody titer against JEV while high titers of antibodies were detected by neutralization test against TBEV.

Because the patient was a dairy farmer with no history of overseas travel, it was concluded that she had been infected with TBEV by a tick in her living area in Hokkaido. Epizootiological surveys were conducted in Hokkaido, antibodies against TBEV were detected in dogs, horses, racoons, deer and wild rodents in the wide areas of Hokkaido.[3-12] TBEV was isolated from dogs, wild rodents and from *Ixodes ovatus* ticks, which are the predominant ticks in the area. Sequence and phylogenetic analysis classified the TBEV isolates as Far-Eastern subtype. Besides, antibodies against TBEV were detected in deer and wild rodents in the Tochigi, the Shimane and the Nagasaki prefectures, and antibodies against the TBEV-serocomplex were also detected in wild boars in wide areas of Japan (the Yamaguchi, Wakayama, Hyogo, Oita, Gifu, Toyama and Chiba prefecture), indicating wide distribution of TBEV all over Japan.[4,11,13,14]

Ever since the first confirmed TBE case in 1993, only four additional cases of TBE were reported from Japan, the last one in 2018, although endemic foci of TBEV were detected in various parts of the country, not only in Hokkaido. It is possible that TBE patients are missed in Japan. One major problem is the low awareness for the disease in Japan, even among physicians. Another problem is that commercial tests for diagnostic confirmation of TBEV-infections are not available due to low awareness and due to the restrictions to handle TBEV in high biosafety level laboratories (BSL 3) only. In Japan, no TBE vaccine is licensed, and it is an urgent medical need to conduct a serological survey among residents in TBEV-endemic areas and to establish preventive measures for residents as well as for travelers to Europe and Russia.

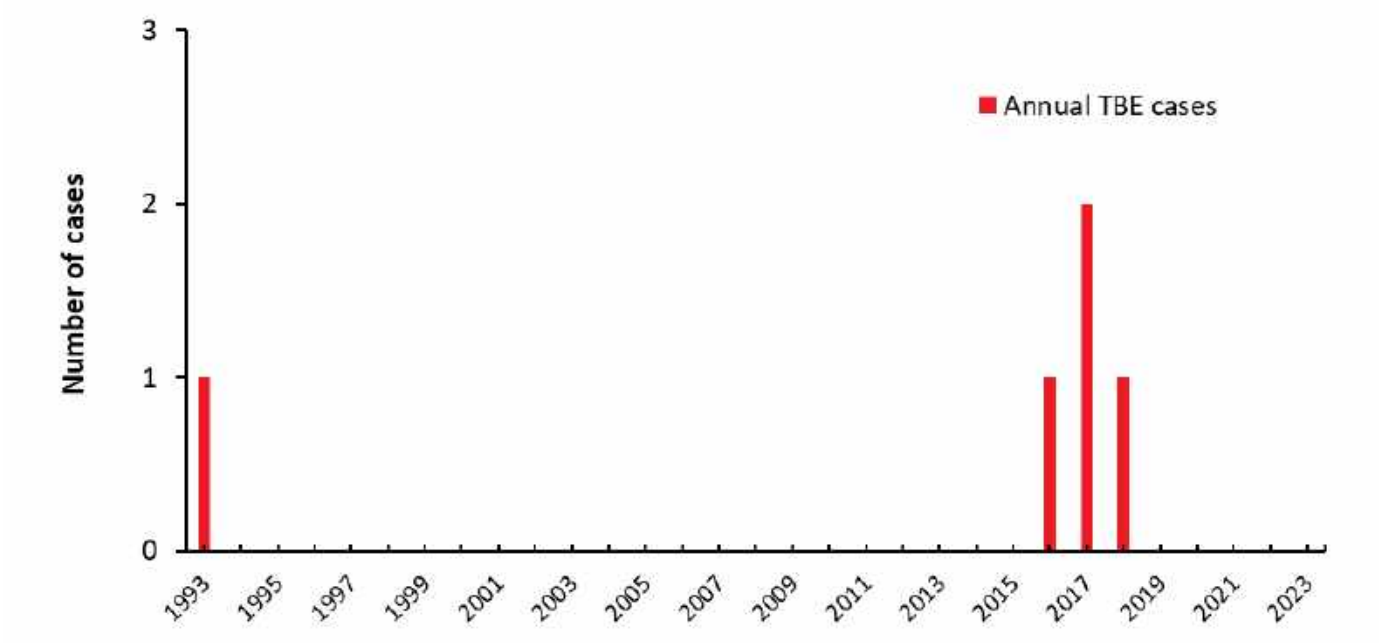
Table 1: TBE in Japan	
Viral subtypes isolated	Far-Eastern subtype[5-9,12]
Reservoir animals	Wild rodents[5,9,11]
Percentage infected ticks	*I. ovatus* (0.05%–0.33%)[7,8]
Dairy product transmission	Not reported
Case definition used by authorities	Isolation of TBEV or detection of TBEV genomic ribonucleic acid by RT-PCR from blood or cerebrospinal fluid; detection of IgM antibodies against TBEV from blood or cerebrospinal fluid; detection of significant increase in neutralizing antibodies against TBEV in paired serum. Based on the Infectious Diseases Control Law
Completeness of case detection and reporting	Unknown
Type of reporting	Mandatory
Other TBE surveillance	Detection in ticks, wild and companion animals[3-13]
Special clinical features	Encephalitis and meningitis with typical neurological symptoms.[6,15-17]
Licensed vaccines	No licensed vaccine
Vaccination recommendations	No
Vaccine uptake	No
National Reference center for TBE	NATIONAL INSTITUTE OF INFECTIOUS DISEASE, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan, info@nih.go.jp

Overview of TBE in Japan

Only five confirmed cases of TBE have been reported from Japan to date. The first patient was a 37-year-old female in 1993,[2,6] and the second patient was a male person in his 40s (2016).[15] The third and fourth patients were male in their 70s (2017).[16] The fifth patient was a female in her 40s (2018).[17]

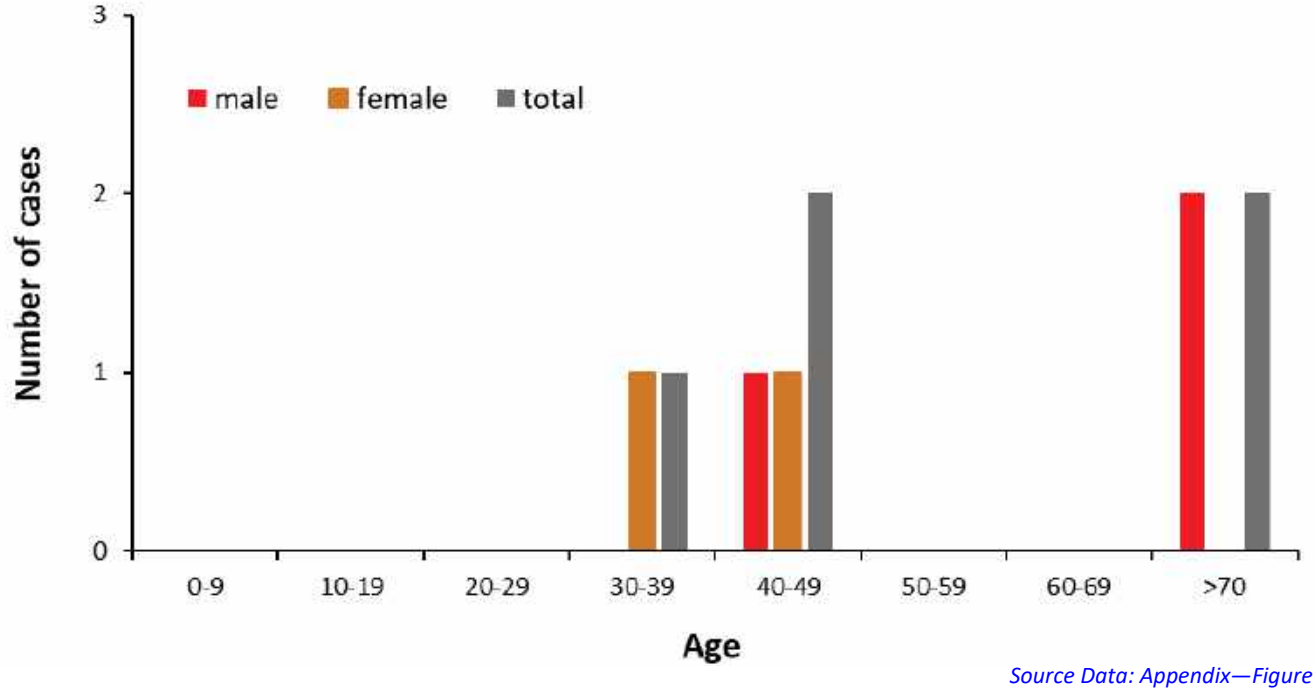
Retrospective survey revealed infection with TBEV in one Lyme disease-suspected patient with meningoencephalitis[18], seven patients with neurological disorders[19] and two asymptomatic cases in Japan Self-Defense Forces members in Hokkaido.[20] Other surveys also revealed infection with TBEV in three patients hospitalized with encephalitis or meningitis of unknown etiology outside Hokkaido.[21]

Figure 1: Reported TBE cases in Japan 1993–2023[6,15-17]



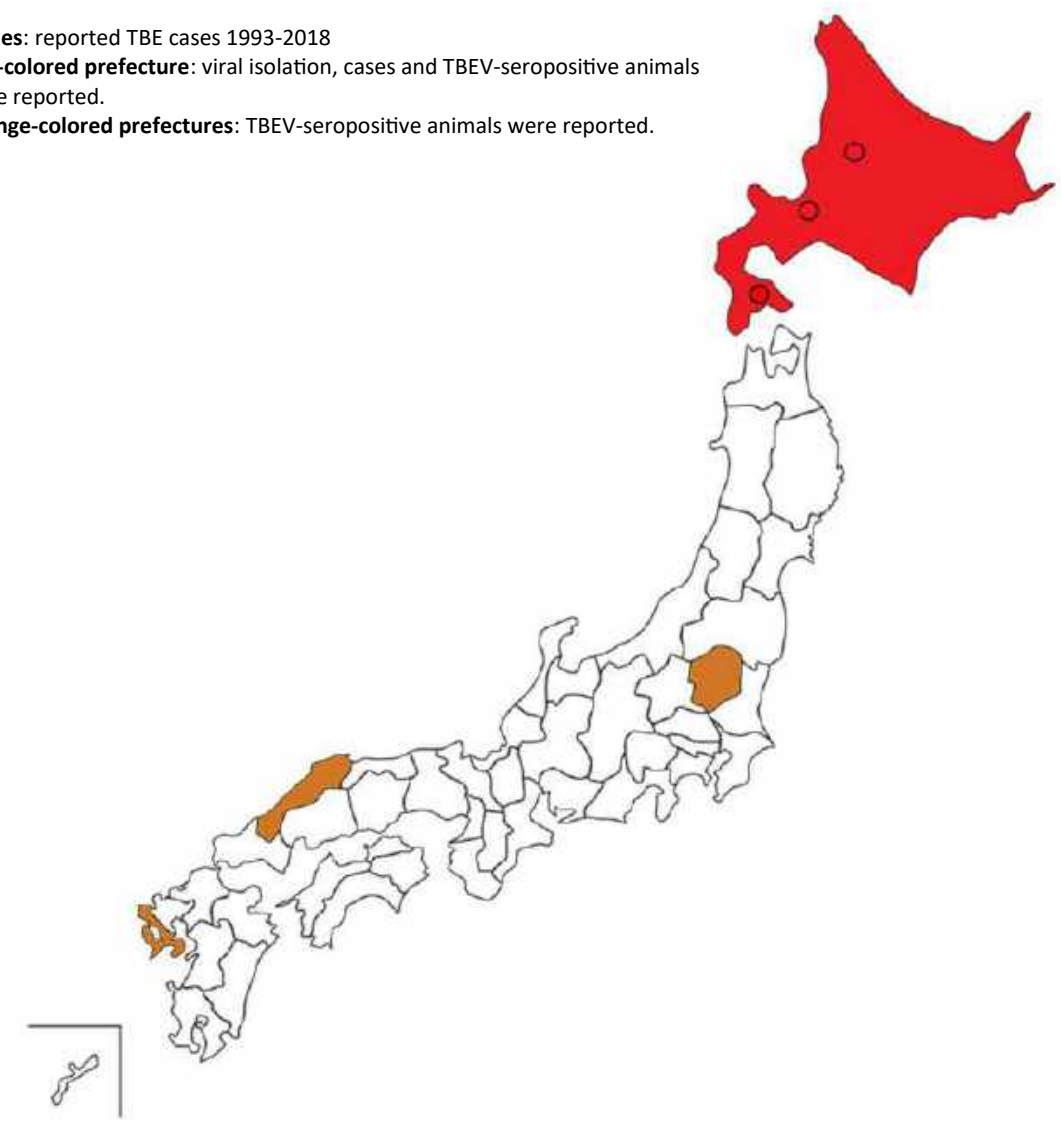
Source Data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE in Japan, 1993-2023[6,15-17]



Source Data: Appendix—Figure 2

Figure 3: Geographic distribution of TBE in Japan[4,5,8,11,13,15,16]



Authors and Affiliations

Kentaro Yoshii
National Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University, Nagasaki, Japan.

Contact: kyoshii@nagasaki-u.ac.jp

Citation:
Yoshii K. TBE in Japan. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-16-7

Appendix

Source data: Figure 1

Year	Number of cases	Year	Number of cases
1993	1	2009	0
1994	0	2010	0
1995	0	2011	0
1996	0	2012	0
1997	0	2013	0
1998	0	2014	0
1999	0	2015	0
2000	0	2016	1
2001	0	2017	2
2002	0	2018	1
2003	0	2019	0
2004	0	2020	0
2005	0	2021	0
2006	0	2022	0
2007	0	2023	0
2008	0		

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	0	0	0
10-19	0	0	0
20-29	0	0	0
30-39	0	1	1
40-49	1	1	2
50-59	0	0	0
60-69	0	0	0
>70	2	0	2

References

1. Arai S, Matsunaga Y, Takasaki T, et al. Japanese encephalitis: surveillance and elimination effort in Japan from 1982 to 2004. *Jpn J Infect Dis*. 2008;61(5):333-8.
2. Morita K, Igarashi A, Sato T, Takezawa T. A suspected case of tick-borne encephalitis in Hokkaido. Vol. 15. 1994:273-4. *Infect Agents Surv Rep.*
3. Hayasaka D, Suzuki Y, Kariwa H, et al. Phylogenetic and virulence analysis of tick-borne encephalitis viruses from Japan and far-Eastern Russia. *J Gen Virol*. 1999;80 (Pt 12):3127-3135. doi:10.1099/0022-1317-80-12-3127
4. Jamsransuren D, Yoshii K, Kariwa H, et al. Epidemiological survey of tick-borne encephalitis virus infection in wild animals on Hokkaido and Honshu islands, Japan. *Japanese Journal of Veterinary Research*. 2019;67(2):166-72. doi:10.14943/jjvr.67.2.163
5. Kentaro Y, Yamazaki S, Mottate K, et al. Genetic and biological characterization of tick-borne encephalitis virus isolated from wild rodents in southern Hokkaido, Japan in 2008. *Vector Borne Zoonotic Dis*. 2013;13(6):406-14. doi:10.1089/vbz.2012.1231
6. T Takashima I, Morita K, Chiba M, et al. A case of tick-borne encephalitis in Japan and isolation of the the virus. *J Clin Microbiol*. 1997;35(8):1943-1947. doi:10.1128/jcm.35.8.1943-1947.1997
7. Takahashi Y, Kobayashi S, Ishizuka M, et al. Characterization of tick-borne encephalitis virus isolated from a tick in central Hokkaido in 2017. *J Gen Virol*. 2020;101(5):497-509. doi:10.1099/jgv.0.001400
8. Takeda T, Ito T, Chiba M, Takahashi K, Niioka T, Takashima I. Isolation of tick-borne encephalitis virus from Ixodes ovatus (Acari: Ixodidae) in Japan. *J Med Entomol*. 1998;35(3):227-231. doi:10.1093/jmedent/35.3.227
9. Takeda T, Ito T, Osada M, Takahashi K, Takashima I. Isolation of tick-borne encephalitis virus from wild rodents and a seroepizootiologic survey in Hokkaido, Japan. *Am J Trop Med Hyg*. 1999;60(2):287-291. doi:10.4269/ajtmh.1999.60.287
10. Uchida L, Hayasaka D, Ngwe Tun MM, Morita K, Muramatsu Y, Hagiwara K. Survey of tick-borne zoonotic viruses in wild deer in Hokkaido, Japan. *J Vet Med Sci*. 2018;80(6):985-988. doi:10.1292/jvms.18-0017
11. Yoshii K, Mottate K, Omori-Urabe Y, et al. Epizootiological study of tick-borne encephalitis virus infection in Japan. *J Vet Med Sci*. 2011;73(3):409-412. doi:10.1292/jvms.10-0350
12. Takahashi Y, Kobayashi S, Nakao R, Kariwa H, Yoshii K. Characterization of tick-borne encephalitis virus isolated from tick infesting dog in central Hokkaido in 2018. *Ticks Tick Borne Dis*. 2022;13(2):101900. doi:10.1016/j.ttbdis.2022.101900
13. Luvai EAC, Uchida L, Tun MMN, et al. Seroepidemiological surveys of tick-borne encephalitis virus and novel tick-borne viruses in wild boar in Nagasaki, Japan. *Ticks Tick Borne Dis*. Jan 2022;13(1):101860. doi:10.1016/j.ttbdis.2021.101860
14. Shimoda H, Hayasaka D, Yoshii K, et al. Detection of a novel tick-borne flavivirus and its serological surveillance. *Ticks Tick Borne Dis*. Jun 2019;10(4):742-748. doi:10.1016/j.ttbdis.2019.03.006
15. Tajima Y, Yaguchi H, Mito Y. Fatal Meningoencephalomyelitis due to the Tick-borne Encephalitis Virus: The First Detailed Neurological Observation in a Japanese Patient from the Central Part of Hokkaido Island. *Intern Med*. 2018;57(6):873-876. doi:10.2169/internalmedicine.8437-16
16. Yamaguchi H, Komagome R, Miyoshi M, et al. tick-borne encephalitis in Hokkaido in 2017. Vol. 39. 2018:46–7. *Infect Agents Surv Rep*.
17. Tanaka D, Abe M, Kuroshima K, Ura S, Yoshida K, Yabe I. [A case of tick-borne encephalitis without any sequelae]. *Rinsho Shinkeigaku*. 2021;61(5):310-313. doi:10.5692/clinicalneurol.cn-001555
18. Yoshii K, Sato K, Ishizuka M, Kobayashi S, Kariwa H, Kawabata H. Serologic Evidence of Tick-Borne Encephalitis Virus Infection in a Patient with Suspected Lyme Disease in Japan. *Am J Trop Med Hyg*. 2018;99(1):180-181. doi:10.4269/ajtmh.18-0207

19. Yoshii K, Takahashi-Iwata I, Shirai S, Kobayashi S, Yabe I, Sasaki H. A Retrospective Epidemiological Study of Tick-Borne Encephalitis Virus in Patients with Neurological Disorders in Hokkaido, Japan. *Microorganisms*. Oct 28 2020;8(11). doi:10.3390/microorganisms8111672

20. Yoshii K, Kojima R, Nishiura H. Unrecognized Subclinical Infection with Tickborne Encephalitis Virus, Japan. *Emerg Infect Dis*. Oct 2017;23(10):1753-1754. doi:10.3201/eid2310.170918

21. Ohira M, Yoshii K, Aso Y, et al. First evidence of tick-borne encephalitis (TBE) outside of Hokkaido Island in Japan. *Emerg Microbes Infect*. Dec 2023;12(2):2278898. doi:10.1080/22221751.2023.2278898

Chapter 13

TBE in Kazakhstan

Andrey Dmitrovskiy and Zhanna Shapiyeva

E-CDC risk status: endemic (data as of end 2023)

History and current situation

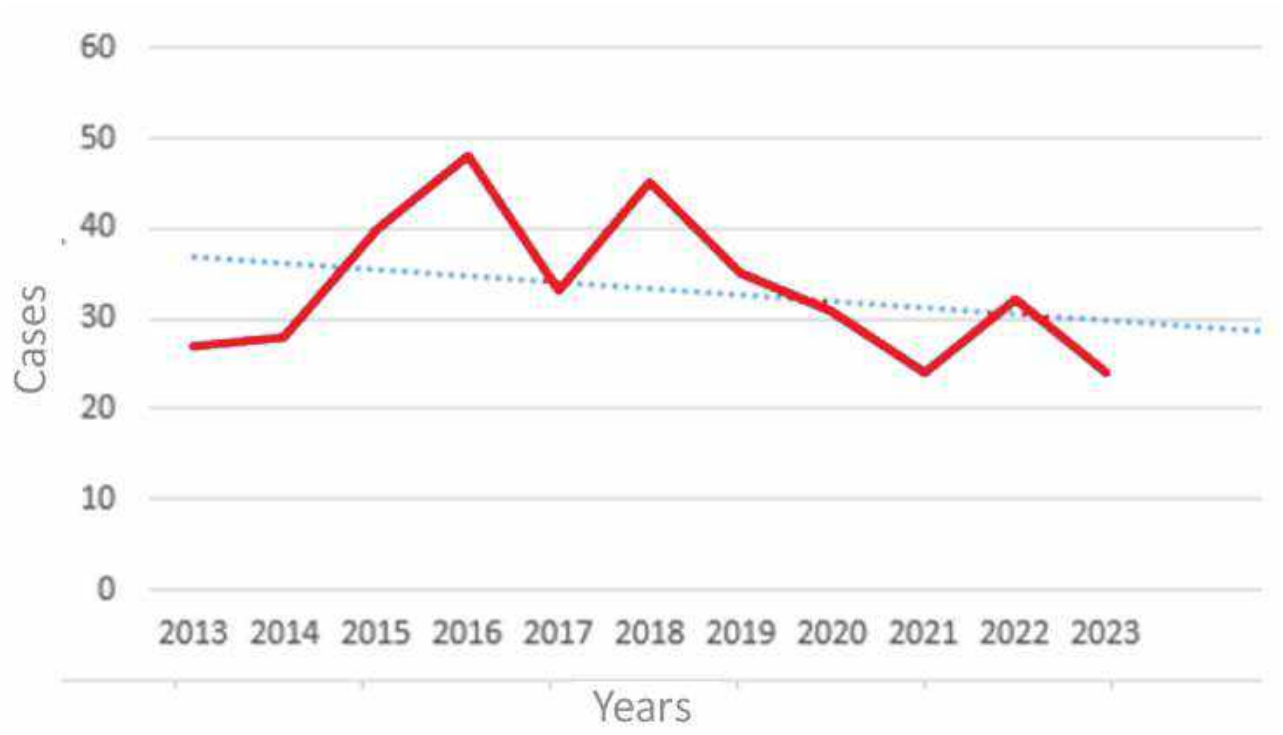
The first isolation of TBEV in Kazakhstan was in the Almaty region by M.P. Chumakov in 1941 (one strain from patient). This is proof that the clinically well-described "spring-summer encephalitis" in the Almaty region was in fact TBE. Later in 1943, 1944 and 1945 the TBEV was isolated from patients by local scientists from the Institute of Epidemiology and Microbiology (IEM), in Alma-Ata by E. I. Demikhovsky from CSF samples and brain tissue.[1] TBE clinical manifestations were first described by E.M. Steblov in the Almaty region, and the disease had been named "Almaty encephalitis". Moreover, Steblov described a chronic variant of TBE as "Kojevnikov's Epilepsy".[2] In 1954, the TBEV was isolated from Ixodes persulcatus ticks.[3] The endemic zone in Eastern Kazakhstan was first characterized by Zhumatov in 1957.[4] In 1959, a total of 5 TBEV strains were isolated from 315 Dermacentor reticulatus ticks (in 11 pools - minimal infection rate (MIR)- %14,3) in Zailiysky Alatau and 12 additional strains in Jungarsky Alatau (720 ticks – 12 pools – minimal infection rate (MIR) %13,9).[5] In the 1960s the Arbovirus Infections Laboratory of the Institute of Epidemiology, Microbiology and Hygiene (Alma-Ata) under the direction of Zhumatov conducted extensive work to study the natural foci of TBE in Kazakhstan. In particular, for several years, they examined birds for TBEV antibodies in Eastern Kazakhstan using a Hemagglutination Inhibition Assay). In 1961, during the examination of the sera of 46 birds, anti-TBEV antibodies were found in 4 local (non-migratory) species of birds (including jackdaw and starling). In 1962, 2 starlings out of 260 were also found with antibodies to the TBEV, whereas testing of 174 farm animal sera turned out to be negative. At the same time, studies of humans in Eastern Kazakhstan demonstrated seropositivity rates from 1.9% to 19.4%.[6] The study of human sera in different endemic regions showed that in mountain foci, where *I. persulcatus* is common, antibodies were detected in 12.0% of patients whereas in steppe foci it was 4.7%. Of persons between the ages of 11–15 years, antibodies were detected in 0.7%, between 16–25 years in 7.8%, between 26–35 years in 9.9% and over 35 years in 8.3%.

When studying human TBEV infection by different genera of ticks in different endemic territories of Kazakhstan, researchers concluded that in those places with no *I. persulcatus* ticks patients were infected by *Dermacentor reticulatus* or *Dermacentor marginatus* and such infections did not result in any symptoms of TBE.[7] All this work resulted in the creation of an epidemiological surveillance network for TBE, including the annual collection and study of ticks for infection rate, tick treatment of farm and domestic animals, as well as in areas where humans are concentrated, and in addition vaccination of the population in endemic areas. Local medical organizations are officially advised to conduct timely identification, recording and reporting of cases, including all individuals affected by tick bites, and this documentation includes diagnostic measures taken, hospitalization, medical examination and treatment of patients with TBE. Clinical supervision for patients who recovered from TBE must be conducted by a neurologist for a two-year period or longer, depending on the patient's health status. Routine immunization against tick-borne encephalitis must be carried out by medical organizations and must be provided for individuals whose activities are connected with being in a natural focus of TBE.[16] The Kazakh Institute of Epidemiology, Microbiology and Hygiene Research defines TBE-endemic areas in the 27 districts and 6 regions of Kazakhstan (Almaty, Eastern Kazakhstan, Akmola, Kostanai, Karaganda and Northern Kazakhstan).[13] In 2016, new cases appeared in "old" endemic zones in the Akmola region, in 2020, cases appeared in Northern Kazakhstan region, and in 2022 – in Zhambyl region.[17-20] In 2023, the number of confirmed TBE cases had decreased to 24 (32 in 2022), half of cases were registered in Eastern Kazakhstan Region (12). The incidence was still registered in the "new" endemic regions – Akmola - 4 cases and in the Northern Kazakhstan Region - 3 cases. Thus, the data of the former Kazakh Institute of Epidemiology and Microbiology on the wider endemicity of TBE, in addition to the Almaty and East Kazakhstan regions, are confirmed. In this regard, the Ministry of Health of the Republic of Kazakhstan transferred two more regions - Akmola (1 district) and North Kazakhstan (1 district) to the status of endemic regions.[21]

Overview of TBE in Kazakhstan

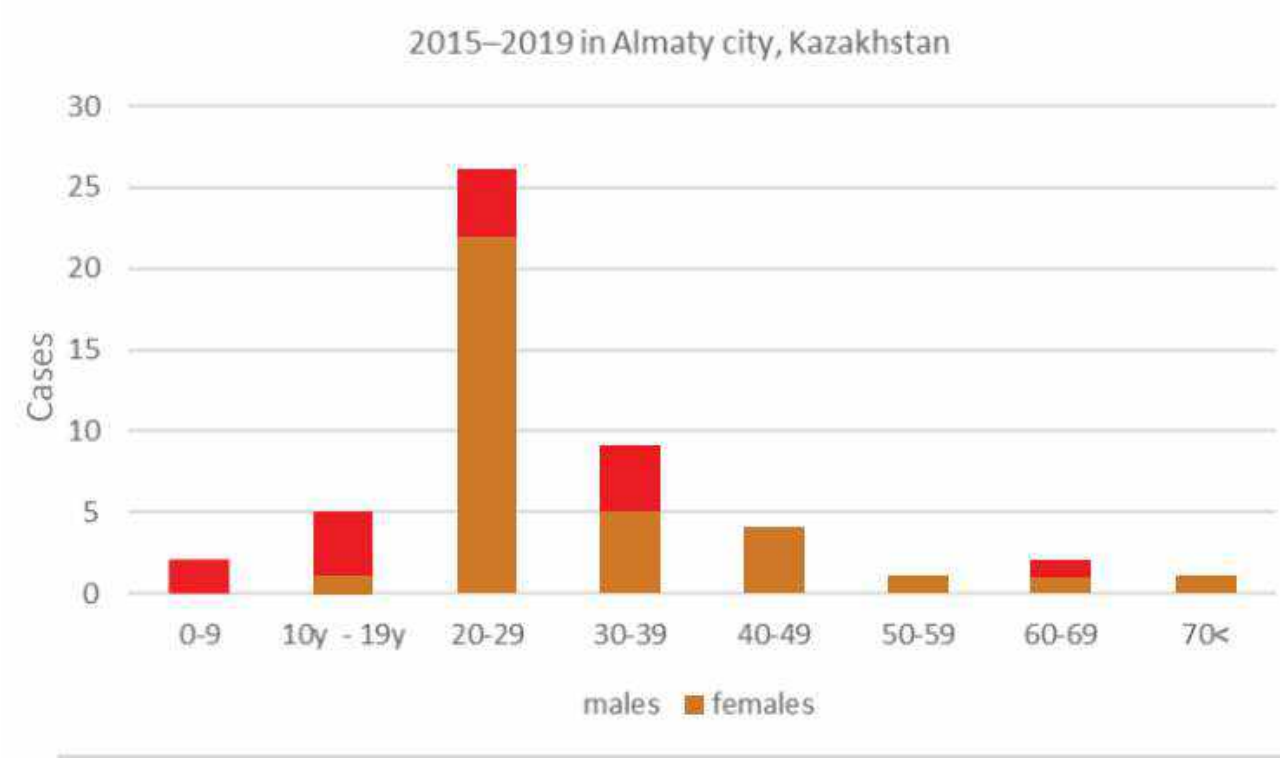
Table 1: TBE in Kazakhstan	
Viral subtypes isolated	Siberian subtype, Almaty region[12,13]
Reservoir animals	*No information available*
Percentage infected ticks	The tick infection rate of long-term data (1970)[14] • *I. persulcatus* – 31.3% positive pools • *D. reticulatus* — 29.2% positive pools • *D. marginatus* –15/5 – 33.3% positive pools By ELISA on TBEV Ag in Almaty region (2014–2015):[15] *I. persulcatus* 18.6%–21.8% positive pools *D. marginatus* 32.1%–74.2% positive pools *D. reticulatus* 33.3%–33.3% positive pools *D. niveus* 34.8%–45.4% positive pools *H. punctata* 33.3%–47.0% positive pools *R. turanicus* 14.8%–15.7% positive pools By PCR in Almaty region (2014–2016)[16] *I. persulcatus* - 15.4%-29.4% pools pos.; *D. marginatus* 8.3%; *Haemophysalis punctata* - 1.0%[19]
Dairy product transmission	Not documented—rare—frequent
Case definition used by authorities	Original
Completeness of case detection and reporting	The study of human sera in different endemic regions showed that in mountain foci where *I. persulcatus* is common, antibodies were detected in 12.0% of unvaccinated people whereas in steppe foci it was 4.7%.[6]
Type of reporting	Mandatory
Other TBE surveillance	Detection in ticks in ELISA and PCR
Special clinical features	Monophasic. Risk groups - the local population in endemic regions and those who visit them Clinical manifestation (%) - no information available
Licensed vaccines	Tikovak, Baxter AG, Austria, Pfizer Manufacturing Belgium N.V. EnceVir, Microgen, Russia
Vaccine recommendations	Local population in endemic regions, and the people working in this area
Vaccine uptake	No information available
National Reference center for TBE	There is no TBE Reference center in Kazakhstan

Figure 1: TBE case numbers over time against the background of vaccination



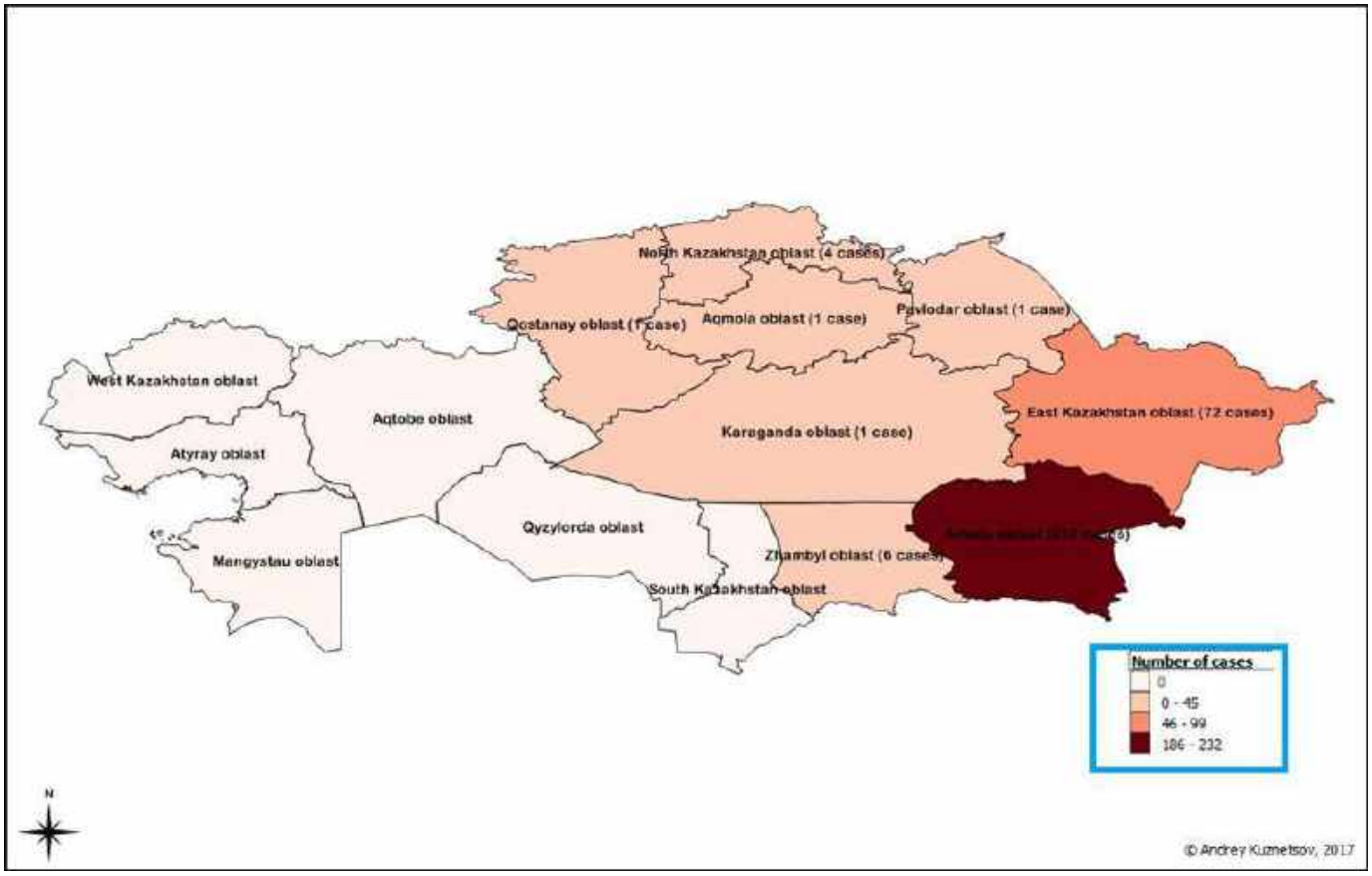
Source Data: Appendix Figure 1

Figure 2: Age and Gender distribution of TBE in Almaty city of Kazakhstan, 2015 - 2019



Source Data: Appendix Figure 2

Figure 3: TBEV-isolation and TBE cases in Kazakhstan



Maps were created in open source GIS, QGIS ver. 2.8.6 (Wien).

Appendix

Source data: Figure 2

Age group (years)	Males	Females	All
0–9	0	2	2
10–19	1	4	5
20–29	22	4	26
30–39	3	4	9
40–49	4	0	4
50–59	1	0	1
60–69	1	1	2
>70	1	0	1

Data for 2015–2019 in Almaty city

Source data: Figure 1

Year	Number of TBE cases	TBE incidence /10^5
1970	17	0.1
1971	12	0.09
1972	26	0.15
1973		
1974		
1975		
1976	22	0.13
1977	11	0.07
1978	11	0.07
1979	21	0.14
1980	7	0.04
1981	7	0.04
1982	8	0.05
1983	14	0.09
1984	18	0.11
1985	12	0.08
1986	11	0.07
1987	11	0.07
1988	14	0.08
1989	25	0.2
1990	14	0.08
1991	20	0.12
1992	19	0.13
1993	12	0.08
1994	17	0.12
1995	22	0.15
1996	30	0.20
1997	43	0.29
1998	38	0.26
1999	60	0.41
2000	44	0.30
2001	35	0.23
2002	55	0.38
2003	30	0.20
2004	50	0.33
2005	49	0.32
2006	33	0.20
2007	32	0.21
2008	34	0.22
2009	49	0.31
2010	30	0.20
2011	40	0.26
2012	33	0.20
2013	27	0.18
2014	28	0.18
2015	49	0.32
2016	48	0.31
2017	34	0.22
2018	46	0.30
2019	35	0.19
2020	31	0.17
2021	24	0.13
2022	32	0.17

Acknowledgments

TBE surveillance was supported by the Science practical Center for sanitary and epidemiological monitoring and expertise.

Authors and Affiliations

Andrey Dmitrovskiy, National Center for Biotechnology, Kazakhstan-Russian Medical University, Almaty branch, Almaty, Kazakhstan

Zhanna Shapiyeva , Science practical Center for sanitary and epidemiological monitoring and expertise, Almaty Kazakhstan

Contact: am_dimitr@mail.ru

Citation:
Dmitrovskiy A. TBE in Kazakhstan. Chapter 12b. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi: 10.33442/26613980_13-17-7

References

1. Demikhovsky EI. About Almaty virus encephalitis (scientific session). Alma-Ata. 1945:23-24.
2. Steblov EM. Clinic, etiology, pathogenesis, differential diagnosis and therapy of Kozhevnikov's epilepsy (scientific session). Alma-Ata. 1945:24-26.
3. Kiryuschenko TV, Akberdin SU. XI Conference of the Kazakh Institute of Epidemiology, Microbiology and Hygiene. AlmaAta. 1970:123-125.
4. Karimov SK, Akberdin SU, Temirbekov TK. Information from the 8th concluding practical science conference of the Kazakh Institute of Epidemiology, Microbiology and Hygiene. AlmaAta. 1967:65-67.
5. Dmitrienko IN, Bondareva RP, Prikhodko ET. Information from the 6th concluding practical science conference of the Kazakh Institute of Epidemiology, Microbiology and Hygiene. AlmaAta. 1964:39-43.
6. Temirbekov ZhT, Akberdin, Tagiltsev AA, Survillo AV, Ustimenko. Information from the 5th concluding practical science conference of the Kazakh Institute of Epidemiology, Microbiology and Hygiene. Alma-Ata. 1963:45,48-51.
7. Tagiltsev AA, Dmitrienko IN, Prikhodko ET. Information from the 7th concluding practical science conference of the Kazakh Institute of Epidemiology, Microbiology and Hygiene.

AlmaAta. 1966:90-94.
8. Karimov GK, Akberdin, Temirbekov ZhT, Usebayeva TK, Zhumatov. Information from the 8th concluding practical science conference of the Kazakh Institute of Epidemiology, Microbiology and Hygiene. Alma-Ata. 1967:65-68.
9. Yegemberdiyeva R. Clinical and epidemiological manifestations of some natural focal vector-borne infections in Kazakhstan. Scientific dissertation for the degree of Doctor of Medical Sciences. Chapter 3.3. Characteristic species composition of ticks and their natural infection in the Republic of Kazakhstan. Almaty. 2011:37-47.
10. Shapieva ZhZh. (republican SES data) Symposium within the framework of the German-Kazakh partnership in diagnostics of dangerous infectious diseases. Almaty, 2016.
11. Abdiyeva KS, Yeraliyeva LT, Turebekov NA, et al. Actual problems of epidemiology, microbiology, natural foci of human diseases. Omsk, 2016:76-78.
12. Decreeof the Committee of Health of the Ministry of Education, Culture and Health of the Republic of Kazakhstan dated March 6, 1998; #111 "On preventive measures for the tick-borne encephalitis".
13. Decree of the Acting Minister of Health of the Republic of Kazakhstan dated December 15, 2006; #623 “About approval of standards of medical practice in identifying cases of especially dangerous infections of humans when they are registering”.
14. Decree of the Acting Minister of Health of the Republic of Kazakhstan dated June 11, 2007; #357 “On approval of sanitary and epidemiological rules and norms. Organization of the sanitary and epidemiological (prophylactic) measures for the prevention of tick-borne encephalitis”.
15. Government Decree #89 from January 17, 2012 "On approval of the Sanitary Rules. Sanitary and epidemiological requirements for the organization and conduct of antiepidemic (preventive) measures for the prevention of parasitic diseases“.
16. Yegemberdiyeva R, Shapiyeva Z, Dmitrovskiy A, Oradova A, Azhdarova G, Zhusupov K. Investigation of tick-borne encephalitis spread in the non-endemic regions of Kazakhstan. In: Book of the 16th Medical Biodefense Conference. Munich, 2018:19,51.
17. Dmitrovskiy AM. TBE Monitoring and Prophylaxis in Kazakhstan. In: Abstract book of 4th Global Conference on Vaccines Research & Development from February 10-11, 2020 at Lisbon, Portugal. p. 31. doi:10.13140/RG.2.2.33000.62723
18. Perfilyeva YV, Shapiyeva ZZ, Ostapchuk YO, et al. Tick-borne pathogens and their vectors in Kazakhstan - A review. Ticks Tick Borne Dis. 2020;11(5):101498. doi:10.1016/j.ttbdis.2020.101498
19. Abdiyeva K, Turebekov N, Yegemberdiyeva R, et al. Vectors, molecular epidemiology and phylogeny of TBEV in Kazakhstan and central Asia. Parasit Vectors. 2020;13(1):504. doi:10.1186/s13071-020-04362-1
20. Ostapchuk Y.O., Dmitrovskiy A.M., Pak E.A., Perfilyeva Y.V. A case of combined infection with tick-borne encephalitis and Lyme borreliosis with severe meningoencephalitis and complete recovery // Journal of Global Infectious Diseases. – 2023. – Vol. 15 (2). – P. 81-83. doi:10.4103/jgid.jgid_76_22
21. The Order of the Ministry of Health of the Republic of Kazakhstan dated May 16, 2022 No. 44. On approval of the Sanitary Rules. “Sanitary and epidemiological requirements for the organization and implementation of sanitary, anti-epidemic and sanitary and preventive measures to prevent parasitic diseases”

Chapter 13

TBE in Kyrgyzstan

Wilhelm Erber

E-CDC risk status: endemic (data as of end 2023)

History and current situation

There is very little information and there are only a few publications on TBE in Kyrgyzstan. A survey by Atkinson[1] references the following: In humans and birds low seropositivity has been demonstrated as early as 1973. In 1978, the TBEV was isolated from ticks, and twelve human cases were reported between 1976–1981.

A more recent publication confirmed virus circulation between 2007 and 2009 in local tick populations in Ala-Archa National Nature Park ≈40 km south of Bishkek, the capital of Kyrgyzstan, as well as serologic evidence of a possible human TBE case.[2]

The TBEV strain isolated from an *Ixodes persulcatus tick* pool and from liver samples from 2 *Apodemus pallipes* mice was shown to be of the Siberian (TBEV-Sib) subtype and most closely related to strains from Novosibirsk.[2]

Overview of TBE in Kyrgyzstan

Table 1: Virus, vector, transmission of TBE in Kyrgyzstan	
Viral subtypes, distribution	Siberian TBEV strains from Bosnia, the Crimean peninsula, Kyrgyzstan and Kazakhstan are clustered into a newly described Bosnia Lineage[3]
Reservoir animals	Rodents, insectivores
Infected tick species (%)	*I. persulcatus*
Dairy product transmission	Not known

Burden of TBE in Kyrgyzstan over time:

no data available

Age and gender distribution of TBE in Kyrgyzstan: no data available

TBEV-isolation and TBE cases in Kyrgyzstan:

no reported cases of TBE in the country

Table 2: TBE reporting and vaccine prevention in Kyrgyzstan	
Mandatory TBE reporting	Not known
Other TBE surveillance	Not known
Special clinical features	Not known
Available vaccines	Not known
Vaccination recommendations and reimbursement	Not known
Vaccine uptake by age group/risk group/general population	Data not available
Name, address/ website of TBE NRC	Not known

Contact: wilhelm.erber@gmail.com

Citation:
Erber W. TBE in Kyrgyzstan. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt, HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-18-7

References

1. Atkinson B, Hewson R. Emerging arboviruses of clinical importance in Central Asia. *J Gen Virol*. 2018;99(9):1172-84.
2. Briggs BJ, Atkinson B, Czechowski DM, et al. Tick-borne encephalitis virus, Kyrgyzstan. *Emerg Infect Dis*. 2011;17(5):876-9.
3. Tkachev SE, Babkin IV, Chicherina GS, et al. Genetic diversity and geographical distribution of the Siberian subtype of the tick-borne encephalitis virus. *Ticks Tick Borne Dis*. 2020;11(2):101327.

Chapter 13

TBE in Latvia

Dace Zavadska and Zane Freimane

E-CDC risk status: endemic (data as of end 2023)

History and current situation

Aggregated data on TBE cases in Latvia are available from 1955,[1] but serological testing for TBE began in the 1970s.[2] Since TBE became notifiable in Latvia, epidemiological changes of disease incidence have been dramatic. Between 1990–2000 Latvia had the highest rates of TBE incidence in the world, ranging from 8 to 53 cases per 100,000 population.[2] Although the incidence has decreased significantly in the past 10 years to about half – from 14.58/100,000 in 2010 to 7.86/100,000 in 2018 – Latvia still ranks very high among all countries in Europe with an annual incidence of 12.67/100,000 in 2022. The distribution of TBE cases in Latvia varies between different regions with the highest incidence usually registered near the northwestern coast.

The Centre for Disease Prevention and Control (CDPC) of Latvia is the governmental institution that provides TBE surveillance in Latvia. Based on national legislation, there is countrywide mandatory but passive case-based reporting, guided by case definition of the European Centre for Disease Prevention and Control (ECDC) since 2012. Adoption of the standardized European case definition for TBE ensures a more specific capture of TBE cases as well as the impact by vaccination.

The main vectors of the TBE virus in Latvia are ticks of the family Ixodidae, mainly *Ixodes ricinus* and *Ixodes persulcatus* in the eastern part of the country.[3] All three main TBEV subtypes are carried by ticks in Latvia – the European, Siberian and Far-Eastern subtype.[4,5,6]

Epidemiological investigations suggest that in Latvia, ticks carry a higher TBEV load than in other at-risk countries, and moreover, up to 20%–40% of ticks are infected in highly endemic areas.[7] Latvia also has one the highest reported rates of TBEV transmission via unpasteurized dairy products, mainly goat milk,[2] which accounts for 0.5%–3.5% of all cases (2011–2019).

The largest recent study of the epidemiology of TBE in Latvia documents on a population basis with active case search in hospitals, documents that mostly persons in the age group 18–59 years are affected, mostly males. This is in line with the general risk factors for TBE, i.e., active lifestyle with increased outdoor activities, travelling, and other factors that increase the risk of tick-human contact.[8] Children (0–17 years) in Latvia make up only 5.6% of all TBE cases.

The most common clinical manifestation of TBE was meningitis, with the highest number of cases in the age group 18–59 years. For children, meningitis was also the most frequent cause of hospitalization.[9] Compared to other age groups, more severe TBE clinical forms (meningo-encephalitis, etc.) were mainly reported among the age group >60 years.

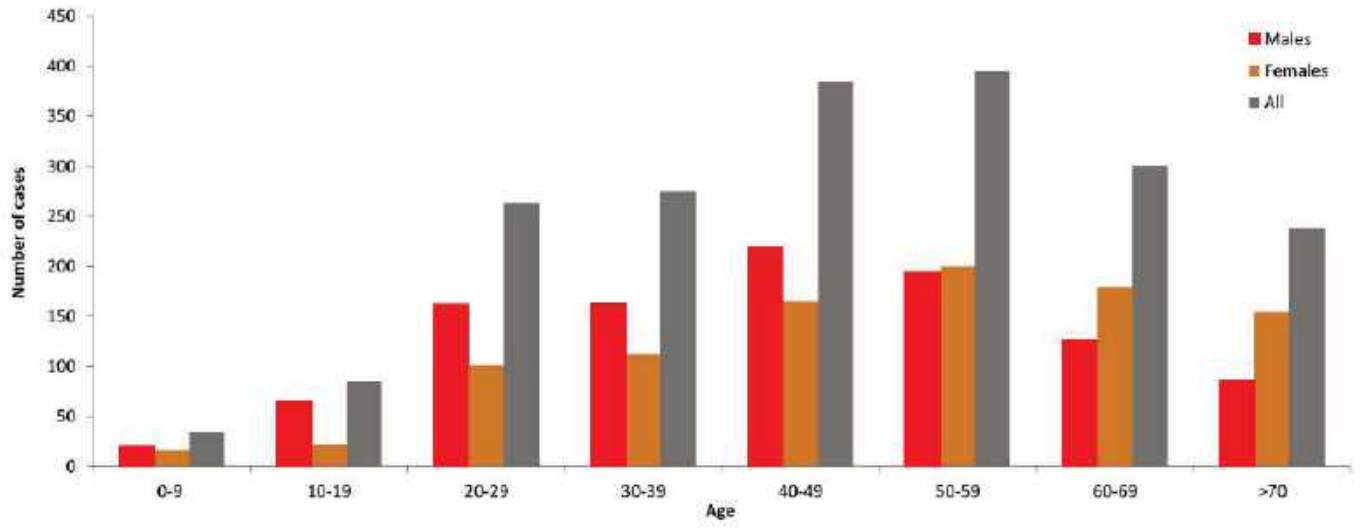
Vaccination remains the most effective protective measure against TBE.[10,11,12] In Latvia, there is only a partial National Immunization Program, which has provided vaccine free of charge for children living in highly endemic areas since 2006 and orphans/children without parental care in the whole country since 2010. Vaccination is mandatory for employees with a high risk of occupational exposure, such as forest workers, military personnel, and lab workers and it is paid by the employer. For other residents of Latvia and travelers, vaccination is strongly recommended but not reimbursed; however, most private insurance companies cover TBE vaccine expenses.[13,14] Because of the National Immunization Program for children, TBE vaccine uptake in children reached up to 77% in highly endemic areas and 22% nationwide, reducing the proportion of TBE cases among children from 12.5% in 2001 to 3.6% in 2010[15] and 2016. Vaccine uptake in the whole population was 39% in 2009[15] and it increased to 52.5% in 2015.[16]

Currently used vaccines in Latvia are *FSME-Immun®* (*TicoVac*, used since 1995) and *Encepur®* (since 2001 for adults and 2002 for children). *FSME-Immun®* is the most commonly used TBE vaccine in Latvia, with a market share of up to 86% in those who had received at least one dose where the brand administered was captured.[17] In the future, uptake data need to be carefully monitored in order to explain epidemiological findings.

Overview of TBE in Latvia

Table 1: Virus, vector, transmission of TBE in Latvia	
Viral subtypes, distribution	In Latvia, all 3 main TBEV sub-types circulate: European, Siberian, and Far Eastern In Latvia 1-96 is a close relative to the Vasilchenko strain (Siberian sub-type), and RK1424 is related to the Sofjin strain (Far Eastern sub-type).[4,5,6]
Reservoir animals	Among the small rodents identified in the most long-term *I. ricinus* monitoring site (Riga region) in 1997–2001 were *Clethrionomys glareolus* (85%), followed by *Sorex araneus, Apodemus flavicollis,* and *Apodemus agrarius*.[19]
Infected tick species (%)[3]	*Ixodes ricinus* ticks are spread in the western and central parts of Latvia, and in small numbers also in the eastern part of the country. *Ixodes persulcatus* dominates only in the eastern part of the country, comprising 58%–99% of all collected ticks. Earlier data reveals that TBEV annual prevalence from 1993 to 2002 in the field-collected adults for *I. ricinus* adults varied between 1.7% and 26.6% and for *I. persulcatus* – between 0% and 37.3%. The infection level in ticks removed from humans was much higher and from 1998 to 2002 reached about 30%.[3,6,7]
Dairy product transmission	Rare

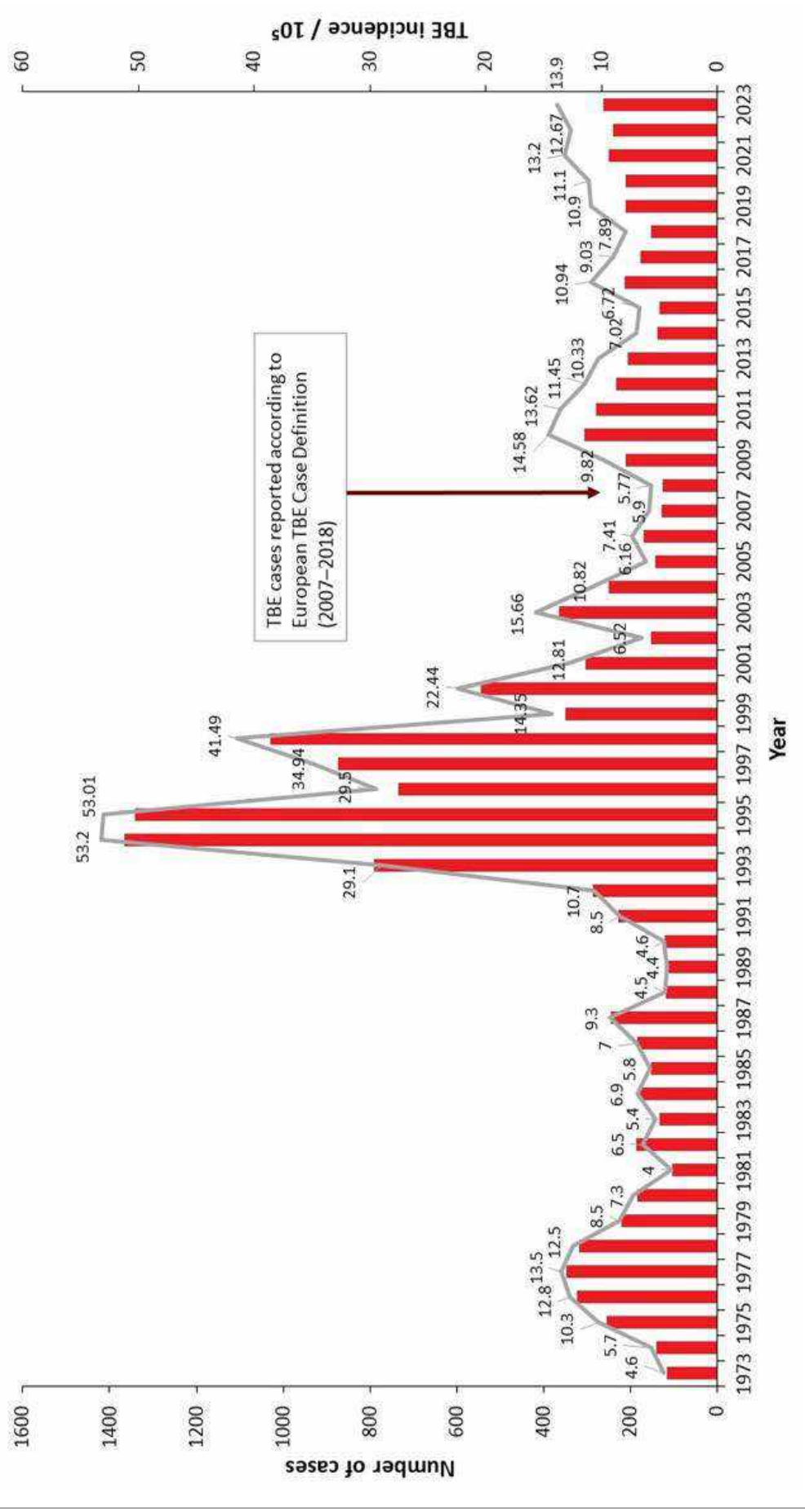
Figure 2: Age and gender distribution of TBE in Latvia (2007–2016, n=1973)[8]



Source Data: Appendix Figure 2

Table 2: TBE reporting and vaccine prevention in Latvia	
Mandatory TBE reporting[3,20]	Mandatory notification since 1955. Based on national legislation, there is countrywide mandatory case-based passive reporting and the European Centre for Disease Prevention and Control (ECDC) case definition for TBE was adapted in Cabinet Regulations in 2012. Aggregated data on TBE cases are available from 1955 and case-based data in electronic format are available from 2007. Prior to 2012, the case definition of TBE in Latvia included (1) hospitalization because of central nervous system disease and (2) confirmation of infection with TBE virus by laboratory diagnosis, usually by the demonstration of specific IgM antibodies by ELISA.
Other TBE surveillance	None
Special clinical features	Study done in Children's Clinical university hospital reveals that Biphasic fever course was presented in 50% (n=41) of children treated in the hospital between 2000–2015.[9] Annual mortality varies from 0% to 1.3% (1973–2009) and is not related to the overall incidence of TBE. Follow-up for 1–13 years of a cohort of 100 patients revealed long-term sequelae in over 50%, more commonly in those suffering focal forms of acute TBE.[3]
Available vaccines[21,22]	TicoVac (0.25 and 0.5 ml) since 1995 (FSME-Immun) • Encepur adults since 2001 - Delivery interruption – 12/2012 till 03/2014, therefore sold fewer doses • Encepur Children since 2002 - Delivery interruption – 04/2013 till 09/2014, therefore sold fewer doses
Vaccination recommendations and reimbursement[16,23]	There is only a partial National Immunization Program in place which recommends vaccination for children and adolescents living in endemic areas since 2007 and has provided vaccine free of charge for children living in highly endemic areas since 2006 and orphans/children without parental care in the whole country since 2010. Vaccination is mandatory for high risk groups and/or those with high occupational exposure such as forest workers, military personnel, and lab workers and is paid by the employer. Vaccination is also recommended, but not reimbursed for adults. Also most insurance companies cover TBE vaccination costs. (https://likumi.lv/doc.php?id=11215 Cabinet Regulations Nr.330. Vaccination regulations)
Vaccine uptake by age group/risk group/general population[17,23]	The vaccination uptake overall was 53% in 2015.* In Latvia, approximately 22% of children had been vaccinated by the end of 2010, most (77%) of whom were living in highly endemic areas, the cost of which was reimbursed by the state. The vaccination rate for the national population was 39% in 2009 and 41% in 2010.
Name, address/website of TBE NRC	Center of Disease Prevention and Control of Latvia www.spkc.gov.lv Duntes iela 22, k-5, Rīga, Latvija, LV 1005 *Diagnostics*: Latvian Centre of Infectious Diseases (Latvijas Infektoloģijas centrs) of the Riga East University Hospital: https://www.aslimnica.lv/en/saturs/latvian-centre-infectious-diseases 3 Linezera Street, Riga, LV-1006

Figure 1: Burden of TBE in Latvia over time[8]



**Although European Case Definition for TBE was officially adapted in Latvia in 2012, surveillance study[8] has reported TBE cases according to Case Definition for 2007–2011 as well.*

Source Data: Appendix Figure 1

Figure 3: TBEV-isolation and TBE cases in Latvia (2007–2016, n=1973)[8]

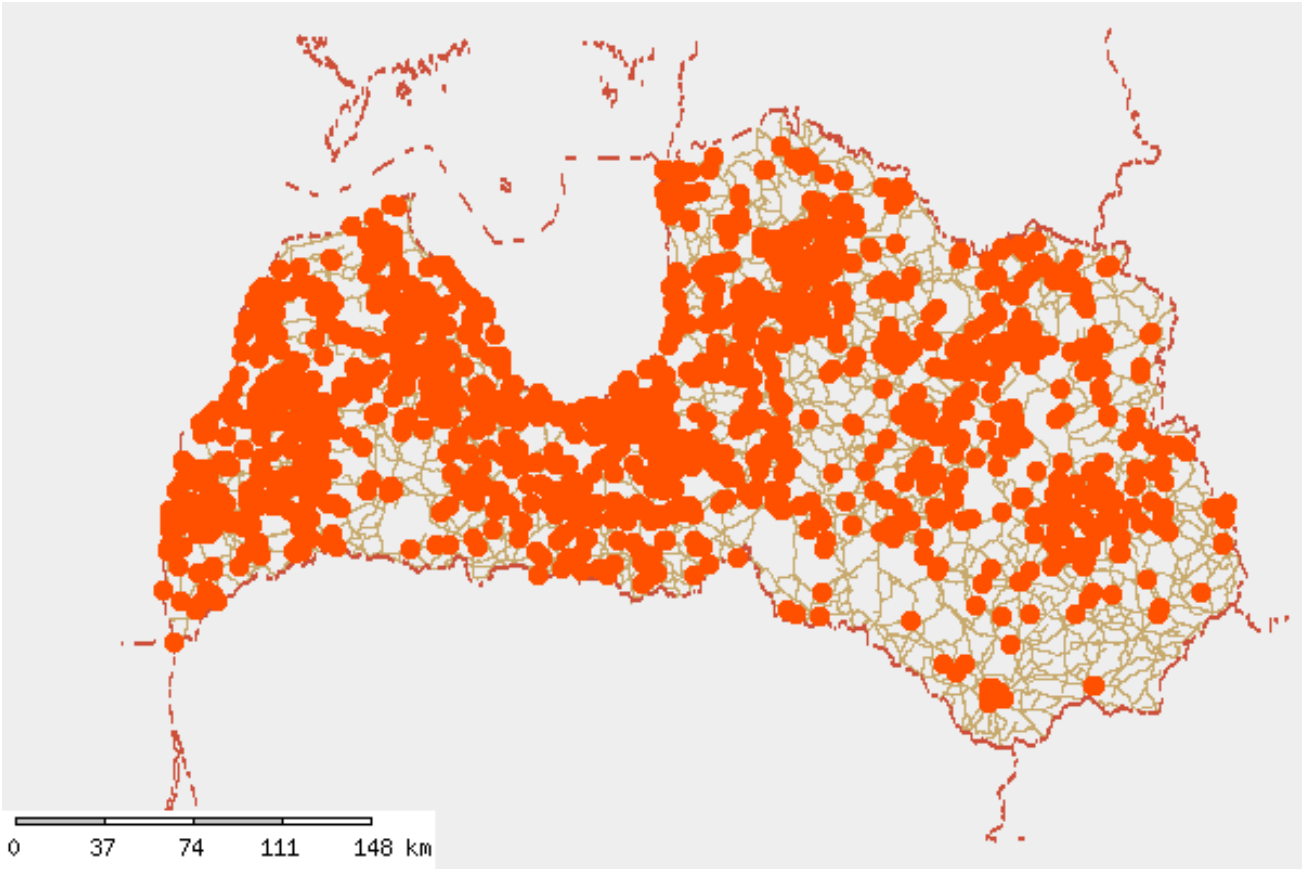
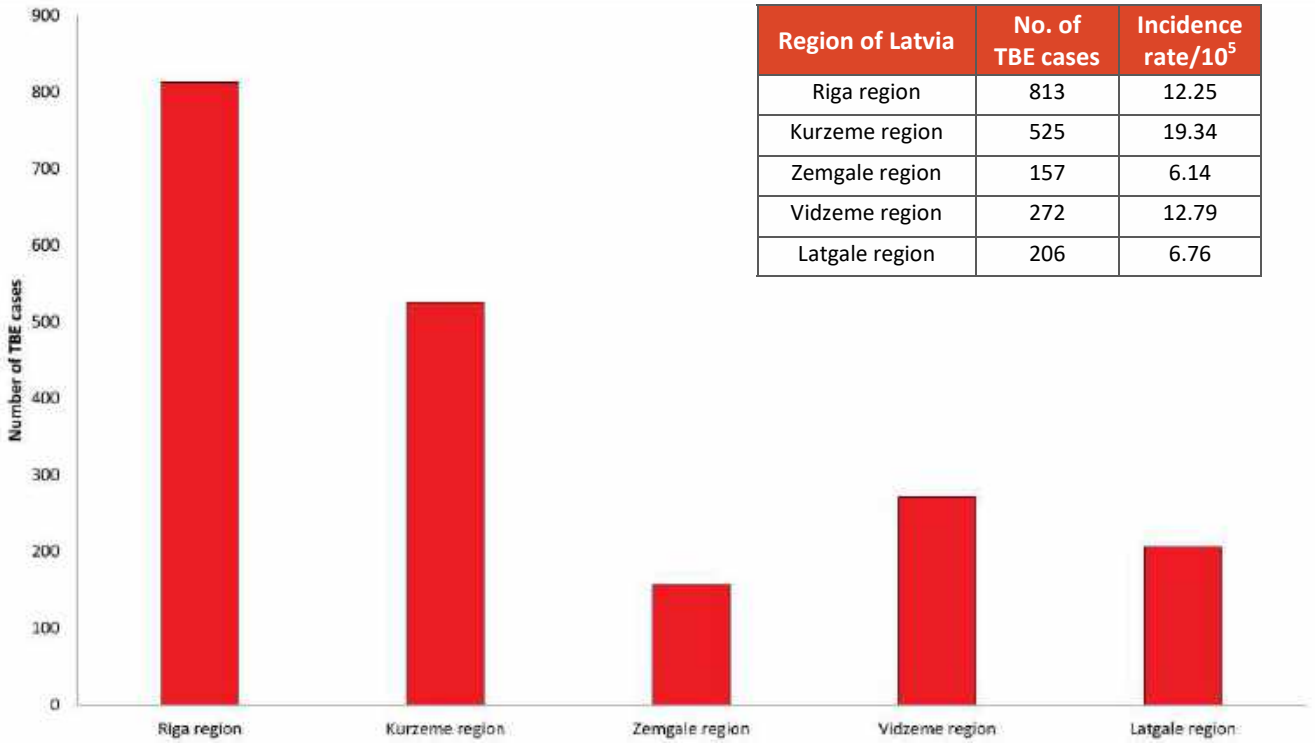


Figure 4: Burden of TBE ("CNS disease") by 5 regions of Latvia (2007–2016, n=1973)[8]



Region of Latvia	No. of TBE cases	Incidence rate/10^5
Riga region	813	12.25
Kurzeme region	525	19.34
Zemgale region	157	6.14
Vidzeme region	272	12.79
Latgale region	206	6.76

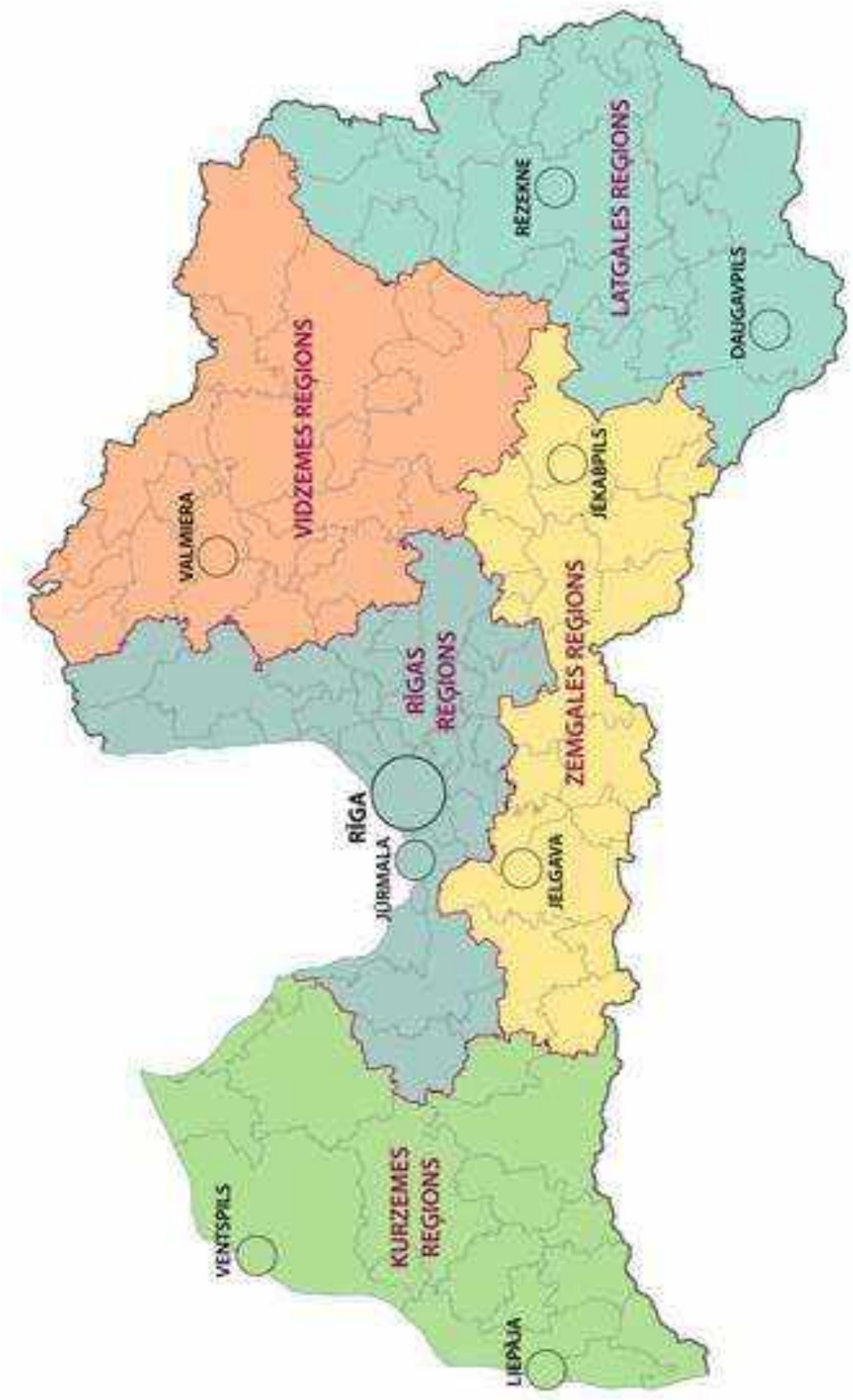


Figure 5: Regions of Latvia[18]

Appendix

Source data: Figure 1

Year	Number of TBE cases (including "no CNS disease" forms)	TBE incidence /10^5
1973	116	4.6
1974	141	5.7
1975	256	10.3
1976	322	12.8
1977	347	13.5
1978	318	12.5
1979	220	8.5
1980	184	7.3
1981	103	4
1982	186	6.5
1983	133	5.4
1984	179	6.9
1985	152	5.8
1986	184	7
1987	246	9.3
1988	119	4.5
1989	117	4.4
1990	122	4.6
1991	227	8.5
1992	287	10.7
1993	791	29.1
1994	1366	53.2
1995	1341	53.01
1996	736	29.5
1997	874	34.94
1998	1029	41.49
1999	350	14.35
2000	544	22.44
2001	303	12.81
2002	153	6.52
2003	365	15.66
2004	251	10.82
2005	142	6.16
2006	170	7.41
2007	129	5.90
2008	125	5.77
2009	210	9.82
2010	306	14.58
2011	280	13.62
2012	232	11.45
2013	207	10.33
2014	139	7.02
2015	132	6.72
2016	213	10.94
2017	176	9.03
2018	152	7.89
2019	211	10.9
2020	210	11.1
2021	249	13.2
2022	240	12.67
2023	262	13.9

*Although European Case Definition for TBE was officially adapted in Latvia in 2012, surveillance study[8] has reported TBE cases according to Case Definition for 2007–2011 as well.

*Source data: Figure 2***

Age group (years)	Males	Females	All
0–9	18	16	34
10–19	63	22	85
20–29	162	101	263
30–39	163	112	275
40–49	219	165	384
50–59	194	200	394
60–69	126	179	300
>70	84	154	238

**Number of TBE cases ("CNS disease") by age and gender.

Contact: dzavadska@apollo.lv

Citation:
Zavadska D, Freimane Z. TBE in Latvia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-19-7

References

1. Lucenko I, Jansone I, Velicko I, Pujate E. Tickborne encephalitis in Latvia. *Euro Surveill.* 2004;(26):pii=2495.
2. Sumilo D, Bormane A, Vasilenko V, Golovljova I, Asokliene L, Zygutiene M, Randolph S. Upsurge of tick-borne encephalitis in the Baltic States at the time of political transition, independent of changes in public health practices. *Clin Microbiol Infect*. 2009;15(1):75-80. doi:10.1111/j.1469-0691.2008.02121.x
3. Karelis G, Bormane A, Logina I, Lucenko I, Suna N, Krumina A, Donaghy M. Tick-borne encephalitis in Latvia 1973-2009: epidemiology, clinical features and sequelae. *Eur J Neurol*. 2012;19(1):62-8. doi:10.1111/j.1468-1331.2011.03434.x
4. Süss J, Schrader C, Abel U, Bormane A, Duks A, Kalnina V. Characterization of tick-borne encephalitis (TBE) foci in Germany and Latvia (1997-2000). *Int J Med Microbiol.* 2002;291 Suppl 33:34-42. doi:10.1016/s1438-4221(02)80007-8
5. Mavtchoutko V, Vene S, Haglund M, Forsgren M, Duks A, Kalnina V, Hörling J, Lundkvist A. Characterization of tick-borne encephalitis virus from Latvia. *J Med Virol*. 2000;60:216-22
6. Bormane A, Zeltiņa A, Lucenko I, Mavčutko V, Duks A, Pujate E, Ranka R, Baumanis V. Tick-borne encephalitis - pathogen, vectors and epidemiological situation in Latvia 2002-2003. *Acta Universitatis Latviensis*. 2004;670:27-37.
7. Bormane A, Lucenko I, Duks A, Mavtchoutko V, Ranka R, Salmina K, Baumanis V. Vectors of tick-borne diseases and epidemiological situation in Latvia in 1993-2002. *Int J Med Microbiol*. 2004;293 Suppl 37:36-47. doi:10.1016/s1433-1128(04)80007-x
8. Zavadska D, Odzelevica Z, Karelis G, Liepina L, Litauniece ZA, Bormane A, et al. (2018) Tick-borne encephalitis: A 43-year summary of epidemiological and clinical data from Latvia (1973 to 2016). *PLoS One.* 2018;13(11):e0204844. doi:10.1371/journal.pone.0204844
9. Zavadska D, Odzelevica Z. "Tick borne encephalitis in Children's Clinical University Hospital – clinical course and neurological outcome" 2015, presented at 18th meeting ISW-TBE 2016.
10. World Health Organization. Tick-borne encephalitis. 2015. [online] – [Reference 20.01.17.] Available: http://www.who.int/biologicals/vaccines/tick_borne_encephalitis/en/
11. World Health Organization Position Paper. Vaccines against tick-borne encephalitis. 2011. [online] – [Reference 01.03.17.] Available: http://www.who.int/immunization/sage/1_TBE_PP_Draft_13_Mar_2011_SAGE_apr_2011.pdf
12. Heinz F, Stiasny K, Holzmann H, Vitek M, Kriz B, Essl A, Kundi M. Vaccination and Tick-borne Encephalitis, Central Europe. *Emerg Infect Dis*. 2013;19(1):69-76. doi:10.3201/eid1901.120458
13. Republic of Latvia. Cabinet Regulations No. 330. Vaccination regulations. 2000.g. [online] – [reference 15.02.17.] Available: http://likumi.lv/doc.php?id=11215
14. The Centre for Disease Prevention and Control of Latvia.[online] – [reference 15.02.17.] Available: https://spkc.gov.lv/lv/tavai-veselibai/infekcijas-slimibas/vakcinacija/pret-ercu-encefalitu-berniem
15. Zavadska D, Anca I, André F, Bakir M, Chlibek R, Čižman M, Ivaskeviciene I, Mangarov A, Mészner Z, Pokorn M, Prymula R, Richter D, Salman N, Šimurka P, Tamm E, Tešović G, Urbancikova I, Usonis V. Recommendations for tick-borne encephalitis vaccination from the Central European Vaccination Awareness Group (CEVAG). *Hum. Vacc. Immunother*. 2013;9(2):362-74.
16. Zavadska D. Latvia: Success Story of TBE Vaccination. Presentation for 15th ISW-TBE. 2013.
17. TBE Compliance & Coverage Country Report – Latvia; Report prepared for Pfizer by GfK SE. GfK SE. October 2015
18. Ministry of Environmental Protection and Regional Development of the Republic of Latvia. [online]. Available at: http://www.varam.gov.lv/lat/darbibas_veidi/reg_att/pl_reg/?doc=13637
19. Bormane A (2007) *I. ricinus* L. un *I. persulcatus* P. Sch. (Acari: Ixodidae) izplatība, to pārnēsāto infekcijas slimību nozīme un molekulārā epidemioloģija Latvijā. Dissertation, University of Latvia
20. Dumpis U, Crook D, Oksi J. Tick-borne Encephalitis. *Clin Infect Dis*. 1999;28(4):882-90.
21. State Agency of Medicines of Latvia, 2017
22. Sumilo D, Asokliene L, Avsic-Zupanc T, et al. Behavioural responses to perceived risk of tick-borne encephalitis: vaccination and avoidance in the Baltics and Slovenia. Vaccine. 2008;19;26(21):2580-8. doi:10.1016/j.vaccine.2008.03.029
23. Zavadska D, Anca I, André F, et al. Recommendations for tick-borne encephalitis vaccination from the Central European Vaccination Awareness Group (CEVAG). Hum Vaccin Immunother. 2013;9(2):362-374. doi:10.4161/hv.22766

Chapter 13

TBE in Lithuania

Auksė Mickienė

E-CDC risk status: endemic (data as of end 2023)

History and current situation

The first case of tick-borne encephalitis (TBE) in Lithuania, diagnosed by clinical and epidemiologic criteria only, was reported in 1953. A forest worker became ill with the disease in April after a tick bite, had a typical clinical presentation with shoulder girdle muscle paralysis and bulbar syndrome, and died after 12 days from the start of clinical symptoms. Autopsy data were compatible with viral encephalitis.[1] Serological diagnosis of TBE in Lithuania was started in 1970.[2]

In Lithuania, *Ixodes ricinus* is the main vector of tick-borne encephalitis virus (TBEV), which is spread throughout the entire country. In addition, *Dermacentor reticulatus* is also found in Lithuania.[3,4,5] In 1974, 142 of 13,726 field-collected ticks in two northeastern districts of Lithuania (Rokiškis and Biržai) located near the Latvian border were identified as *Ixodes persulcatus*.[6] The recent entomological studies have also detected *I. persulcatus* in the Rokiškis district.[7] TBEV is found from ticks collected in all administrative districts of Lithuania and in 3 urban parks in the country.[3] According to the recent nationwide study conducted in Lithuania in 2017 –2019, which investigated 7,170 *I. ricinus* and 1,676 *D. reticulatus* ticks (questing), collected from 81 locations in all ten counties, TBEV-infected ticks were found at 16 locations in seven counties, with minimum infection rate (MIR) ranging from 0.1% to 1.0%. The MIR of TBEV in the total sample of *I. ricinus* was 0.4 % and for *D. reticulatus* it was estimated to be 0.4 %.4 Sequence analysis of Lithuanian TBEV strains isolated from humans and field-collected ticks has shown that the virus belongs to the European TBEV subtype.[8,9] TBEV seroprevalence in non-vaccinated healthy permanent residents in Lithuania is 3%.[10]

Since 1990, the highest TBE incidence in Lithuania was recorded in 2003 (21.95 per 100 000; 763 cases), 2016 (22.1 per 100 000; 633 cases), and 2019 (25.5 per 100 000; 711 cases).[11] From 1998 to 2012, the highest annual incidence of TBE was recorded in the northern and central parts of the country, mainly in the municipalities of Kaunas, Panevėžys, and Šiauliai. Between 1998 and 2011, when the average incidence of TBE in Lithuania was 11.5 cases per 100,000 people, the average incidence rate in Panevėžys, Šiauliai and Radviliškis districts was 52.1, 45.6, and 33.3, respectively (3–5 times higher than the average incidence in the country).[12] Since 2013, a new trend in the epidemiology of TBE in Lithuania could be observed. While the incidence in the three aforementioned districts remains high, an increase in Vilnius, Alytus and Utena counties is gradually but steadily recorded. During the last 5 years, the highest TBE incidence rate in Lithuania was observed in Utena county (the northeastern part of Lithuania on the border to Latvia): 2019 – 59.5/100 000, 2020 – 66/100 000, 2021 – 31.6/100 000, 2022 – 33.5/100 000, 2023 – 40.5/100 000.[11]

Presently, TBE is the most common viral infection of the CNS in Lithuania[12], with a total of 13,332 TBE cases reported between 1990 and 2023, and 22 lethal TBE cases registered during the last ten years (2013-2023).[11] Children (mainly school children and adolescents) comprise 8.7% of all TBE cases in the country[12]. During the last 5 years (2019-2023), preschool children comprised 0.8% - 2% out of all TBE cases in the Lithuania.[11] Retired and unemployed people are the major risk group for infection with TBEV in Lithuania; 42.4%-56.4% of TBE patients are infected in the immediate areas surrounding their homes.[13,14] 7.8% of TBE cases in Lithuania are milk-borne.[14]

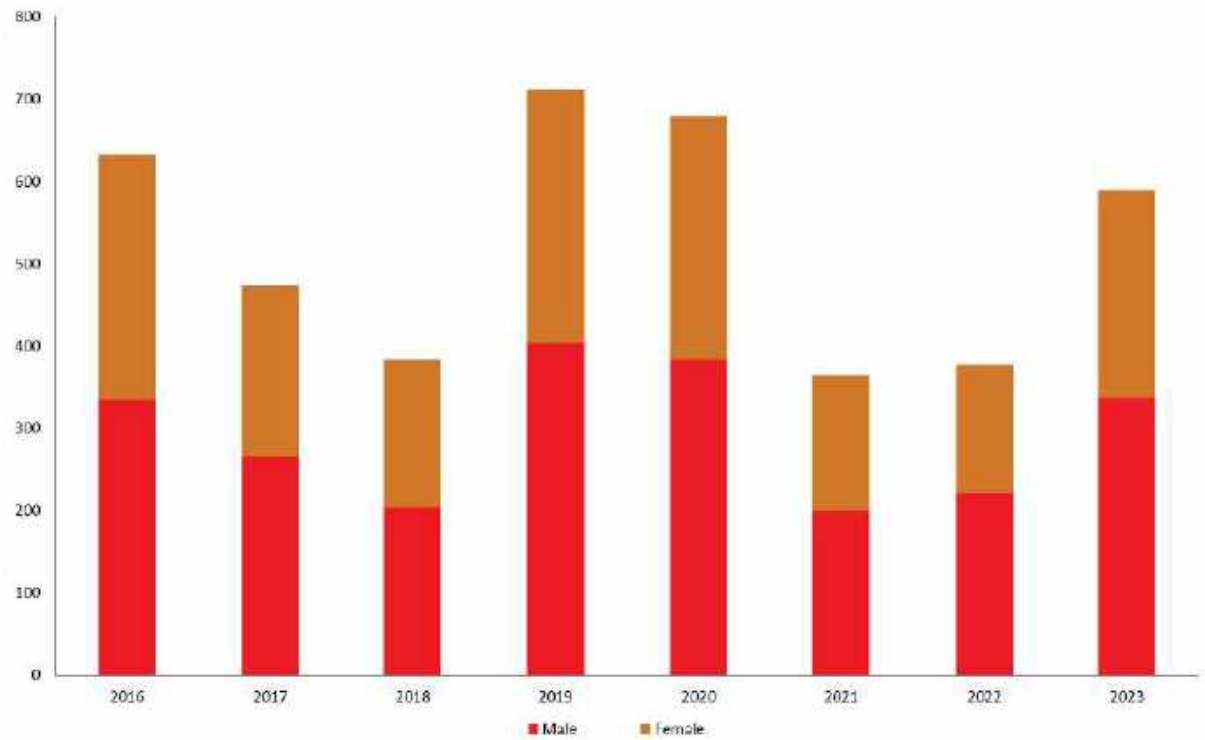
Overview of TBE in Lithuania

Table 1: Virus, vector, transmission of TBE in Lithuania

Viral subtypes, distribution	European TBEV subtype[8,9]
Reservoir animals	Main reservoir animals – *Apodemus agrarius, Apodemus flavicollis, Myodes glareolus*[15]
Infected tick species (%)	*I. ricinus* (0.1%–1.84%), *D. reticulatus* (0.58%)[4]
Dairy product transmission	7.8%[14]

Table 2: TBE reporting and vaccine prevention in Lithuania	
Mandatory TBE reporting	All patients with CNS form of TBEV infection confirmed by serological methods (TBEV IgM ± IgG) are reported to the National Public Health Centre under the Ministry of Health[11]
Other TBE surveillance	N/A
Special clinical features	Biphasic disease in 58%- 72.2%[13,14] Risk groups: retired people, unemployed people, and permanent inhabitants of highly endemic areas[11,13,14,] Moderate and severe sequelae in 30.8%. Mortality 0.75%[13]
Available vaccines	Encepur, Ticovac.[11]
Vaccination recommendations and reimbursement	Vaccination of adults: the recommendations by Lithuanian Society for Infectious Diseases (2022; no reimbursement). Reimbursed for military recruits and forestry workers.[11] Since 2024 – reimbursement for all adults above 50 years of age (starting with cohort of 50-55 years of age in September 2024).[17]
Vaccine uptake by age group/risk group/general population	Vaccine uptake (at least one dose of TBE vaccine) in 2020: 37%[18] Total number of consumed TBE vaccine doses: 2021: 334,664[19] 2022: 327,867[20] 2023: 381,698 (Razmuviene, D. National Public Health Centre under the Ministry of Health. Personal communication)
Name, address/website of TBE NRC	National Public Health Centre under the Ministry of Health[11]

Figure 2: Gender distribution of TBE cases in Lithuania, 2016-2023



Source Data: Appendix—Figure 2

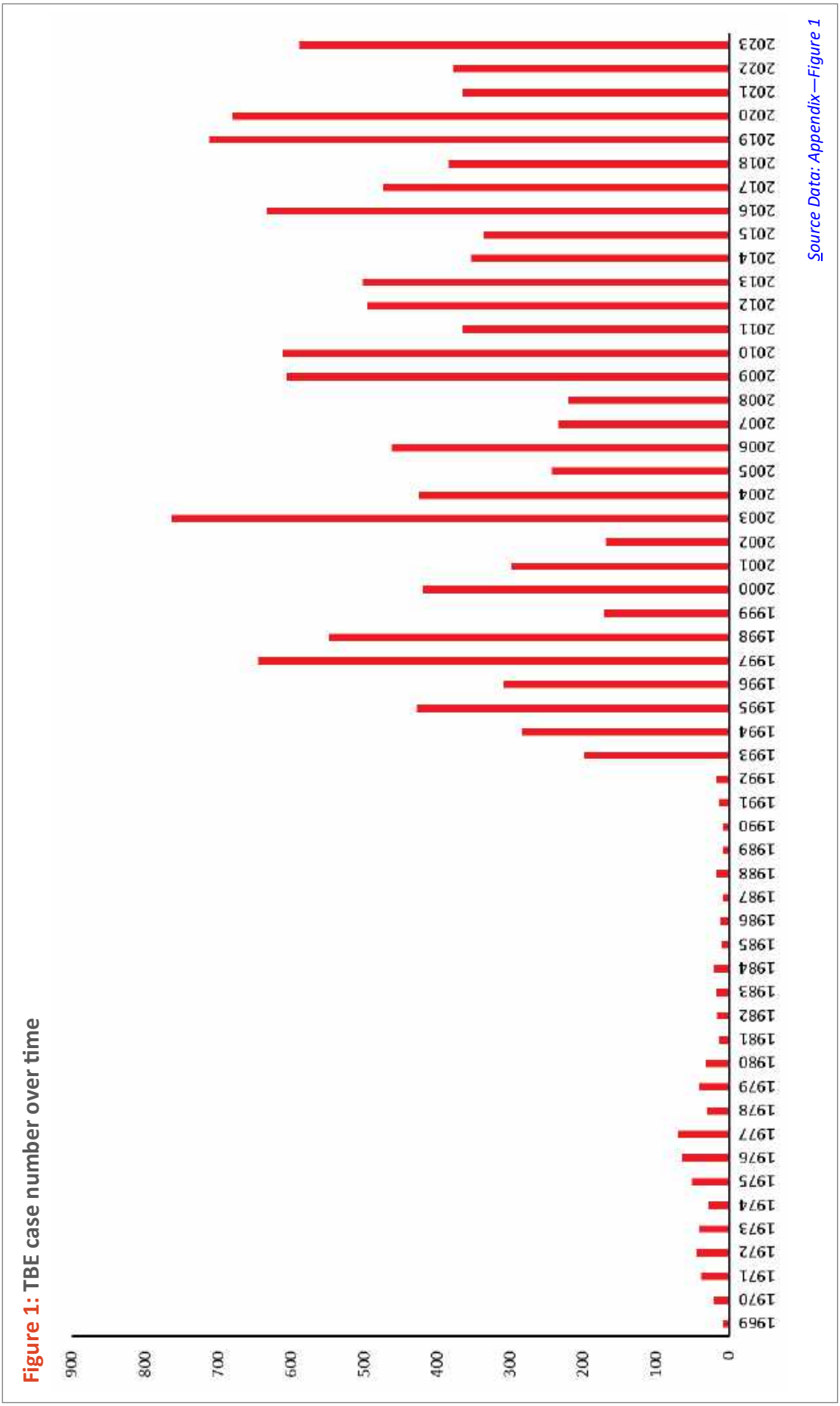


Figure 1: TBE case number over time

Source Data: Appendix—Figure 1

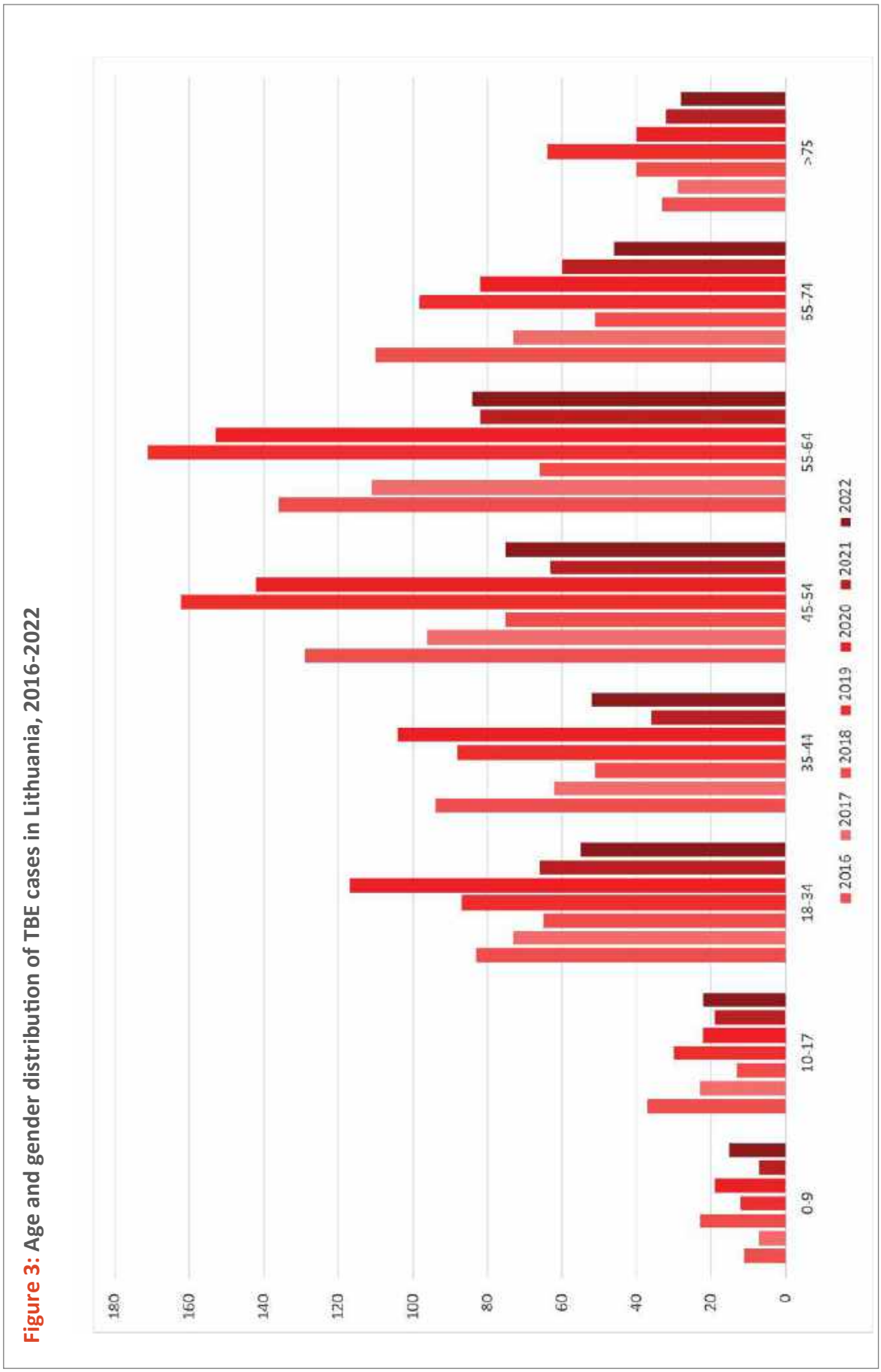


Figure 3: Age and gender distribution of TBE cases in Lithuania, 2016-2022

Appendix

Source data: Figure 1

Year	Number of cases	Incidence / 10^5
1969	9	0.3
1970	21	0.7
1971	38	1.12
1972	44	1.14
1973	40	1.12
1974	28	0.8
1975	51	1.5
1976	65	1.9
1977	70	2.1
1978	30	0.9
1979	41	1.1
1980	32	0.9
1981	13	0.3
1982	16	0.4
1983	18	0.5
1984	21	0.6
1985	10	0.2
1986	12	0.3
1987	9	0.2
1988	17	0.5
1989	8	0.2
1990	9	0.2
1991	14	0.4
1992	17	0.4
1993	198	5.3
1994	284	7.6
1995	427	11.5
1996	310	8.4
1997	645	17.4
1998	548	14.8
1999	171	4.6
2000	419	11.3
2001	298	8.5
2002	168	4.8
2003	763	22
2004	425	12.2
2005	243	7.1
2006	462	13.5
2007	234	6.9
2008	220	6.5
2009	605	17.9
2010	612	18.3
2011	365	11.1
2012	495	16.5
2013	501	16.9
2014	353	12
2015	336	11.5
2016	633	22.1
2017	474	16.8
2018	384	13.7
2019	711	25.8
2020	679	24.3
2021	365	12.8
2022	377	13.4
2023	589	20.8

Source data: Figure 2

Year	Male	Female
2016	334	299
2017	265	209
2018	204	180
2019	404	307
2020	384	295
2021	200	165
2022	222	155
2023	336	253

Contact: amickiene@gmail.com

Citation:
Mickiene A. TBE in Lithuania. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-20-7

References

1. Motiejunas L, Regaliene G. [The outbreak of cow milk origin tick-borne encephalitis]. *Sveikatos apsauga*. 1982;10:28-31.
2. Motiejunas L, Sapranauskaite D, Regaliene G. [Concerning the causes of a different morbidity of tick-borne encephalitis and the quality of patient's laboratory testing in the cities and districts of Lithuanian SSR]. *Sveikatos apsauga*. 1978;10:20-3.
3. Zygutiene M. [Tick-borne pathogens and spread of Ixodes ricinus in Lithuania]. *EpiNorth*. 2009;10:63-71
4. Sidorenko M, Radzijevskaja J, Mickevičius S, Bratčikovienė N, Paulauskas A. Prevalence of tick-borne encephalitis virus in questing Dermacentor reticulatus and Ixodes ricinus ticks in Lithuania. Ticks *Tick Borne Dis*. 2021;12(1):101594. doi:10.1016/j.ttbdis.2020.101594
5. Katargina O, Russakova S, Geller J, et al. Detection and characterization of tick-borne encephalitis virus in

Baltic countries and eastern Poland. *PLoS One*.2013;8 (5):e61374. doi:10.1371/journal.pone.0061374.

6. Motiejunas L , Podenaite B. [Tick species and abundance of ticks in forest landscape in Lithuanian SSR]. *Medical Parasitology and Parasitic Diseases*.1972;41(2):235-7.
7. Paulauskas A, Radzijevskaja J, Mardosaitė-Busaitienė D, Aleksandravičienė A, Galdikas M, Krikštolaitis R. New localities of Dermacentor reticulatus ticks in the Baltic countries. *Ticks Tick Borne Dis*. 2015;6(5):630-635.
8. Sidorenko M, Radzijevskaja J, Mickevičius S, et al. Phylogenetic characterisation of tick-borne encephalitis virus from Lithuania. *PLoS One*. 2024;19 (2):e0296472. Published 2024 Feb 7. doi:10.1371/ journal.pone.0296472
9. Mickiene A, Vene S, Golovljova I, et al. [Tick-borne encephalitis virus in Lithuania]. *Eur J Clin Microbiol Infect Dis*. 2001;20:886-8.
10. Juceviciene A, Vapalahti O, Laiskonis A, Ceplikiene J, Leinikki P. [Prevalence of tick-borne-encephalitis virus antibodies in Lithuania]. *J Clin Virol*. 2002;25(1):23-7.
11. National Public Health Centre under the Ministry of Health. Accessed March 27, 2024. https://nvsc.lrv.lt/ uploads/nvsc/documents/files/EPL%20apzvalga%20 (002).pdf
12. Mickiene A. Tick-Borne Encephalitis – Clinical and Pathogenetic Aspects. University dissertation from Stockholm: Karolinska Institutet, Department of Medicine at Huddinge University Hospital. 2015.

    Accessed March 27,2024. https:// openarchive.ki.se/ xmlui/bitstream/handle/10616/44938/ Thesis_Aukse_Mickiene.pdf?sequence=1&isAllowed=y
13. Mickienė A, Laiškonis A, Günther G, Vene S, Lundkvist Ă, Lindquist L. Tickborne Encephalitis in an Area of High Endemicity in Lithuania: Disease Severity and Long-Term Prognosis. *Clin Infect Dis*. 2002;35(6):650-8.
14. Radzišauskienė D, Žagminas K, Ašoklienė L, et al. Epidemiological patterns of tick-borne encephalitis in Lithuania and clinical features in adults in the light of the high incidence in recent years: a retrospective study. *Eur J Neurol*. 2018;25(2):268-274. doi:10.1111/ ene.13486
15. Paulauskas A, Radzijevskaja J, Turcinaviciene J, Ambrasiene D, Galdikaite E. Data on some ixodid tick species (Acari, Ixodidae) in the Baltic countries. *New and Rare For Lithuania Insect Species*. 2010:22.
16. Lietuvos Infektologu Draugija. [Immunoprophylaxis of adults: recommendations of the Lithuanian Society for Infectious Diseases, 2022].
    Accessed March 27, 2024. https://lid.lt/ storage/2023/01/A5-Suaugusiuju-skiepijimo-Lietuvoje-rekomendacijos_2022.pdf
17. Minister of Health of the Republic of Lithuania. National immunization programme for 2024-2028. Accessed March 27, 2024. https://e-seimas.lrs.lt/ portal/legalAct/lt/TAD/ f827fd31c9e811ee9269b566387cfecb?jfwid=-yvz4v411b
18. Pilz A, Erber W, Schmitt HJ. Vaccine uptake in 20 countries in Europe 2020: Focus on tick-borne encephalitis (TBE). *Ticks Tick Borne Dis*. 2023;14 (1):102059. doi:10.1016/j.ttbdis.2022.102059.
19. National Public Health Centre under the Ministry of Health. Annual report for 2021. Accessed March 27, 2024. https://nvsc.lrv.lt/uploads/nvsc/documents/ files/2022%2B%2BEPL%2Bapzvalga%2Btinklalapis.pdf
20. National Public Health Centre under the Ministry of Health. Annual report for 2022. Accessed March 27, 2024. https://nvsc.lrv.lt/uploads/nvsc/documents/ files/EPL%20apzvalga%20(002).pdf

Chapter 13

TBE in Moldova

Olga Sofronie, Olga Burduniuc, Greta Bălan

E-CDC risk status: affected (last edited: date 22.02.2024)

History and current situation

Tick-borne encephalitis is monitored in the Republic of Moldova with an emphasis on surveillance of ticks with no attention to human cases. The competent national institution responsible for TBE monitoring is the National Agency for Public Health. Official data on vector testing have been recorded since 2011. Ever since, studies on the circulation of the TBEV are conducted annually in spring, summer and autumn by collecting ticks from several regions of the country: Floreşti, Soroca, Bender, Tiraspol, Orhei, Drochia, Hînceşti, Ialoveni, Străşeni, Vadul lui Vodă, Chişinău, Taraclia, and Comrat territorial administrative units. TBEV was detected in most of the regions mentioned above, with highest isolation rates in Chişinău municipality (including Vadul lui Vodă), Străşeni, Comrat, Bender, and Tiraspol (Figure 1).

Tick testing was carried out using commercial ELISA sets for the detection of TBEV antigen (VectoTBEV-antigen; Novosibirsk, Russian Federation; https://en.vector-best.ru/catalog/IFA/kits/tick-borne-and-zoonotic-infections/). Tick species most frequently encountered in the territory of the Republic of Moldova are *Ixodes ricinus*, *Dermacentor marginatus*, *Dermacentor reticulatus*, *Haemaphysalis inermis* and *Haemapysalis punctata*, while *I. ricinus* was present in all of the three geographical areas. The average density index of the species *I. ricinus* in the period 2009-2011 was ~ 21 at the standard 200 m route.[1]

In a study conducted in 2010-2011 the Far Eastern TBEV subtype was detected by PCR in ticks (*I. ricinus*, *Dermacentor* spp. and *Haemaphysalis* spp.) collected from vegetation and domestic animals in Moldova. The regions where the presence of the TBEV-FE subtype was confirmed were Chişinău municipality and Ungheni district.[2]

Generally speaking, commercial ELISA kits for detection of antibodies to the TBEV are available for use in patients with CNS symptoms and a history of a tick bite in Moldova.[3] Studies on TBEV-seroprevalence in humans have not been carried out yet and testing for TBEV-infection is not routinely integrated into medical practice. During 2018 and 2023, a total of only 11 patient sera were tested for antibodies against the TBEV. One was positive for anti-TBEV -IgM and one was positive for both, anti-TBEV-IgG and -IgM. The two patients were adults from different regions of the country (Făleşti, Tiraspol), and none of the two had a history of travel outside Moldova.

In summary, the risk for TBEV-infection in Moldova has been confirmed by[1] the presence of the appropriate vectors - ixodid ticks in different territories of the Republic of Moldova - and[2] by the presence of ticks infected with the TBEV (Far Eastern subtype); as well as[3] by documentation of (some) human cases in the past. There is clearly a need to increase awareness of TBE in Moldova along with appropriate surveillance to better define the circulation of the TBEV in the country.

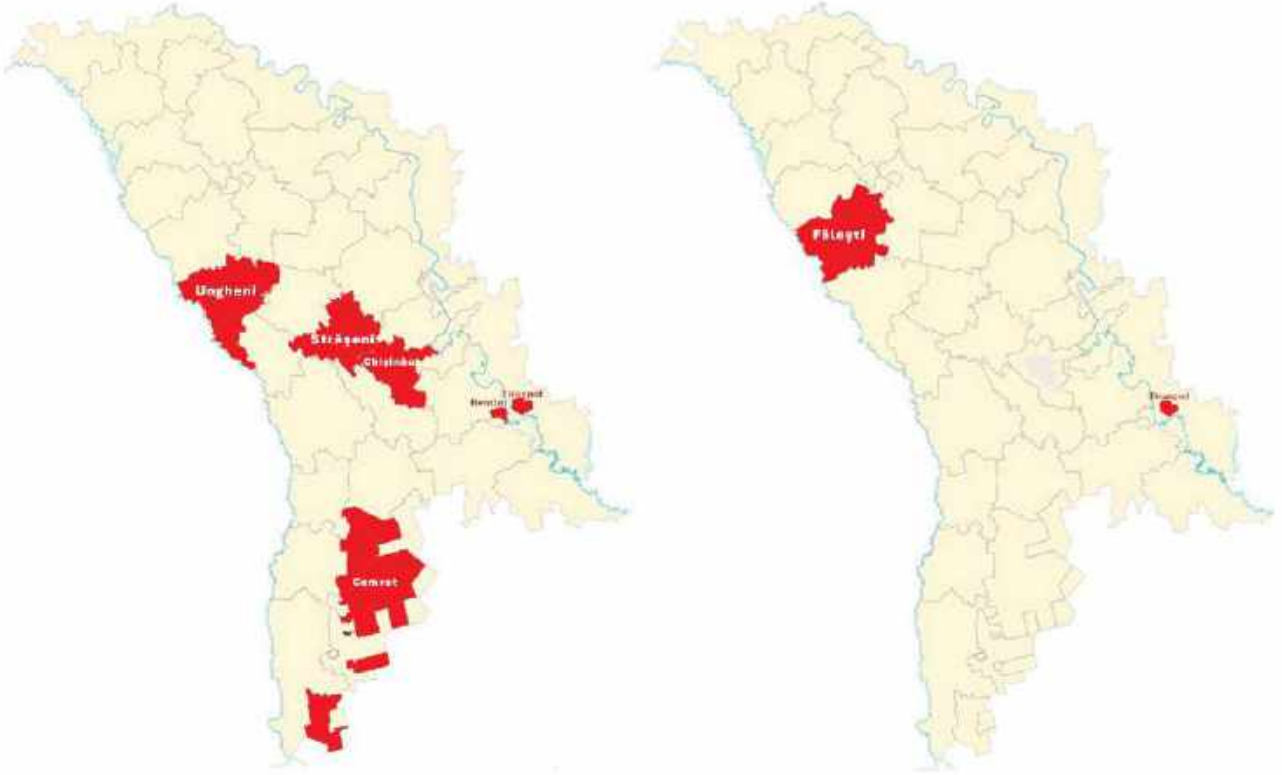
Overview of TBE in Moldova

Table 1: TBE in Moldova

Viral subtypes, distribution	Far Eastern subtype[2]
Reservoir animals	Information not available
Infected tick species (%)	*Dermacentor reticulatus* 3,9% (3/77)[2] *Ixodes ricinus* 3,8% (3/78)[2] *Haemaphysalis punctate* 8,8% (3/34)[2]
Dairy product transmission	Not documented
Completeness of case detection	Unknown
Type of reporting	Not Mandatory
Other TBE surveillance	Not applicable
Special clinical features	Monophasic (limited data) Risk groups (no data) Clinical manifestation (limited data)
Licensed vaccines	None
Vaccination recommendations	None
Vaccine uptake	Unknown
National Reference center for TBE	National Agency for Public Health, Chişinău, MD-2028, 67A Gh. Asachi st. https://ansp.md/

Figure 1: Regions of Moldova with TBEV detection in in ticks and location of 2 confirmed cases

Note: (Ungheni, Străşeni, Comrat - district, Chişinău municipality, Bender, Tiraspol - towns)



Acknowledgments

TBEV surveillance is supported by the National Public Health Agency (NAPH), Republic of Moldova and the *Microbiology Laboratory*, part of this agency. Information on collection and testing of ticks regarding human and non-human samples was provided by NAPH, *Microbiology Laboratory*.

Authors and affiliations

Olga Sofronie, MD, Microbiologist, Assistant Professor, *Nicolae Testemitanu State University of Medicine and Pharmacy of the Republic of Moldova, Microbiology and Immunology Discipline.*

Olga Burduniuc, MD, PhD, Head of Department of the *Laboratory Diagnosis in Public Health, National Agency for Public Health*

Greta Bălan, MD, PhD, Associate Professor, *Nicolae Testemiţanu State University of Medicine and Pharmacy of the Republic of Moldova, Microbiology and Immunology Discipline.*

Contact: olga.sofronie@gmail.com

Citation:

Sofronie O, Burduniuc O, Bălan G. TBE in Moldova. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi: 10.33442/26613980_13-21-7

References

1. Gheorghiţă S, Chicu V, Burlacu V, et al. Rolul căpuşelor Ixodes ricinus (Ixodidae) în menţinerea riscului de contractare a borreliozei Lyme în ecosistemele Republicii Moldova. *Curierul Medical*. 2012, 3(327):195-6.
2. Ponomareva EP, Mikryukova TP, Gori AV, et al. Detection of Far-Eastern subtype of tick-borne encephalitis viral RNA in ticks collected in the Republic of Moldova. *J Vector Borne Dis*. 2015; 52(4):334-6.
3. Sofronie O, Burlacu V. Tick-borne encephalitis virus – an emerging pathogen. *One Health and Risk Management*. 2023;(2023):44.

Chapter 13

TBE in Mongolia

Tserennorov Damdindorj, Uyanga Baasandagva, Uranshagai Narankhuu, Burmaa Khoroljav, Tsogbadrakh Nyamdorj, Burmaajav Badrakh

E-CDC risk status: endemic (data as of end 2023)

History and current situation

In Mongolia, tick-borne encephalitis virus was first isolated (Kraminskii V.A) from marmot liver in Dornod province in 1979 while the *Ixodes persulcatus* tick was identified in 1987 by M.Dash.[1,2] *Ixodes persulcatus* is a taiga tick distributed in coniferous forests consisting mostly of pines, spruces and larches.[3] Much of northern Mongolia is covered in coniferous forest and the southern edge of the Siberian taiga is located along the Khangai and Khentii mountains.

In the 1980s Mongolian scientists worked together with researchers from the Institute of Epidemiology and Microbiology of Irkutsk, Russia to investigate the spread of ticks carrying the TBEV in the forest areas of Khuvsgul, Khentii, Bulgan, Selenge, Orkhon, Central, Dornod, Arkhangai and Uvurkhangai provinces, which had been identified as TBEV-endemic regions.[4] Finally, in 1989, following available local information on diseases suspected to be TBE, Abmed et al. documented natural foci of the TBEV in the administrative districts of Zelter, Bugant and Khuder in the Selenge province and noted that it is important to plan and implement preventive measures.[5]

The physician of the Khuder district in the province of Selenge remembers that she had treated more than 400 patients with clinical signs of tick-borne encephalitis from 1993-2000. Five of them had died and had been recorded as, viral infections". This is the evidence to indicate that TBE was prevalent at that time.[6]

The Selenge province was found to carry the highest counts of *I. persulcatus* ticks frequently infected with the TBEV. *I.persulcatus* ticks were also found to be abundant in Bulgan, Tuv, Khuvsgul and Orkhon provinces of Mongolia.[1,7,10] Human cases of TBE have been officially registered at the national level since 2005.

During 2005-2023, 405 confirmed cases have been registered in Arkhangai, Bayankhongor, Bulgan, Darkhan-Uul, Dundgobi, Dornod, Orkhon, Uvurkhangai, Selenge, Tuv, Uvs, Khunsgul, Khentii, Bayan-Ulgii provinces and Ulaanbaatar city. Most patients remembered a tick bite to have occurred in the areas of Selenge (78%) and the Bulgan (12%) provinces. There were 21 fatal cases (CFR 4.85%) attributed to severe meningoencephalitis (Figure 1).

Since 2005, prevention measures such as vaccination, training and advocation among the population have been administered but human cases continue to registered. Between 2014-2017 the number of reported TBE cases and deaths increased annually, but it was decreasing in the last 5 years (2018-2022). In 2023, human morbidity increased 4.25 times compared to the previous year. TBE cases have been notified from areas without the main vector *I. persulcatus* and moreover the expansion of natural TBE-foci is observed.[8-12]

Most infections occurred among Individuals between 20–49 years of age, and it was 2.7–4.5 times higher than other age groups. Also, men more frequently contracted the disease (2.3, $p<0.001$) than women (Figure 2). The majority of subjects were bitten by ticks when they had been collecting plants and picnicking during May and June.[7]

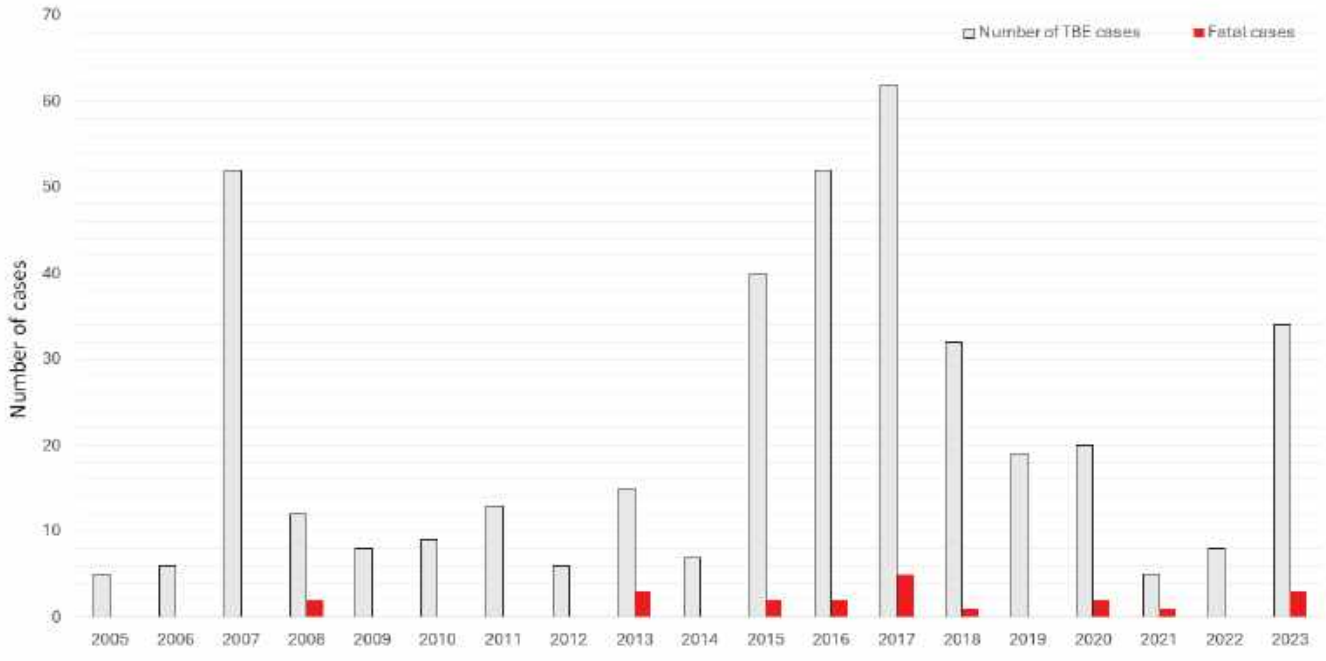
According survey of long-term neurological symptoms in TBE recovered people of Selenge province. In survey, 37 people who recovered TBE were participated. 16.1(5) % of fever form, 19.4 (6)% of paralysis form, 25.8 (8)% of meningoencephalitis and 38.7 (12)% of them meningitis form when they were ill. After recovery between one to twelve years, 78.4% of them having headache, 30-40% of them having fatigue, forgetfulness, decrease ability to concentrate and stiff neck, 10-20% of them hearing loss, paralysis, small percentage (3.2%) of them remained mental change, shoulder muscle atrophy, back muscle tone and muscle tremors convulsions.[24]

Vaccination against TBE has been consistently carried out since 2005 in the risk areas of the country.[13-15] A molecular biological study of TBEV was performed in collaboration with researchers from Germany and Russia and determined the prevalent viral subtypes by genetic sequencing.[7,15-20,22]

Overview of TBE in Mongolia

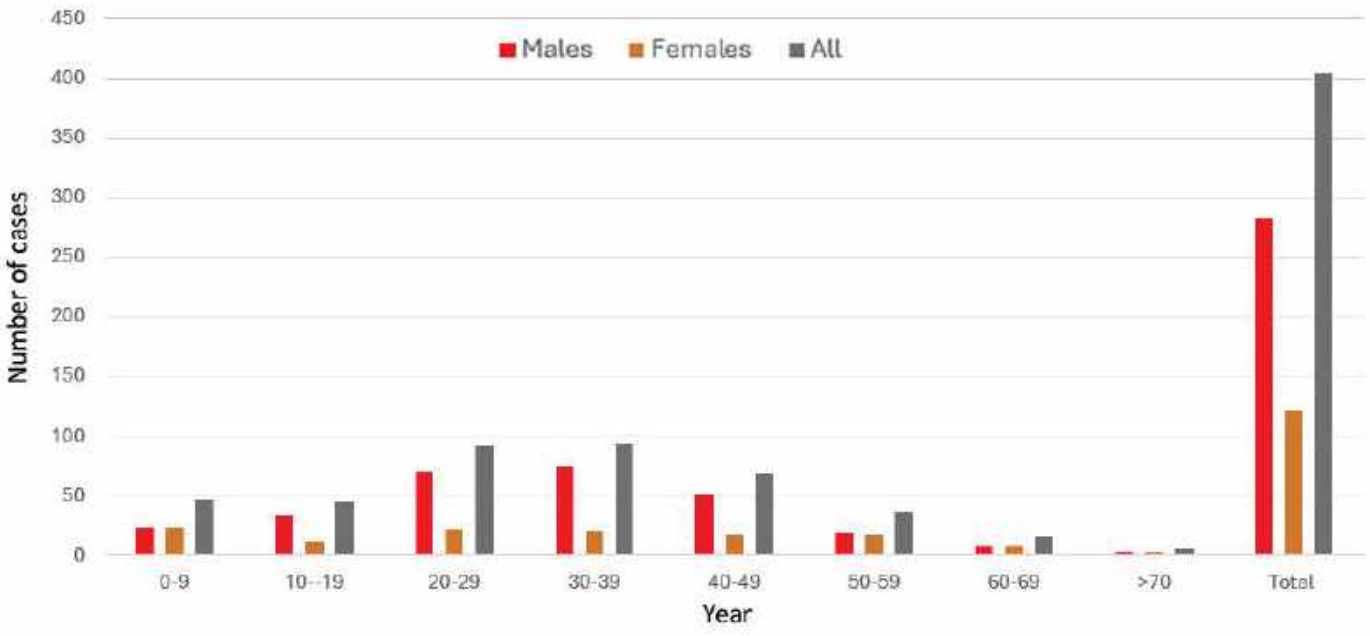
Table 1: TBE in Mongolia	
Viral subtypes, distribution[8,16-21]	Far Eastern subtype isolated from fatal cases Siberian subtype isolated from *I. persulcatus*
Reservoir animals	Not documented
Infected tick species (%)[7,8]	*I. persulcatus* (3.18 ± 2.5%) *D. silvarum* (2.9 ± 2.6%) *D. nuttalli* (0.6%)
Dairy product transmission	Not reported
Mandatory TBE reporting	Patients with clinical suspected TBE are reported to the National Center for Zoonotic Diseases (NCZD) where the diagnosis can be microbiologically confirmed (anti-TBEV-IgG and IgM by ELISA). Any patient with serologically confirmed TBE or by PCR is reported to the Center for Health Development and also to the Ministry of Health, Mongolia *(Source: http://hdc.gov.mn/)*
Other TBE surveillance	National Center for Zoonotic Diseases and its local branches (15 Centers for zoonotic diseases in provinces) are conducting TBE surveillance in ticks in the population of endemic areas.[4,6,9,10,11]
Special clinical features	Clinically, 37.7% of patients have fever only, 34.6% suffer from meningitis, 26.5% from meningoencephalitis and 1.2% from encephalomyelitis. By age, fever dominates in age groups 0–9 and 40–49 years, meningitis in the age groups of 10–39 and 50–59 years and meningoencephalitis in those >60 years.[7,11,12] In terms of age and sex, 20–49 year olds (65.6%) and males (69.3%) are the most affected groups. Among all affected males, those aged 10–49 years (81.8%) comprised the majority of male cases.[7,8] The overall CFR was 4.85% between 2005 and 2022 with an annual range between 3.1%–20%.
Available vaccines	Russian vaccine - EnceVir and TBE-Moscow.
Vaccination recommendations and reimbursement	Persons in a risk population of most endemic provinces can receive TBE vaccination free of personal charge. Vaccination is also recommended for anybody living in or visiting known endemic areas with a risk for tick bites. *(Source: The Order A160 on 21 April 2017 approved by the Minister of Health Annex 4: Guidelines for prevention and control of tick-borne diseases)*
Vaccine uptake by age group/ risk group/general population	TBE vaccination is organized since 2005. As of 2017, 51,000 persons from 13 provinces and the capital have been vaccinated, i.e., 2.1% of the total population. Vaccine uptake in endemic provinces ranges between 0.2%–23%.[13-15]
Name, address/website of TBE NRC	National Center for Zoonotic Diseases, Songinokhairkhan District, 20 khoroo, Ulaanbaatar, 18131, Mongolia *(Source: www.nczd.gov.mn)*

Figure 1: Reported TBE cases in Mongolia 2005-2023 (n=405)



Source data: Appendix - Figure 1

Figure 2: Age and gender distribution of TBE in Mongolia (2005–2023, n=405)



Source data: Appendix - Figure 2

Table 3: TBEV-isolation and TBE cases in Mongolia

Year of isolation	Strain name	Source of isolation	Location of isolation
2004[19]	Siberian	*I. persulcatus*	Selenge province
2008[16]	Far-Eastern	Patient brain	Bulgan province
2010[15]	Siberian	*I. persulcatus*	Bulgan province
2012[17]	Siberian	*I. persulcatus*	Selenge province
2013[17]	Siberian	*I. persulcatus*	Selenge province
2014[20]	Siberian	*I. persulcatus*	Selenge province
2020[22]	Far-Eastern	Patient brain	Bulgan province

57% of TBE cases (incidence 9.51/100,000) occurred in the forest-taiga range, 40% (incidence 0.56/100,000) in the forest-steppe range, 0.7% (incidence 0.12/100,000) in steppe range, and 2.8% (incidence 0.1–0.27/100,000) in other ranges, including steppe-desert, Gobi and high mountain (Figure 3).

According to the surveillance efforts since 2006, 10,464 ticks were collected. Following species identification, 14.7% (1,540) were classified as *Ixodes persulcatus*, 79.3% (8,300) were *Dermacenter nutalli*, 3.2% (341) were *Dermacenter silvarum*, and 2.8% (283) were *Hyalomma asiaticum*.[8]

I. persulcatus ticks were collected from 13 districts of Selenge, Bulgan, Orkhon, Darkhan-Uul, Khentii and Khuvsgul provinces. Most cases were found in Selenge (66%) and Bulgan (23%) provinces. The total tick infection rate was 3.18±2.5% and the highest infection rates were found in Bugat district of Bulgan Province (7.5%) and in the Mandal district (6.3%) and Khuder district (3.75%) of Selenge province.

D. nuttalli ticks were collected from 43 districts of 12 provinces and Ulaanbaatar city. The total tick infection rate for the entire country was 0.61% with the highest infection rates (3.3-7.8) in Khentii, Selenge, Arkhangai and Dornod province.

D. silvarum ticks were collected from Dornod and Khentii provinces and the tick infection rate was 2.9±2.6% (Figure 4).

Figure 3: Geographical distribution of TBE human cases

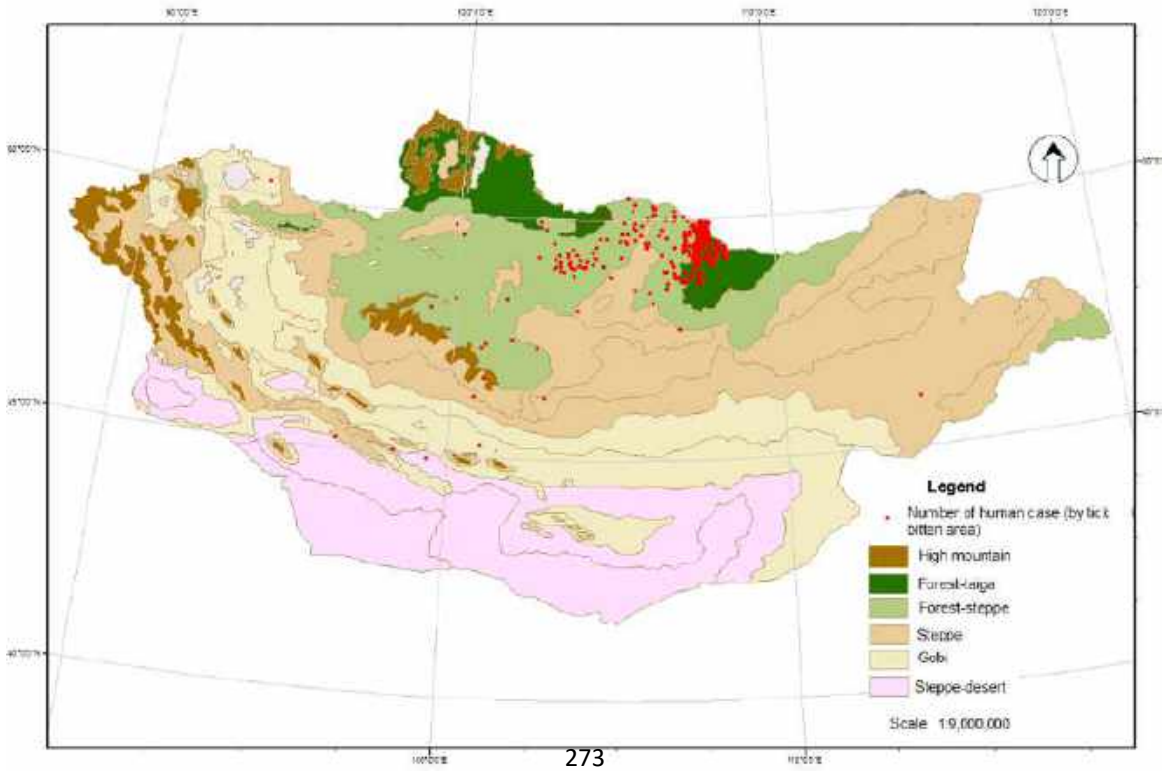
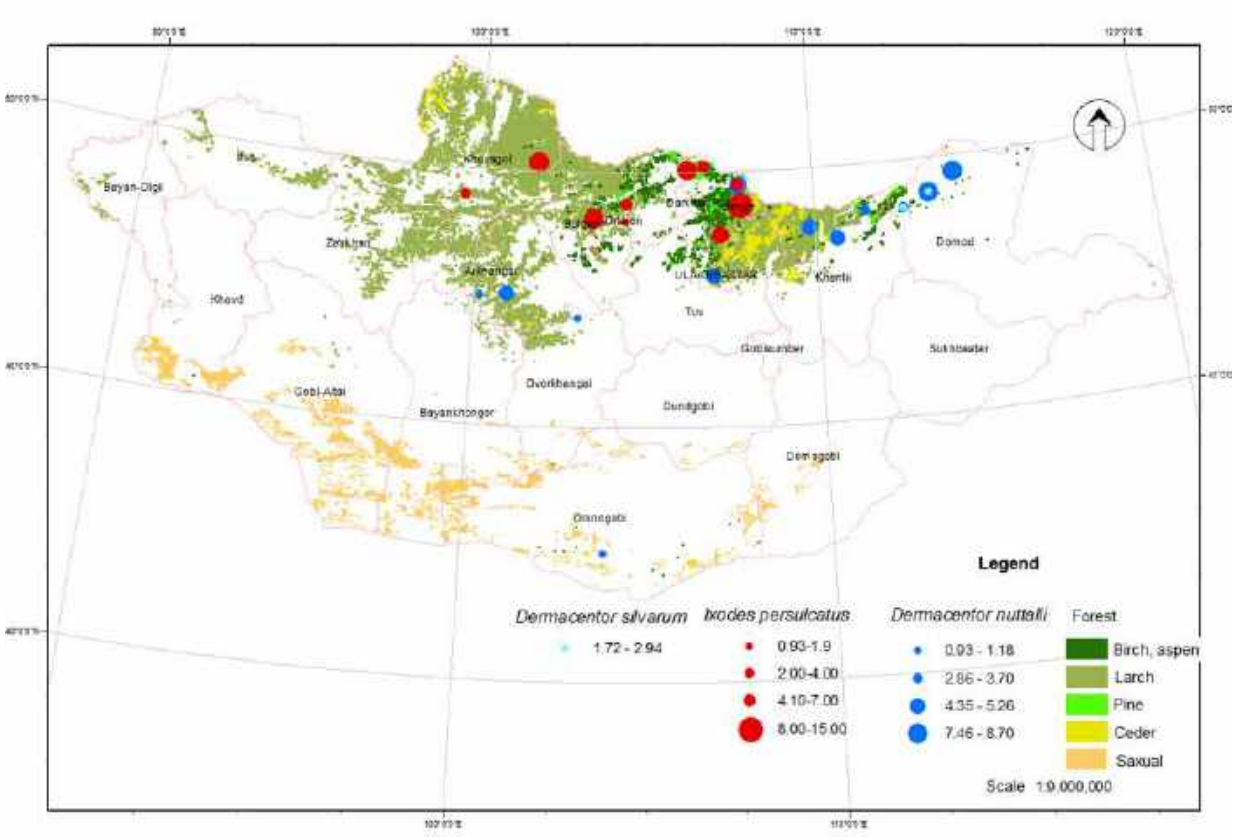


Figure 4: TBEV infection rate of three tick species



Appendix

Source data: Figure 1

Year	Number of cases	Fatal cases	Incidence/10^5
2005	5	0	0.21
2006	6	0	0.23
2007	52	0	2.06
2008	12	2	0.47
2009	8	0	0.3
2010	9	0	0.33
2011	13	0	0.46
2012	6	0	0.21
2013	15	3	0.5
2014	7	0	0.23
2015	40	2	1.33
2016	52	2	1.8
2017	62	5	2.0
2018	32	1	0.97
2019	19	0	0.57
2020	20	2	0.60
2021	5	1	0.15
2022	8	0	0.23
2023	34	3	0.98

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	24	23	47
10-19	34	11	45
20-29	70	22	92
30-39	74	20	94
40-49	51	17	68
50-59	19	18	37
60-69	8	8	16
≥70	3	3	6
Total	283	122	405

Contact: dnorov09@gmail.com

Citation:

Damdindorj T, Baasandagva U, Narankhuu U, Nyamdorj T, Badrakh B, Khoroljav B. TBE in Mongolia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-22-7

References

1. Bataa J, Abmed D. Tick-borne diseases Handbook. Ulaanbaatar, Mongolia. 2007:20-25.
2. Dash M, Byambaa B, Trasevich IV. Handbook of New rickettsial diseases. Ulaanbaatar, Mongolia: Ma: Esun Erdene press; 1994:9.
3. Wikipedia contributors. *Ixodes persulcatus*. Wikipedia, The Free Encyclopedia. April 10, 2021, at 21:59 UTC. Available at: https://en.wikipedia.org/wiki/Ixodes_persulcatus. Accessed June 1, 2016
4. Abmed D, Bataa J, Tserennorov N, et al. Natural foci of transmissible tick-borne infections in northern and central Mongolia. Actual aspects of natural focal diseases. Materials of the interregional scientific-practical conference, Omsk. 2001:23.
5. Abmed D, Ganbold D, Andreev VN, Lvov S, Dmitriev DB. The study of tick-borne encephalitis in Selenge aimag (province). *The Center for Research of infectious diseases with natural foci, research book.* 1990;(6):67-69.
6. Veteran doctor D. Renchenkhand's memoirs. *Mongolian Journal of infectious disease research*. 2018;3(80):74-75.
7. Uyanga B. Epidemiological characteristics and prevalence of tick-borne encephalitis in Mongolia 2005-2017. *Dissertation*. 2019.
8. Uyanga B, Burmaajav B, Tserennorov D, et al. Geographical distribution of Tick-borne encephalitis and its vector in Mongolia, 2005-2016. *Central Asian Journal of medical sciences.* 2017;3(3):250-258.
9. Uyanga B, Tserennorov D, Badrakh B, Damdin O, Baatar U, Tsogbadrakh N. The becoming importance species of *Dermacentor.spp* tick in tick-borne encephalitis of Mongolia. 16th Medical Biodefense Conference Munich, 28—31 October, 2018 organized by Bundeswehr Institute of Microbiology ABSTRACTS –P. 111
10. Uyanga B, Burmaajav B, Tserennorov D, Undraa B. Epidemiological features of tick-borne encephalitis registered in Mongolia, 2005-2016. *Mongolian Journal of infectious disease research.* 2017;5(76):36-41.
11. Uyanga B, Tserennnorov D, Purevdulam L, Tsogbadrakh N. Tick -borne encephalitis in Mongolia. *Mong J Infect Dis Res.* 2015;5 (64):13-34.
12. Tserennorov D, Uyanga B, Battsetseg J, Bayar C, Baigalmaa B, Njamsuren M. Results of a study of tick-borne encephalitis and analysis of human cases in Mongolia. *Far Eastern Journal of Infectious Pathology (Medical Scientific Review Journal).* 2014;25:36-39.
13. Uyanga B, Unursaikhan U, Undraa B, Tsogtsaikhan S, Davaalkham D. Epidemiological characteristics of tick-borne encephalitis and vaccination results. *J Infect Pathol.* 2012;19 (3):114
14. Uyanga B, Adiyasuren Z, Tsogtsaikhan S, Davaalkham D. Results of tick-borne encephalitis vaccination. *J Mong Med Sci*. 2010;3(153):64-70.
15. Bataa J, Abmed D, Tsend N, et al. Results of immunization against tick-borne encephalitis in Mongolia. *Biotechnical research, production and use.* 2004:20-24.
16. Frey S, Mossbrugger I, Altantuul D, et al. Isolation, preliminary characterization, and full-genome analyses of tick-borne encephalitis virus from Mongolia. *Virus Genes.* 2012;45(3):413-425
17. Khasnatinov MA, Danchinova GA, Kulakova NV, et al. Genetic characteristics of the causative agent of tick-borne encephalitis in Mongolia. *Vopr Virusol.* 2010;55(3):27-32.
18. Tserennorov D, Höper D, Binder K, et al. Epidemiological and Molecular Biological Characterization of TBEV in Mongolia. 15th Medical Biodefence Conference, Munich, Germany. 2016:30-31
19. Tserennorov D, Uyanga B, et al. Study of Tick-borne Encephalitis Virus in Mongolia. 14th Medical Biodefence Conference, Munich, Germany. 2013:29-30
20. Abmed D, Khasnatinov M, Bataa J, et al. Molecular, epidemiological, ecological study of tick-borne encephalitis virus in Mongolia. *Mong J Infect Dis Res*. 2005;4(7):22-5.
21. Erdenechimeg D, Boldbaatar B, Enhmandakh Y, Myagmarsukh Y, Oyunnomin N, Purevtseren B. Identification of the Siberian type of the tick-borne encephalitis virus and serological surveillance in Mongolia. *Mong J Agric Sci.* 2014;13(2):19-26.
22. Walder G, Lkhamsuren E, Shagdar A, et al. Serological evidence for tick-borne encephalitis, borreliosis, and human granulocytic anaplasmosis in Mongolia. *Int J Med Microbiol.* 2006;296 Suppl 40:69-75.
23. Uyanga B, Burmaajav B, Natsagdorj B, et al. A case series of fatal meningoencephalitis in Mongolia: epidemiological and molecular characteristics of tick-borne encephalitis virus. *Western Pacific Surveillance and Response Journal.* 2019;10 (1):1-7. doi: 10.5365/wpsar.2018.9.1.003
24. Uyanga B, Oyun B, Oyunchimeg S, Ganzorig G, Rolomjav L, Erdenebat N, et al. Long-term neurological outcome of tick-borne encephalitis in Mongolia. *Mong J Infect Dis Res*. 2021;4 (99):78/28

Chapter 13

TBE in the Netherlands

Johannes H.J. Reimerink, Hein Sprong, Margriet Harms and Chantal B.E.M. Reusken

E-CDC risk status: endemic (data as of end 2023)

History and current situation

Until 2015, tick-borne encephalitis virus (TBEV) was presumed not to be endemic in the Netherlands.[1,2] Consequently, the number of diagnostic requests for detection of tick-borne encephalitis (TBE) infection has been low. Between 2006 and 2015, the laboratory of the Netherlands Centre for Infectious Disease Control (CIb), 1 of the 2 laboratories that performed TBEV diagnostics in the Netherlands at the time, received an average of 20 (range 12–27) requests for TBEV diagnostics per year. In the same period, TBE was diagnosed in 7 Dutch patients. All cases were considered to be travel-related. Indeed, 6 out of 7 patients reported that they had recently travelled to TBEV-endemic countries such as Austria (4), Germany (1), and Sweden (1).

In 2015, however, six of 297 (2%) roe deer sera, collected in 2010, were found serologically positive for TBEV-infection.[2,3] Five of 6 sera were collected at the national park 'Sallandse heuvelrug' in the province of Overijssel, in the east of the Netherlands. The other TBEV-positive roe deer serum was collected in the south of the Netherlands, in the province of Noord-Brabant. Based on these findings, *I. ricinus* ticks were collected for screening for the presence of TBEV at the 'Sallandse heuvelrug' in 2015. From the approximately 1,460 ticks collected in 2015, one pool of nymphs (0.09%) and one pool of female adult ticks (0.33%) were RT-PCR-positive for TBEV.[3,4] Sequencing of the viral genome revealed that the virus grouped with the European (Western) subtype but was genetically distinct from all known Western European TBEV strains. Based on the near complete genome, the 'Salland' strain diverged from currently known TBEV-Eu strains by 9% on nucleotide and 2% on amino acid level, respectively.

In 2016, soon after the Netherlands Centre for Infectious Disease Control raised general awareness of the presence of TBEV in the Netherlands, the first 2 autochthonous TBE cases were reported.[5,6] Both patients were positive for TBEV-specific antibodies by ELISA and virus neutralization test. The first patient most likely acquired TBEV when hiking at the national park 'Utrechtse Heuvelrug'[2,5] located in the center of the Netherlands (Figure 3). A tick collected from this patient was RT-PCR-positive for TBEV RNA. Interestingly, the virus strain from this tick was genetically similar to known Western European TBEV strains and differed considerably from the Salland strain (9% on nucleotide level, 2% on amino acid level). The second patient lived near the national park Sallandse heuvelrug and frequently visited this park. Moreover, twenty additional autochthonous human cases have been reported since (till December 2023). From the five autochthonous cases reported in 2023, three patients were from two of the endemic regions: Salland-region and the island of Terschelling. The other patients were reported outside the known TBEV loci: one in the province of Gelderland (Ermelo), and one in Noord-Holland (Bloemendaal) (Figure 3). The presence of the TBEV on Terschelling and in Bloemendaal could be confirmed by the detection of viral RNA in questing ticks. Additionally, three travel-associated TBEV infections were diagnosed in 2023 and most probably infected in Italy, Sweden and Austria.

The number of laboratories implementing routine TBEV diagnostics stagnates at five with virus neutralization tests implemented at the two National Reference laboratories for arboviruses. Despite the general availability of routine diagnostics in the Netherlands the number of diagnosed cases is still low.

A One-Health approach is conducted in The Netherlands, where ticks will be collected and tested from locations outside endemic areas, where TBE-patients were when they contracted a tick bite. With this approach we could confirm the presence of the virus near Bloemendaal and on Terschelling. Phylogenetic analyses indicates that at least 5 different variants of the TBEV-Eu subtype circulate in the Netherlands, suggesting multiple independent introductions. Combined with data on human cases and from roe deer, our impression is that the distribution of TBEV in the Netherlands is more widespread than previously thought.[11]

As it is not mandatory to report TBEV in the Netherlands,[8] the exact number of requests for TBEV diagnostics and confirmed cases per year is currently not available.

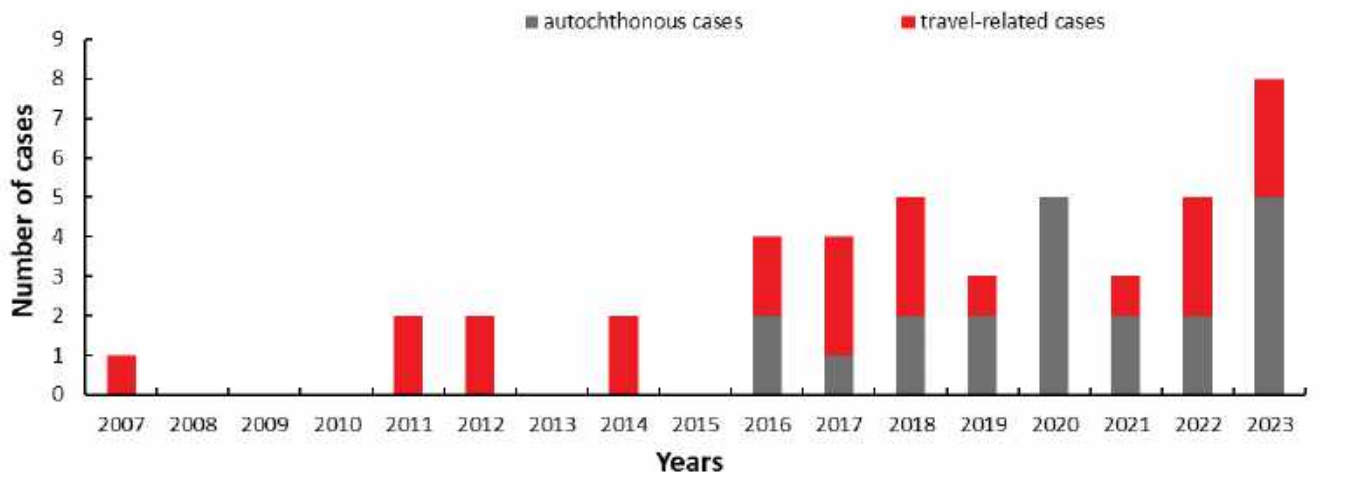
In summary, in 2016, the first autochthonous TBE cases were reported in the Netherlands. Since then autochthonous cases have been recognized mainly in or close to the two known foci of presence. In 2020 we saw three TBE cases outside the known endemic regions which might be indicative of an expanding presence. However,

TBEV was likely already present in these areas before 2020 according to the roe dear seroprevalence study in 2017. Awareness for TBEV is increasing in the Netherlands as reflected in the increasing number of labs that implemented diagnostics and the increase in requests for TBEV diagnostics at the CIb. Two different Western European TBEV strains have been detected in the Netherlands. Based on the fact that two autochthonous cases were infected near national park 'Sallandse heuvelrug', it is highly likely that the divergent 'Salland' strain found in this area can cause disease in humans, but this remains to be confirmed.

Overview of TBE in the Netherlands

Table 1: TBE in the Netherlands	
Viral subtypes, distribution	TBEV-EU (Utrechtse Heuvelrug)[5,6] TBEV-EU "Salland" (Sallandse Heuvelrug)[3]
Reservoir animals	Unknown (Roe deer were found to be sentinels and are likely dead-end hosts)[3]
Infected tick species (%)	*I. ricinus*[3-5]
Dairy product transmission	No information available
Mandatory TBE reporting	It is not mandatory to report TBE in the Netherlands[8]
Other TBE surveillance	-
Special clinical features	No information available
Available vaccines	FSME-Immun® and FSME-Immun® Junior[8]
Vaccination recommendations and reimbursement	Upon travel to TBEV-endemic areas vaccination can be considered[8]
Vaccine uptake	No information available
Name, address/website of TBE NRC	-

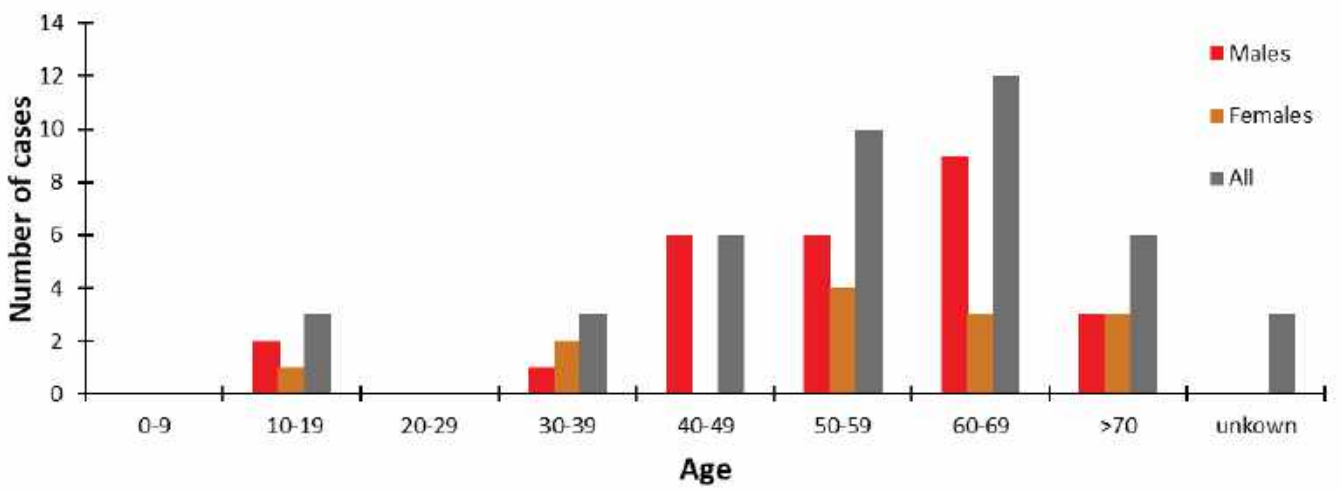
Figure 1: Burden of TBE in the Netherlands over time



Due to the low numbers of diagnostic requests and diagnosed infections, a reliable number for the incidence is difficult to provide.

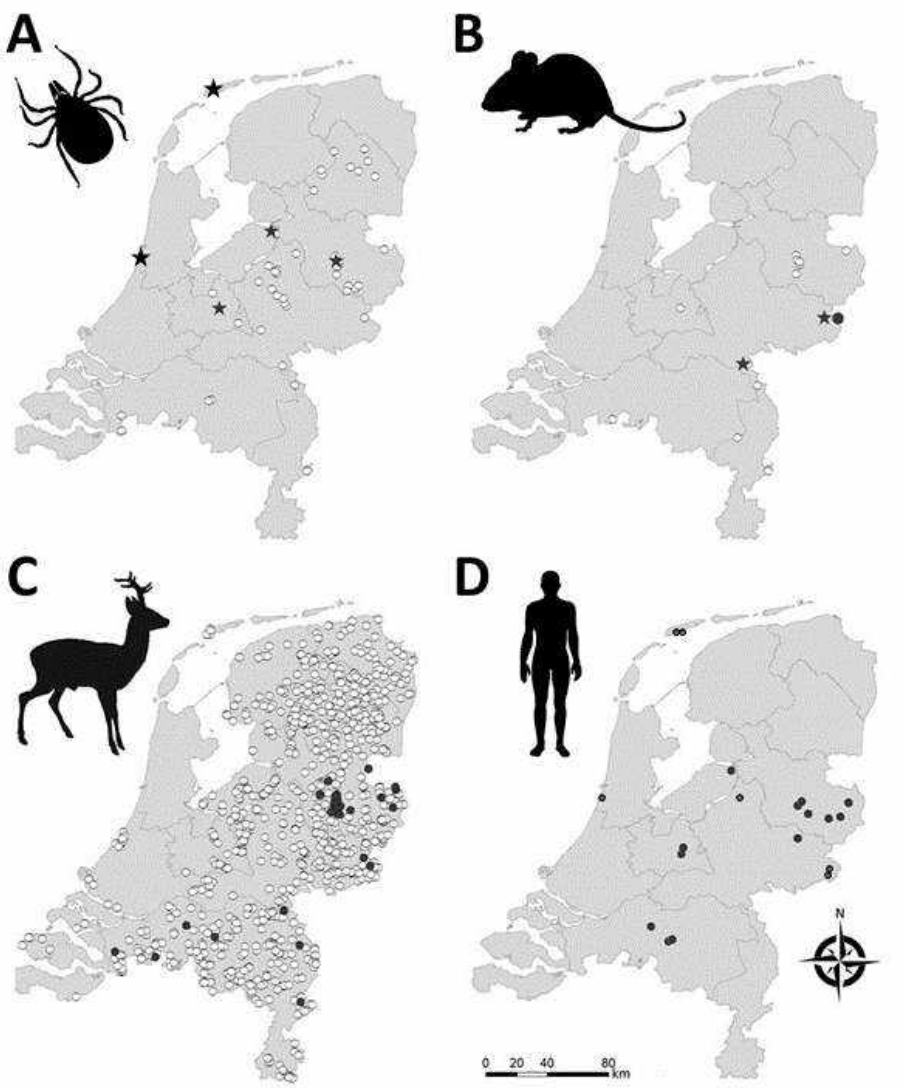
Source Data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE in the Netherlands



Source Data: Appendix—Figure 2

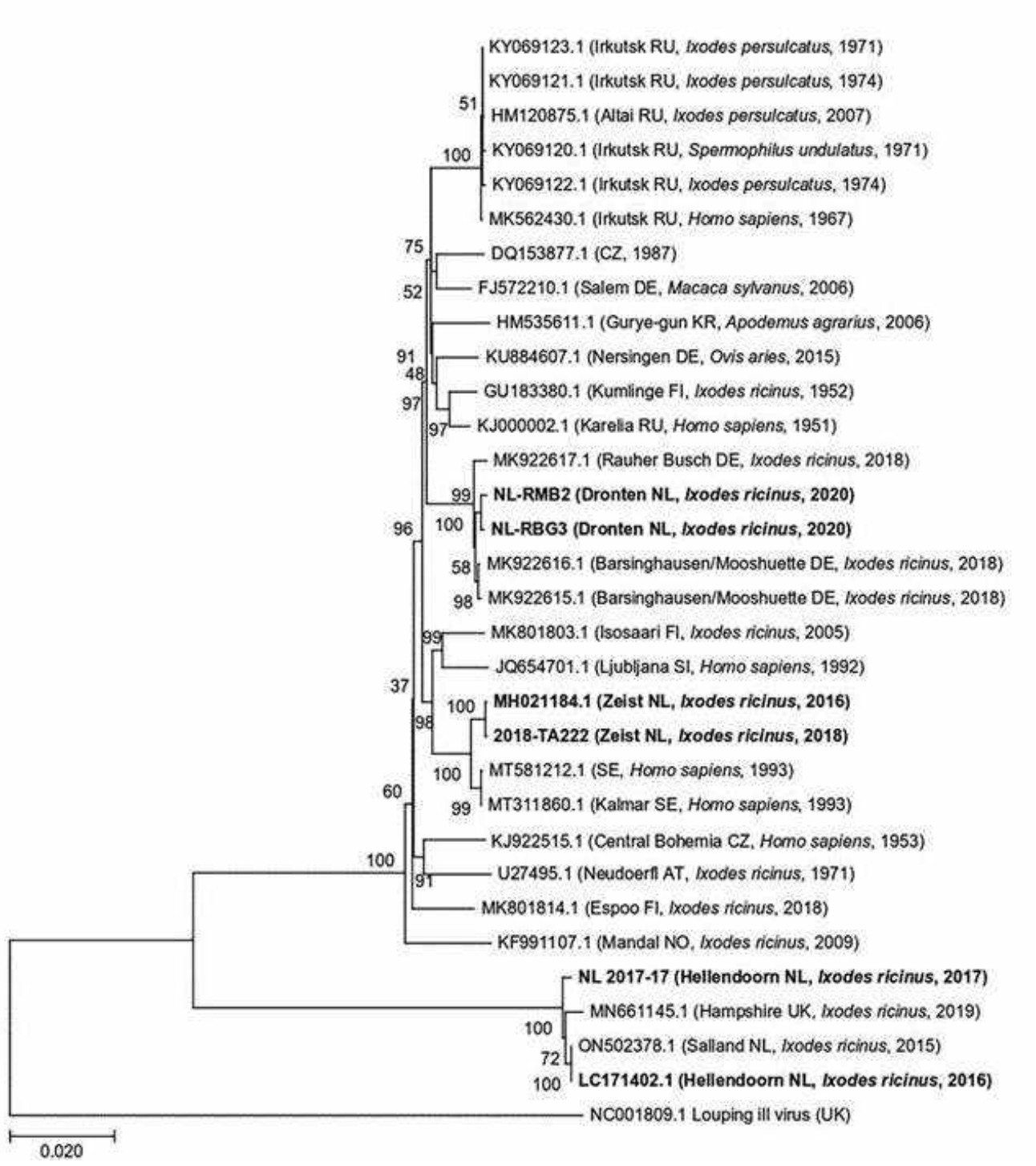
Figure 3



Geographic distribution of tick-borne encephalitis virus (TBEV) in the Netherlands based on sampling of ticks (A), rodents (B), roe deer (C), and reported human (D) tick-borne encephalitis cases. Stars indicate TBEV RNA–positive tick pools or rodent samples.

Closed circles indicate serum samples that tested positive in TBEV serum neutralization tests. White circles indicate negative test results. Figure is partially based on (Esser et al.)[11]

Figure 4



Maximum-likelihood phylogenetic tree of polyprotein sequences obtained from tick-borne encephalitis virus RNA–positive Ixodes ricinus ticks collected from 3 locations in the Netherlands during 2016–2020 (in bold). Additional published sequences obtained from GenBank are included for reference. Louping ill virus is used as the outgroup. Sample ID or GenBank accession numbers are indicated for each sequence, with location in brackets (if known) and country code, original isolation source, and collection year of each sample. Numbers next to each branch indicate the percentage of trees resulting from bootstrapping on the basis of 1,000 pseudoreplicate datasets for which the associated taxa clustered together. Scale bar represents the percentage of genetic variation along tree branches.

Appendix

Source data: Figure 1

Year	Number of cases
2007	0 (+1 travel-related)
2008	0
2009	0
2010	0
2011	0 (+2 travel-related)
2012	0 (+2 travel-related)
2013	0
2014	0 (+2 travel-related)
2015	0
2016	2 (+2 travel-related)
2017	1 (+3 travel-related)
2018	2 (+3 travel-related)
2019	2 (+1 travel-related)
2020	5 (0 travel-related)
2021	2 (+1 travel-related)
2022	2 (+3 travel-related)
2023	5 (+3 travel-related)

Source data: Figure 2

Age group (years)	Males	Females	All
0–9	0	0	0
10–19	2	1	3
20–29	0	0	0
30–39	1	2	3
40–49	6	0	6
50–59	6	4	10
60–69	9	3	12
>70	3	3	6
Unknown			3

Contact: johan.reimerink@rivm.nl

Citation:
Johan H.J. Reimerink, Hein Sprong, Margriet Harms and Chantal B.E.M. Reusken. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-23-7

References

1. Reusken C, Reimerink J, Verduin C, Sabbe L, Cleton N, Koopmans M. Case report: tick-borne encephalitis in two Dutch travellers returning from Austria, Netherlands, July and August 2011. *Euro Surveill.* 2011;16(44).
2. Schimmer B, Reimerink JH, Hira V, et al. First autochthonous cases of tick-borne encephalitis detected in the Netherlands, July 2016.
3. Jahfari S, de Vries A, Rijks JM, et al. Tick-Borne Encephalitis Virus in Ticks and Roe Deer, the Netherlands. *Emerg Infect Dis*. 2017;23(6):1028-1030. doi:10.3201/eid2306.161247
4. [Tick-borne encephalitis virus found in Dutch ticks]. Signaleringsoverleg 26/2016. Bilthoven: *Rijksinstituut voor volksgezondheid en milieu*; 2016.
5. de Graaf JA, Reimerink JH, Voorn GP, et al. First human case of tick-borne encephalitis virus infection acquired in the Netherlands, July 2016 [published correction appears in Euro Surveill. 2016 Aug 25;21(34):]. *Euro Surveill*. 2016;21 (33):30318. doi:10.2807/1560-7917.ES.2016.21.33.30318
6. Hira V, de Graaf JA, Rockx B; authors of the original article. Author's reply: The first tick-borne encephalitis case in the Netherlands: reflections and a note of caution. *Euro Surveill.* 2016;21(39):30356. doi:10.2807/1560-7917.ES.2016.21.39.30356
7. Rijks JM, Montizaan MGE, Bakker N, de Vries A, Van Gucht S, Swaan C, et al. Tick-borne Encephalitis Virus Antibodies in Roe Deer, the Netherlands. *Emerg Infect Dis*. 2019;25(2). doi:10.3201/eid2502.181386
8. Dekker M, Laverman GD, de Vries A, Reimerink J, Geeraedts F. Emergence of tick-borne encephalitis (TBE) in the Netherlands. *Ticks Tick Borne Dis*. 2019 Jan;10(1):176-179. doi:10.1016/j.ttbdis.2018.10.008
9. Geeraedts F, van der Kroft E, Reimerink J. First paediatric case of autochthonous tick-borne encephalitis in the Netherlands, 2018. *New Microbes New Infect.* 2019;32:100603. Published 2019 Sep 19. doi:10.1016/j.nmni.2019.100603
10. LCI-richtlijn Tekenencephalitis: RIVM; 2016. Accessed 17 March, 2024. http://www.rivm.nl/Documenten_en_publicaties/Professioneel_Praktisch/Richtlijnen/Infectieziekten/LCI_richtlijnen/LCI_richtlijn_Tekenencefalitis.
11. Esser HJ, Lim SM, de Vries A, et al. Continued Circulation of Tick-Borne Encephalitis Virus Variants and Detection of Novel Transmission Foci, the Netherlands. *Emerg Infect Dis*. 2022;28 (12):2416-2424. doi:10.3201/eid2812.220552

Chapter 13

TBE in Norway

Rose Vikse, Urusha Maharjan, Arnulf Soleng, Heidi H. Lindstedt, Rikard Rykkvin, Kristian Alfsnes and Åshild K. Andreassen

E-CDC risk status: endemic (data as of end 2023)

History and current situation

In Norway, tick-borne encephalitis (TBE) has been a mandatory notifiable disease since 1975 (Norwegian Surveillance system for communicable diseases, MSIS).[1] According to ECDCs classification, coastal areas in southern Norway (counties of Agder, Vestfold and Telemark) are endemic for TBE. Further, the counties of Østfold, Akershus and Buskerud, and western and northern Norway to Brønnøy municipality are imperiled.[2-9]

The first reported case of TBE occurred in 1997 at Tromøy in Agder County.[10] This is a region with holiday cabins and outdoor recreation areas for both local inhabitants and tourists, and it is known for high temperatures during spring and summer. In addition, TBE antibodies in dogs and tick-borne encephalitis virus (TBEV) in ticks have been detected in this area.[8,10-13]

A total number of 519 TBE cases have been reported to MSIS as of February 2024 (Fig. 1). Of these, 420 cases are autochthonous infections, while 99 cases were infected abroad or have an unknown infection history. The number of cases varies annually between 1 and 86 (Table 1 and Fig. 1). Data for 2018 to 2023 shows an increase in the number of cases, especially in the counties of Vestfold and Telemark (Fig. 5, MSIS, February 2023). In 2023 the first case from Vestland County was reported to MSIS. The TBE patients' age distribution is in accordance with other European studies, with a higher infection rate for those older than 30 years (Table 2 and Fig. 2).[14-15] According to MSIS, the reported cases in Norway are represented by the counties of Agder, Vestfold and Telemark, and Viken, all located in the southern part of the country (Fig. 3). No cases are reported from the northern coastal areas but a few cases are reported from the western areas and the area east of the Oslofjord, even though outdoor recreation activities are common in the whole country.

Ticks and TBEV in Norway

The castor bean tick (*Ixodes ricinus*) is the most common tick species in Europe,[16] and considered to be the major vector of the European TBE-virus.[17-18] The geographical distribution of *I. ricinus* in Norway has been investigated in several studies.[2,19-23] Both Tambs-Lyche (1943) and Mehl (1983) found *I. ricinus* to be mainly distributed in the coastal areas of Norway, from the southeastern border to Sweden, along the southern and western coastline, up to Nordland County at ~65.1°N.[19-20] The density of ticks varies between locations, even when separated by short distances. This is probably caused by differences in microclimatic conditions, vegetation, and density of vertebrate hosts. However, locations with a high density of ticks are found all over the major distributional range. The density of ticks declines rapidly with both increasing distance from the coast and increasing altitude. In a multi-source study, Jore et al. (2011) suggested that tick populations in Norway had undergone recent shifts in latitudinal and altitudinal range.[24] This result is, however, disputed in recent studies.[2,21]

Although ticks are reported far outside (i.e. northeast) of the hitherto established distribution limit of *I. ricinus* in Norway, the vast majority of these are engorged females.[22-23] Migratory birds may deposit engorged larvae or nymphs in areas where temperatures permit development to the next stage but not completion of the life cycle. Thus, such records do not constitute evidence for established and sustainable tick populations as this requires the presence of all the active stages (larvae, nymphs, and adults) in a locality for at least two consecutive seasons.[25-26] Using flagging and dragging, Soleng et al. (2018) found tick larvae, nymphs and adults to be abundant at 64.5 and 65.1°N. Only a few tick nymphs and adults, and no larvae, were found at locations close to 66°N. At several locations from 66.3°N up to 67.5°N no ticks were found.[2] In a recent study by Hvidsten et al. (2020), the occurrence of ticks in northern Norway was examined by dragging in 109 separate locations between the latitudes of 64°N and 70°N. The northernmost location with a permanent *I. ricinus* population was at 66.2°N on the Island of Dønna (Fig. 4).[21] It is noteworthy that the taiga tick (*Ixodes persulcatus*) and the meadow tick (*Dermacentor reticulatus*) were not detected in a large screening of ticks collected in the southern part of Norway in 2016.[27]

Studies of *I. ricinus* in Norway have detected TBEV in nymphs with prevalence ranging from 0% to 1.1%. In adult ticks collected from the same areas, the prevalence ranges from 0% to 20.6%. TBEV positive ticks have been found in sampling areas along the Norwegian coastline from the east of Østfold County to Brønnøy in Nordland County.[6] The highest estimated TBEV prevalence in adult ticks has been found in the counties of Rogaland and Vestfold and

Telemark. In nymphs, the highest prevalence has been found in Vestfold, Telemark, Agder and Rogaland.[6]

Historically, the first suggested TBEV isolate from Norway was collected in *I. ricinus* from Vestland County (former Sogn and Fjordane) in June 1976 as described by Traavik and co-workers. Five virus strains with a close serological relationship to the TBEV complex were detected in this study.[28]

One pool of ten nymphs collected from southern Norway has been whole-genome sequenced and phylogenetically characterized. The strain, "Mandal 2009", was found to belong to the Scandinavian group of the European TBEV subtype. Interestingly, "Mandal 2009" revealed a shorter form of the TBEV genome within the 3' non-coding region, like the highly virulent "Hypr" strain.[29] Recent unpublished findings indicate circulation of at least one new TBEV variant in Norway from two new areas. This variant in the TBEV sequence is detected in a tick and one patient sample, both different from the previous Mandal 2009 strain.

Seroprevalence in animals

In addition to tick studies, a seroprevalence study has detected TBE antibodies in specimens from cervids (deer) collected in Farsund (Agder County) and Molde (Møre and Romsdal County). In Farsund, located on the southern coast of Norway, 41% (22 of 54 animals) were TBE-positive. This contrasts with Molde, situated midwest, where the prevalence was 1.6% (1 of 64 animals). The same study detected antibodies to Louping ill virus (LIV), a closely related flavivirus, in 14.8% (8 of 54) of the analyzed cervid sera from Farsund.[30]

A recent seroprevalence study of cervids where serum samples were collected across Norway found TBEV antibodies in the municipalities of Steinkjer, Vindafjord, Søgne, Birkenes, Lardal, Larvik and Halden (Fig. 4). The overall seroprevalence was 4.6%. Antibodies against TBEV detected by serum neutralization test were present in 9.4% of the moose samples, 1.4% in red deer, 0.7% in roe deer, and 0% in reindeer.[4]

Ticks (6850 nymphs and 765 adults) from eastern, western, and northern Norway were analyzed for LIV using an in-house real-time polymerase chain reaction (PCR), none of these were positive (unpublished data). However, a recent study by Ytrehus et al. detected antibodies against LIV in willow ptarmigan (*Lagopus lagopus lagopus*) across the whole country. The study suggested that either LIV or a cross- reacting virus infects ptarmigan in Norway, also at high altitudes and latitudes.[31]

There is limited knowledge of TBEV in domestic animals in Norway. A recent study reported TBEV RNA in unpasteurized cow milk from three farms located in southern and northern Norway in 5.4% of the tested animals. Seropositive animals were only detected at one farm in southern Norway, in 88.2% of the tested animals.[5] This is higher than in a previous study by Traavik (1973), where a seroprevalence of 17.7% was detected in bovine sera in western Norway.[32]

Seroprevalence in humans

In Søgne municipality, a TBE endemic area of southern Norway, a TBEV seroprevalence of 3.1% (45/1,453) was found in the general adult population. Among individuals not vaccinated against TBEV and/or yellow fever, the seroprevalence of IgG antibodies to TBEV was 1.4% (6/419).[33] A recent blood donor study from TBE endemic areas in Vestfold and Telemark found a low seroprevalence of 0.4% (4/1,123). Out of the 1,123 analyzed samples, 21 had neutralizing antibodies to TBEV, of which 17 reported a previous TBE vaccination.[34]

Three seroprevalence studies in humans from presumed non- endemic areas have been published. Larsen et al. detected TBE immunoglobulin G (IgG) antibodies among 0.65% of blood donors in Viken County (former Østfold) in southeastern Norway.[9] The second study in 1,213 blood donors was performed in Vestland County (former Sogn and Fjordane), located in western Norway. TBE IgG antibodies (ELISA) were detected in five (0.4%) of these samples. However, four of these were reported to be vaccinated against flaviviruses and one was negative by neutralization test.[35] In 1979, Traavik detected a 19.6% seroprevalence from Vestland County. However, these results were not confirmed with a neutralization test and thus, may be explained by cross- reactions to LIV, vaccine-related flaviviruses, or nonspecific binding in the test.[36]

TBEV in ticks in Norway is widely distributed (Fig. 4). It has been a puzzle why there have been no reports of patients outside the endemic areas. However, this seems to undergo a change with increasing incidence and the geographical expansion of cases towards north and east as illustrated (Appendix Fig. 1; Fig. 5).

Conclusion

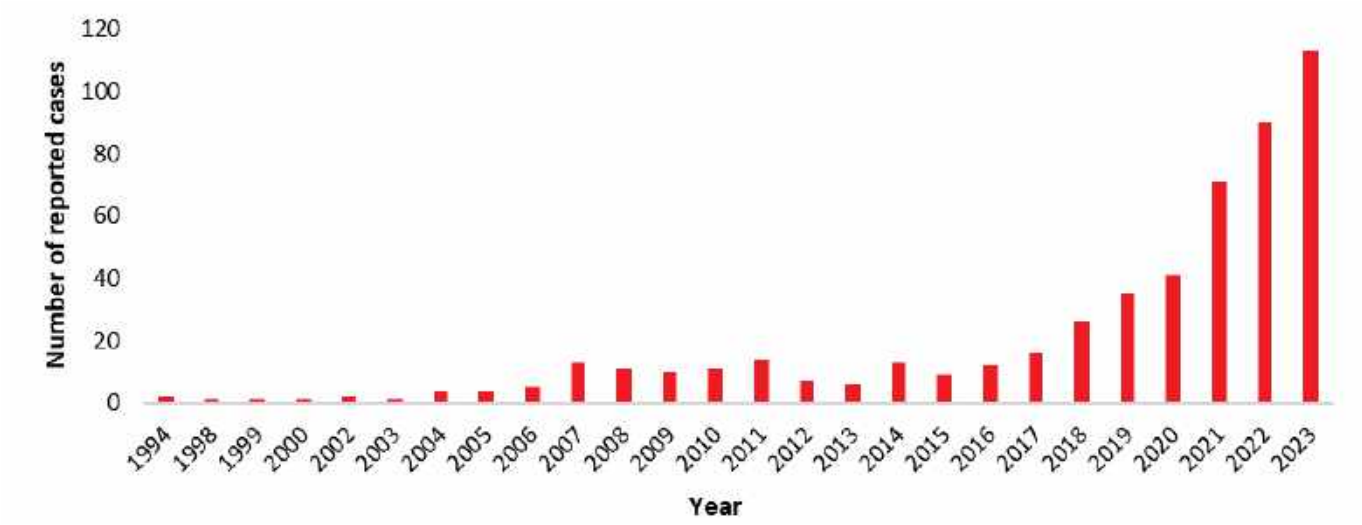
In summary, TBE is endemic in parts of Norway and the number of human TBE cases has been increasing in recent years. Clinical TBE cases are only found in southern parts of Norway; however, the results from both prevalence studies in ticks and seroprevalence studies in humans and animals indicate that TBEV might be widespread in the country, and not limited to the southern region. This is highly relevant information for public health considerations and risk evaluation. Further studies on tick distribution and prevalence of TBEV in ticks, humans and animals in Norway are currently ongoing.

Overview of TBE in Norway

Table 1: Virus, vector, transmission of TBE in Norway	
Viral subtypes, distribution[2-3,5-11]	Western subtype. TBEV is distributed in *Ixodes ricinus* ticks in the following counties: Buskerud, Akershus, Østfold, Vestfold, Telemark, Agder, Rogaland, Vestland, Møre and Romsdal, Trøndelag, and Nordland. Human TBE cases have been reported in the following counties: Agder, Vestfold, Telemark, Buskerud, Akershus, Østfold. *Source: www.fhi.no Norwegian Surveillance System for Communicable Diseases (MSIS)*
Reservoir animals	Small rodents in the genera *Shrew*, *Apodemus* and *Myodes.*[37]
Infected tick species (%)	*Ixodes ricinus* (0–1.1% in nymphs and 0–20.6% in adults).[6]
Dairy product	Not documented.

Table 2: TBE-reporting and vaccine prevention in Norway	
Mandatory TBE-reporting	Hospitals and General Practitioners Only cases affecting the central nervous system (e g meningitis/encephalitis) are notifiable. Criteria: - Detection of specific antibody response in serum and/or cerebrospinal fluid and/or - Detection of TBEV in cerebrospinal fluid by isolation and/or nucleic acid detection *Source: www.fhi.no*
Other TBE-Surveillance	Ongoing studies: The Barents and Arctic region projects: Health and climate in Arctic (HEKLA-TBE ID A2306), and Surveillance of emerging infections (SE-TBE ID B 2306). TBFVnet (EEA-project): surveillance and research on tick-borne flaviviruses Development of pipeline for whole genome sequencing of TBEV[38]
Special clinical features	TBE has been mandatorily notifiable to MSIS (Norwegian Surveillance System for Communicable Diseases) since 1975. *Source: www.fhi.no*
Available vaccines	TicoVac, Pfizer TicoVac Junior, Pfizer *Source: The Norwegian Medicines Agency*
Vaccination recommendations and reimbursement	TBE vaccination should be considered for children and adults who often experience tick bites in coastal areas where human TBE cases have been reported: - Sørlandet and the west coast of Oslofjorden from Flekkefjord to Drammen - The east coast of Oslofjorden from Vestby to the Swedish border *Source: www.fhi.no*
Vaccine uptake by age group/risk group/general population	In Norway, all immunizations should be registered in the national immunization register, SYSVAK. According to SYSVAK, about 108 078 persons have received at least 3 doses of TBE vaccine. There is no information about risk factors in the register. For vaccines outside the childhood immunization program, registration in SYSVAK was consensual up to 1.1.2020. The number of TBE vaccine doses given could therefore be higher than the numbers registered. *Source: Norwegian Immunization Registry (SYSVAK)*
Name, address/ website of TBE NRC	Norwegian Institute of Public Health. *Source: www.fhi.no*

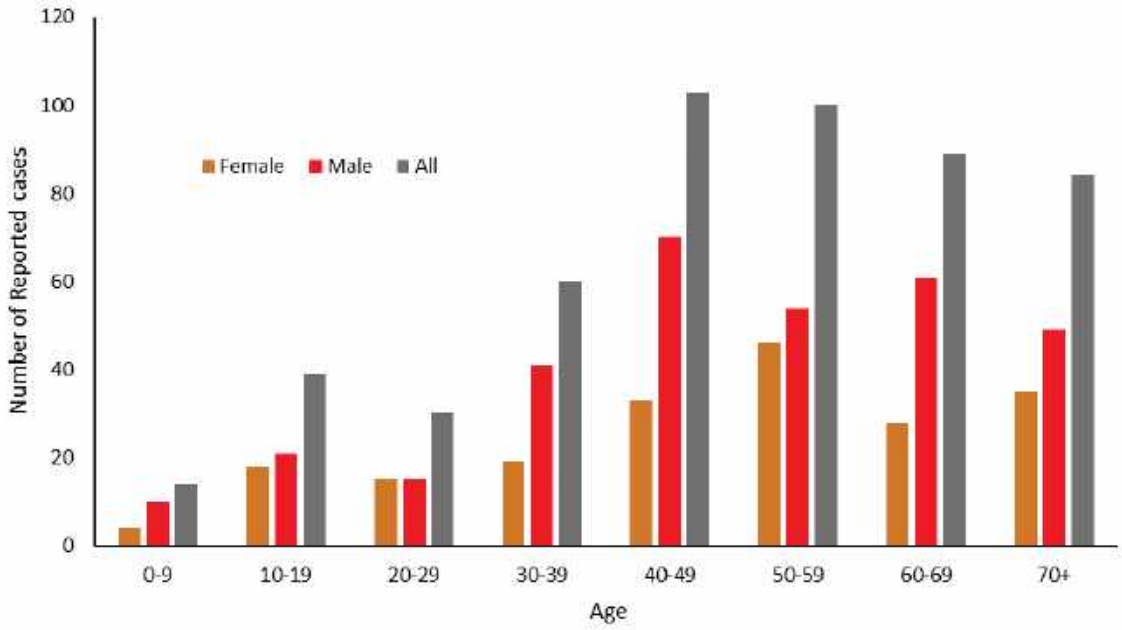
Figure 1: Burden of TBE in Norway 1994-2023*



*data per February 2024 (MSIS).
These data include 99 cases that have been infected abroad or have an unknown infection history. The 1997 case was registered in 1998.

Source Data: Appendix—Figure 1

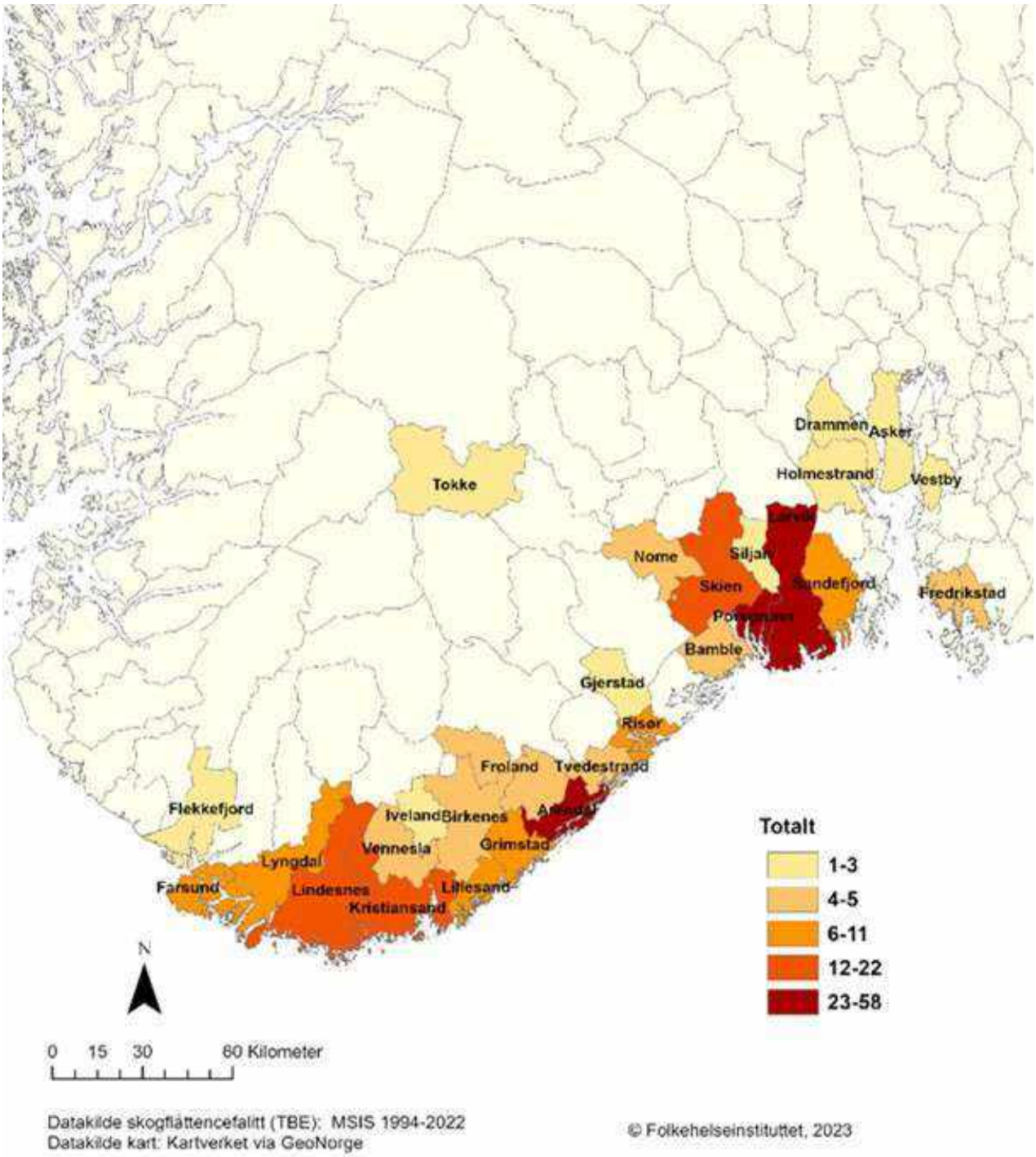
Figure 2: Age and gender distribution of TBE in Norway 1994–2023*



*data per February 2024 (MSIS).
These data include 99 cases that have been infected abroad or have an unknown infection history.

Source Data: Appendix—Figure 2

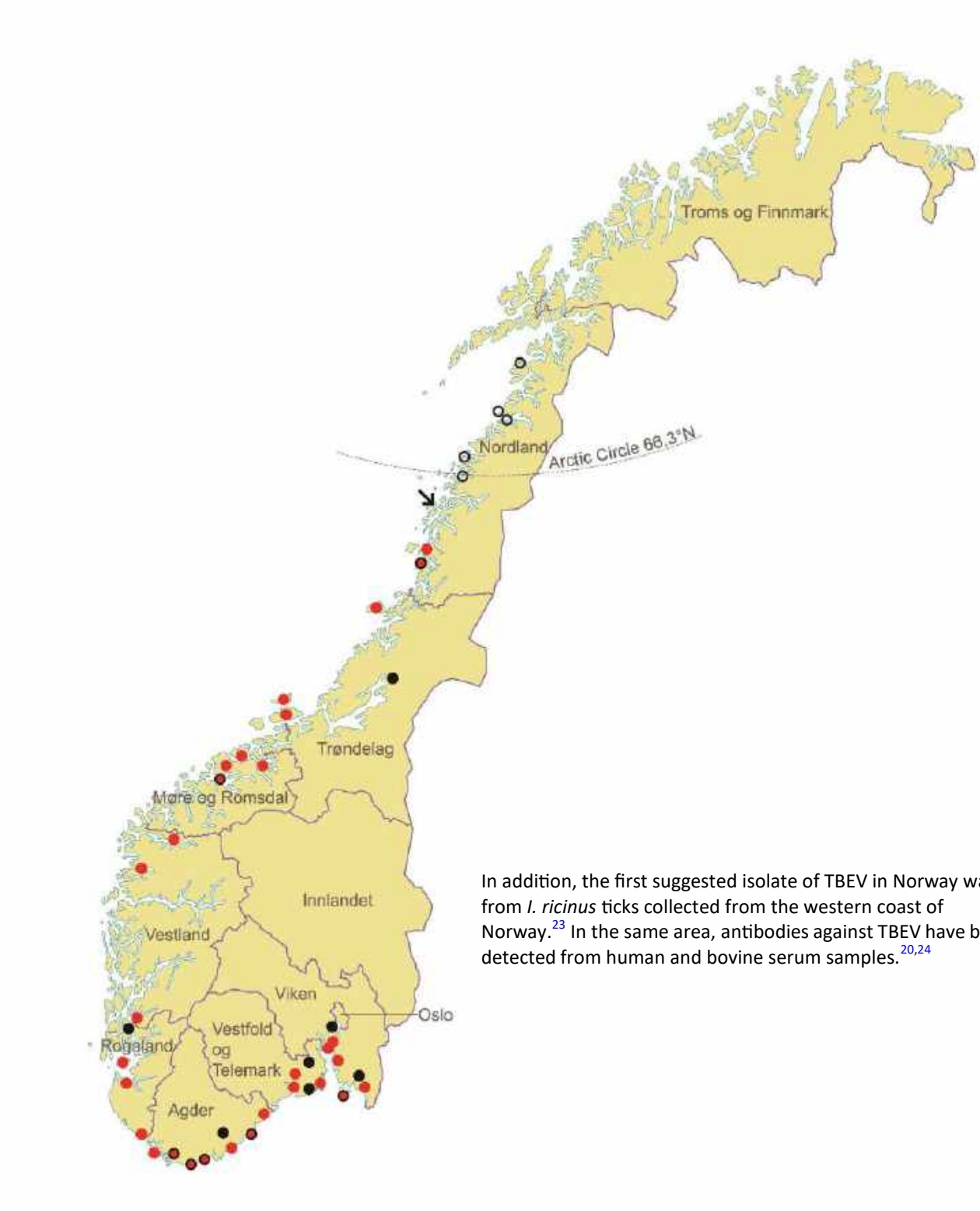
Figure 3: TBE cases in Norway 1994–2022 (MSIS)



Source: https://www.fhi.no/sm/smittevernveilederen/sykdommer-a-a/skogflattencefalitt-tbe-virusinfeksjoner/?term=#forekomst-i-norge

Figure 4: Geographical locations where tick-borne encephalitis virus has been detected in Norway from 2004 to 2020:
○ No ticks found, ● Ticks with TBEV, ● TBEV antibodies in animals, ● TBEV in ticks, cow milk, and TBEV antibodies in animals

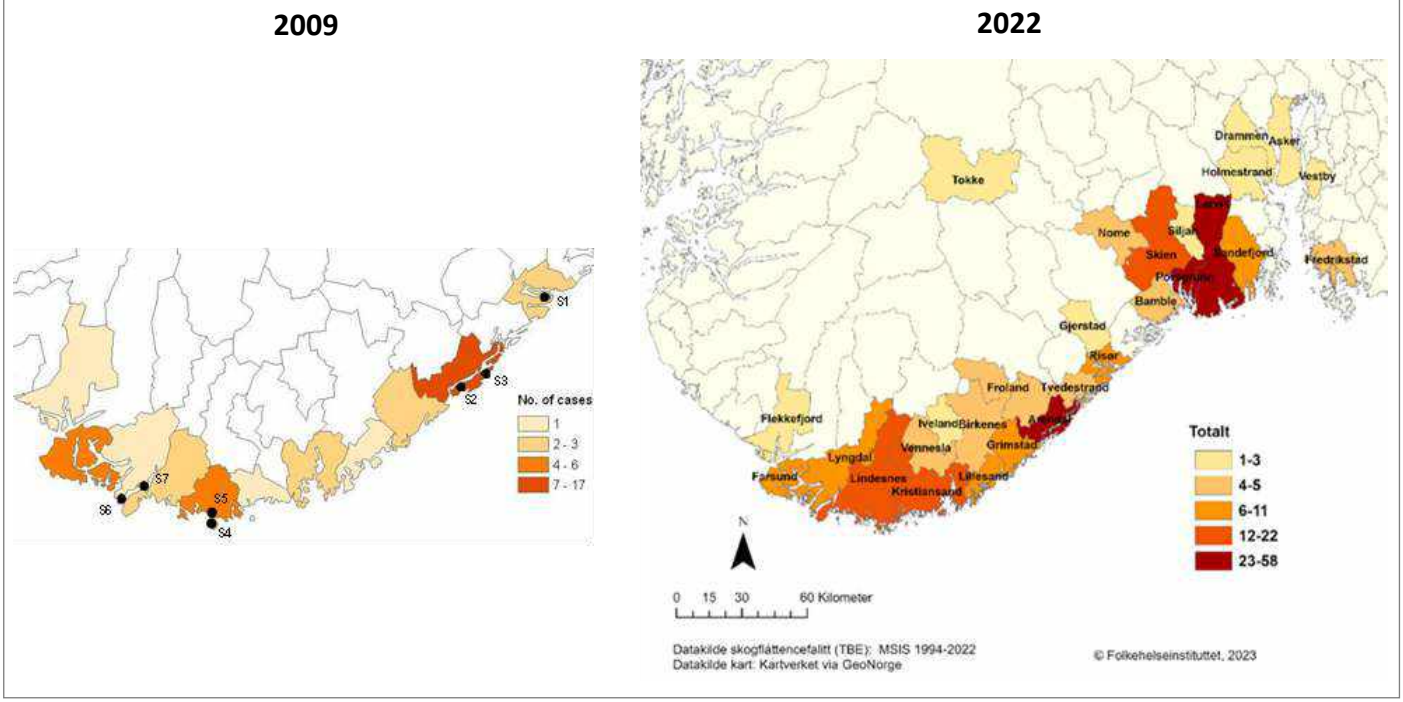
Arrow indicates the northernmost established and viable population of *I. ricinus* in Norway.[2-7,9,21,30]
In addition, the first suggested isolate of TBEV in Norway was from *I. ricinus* ticks collected from the western coast of Norway.[28] In the same area, antibodies against TBEV have been detected from human and bovine serum samples.[32,36]



In addition, the first suggested isolate of TBEV in Norway was from *I. ricinus* ticks collected from the western coast of Norway.[23] In the same area, antibodies against TBEV have been detected from human and bovine serum samples.[20,24]

Map from © Kartverket (https://www.kartverket.no/ Attribution 4.0 International (CC BY 4.0))

Figure 5: Expanded geographical distribution of reported TBE cases in Norway between 2009 and 2022. The red line shows the distribution border in 2009, the areas north and east of this, represents areas where new cases have been reported after 2009.



Appendix

Source data: Figure 1

Year	Number of cases	Incidence / 10^5
1994	2	<0.1
1995	0	0
1996	0	0
1997	0	0
1998	1	<0.1
1999	1	<0.1
2000	1	<0.1
2001	0	0
2002	2	<0.1
2003	1	<0.1
2004	4	<0.1
2005	4	<0.1
2006	5	0.1
2007	13	0.2
2008	11	0.2
2009	10	0.2
2010	11	0.2
2011	14	0.3
2012	7	0.1
2013	6	0.1
2014	13	0.2
2015	9	0.2
2016	12	0.2
2017	16	0.3
2018	26	0.5
2019	35	0.7
2020	41	0.8
2021	71	1.3
2022	90	1.6
2023	113	2.0

Source data: Figure 2

Age group (years)	Females	Males	All
0-9	4	10	14
10-19	18	21	39
20-29	15	15	30
30-39	19	41	60
40-49	33	70	103
50-59	46	54	100
60-69	28	61	89
>70	35	49	84

Acknowledgements
We thank Trude Marie Lyngstad for making Figure 3.

Authors and affiliations

Rose Vikse, Urusha Maharjan, Arnulf Soleng, Heidi H. Lindstedt, Rikard Rykkvin and Åshild K. Andreassen

Norwegian Institute of Public Health, Department of Virology

Kristian Alfsnes
Norwegian Institute of Public Health, Department of Bacteriology

Contact: ashildkristine.andreassen@fhi.no

Citation:
Vikse R, Maharjan U, Soleng A, Lindstedt HH, Rykkvin R, Alfsnes K, Andreassen KA. TBE in Norway. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-24-7

References

1. Norwegian Surveillance System for Communicable Diseases (MSIS). 2021; Available from: www.MSIS.no, 02.21.

2. Soleng A, et al. Distribution of *Ixodes ricinus* ticks and prevalence of tick-borne encephalitis virus among questing ticks in the Arctic Circle region of northern Norway. *Ticks Tick Borne Dis*. 2018;9(1):97-103. doi:10.1016/j.ttbdis.2017.10.002

3. Paulsen KM, Pedersen BN, Soleng A, Okbaldet YB, Pettersson JH, Dudman SG, Ottesen P, Vik IS, Vainio K, Andreassen A. Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* ticks from three islands in north-western Norway. *APMIS*. 2015; 123(9):759-64. doi: 10.1111/apm.12412

4. Paulsen KM, das Neves CG, Granquist EG, Madslien K, Stuen S, Pedersen BN, Vikse R, Rocchi M, Laming E, Stiasny K, Andreassen AK. Cervids as sentinel-species for tick-borne encephalitis virus in Norway - A serological study. *Zoonoses Public Health.* 2019. doi:10.1111/zph.12675.

5. Paulsen KM, Stuen S, das Neves CG, Suhel F, Gurung D, Soleng A, Stiasny K, Vikse R, Andreassen AK, Granquist EG. Tick-borne encephalitis virus in cows and unpasteurized cow milk from Norway. *Zoonoses Public Health.* 2019;66(2):216-222. doi: 10.1111/zph.12554

6. Vikse R, Paulsen KM, Edgar KS, Pettersson JH-O, Ottesen PS, Okbaldet YB, Kiran N, Lamsal A, Lindstedt HEH, Pedersen BN, Soleng A, Andreassen AK. Geographical distribution and prevalence of tick-borne encephalitis virus in questing *Ixodes ricinus* ticks and phylogeographic structure of the *Ixodes ricinus* vector in Norway. *Zoonoses Public Health.* 2020:1-12. doi:10.1111/zph.12696.

7. Kjelland V, Paulsen KM, Rollum R, Jenkins A, Stuen S, Soleng A, Edgar KS, Lindstedt HH, Vaino K, Gibory M, Andreassen AK. Tick-borne encephalitis virus, *Borrelia burgdorferi sensu lato, Borrelia miyamotoi, Anaplasma phagocytophilum* and *Candidatus Neoehrlichia mikurensis* in *Ixodes ricinus* ticks collected from recreational islands in southern Norway. *Ticks Tick Borne Dis.* 2018;9(5):1098-1102.

8. Andreassen A, Jore S, Cuber P, Dudman S, Tengs T, Isaksen K, Hygen HO, Viljugrein H, Anestad G, Ottesen P, Vainio K. Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. *Parasit Vectors*. 2012;5:177. doi:10.1186/1756-3305-5-177.

9. Larsen AL, Kanestrom A, Bjorland M, Andreassen A, Soleng A, Vene S, Dudman SG. Detection of specific IgG antibodies in blood donors and tick-borne encephalitis virus in ticks within a non-endemic area in southeast Norway. *Scand J Infect Dis*. 2014;46(3):181-4. doi: 10.3109/00365548.2013.865140.

10. Skarpaas T, Ljøstad U, Sundøy A. First human cases of tickborne encephalitis, Norway. *Emerg Infect Dis*. 2004;10 (12):2241-3. doi:10.3201/eid1012.040598.

11. Skarpaas T, Golovljova I, Vene S, Ljøstad U, Sjursen H, Plyusnin A, Lundkvist A. Tickborne encephalitis virus, Norway and Denmark. *Emerg Infect Dis*. 2006;12(7):1136-8. doi:10.3201/eid1207.051567

12. Skarpaas T, et al. [Tick-borne encephalitis in Norway]. *Tidsskr Nor Laegeforen*. 2002;122(1):30-2.

13. Csángó PA, Blakstad E, Kirtz GC, Pedersen JE, Czettel B. Tick-borne encephalitis in southern Norway. *Emerg Infect Dis*. 2004;10(3):533-4. doi:10.3201/eid1003.020734

14. Radzisauskiene D, Zagminas K, Asokliene L, Jasionis A, Mameniskiene R, Ambrozaitis A, Jancoriene L, Jatuzis D, IPetraityte I, Mockiene E. Epidemiological patterns of tick-borne encephalitis in Lithuania and clinical features in adults in the light of the high incidence in recent years: a retrospective study. *Eur J Neurol*. 2018;25(2):268-74. doi:10.1111/ene.13486

15. Kaminski M, Grummel V, Hoffmann D, Berthele A, Hemmer B. The spectrum of aseptic central nervous system infections in southern Germany - demographic, clinical and laboratory findings. *Eur J Neurol*. 2017;24(8):1062-70. doi:10.1111/ene.13335

16. Chitimia-Dobler L, et al. Repeated isolation of tick-borne encephalitis virus from adult Dermacentor reticulatus ticks in an endemic area in Germany. *Parasit Vectors*. 2019;12(1):90. doi:10.1186/s13071-019-3346-6

17. Suss J. Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine*. 2003;21 Suppl 1:S19-35. doi:10.1016/s0264-410x(02)00812-5

18. Rizzoli A, et al. Ixodes ricinus and Its Transmitted Pathogens in Urban and Peri-Urban Areas in Europe: New Hazards and Relevance for Public Health. *Front Public Health*. 2014;2:251. doi:10.3389/fpubh.2014.00251

19. Tambs-Lyche. Ixodes ricinus og piroplasmosen i Norge (Meddelelse fra Bergen Museums zoologiske avdeling). 1943;55:337-366.

20. Mehl R. The distribution and host relations of Norwegian ticks (Acari, Ixodides). *Fauna norwgica series B*. 1983;30:46-51.

21. Hvidsten D, Frafjord K, Gray JS, Henningsson A, Jenkins A, Kristiansen BE, Lager M, Rognerud B, Slåtsve AM, Stordal F, Stuen S, Wilhelmsson P. The distribution limit of the common tick, Ixodes ricinus, and some associated pathogens in north-western Europe. *Ticks Tick Borne Dis*. 2020;3:101388. doi:10.1016/j.ttbdis.2020.101388

22. Jenkins A, Hvidsten D, Matussek A, Lindgren PE, Stuen S, Kristiansen BE. Borrelia burgdorferi sensu lato in Ixodes ricinus ticks from Norway: evaluation of a PCR test targeting the chromosomal flaB gene. *Exp Appl Acarol*. 2012; 58(4):431-9. doi:10.1007/s10493-012-9585-2

23. Hvidsten D, Stuen S, Jenkins A, Dienus O, Olsen RS, Kristiansen BE, Mehl R, Matussek A. Ixodes ricinus and Borrelia prevalence at the Arctic Circle in Norway. *Ticks Tick Borne Dis*. 2014;5 (2):107-12. doi:10.1016/j.ttbdis.2013.09.003

24. Jore S, et al. Multi-source analysis reveals latitudinal and altitudinal shifts in range of Ixodes ricinus at its northern distribution limit. *Parasit Vectors*. 2011;4:84. doi:10.1186/1756 -3305-4-84

25. Anonymous. Consensus conference on Lyme disease. *Cmaj*. 1991;144(12):1627-32.

26. Piesman J. Field studies on Lyme disease in North America. *Can J Infect Dis*. 1991;2(2):55-7. doi:10.1155/1991/394041g

27. Kjaer LJ, et al. A large-scale screening for the taiga tick, Ixodes persulcatus, and the meadow tick, Dermacentor reticulatus, in southern Scandinavia, 2016. *Parasit Vectors*. 2019;12(1):338. doi:10.1186/s13071-019-3596-3

28. Traavik T, Mehl R, Wiger R. The first tick-borne encephalitis virus isolates from Norway. *Acta Pathol Microbiol Scand B*. 1978;86(4):253-5. doi:10.1111/j.1699-0463.1978.tb00040.x

29. Asghar N, Lindblom P, Melik W, Lindqvist R, Haglund M, Forsberg P, Overby AK, Andreassen A, Lindgren PE, Johansson M. Tick-borne encephalitis virus sequenced directly from questing and blood-feeding ticks reveals quasispecies variance. *PLoS One*. 2014;9(7):e103264. doi:10.1371/ journal.pone.0103264

30. Ytrehus B, Vainio K, Dudman SG, Gilray J, Willoughby K. Tick-borne encephalitis virus and louping-ill virus may co-circulate in Southern Norway. *Vector Borne Zoonotic Dis*. 2013;13 (10):762-8. doi:10.1089/vbz.2012.1023

31. Ytrehus B, et al. Louping-Ill Virus Serosurvey of Willow Ptarmigan (Lagopus lagopus lagopus) in Norway. *J Wildl Dis*. 2021;57(2):282-291. doi:10.7589/JWD-D-20-00068.

32. Traavik T. Serological investigations indicating the existence of tick-borne encephalitis virus foci along the Norwegian coast. *Acta Pathol Microbiol Scand B Microbiol Immunol*. 1973;81 (1):138-42. doi:10.1111/j.1699-0463.1973.tb02197.x

33. Thortveit ET, Aase A, Petersen LB, Lorentzen ÅR, Mygland Å, Ljøstad U. Human seroprevalence of antibodies to tick-borne microbes in southern Norway. *Ticks Tick Borne Dis*. 2020;11(4):101410. doi: 10.1016/j.ttbdis.2020.101410.

34. Marvik Å, et al. Low prevalence of tick-borne encephalitis virus antibodies in Norwegian blood donors. *Infect Dis (Lond)*. 2021;53(1):44-51. doi:10.1080/23744235.2020.1819561

35. Hjetland R, Henningsson AJ, Vainio K, Dudman SG, Grude N, Ulvestad E. Seroprevalence of antibodies to tick-borne encephalitis virus and Anaplasma phagocytophilum in healthy adults from western Norway. *Infect Dis (Lond)*. 2015;47(1):52-6. doi:10.3109/00365548.2014.959044

36. Traavik T. Antibodies to tick-borne encephalitis virus in human sera from the western coast of Norway. *Acta Pathol Microbiol Scand B*. 1979;87B(1):9-13. doi:10.1111/j.1699-0463.1979.tb02396.x

37. Jaenson TG, Hjertqvist M, Bergstrom T, Lundkvist A. Why is tick -borne encephalitis increasing? A review of the key factors causing the increasing incidence of human TBE in Sweden. *Parasit Vectors*. 2012;5:184. doi:10.1186/1756-3305-5-184

38. Paulsen KM, et al. High-throughput sequencing of two European strains of tick-borne encephalitis virus (TBEV), Hochosterwitz and 1993/783. *Ticks Tick Borne Dis*, 2021;12 (1):101557. doi:10.1016/j.ttbdis.2020.101557

Chapter 13

TBE in Poland

Katarzyna Pancer

E-CDC risk status: endemic (last edited: date 08.04.2024, data up to 2019-2022)

History and current situation

The history of tick-borne encephalitis (TBE) in Poland started in 1948, when clinical symptoms of TBE were described by Demiaszkiewicz.[7] Disease reporting has been mandatory since 1970. In the years between 1970-1992, a total of 576 TBE cases were reported; the annual number varied from 4 (1991) to 60 (1970), and the incidence in that period ranged from 0.01/100,000 population to 0.2/100,000 inhabitants, respectively. In 1993, however, the number of reported TBE cases increased rapidly, probably because of the first introduction of commercial tests serologically to confirm the diagnosis of TBE by ELISA, which rapidly replaced the older HI assay (Fig.1).[2,3,15] As in other European countries, TBE cases occur mainly in men aged 30 -60 y. (Fig.2).

This trend continued through the 1990s into the beginning of the 21st century. The number of reported TBE cases ranged from 149 in 2015 to 315 cases in 2009. In total, 4,690 cases of TBE were reported in Poland between 2000 and 2019. The respective incidence varied from 0.33 to 0.92/100,000. Possibly, a 3–4-year cycle was identified based on the reported numbers of TBE cases, with peaks observed in 2003, 2006, and 2009, but in the next years the cycle varied and peaks were observed in 2016, 2017 and 2019 (Fig.1).[2,15]

During the early 2020s strong effects of the COVID-19 pandemic were observed. In contrast to neighboring Germany and Sweden[15] there was a decrease in reported case numbers in Poland. However, data from another independent surveillance system, the Nationwide General Hospital Morbidity Study (NGHMS), which collects data about hospitalizations for TBEV and other viral neuro-infections, indicated a large increase of clinical TBE detections at the same time. An analysis of data collected from different databases indicated that the sensitivity of the Polish epidemiological surveillance system for TBE still needs to improve and that the suboptimal use of laboratory diagnostics for identification of the etiological agent in patients with presumed viral CNS-infection is probably the main reason for the underestimation of TBE in Poland.[16] The same conclusion was drawn based on the results of a project that retrospectively verified diagnoses in cases of viral neuro-infections.[21] It is necessary to expand the scope of diagnostics of neuro-infections to include tests for TBEV, particularly outside known endemic areas.

Over the last 4 years (2020-2023), a constant and significant increase in the number of TBE cases has been observed in Poland, reaching up to 663 cases with an incidence of 1.76/100,000 population in 2023.[2] Moreover changes in the geographic distribution of TBE cases were observed in this period: while in previous decades each year more than 60% of TBE cases were detected in just 2 provinces in northeastern Poland (Podlaskie, >45% reported TBE cases; Warmińsko-Mazurskie, 15%-25% of reported cases), in the last 4 years, the predominance of reported cases in the Podlasie Province was reduced to 32%, whereas the proportion of TBE cases in Mazowieckie voivodeship increased from 10% to 15.8%. The ratio of TBE cases in Warmińsko-Mazurskie was stable (15%). The lowest incidence was observed in Lubuskie voivodeship: usually there were no reported TBE cases, with exception of 2023 (3 cases) (Fig.3).[2]

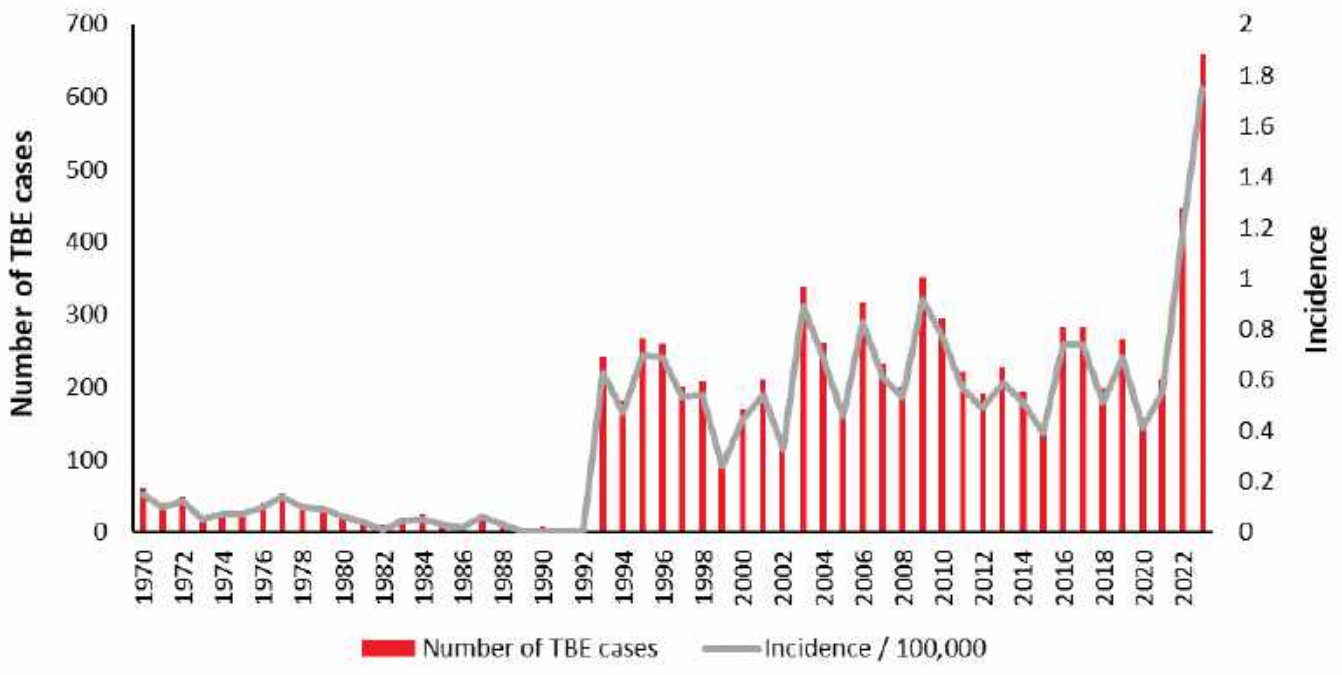
Moreover, more cases were diagnosed in autumn and early winter in the recent years and the percentage of TBE cases reported between October and December increased in comparison to other seasons (2018: 50%; 2022: 42%). One possible explanation for this phenomenon is climate change, with higher temperatures than in previous periods, longer heat waves, periods of drought and violent atmospheric phenomena occurring with varying intensity in Poland.

Vaccination against TBE in Poland started in the 1970s. Vaccines using the TBEV-European strain have been available since 1993 and are recommended for persons staying in endemic TBE areas, specifically forest workers, soldiers, hunters, border guards, firefighters, farmers, tourists and campers of any age as of one year of age. There is no reimbursement.[3] Vaccine uptake was low before 2019 (0.05-0.12%). Since 2019, the number of vaccinations has increased twice, especially among children and young adults <19 years of age. Today, the total number of adults and children vaccinated each year are similar – in 2022 – 41,728 vs 41,292.[1]

Overview of TBE in Poland

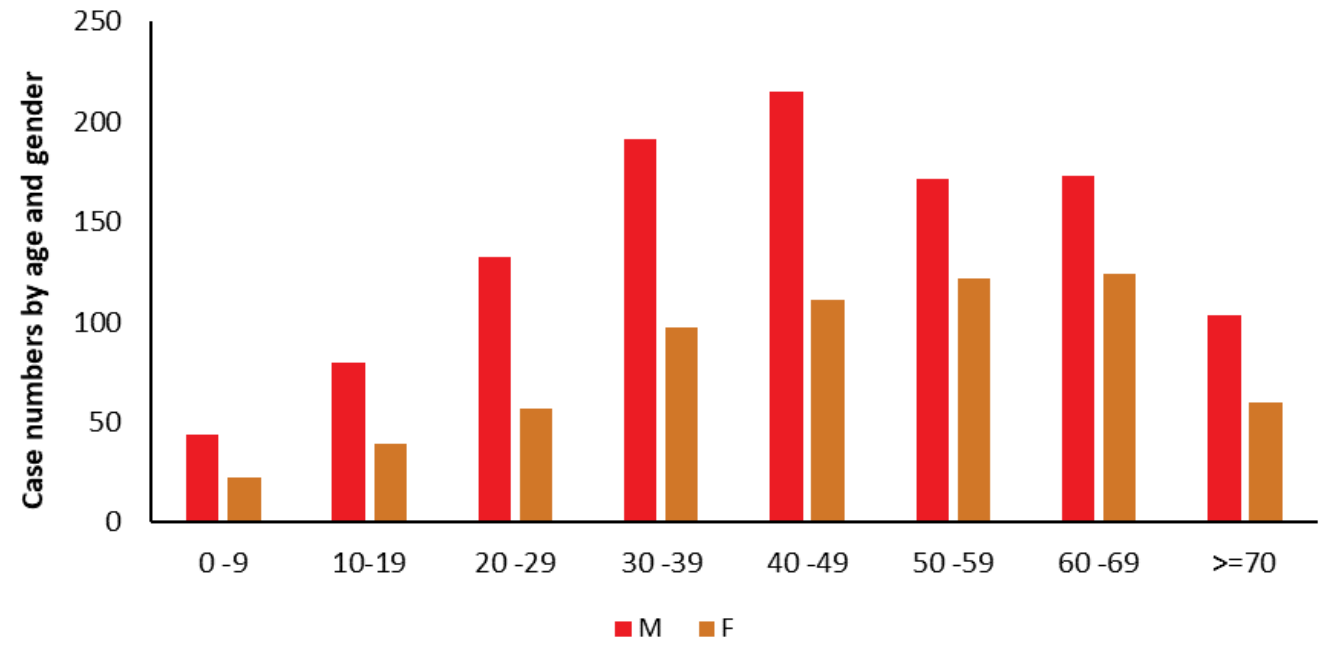
Table 1: TBE in Poland[1,3-6,8-21]	
Viral subtypes isolated	European subtype (TBEV-EU)[9,11,14]
Reservoir animals	Mainly small mammals like: *Apodemus sylvaticus*, *Apodemus flavicollis*, *Rinaceus roumanicus*, *Myodes glareolus*, *Microtus agrestis*, *Sciurus vulgaris*, *Sorex araneus*, *Talpa europaea*[8]
Infected tick species (%)	Varied depending on regions and vector:[4,13,17,19,20] • from 0 to 1.6% in *I.ricinus*, mainly found in North-Eastern and Eastern Poland. • from 0.99 to 12.5% in *D.reticulatus* (Central Poland -7.6%; Eastern – up to 10.8%; North-Eastern - 0.99-12.5%).
Dairy product transmission	Sporadic cases and limited outbreaks[5,6,10,18]
Case definition used by authorities	Based on ECDC[15]
Completeness of case detection and reporting	Comparison of surveillance data and other data from hospitalization and National Health Fund databases indicated strong underreporting of TBE in 2020[16] Retrospective verification of clinical recognition - undetected cases of TBE were found in 13.9% of examined patients[21]
Type of reporting	Mandatory reporting of all cases with neuroinfection. Passive surveillance; obligatory reporting of TBE detection by clinicians as well as positive results of laboratory diagnostics by labs[15]
Other TBE-surveillance	No available data
Special clinical features	70-80% Biphasic Clinical manifestation: fever 95.3%; headache 95%, muscle pain 43%, dizziness 6.3%, vomiting 42%, neurological disorders 11%, meningeal symptoms 70%[15,21]
Licensed vaccines	Commercially available products are: FSME-IMMUN (FSME-IMMUN 0,25-ml Junior, FSME-IMMUN 0,5-ml) and Encepur (Encepur K for children >1 year old; Encepur Adults >12 years
Vaccination recommendations	Risk groups related to occupation or habits; no reimbursement[3] Vaccination for TBE is recommended for persons employed in forest exploitation; military; firefighters and border guards; farmers; people engaging in particularly frequent physical activity outdoors.
Vaccine uptake	Vaccine uptake differs by region; highest usually in the highly affected regions with an incidence >5/100,000; in 2021, 0.5% of the general population in Podlaskie voivodeship was vaccinated in comparison to 0.18% in the general population of Poland[1]
National Reference center for TBE	Since 2004 Poland has had no National Reference Center for TBE
Additional relevant information	Two fatal cases due to organs transplanted from donors with TBE viremia were described.[12] The cases may indicate a potential risk of TBEV transmission by transplantation and transfusion

Figure 1: TBE case numbers and incidence in Poland (1970-2023) (NIPH NIH-NRI data,[2])



Source Data: Appendix Figure 1

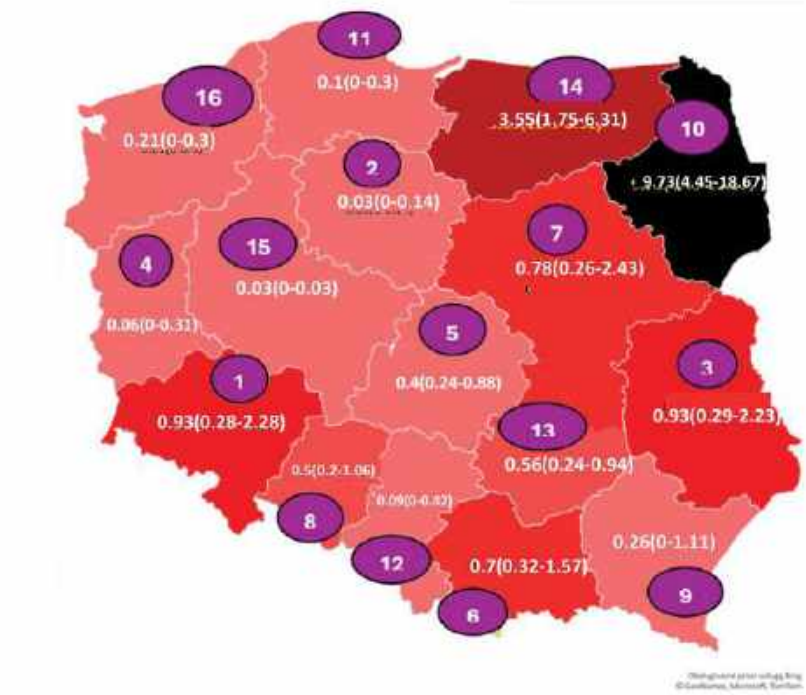
Figure 2: Age and gender distribution of TBE in Poland, 2019-2023 (NIPH NIH-NRI data,[2])



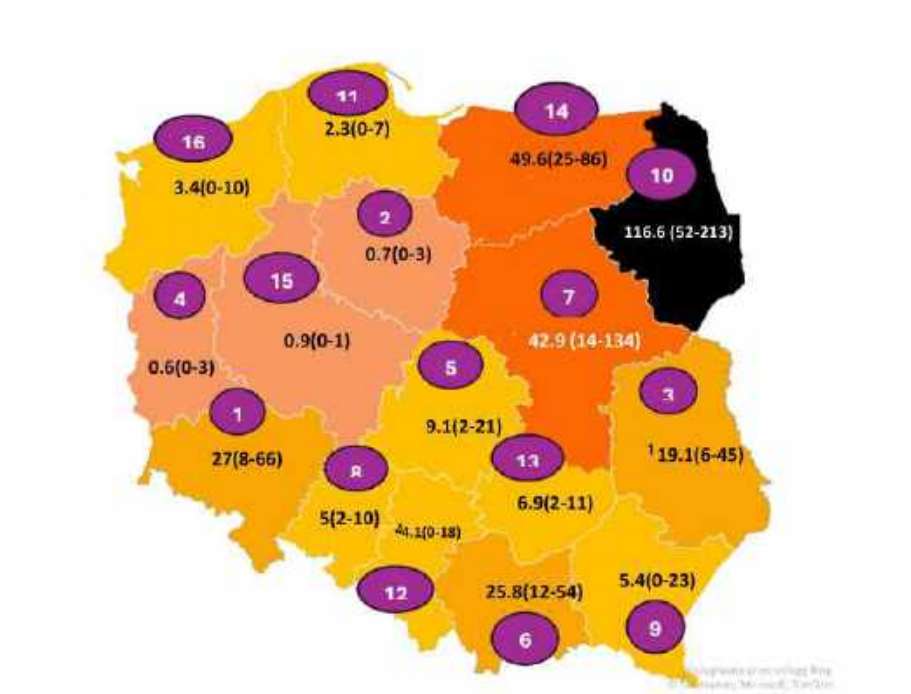
Source Data: Appendix Figure 2

Figure 3: Average annual incidence of TBE and cumulative case numbers in Poland, 2017-2023, by region (voivodeship) (NIPH NIH-NRI)

A. The average Incidence of reported TBE cases in Poland in 2017-2023y.by voivodeships. In brackets - range of annually TBE incidence from min to max



B. The average number of reported TBE cases in Poland in 2027-2023y. - by voivodeships. In brackets -range of annually reported cases from min to max



Voivodeships

1	Dolnośląskie	7	Mazowieckie	13	Świętokrzyskie
2	Kujawsko-Pomorskie	8	Opolskie	14	Warmińsko-Mazurskie
3	Lubelskie	9	Podkarpackie	15	Wielkopolskie
4	Lubuskie	10	Podlaskie	16	Zachodniopomorskie
5	Łódzkie	11	Pomorskie		
6	Małopolskie	12	Śląskie		

Source Data: Addendum

Appendix

Source data: Figure 1

Year	Number of TBE cases	TBE incidence /10^5
1970[a]	60	0.15
1971	41	0.10
1972	50	0.125
1973	22	0.05
1974	27	0.07
1975[b]	26	0.07
1976	40	0.10
1977	54	0.14
1978	36	0.10
1979	35	0.09
1980	25	0.06
1981	17	0.04
1982	9	0.007
1983	20	0.045
1984	25	0.05
1985[#]	14	0.03
1986	10	0.02
1987	24	0.06
1988	15	0.03
1989	6	0.04
1990	8	0.006
1991	4	0.003
1992	8	0.006
1993[c]	241	0.63
1994	181	0.47
1995	267	0.70
1996	259	0.69
1997	201	0.53
1998	208	0.54
1999	208	0.54
2000	170	0.44
2001	210	0.54
2002	126	0.33
2003[d]	339	0.89
2004	262	0.69
2005	177	0.46
2006	317	0.83
2007	233	0.61
2008	202	0.53
2009	351	0.92
2010	294	0.77
2011	221	0.57
2012	190	0.49
2013	227	0.59
2014	195	0.51
2015	149	0.39
2016	284	0.74
2017	283	0.74
2018	197	0.51
2019	265	0.69
2020	158	0.42
2021	210	0.56
2022[e]	445	1.18
2023	663	1.62

Notes:

[a] 1970: Start of registration of TBE in Poland; 1970–1984 recommended vaccination with Russian anti-TBEV Siberian type (not reimbursed)

[b] 1975: Establishment of National Arbovirus Laboratory, National Institute of Public Health – National Institute of Hygiene (NIPH-NIH) and production of hemagglutination inhibition (HI) antigen for surveillance service to the end of 1984

[c] Diagnostics based on ELISA method in hospital and Sanitary Service laboratories with confirmation in Reference Laboratory NIH; 1993–2003 recommended vaccination against TBEV-EU (not reimbursed)

[d] Lack of reference laboratory because of expiry of the mandate and law regulation – from that time there is no necessity to confirm positive serological results for TBEV

[e] Data for 2022 is not verified

[#] From 1970 to 1985 confirmation based on HI test; since 1993, IgM ELISA for confirmation (and local synthesis of TBEV-specific IgG in CSF)

Source data: Figure 2

Age group (years)	Males	Females	All 2015	All 2016	All 2017
0-9	-	-	4	3	18
10-19	-	-	17	13	18
20-29	-	-	20	31	28
30-39	-	-	21	50	42
40-49	-	-	26	50	42
50-59	-	-	32	63	55
60-69	-	-	17	57	50
>70	-	-	12	19	18

Addendum: Table with incidence of TBE per 100,000 inhabitants in voivodeships in Poland in 2017-2023*

Voivodeship	2017	2018	2019	2020	2021	2022	2023*	Average Inc
Dolnośląskie	0.52	0.62	0.93	0.28	0.66	2.28	1.25	0.93
Kujawsko-pomorskie	0	0	0.14	0.05	0.05	0	0	0.03
Lubelskie	0.42	0.47	0.76	0.29	0.91	1.43	2.23	0.93
Lubuskie	0	0	0	0	0.1	0	0.31	0.06
Łódzkie	0.24	0.24	0.49	0.08	0.08	0.88	0.63	0.38
Małopolskie	0.32	0.5	0.35	0.35	0.5	1.25	1.57	0.7
Mazowieckie	0.47	0.46	0.31	0.26	0.66	0.59	2.43	0.74
Opolskie	0.2	0.81	0.3	0.2	0.31	1.06	0.75	0.52
Podkarpackie	0.09	0.09	0.05	0	0.14	0.34	1.11	0.26
Podlaskie	13.5	6.17	9.16	6.63	1.45	11.52	18.67	9.6
Pomorskie	0	0	0.04	0.09	0.13	0.13	0.3	8.86
Ślqskie	0	0.04	0.09	0	0.02	0.09	0.42	0.09
Świętokrzyskie	0.48	0.72	0.65	0.24	0.16	0.76	0.94	0.56
Warmińsko-mazurskie	3.14	1.75	3.3	2.11	3.33	4.89	6.31	3.55
Wielkopolskie	0.03	0.03	0.03	0	0.03	0.03	0.03	0.03
Zachodniopomorskie	0.06	0.06	0.24	0	0.18	0.3	0.61	0.21

**temporary data*

Acknowledgments

Data on reported TBE cases come from the database maintained by the Department of Infectious Diseases Epidemiology and Surveillance financed by the National Health Programme (NPZ) 2021-2025 (6/8/85195/NPZ/2021/1094/827).

Authors and affiliation

Katarzyna Pancer, Department of Virology, National Institute of Public Health NIH-National Research Institute, Chocimska, 24 str., 00-791 Warsaw, Poland

Contact: kpancer@pzh.gov.pl

Citation:
Pancer K, Gut W. TBE in Poland. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-25-7

References

1. National Institute of Public Health NIH – NRI, GIS. Vaccinations in Poland; 2000-2022. Accessed April 8, 2024. http://wwwold.pzh.gov.pl/oldpage/epimeld/index_p.html
2. National Institute of Public Health NIH – NRI, GIS. Infectious diseases and poisonings in Poland; 1990-2023. Accessed April 8, 2024. http://wwwold.pzh.gov.pl/oldpage/epimeld/index_p.html
3. Act on Prevention and combating infections and infectious diseases in humans [Art. 20. Recommended protective vaccinations when performing specific professional activities] *Journal of Laws*. 2020 Pos. 567
4. Biernat B, Karbowiak G, Werszko J, Stańczak J. Prevalence of tick-borne encephalitis virus (TBEV) RNA in Dermacentor reticulatus ticks from natural and urban environment, Poland. *Exp Appl Acarol.* 2014;64(4):543-551. doi:10.1007/s10493-014-9836-5
5. Buczek AM, Buczek W, Buczek A, Wysokińska-Miszczuk J. Food-Borne Transmission of Tick-Borne Encephalitis Virus-Spread, Consequences, and Prophylaxis. *Int J Environ Res Public Health*. 2022;19(3):1812. Published 2022 Feb 5. doi:10.3390/ijerph19031812
6. Cisak E, Wójcik-Fatla A, Zając V, Sroka J, Buczek A, Dutkiewicz J. Prevalence of tick-borne encephalitis virus (TBEV) in samples of raw milk taken randomly from cows, goats and

sheep in eastern Poland. *Ann Agric Environ Med*. 2010;17 (2):283-286.
7. Demiaszkiewicz W. [Spring-summer tick encephalitis in the Bialowieza Forest]. *Pol Tyg Lek (Wars)*. 1952;7(24):799-801.
8. Gliński Z, Kostro K, Grzegorczyk K. [Rodents as potential carriers of pathogenic microorganisms]. *Zycie Weterynaryjne*. 2017; 92(11): 799-804.
9. Katargina O, Russakova S, Geller J, et al. Detection and characterization of tick-borne encephalitis virus in Baltic countries and eastern Poland. *PLoS One*. 2013;8(5):e61374. Published 2013 May 1. doi:10.1371/journal.pone.0061374
10. Monika Emilia Król, Bartłomiej Borawski, Anna Nowicka-Ciełuszecka, Jadwiga Tarasiuk, Joanna Zajkowska. Outbreak of alimentary tick-borne encephalitis in Podlaskie voivodeship, Poland. *Przegl Epidemiol*. 2019;73(2):239-248. doi:10.32394/pe.73.01
11. Kunze M, Banović P, Bogovič P, et al. Recommendations to Improve Tick-Borne Encephalitis Surveillance and Vaccine Uptake in Europe. *Microorganisms*. 2022;10(7):1283. Published 2022 Jun 24. doi:10.3390/microorganisms10071283
12. Lipowski D, Popiel M, Perlejewski K, et al. A Cluster of Fatal Tick-borne Encephalitis Virus Infection in Organ Transplant Setting. *J Infect Dis*. 2017;215(6):896-901. doi:10.1093/infdis/jix040
13. Mierzejewska EJ, Pawełczyk A, Radkowski M, Welc-Falęciak R, Bajer A. Pathogens vectored by the tick, *Dermacentor reticulatus*, in endemic regions and zones of expansion in Poland. *Parasit Vectors*. 2015;8:490. Published 2015 Sep 24. doi:10.1186/s13071-015-1099-4
14. Moraga-Fernández A, Muñoz-Hernández C, Sánchez-Sánchez M, Fernández de Mera IG, de la Fuente J. Exploring the diversity of tick-borne pathogens: The case of bacteria (Anaplasma, Rickettsia, Coxiella and Borrelia) protozoa (Babesia and Theileria) and viruses (Orthonairovirus, tick-borne encephalitis virus and louping ill virus) in the European continent. *Vet Microbiol.* 2023;286:109892. doi:10.1016/j.vetmic.2023.109892
15. Paradowska-Stankiewicz I., Zbrzezniak J. Tick-borne encephalitis in Poland and worldwide. Assessment of the epidemiological situation of TBE in Poland in 2015-2019 based on epidemiological surveillance data. NIPH NIH-NRI Report. 2021. Accessed April 8, 2024. https://www.pzh.gov.pl/wp-content/uploads/2021/03/KleszczoweZapalenieMozgu-raport-PZH_2021.pdf
16. Paradowska-Stankiewicz I, Pancer K, Poznańska A, et al. Tick-borne encephalitis epidemiology and surveillance in Poland, and comparison with selected European countries before and during the COVID-19 pandemic, 2008 to 2020. *Euro Surveill*. 2023;28(18):2200452. doi:10.2807/1560-7917.ES.2023.28.18.2200452
17. Stefanoff P, Pfeffer M, Hellenbrand W, et al. Virus detection in questing ticks is not a sensitive indicator for risk assessment of tick-borne encephalitis in humans. *Zoonoses Public Health*. 2013;60(3):215-226. doi:10.1111/j.1863-2378.2012.01517.x
18. Wójcik-Fatla A, Krzowska-Firych J, Czajka K, Nozdryn-Płotnicka J, Sroka J. The Consumption of Raw Goat Milk Resulted in TBE in Patients in Poland, 2022 "Case Report". *Pathogens*. 2023;12 (5):653. Published 2023 Apr 27. doi:10.3390/pathogens12050653
19. Wójcik-Fatla A, Cisak E, Zając V, Zwoliński J, Dutkiewicz J. Prevalence of tick-borne encephalitis virus in Ixodes ricinus and *Dermacentor reticulatus* ticks collected from the Lublin region (eastern Poland). *Ticks Tick Borne Dis*. 2011;2(1):16-19. doi:10.1016/j.ttbdis.2010.10.001
20. Zając V, Wójcik-Fatla A, Sawczyn A, et al. Prevalence of infections and co-infections with 6 pathogens in *Dermacentor reticulatus* ticks collected in eastern Poland. *Ann Agric Environ Med.* 2017;24(1):26-32. doi:10.5604/12321966.1233893
21. Zajkowska J, Waluk E, Dunaj J, et al. Assessment of the potential effect of the implementation of serological testing tick-borne encephalitis on the detection of this disease on areas considered as non-endemic in Poland - preliminary report. *Przegl Epidemiol*. 2021;75(4):515-523. doi:10.32394/pe.75.48

Chapter 13

TBE in Romania

Lidia Chitimia-Dobler, Adriana Hristea, Wilhelm Erber and Tamara Vuković-Janković

E-CDC risk status: endemic (no new data available as of May 2023)

History and current situation

Based on an epidemiological survey performed,[1] human TBEV neuroinfections may have an endemic emergent course, and natural foci are in full territorial expansion. Identified risk areas are Tulcea district, Transylvania, at the base of the Carpathian Mountains and the Transylvanian Alps.[2,3] TBE has been a notifiable disease since 1996. Surveillance of TBE is not done at the country level, only regionally in some counties (northern/central/western part, close to Hungary). The passive surveillance system was implemented in 2008. However, there is no regular screening and the relative risk of contracting this disease is unknown. In 1999, an outbreak of TBE in humans was recorded with a total of at least 38 human cases.[4] The probable cause of the outbreak was goat milk and raw goat milk products. Subsequent studies to detect TBEV in ticks in the affected regions resulted in a non-specified number of TBEV isolates, which were described as belonging to the European subtype of TBEV. A publication of the neighboring Republic of Moldova described the existence of the Far-eastern subtype of TBEV just at the border to Romania.[5]

In 2001–2006, an epidemiological survey of TBEV infection in 1,669 individuals from 11 Transylvanian counties showed a seroprevalence rate in the general population of 0.6%; higher rates were found in at-risk populations: 5.8% in those living around natural foci and up to 41.5% in those with known occupational risks.[1,6]

In 2008, a seroprevalence study was published testing 5,063 sera from humans and 2,336 sera from animals derived from a total of 20 counties all over Romania during the years 1985 to 1993. The overall seroprevalence rate was found to be 6.5% for humans and 10.0% for animals with ranges from 0% to 19.4% for individual counties. The testing was done using hemagglutination inhibition testing without further confirmation by neutralization test.[7] A recent prevalence antibody study published in 2017, which studied by serum neutralization test, 519 sheep samples from 5 Romanian counties provided a total seroprevalence rate of 15.2% with ranges from 2.0% to 27.7%. The data are summed up in Table 3.

During an unpublished study from 2011–2012, a total of 6,548 nymphs and 853 adult ticks of the species *Ixodes ricinus* from the Romanian counties Alba, Cluj, Ilfov, Mures and Sibiu, including the region of outbreak in 1999, were tested by real time-RT-PCR. All ticks were found to be negative. Testing of 74 sheep sera by TBEV neutralization test gave 6/60 (10%) sera from sheep from Sibiu county, while all other sera were found negative.[7] In the same study the goat flock, which presumably caused the milk-borne outbreak in 1999 in the county of Sibiu was serologically tested by neutralization test. 10/10 (100%) goats of the flock showed positive antibody titers for TBEV.[7]

In the period between 2006–2015 the studies undertaken showed that the most frequent species of ticks in Romania is *I. ricinus*. Three Romanian counties were selected as ticks sampling sites (Sibiu, Tulcea and Giurgiu), collected from vegetation, livestock and reptiles. Specific RNAs from TBEV were detected (3' UTR-genomic region) in <1% of *I. ricinus* pools.[8]

A seroprevalence study tested 1,116 sera collected from humans in 15 localities from 10 counties. The overall seroprevalence was 0.62% (7/1,116). All positive sera were from one single locality from Sibiu county with 4.9% prevalence for the county and 9.7% for that site.[9]

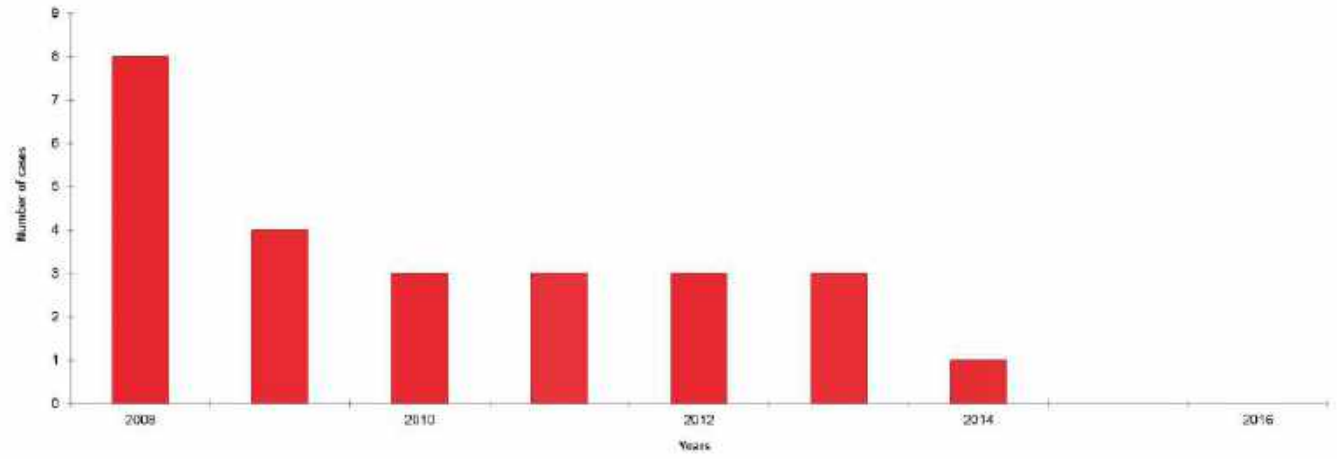
Overview of TBE in Romania

Table 1: Virus, vector, transmission of TBE in Romania	
Viral subtypes, distribution	European subtype; possibly Far-Eastern subtype (?)[1,5]
Reservoir animals	No data
Infected tick species (%)	*I. ricinus* - estimated prevalence of TBE virus <1%[8]
Dairy product transmission	Outbreak in 1999 in Sibiu county with at least 38 human cases[4]

Table 2: TBE reporting and vaccine prevention in Romania	
Mandatory TBE reporting	Since 2008
Other TBE surveillance	No data
Special clinical features	No data
Available vaccines	FSME-IMMUN
Vaccination recommendations and reimbursement	No national TBE vaccination policy and/or recommendations implemented
Vaccine uptake by age group/risk group/general population	Unknown
Name, address/website of TBE NRC	Centrul de Prevenire si Control a Bolilor Transmisibile, Bucarest; https://cnscbt.ro/

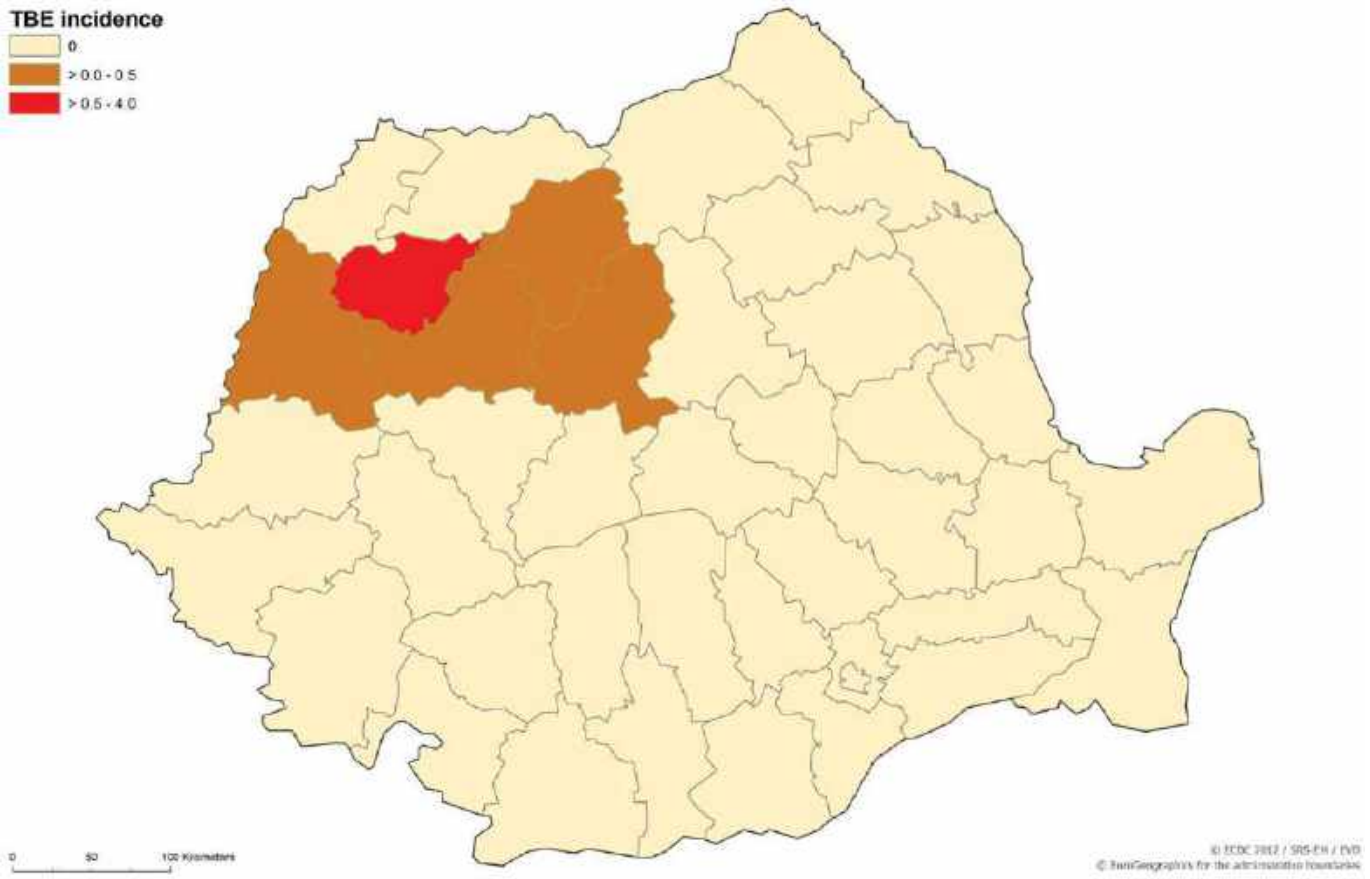
Table 3: Seroprevalence rates against TBEV in humans and animals in different counties of Romania			
County	**No. of sera**	**Study Ionescu et al. 2008[6]**	**Study Salat et al. 2017[10]**
Alba	49 human	4.0%	
	190 animal	0%	
Bihor	119 sheep		27.7%
Bistrita-Nasaud	626 human	4.6%	
	100 sheep		12.0%
Caras Severin	52 human	3.8%	
	241 animal	2.0%	
Calarasi	651 human	1.6%	
	501 animal	0%	
Cluj	328 human	4.5%	
	100 sheep		11.0%
Constanta	433 human	1.1%	
Dolj	117 human	2.5%	
Gorj	75 human	4.0%	
Hunedoara	52 human	3.8%	
	108 animal	18.5%	
Iasi	41 human	0%	
Maramures	873 human	19.4%	
	492 animal	17.4%	
Mures	82 human	7.3%	
	354 animal	14.4%	
	100 sheep	0%	2.0%
Olt	54 human	9.2%	
Prahova	86 human	5.8%	
Sibiu	74 human	3.0%	
Salaj	100 sheep		20.0%
Suceava	407 human	83%	
	213 animal	23.4%	
Timis	168 human	2.3%	
Tulcea	180 human	7.7%	
	202 animal	9.4%	
Valcea	81 human	3.7%	
	35 animal	11.4%	
Bucuresti	186 human	2.6%	

Figure 1: Burden of TBE in Romania over time[7]



Source Data: Appendix—Figure 1

Figure 2: TBEV-isolation and TBE cases in Romania



Source: European Centre for Disease Prevention and Control. Epidemiological situation of tick-borne encephalitis in the European Union and European Free Trade Association countries. Stockholm: ECDC; 2012.

Appendix

Source data: Figure 1

Year	Number of TBE cases	TBE incidence /10^5
2008	8	0.04
2009	4	0.02
2010	3	0.01
2011	3	0.01
2012	3	0.01
2013	3	0.01
2014	1	0.00
2015	0	0.00
2016	0	0.00
2017		
2018		
2019		
2020	0	0.00
2021	No data	
2022	No data	
2023	No data	

Contact: lydiachitimia@gmail.com

Citation:
Chitimia-Dobler L, Hristea A, Erber W, Vuković-Janković T. TBE in Romania. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press;2024. doi: 10.33442/26613980_13-26-7

References

1. Molnar GB, Perseca T, Feder A, Pacuraru D, Marialaki E, Cojan A. Epidemiological assessment of morbidity and natural foci of TBE-CEE virus infection in Transylvania. *Rev Med Chir Soc Med Nat Iasi.* 2008;112:471-7. doi: 10.3201/eid2312.170166
2. Kollaritsch H, et al. Background document on vaccines and vaccination against tick–borne encephalitis. Geneva, WHO Strategic Advisory Group of Experts on Immunization. Available at: http://www.who.int/immunization/sage/6_TBE_backgr_18_Mar_net_apr_2011.pdf [Accessed 17 May 2024]
3. Süss J. Tick-borne encephalitis in Europe and beyond--the epidemiological situation as of 2007. *Euro Surveill*. 2008;13 (26). pii:18916.
4. Ionescu L, Alexse A, Ceianu C, Necsulescu M, Popescu D, Bicheru S, Dumitrescu G, Cumpanasoiu CE, Cumpanasoiu C, Pasat L, Tirziu E. Investigation methods used for identifying the presence of tick-borne encephalitis virus (TBEV) in vector arthropods. *Lucr Stiin Med Vet*. 2009;17(9):288-93.
5. Ponomareva EP, Mikryukova TP, Gori AV, Kartashow MY, Protopopova EV, Chausov EV, Konovalova SN, tupota NL, Gheorghita SD, Burlacu VI, Ternovoi VA, Loktev VB. Detection of Far-Eastern subtype of tick-borne encephalitis viral RNA in ticks collected in the Republic of Moldova. *J Vector Borne Dis*. 2015;52:334-6.
6. Ionescu L, Necsulescu M, Alexse A, Ceianu C, Popescu D, Bicheru S, Ordeanu V; Nicolescu G, Vladimirescu AL, Postoarca A. Infection with tick-borne encephalitis virus in Romania (in Romanian). *Rev Rom Med Vet.* 2008;3:69-79.
7. Kahl O, Chitimia-Dobler L, Süss J. unpublished data.
8. Vladimirescu A, Dumitrescu G, Ionescu L, et al. Real-Time PCR studies regarding the *Borrelia burgdorferi*, *Francisella tularensis*, tick-borne encephalitis virus (TBEV) and crimeean congo hemorrhagic fever virus (CCHFv) occurrence in the Romanian ticks. *Int J Infect Dis*. 2016;45S:193.
9. Panciu AM, Cheran CA, Militaru ED, Rîciu CD, Hristea A. Serosurvey of tick-borne encephalitis virus infection in Romania. *Pathogens*. 2024;13:231. doi:10.3390/pathogens13030231
10. Salat J, Mihalca AD, Mihaiu M, Modrý D, Ruzek D. Tick-borne encephalitis in sheep, Romania. *Emerg. Infect. Dis*. 2017;23:2065–7. doi:10.3201/eid2312.170166

Chapter 13

TBE in Russia

Sergey Tkachev, Maria Esyunina, Maria Syrochkina

E-CDC risk status: endemic (data as of end 2023)

History and current situation

Tick-borne encephalitis (TBE) was first revealed in the Far-East Taiga Forest in the Soviet Union in the spring-summer season 1933-1935[1] and further investigated as of 1937 in a large multidisciplinary expedition led by Professor Lev Zilber, the Head of the Moscow Medical Virology laboratory.[2,3] The expedition demonstrated that the disease develops in humans after a tick-bite[4], and the "Taiga Tick" Ixodes persulcatus was established as the virus carrier. The viral etiology of the disease was confirmed and for the first time a strain of the TBE virus (TBEV) was isolated. The natural clinical disease spectrum in humans and the respective pathology were described and the effectiveness of "immunoglobulin-therapy" was demonstrated.[5] In 1937, based on morphological studies TBE was assigned to the group of neuro-infections as an independent nosological entity.[6,7]

Vaccines against TBE have been available in Russia since 1939. Already in 1938 Kagan et al. developed the first mouse-brain propagated, formalin-inactivated vaccine from the Far East TBEV subtype "Sof'in" (1st generation of vaccines).[8,9] Vaccine field effectiveness was established at the level of 98%, but the vaccine frequently induced serious adverse events. Another vaccine, a live attenuated product based on the Elantsev strain had not been licensed due to severe complications (encephalitis) in the vaccinated group.[10] In 1950-1960 a 2nd generation of TBE vaccine was introduced which used chicken embryonic cell culture for virus reproduction.[11] Finally, in the 1980s another new type of TBE vaccine was licensed in Russia which is currently still in use – a concentrated purified lyophilized 3rd generation vaccine.[12,13]

Only two species of ticks are epidemiologically significant in Russia: *Ixodes persulcatus* in the Asian part and some additional areas in the Urals and European part (Sverdlovsk and Yaroslavl regions) and *I. ricinus* in the European part. In some regions, Dermacentor tick species were found to be the main TBEV vectors (for example, *Dermacentor reticulatus* (previously known as *D. pictus*) in Udmurtia[14-19]; *D. silvarum* and *D. nuttalli* in the Altai Republic[20] and the Republic of Tuva[21]; *D. reticulatus*, and *D. marginatus* in the Zhiguliovsk Reserve, which is located in the central part of Russia and inhabited by three species of ticks (*I. persulcatus*, *D. reticulatus*, and *D. marginatus*), and the abundance and TBEV infection rate of Dermacentor ticks were found even to be higher than those of *I. persulcatus* (4.3% vs. 1.4%, respectively).[22] Moreover, in a number of regions *I. pavlovskyi* ticks have been described as TBEV vectors.[23,24]

Currently, TBEV is subdivided into three main subtypes- the European (TBEV-Eu), the Far-Eastern (TBEV-FE), and the Siberian (TBEV-Sib). The Siberian subtype dominance of the TBEV (over 60% of endemic areas) in the Russian Federation has been demonstrated by numerous virological and molecular-genetic studies.[25-27] The Far Eastern subtype is found predominantly in the Far East, although it has been found in other territories, including Western Siberia, where it has been detected also in the blood of patients with tick-borne encephalitis.[28] The European subtype is most commonly found in the European part of Russia, although foci of the pathogen have been found in Western and Eastern Siberia.[23,29] Also, two putative TBEV subtypes (Baikalian and "178–79-like" subtypes) were described in East Siberia near Lake Baikal.[25,30] It is believed that TBEV-Eu infection usually results in a rather mild form of TBE with a case fatality rate of <2%, TBEV-Sib infection is believed to result in a generally mild illness associated with a non-paralytic febrile form of encephalitis with the tendency towards persistent TBE caused by chronic viral infection in some cases, and TBEV-FE infection causes the most severe forms of TBE.[31] Importantly, viral subtype is not the only factor that may contribute to TBE severity, and both mild and severe cases of TBE could be associated with the infection by any of the TBEV subtypes.

Official reporting of TBE cases in the USSR started in 1944. Fluctuations in TBE incidence had been observed because of the changes within the natural and anthropogenic focies, increased exposure to infected ticks, changes in the social behavior (outdoors activities, extension of the "cultured" areas, etc.), advances in diagnostics and well-designed implemented preventive measures.[18] Over time, two disease peaks were observed in Russia (Fig. 1). In the mid-1950s over 5000 cases were reported followed by a gradual decrease of the incidence until 1970. This was explained by human expansion into natural TBE foci as well as by considerable progress in establishing the diagnosis by improved laboratory methods. In 1965–1971 morbidity decreased year by year mainly due to broadly used acaricides (including DDT). From 1972 to 1991, however, morbidity increased again to the level recorded in 1964,

perhaps because vector control had been canceled. Since 1992, a number of socioeconomic factors, including large-scale allotment of land for garden plots and the growing popularity of outdoor activities, have entailed a high risk of tick bites for the urban population. As a result, the TBE incidence reached the highest values ever recorded.[19] TBE peaked in 1996 and 1999 with incidence rates in these years around 7.0 per 100,000 persons, resulting in more than 10,000 cases per year in the country.

Over the past 10 years, there has been a steady decline in the incidence of TBE in Russia, for the period 2012–2022, the average long-term incidence of TBE in the Russian Federation was 1.3 per 100,000 - a decrease of 9.7%. The share of children remained constant at 12–14% annually. Forty-eight Russian regions with a population of about 66 million people are endemic for TBE. The following federal districts play a decisive role in the formation of the incidence of TBE in the country: Siberian, Volga Federal District, Ural Federal District (Figure 1, 2).

The Reference Center for Monitoring TBE ranked the regions of the Russian Federation by long-term average of the incidence of TBE in 2012–2021, which made it possible to distinguish groups of regions: 16 regions with high epidemiological risk; 14 regions with medium TBE incidence; 17 regions with low TBE incidence; 18 regions where TBE cases were not registered. (MAP)[30].

To summarize the current TBE epidemiology data in Russia, in 2022 there were 502,764 visits to medical centers due to tick-bites (345,40 per 100,000), an increase of 12.6% compared to 2021 (446,282 visits) that is also 6.7% above the long-term average (469,950).[32] Approximately 25% of the cases occurred in children.

In the 2022 epidemic season, from April to October, 331,972 ticks taken from humans after tick bite and 62,706 ticks from environmental objects were examined for the presence of TBEV markers by ELISA and RT-PCR tests. The rates of TBEV infected ticks in those removed from humans was 1.22% (long-term average: 2.12%); and in those from the environment it was 1.57% (long-term average – 1.42%)/ (Fig. 3)[32]

In 2022 TBE incidence in Russia almost doubled compared to the previous year (2021), amounting to 1.34 per 100 thousand population (in 2021 - 0.69 per 100 thousand population)[33], 1957 TBE cases were registered in 48 subjects, including 280 children under 17 years of age (0.92 per 100,000). In the structure of TBE cases, the age group of 50 years and older prevailed (47.2%), the share of children under 17 years of age was 14.2%, the urban population was 65.5%, and the rural population was 34.5%. The main route of transmission of TBE is by tick bites, 12 cases of alimentary route TBEV infection were registered.[32]

In the structure of TBE clinical manifestations, as in previous years, the febrile form prevailed (61.9%), the second most common form was meningeal (22.2%), and the share of focal forms was 13.3%.32 In the period 2007-2022 342 deaths from TBE were registered, in 2022 – 60 deaths, in 2021 -17 deaths.[33-36]

In 2022, 3.5 million people were vaccinated against TBE (1,153,697 vaccinated and 2,347,877 revaccinated). At the same time, 34 cases of the disease were registered in vaccinated persons (11 of them in children), which constitutes 1.7% of the total number of cases.[32]

Nonspecific prevention is common to all tick-borne infections: acaricidal treatment of endemic territories by special substances (cipermetrin 25% or analogues) is regarded to be the main measure nowadays.[37] In Russia, in 2012–2022 there was a trend towards an increase in the area of acaricidal treatments of the most populated and actively used by people areas (i.e. parks, camps and recreation zones, hospital, hotels, school and kindergarten territories) in endemic regions. The minimum coverage was in 2012 (81,193 hectares), the maximum - in 2022 (246,255 hectares).[32]

Regional experience

The Middle Ural area is an active natural focus of TBE; TBE cases have been recorded since the 1930s. The Sverdlovsk region is a good example of a typical Russian TBE endemic area. At present, all 94 administrative territories of the Sverdlovsk Region are endemic for the TBE. In the 1990s in the Sverdlovsk Region TBE changed from an occupational disease to an infection connected to the course of human household activities. TBE incidences in cities began to exceed the incidence in the rural population. Long-term TBE incidence dynamics in the Sverdlovsk region can be separated into 5 periods:

- 1st period (1944-1953) – the incidence is recorded mainly among rural residents; registered only clinical forms; laboratory diagnostics was absent, there were 100-300 TBE cases annually;
- 2nd period (1953-1986) – TBE incidence increasing; laboratory diagnostics detection of the subclinical (inapparent) forms; increased number of TBE cases in people in the cities; 200-750 TBE cases annually;
- 3rd period (1986-1989) – the period of acaricidal (DDT) air spraying of the forests, TBE incidence decrease, ≤200 TBE cases per year;
- 4th period (1990-2000) – new TBE incidence increase due to the restoration of the ticks population post-abortion of the acaricidal air spraying. Change in the

immune status (both natural immunity obtained after the contact with the virus and adaptive immunity due to vaccination) of the population, change in patients' characteristics. Identification of subclinical TBE forms, immunization of occupational risk group and start of the routine adult immunization;

- 5th period (2000 to present) - TBE incidence decrease associated with routine TBE vaccination of the adult population and universal routine immunization of children.[38]

Given the high incidence of TBE, vaccination has become a leading preventative measure in the Sverdlovsk region. Four tactics of vaccination were implemented in the Regional Immunization Program (Fig 4):

- 1990-1996 - Selective specific TBE vaccination - immunization of the occupational risk groups;

- 1997-2001 - Adult population routine TBE vaccination;

- 2001-2008 - Routine children ≥ 7 years of age vaccination and mass immunization of adults;

- 2008 to present - Universal routine vaccination of children from 15 months of age and mass immunization of adults.[38]

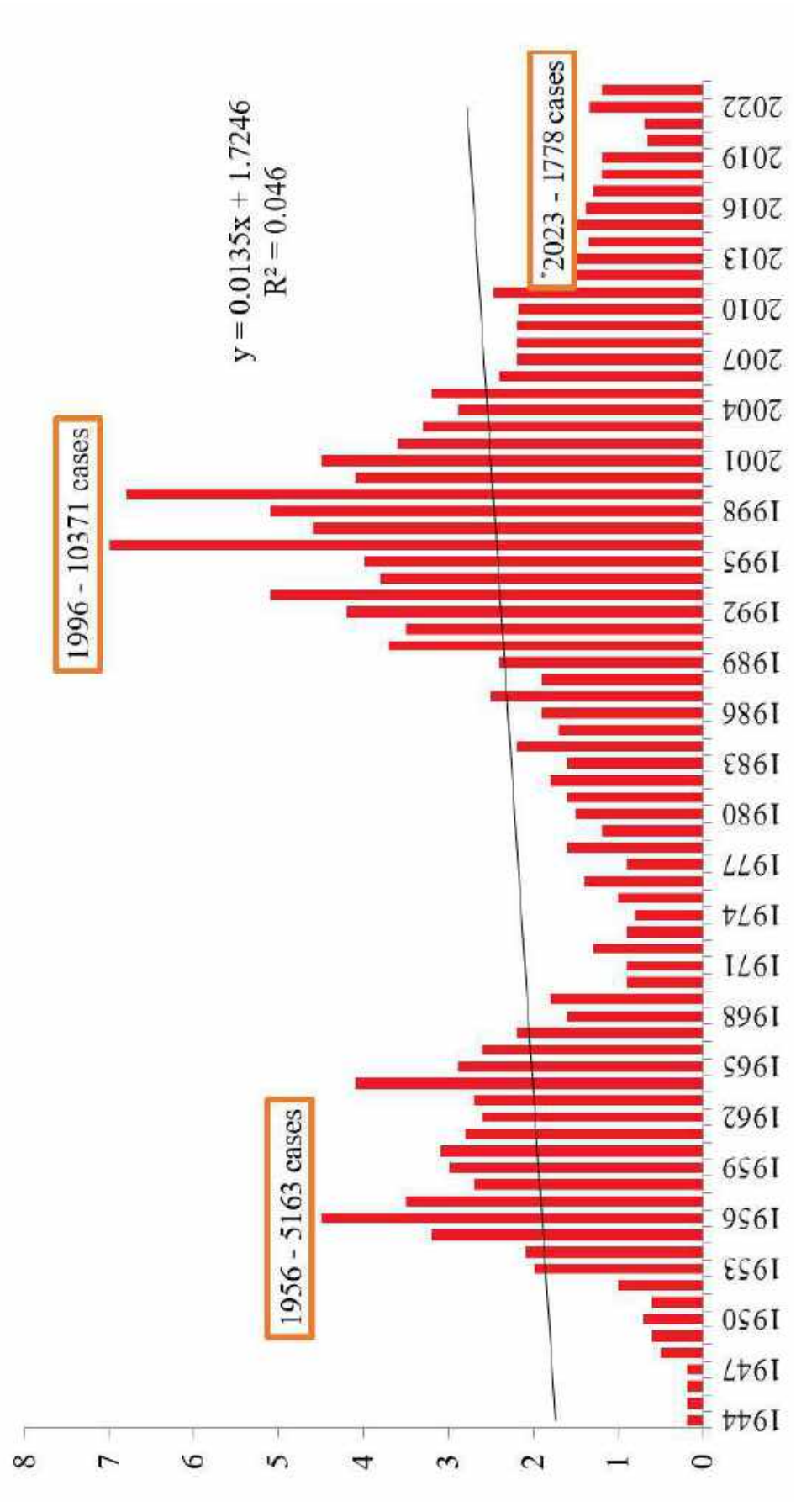
The tactics of universal routine immunization of the population over the age of 15 months in combination with "catch-up" immunization of adults provided an increase in the level of vaccination against TBE from 35 to 87% (Fig. 5) and led to an TBE incidence decrease. 98% TBE vaccination field effectiveness in 2016 (Fig 6).[36,39,40]

Overview of TBE in Russia

Table 1: TBE in Russia	
Viral subtypes, distribution	European, Siberian, and Far Eastern TBEV subtypes
Reservoir animals	Vertebrate reservoir animals assumed
Infected tick species (%)	2,1% infected tick from people after tick bite 1,6% infected tick from natural foci[32]
Dairy product transmission	Rare (goat, cow milk)
Mandatory TBE reporting	**TBE case definition**: The diagnosis of tick-borne encephalitis is made based on information about tick bite, outdoor activities in the TBE season (potential contact with natural foci), clinical course and the results of laboratory tests confirming the etiology of the disease. **Laboratory criteria for case confirmation:** The clinical diagnosis of TBE is considered confirmed in the following cases: - detection of IgM to the TBEV in blood serum in the acute period of the disease in conjunction with the detection of IgG in paired serum; - detection of a 4-fold or more increase in the IgG titer to the TBEV in paired serums, or seroconversion; - detection of a specific fragment of TBEV RNA in the blood and/or cerebrospinal fluid samples; - isolation of the TBEV. All TBE cases with laboratory confirmation are reported to the *Rospotrebnadzor* **Virology is performed in ticks only** – ELISA or multiplex PCR for TBEV, *Borrelia burgdorferi sl*, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis* / *Ehrlichia muris* *(Source: Sanitary regulations "Prevention of tick-borne encephalitis" 3.3686-21)*
Other TBE surveillance	**Endemicity definition**: An administrative territory shall be considered endemic for TBE if the features provided for in one of the following subparagraphs of this paragraph are present together: 1) the presence of vectors of the TBEV (in natural and anthropourgic foci); laboratory-confirmed circulation of the TBEV or detection of TBEV antigen/RNA in ticks from natural foci and removed from humans; immunity to the TBEV in the unvaccinated population; immunity to the TBEV among animals, provided that ixodes ticks have been distributed in the territory for at least a 5-year period; 2) registration of laboratory-confirmed TBE cases in humans during active examination of febrile patients with undetermined diagnosis, patients with meningeal conditions and with symptoms of focal lesions of the brain and spinal cord of unknown etiology; the presence ixodes ticks in the territory; laboratory-confirmed presence of TBEV or TBEV antigen/RNA in ticks collected in natural foci and removed from humans; immunity to the TBEV in the unvaccinated population; 3) registration of confirmed TBE cases; the presence of ixodes ticks in the territory, the presence of the TBEV or TBEV antigen/RNA in ticks selected in natural foci and removed from humans; the presence of immunity to the TBE virus in the unvaccinated population. *(Source: Sanitary regulations "Prevention of tick-borne encephalitis" 3.3686-21)*
Special clinical features[32]	13.3% - TBEV meningoencephalitis or meningoencephalomyelitis, 22.2% - TBEV meningitis 61.9% - fever + anti-TBEV IgM or IgG increase Case fatality rate is 1-2%

Registered vaccines	**Russian TBE vaccines (available in the market):** • **Klesch-E-Vac** for children 0.25 ml and for adults 0.5 ml; (*Source: http://chumakovs.ru/en/products*) • **TBE vaccine concentrated purified inactivated adsorbed culture dry** 0.5ml (Chumakov's Polio Institude); • **EnceVir®Neo** for children 0.25 ml, **EnceVir®** for adults 0.5 ml (Microgen) **European vaccines (not available in the market):** • **Encepur adult** 0.5ml ; • **Encepur baby** 0.25ml (GSK); • **FSME-IMMUN** 0.5ml; • **FSME-IMMUN junior** 0.25ml (Pfizer) *(Source: http://www.microgen.ru/en/)*
Vaccination recommendations and reimbursement	National immunization Calendar for epidemic indications (Order of the Ministry of Health of Russian Federation dated 06.12.2021 No. 1122n, part 2): endemic regions have the right to implement local immunization program (RegIP) with vaccination rates determined by financial conditions in the region (universal vaccination or vaccination of risk groups only – i.e. infants and elderly) Vaccination is indicated for: • persons living in endemic areas (all ages) • persons with occupational risk (forest workers, etc.) • persons traveling to endemic areas *(Source: Sanitary regulations "Prevention of tick-borne encephalitis" 3.1.3.2352-08; Ministry of Health Order #125-n part 2 "National Immunization Calendar for epidemic indications")*
	Vaccinations against TBE is recommended for: 1. Persons under 18 years of age living in administrative territories endemic for TBE, with coverage of at least 95%; 2. Adult population living in administrative territories with a high risk of the disease, taking into account the differentiation of administrative territories according to the risk of infection of the population with the TBEV, with coverage of at least 95%; 3. Adult population, by type of activity or occupation associated with staying in natural stations, as well as in horticultures located in administrative areas endemic for TBE; 4. Populations travelling to administrative areas where TBE is endemic; 5. Persons associated with labor activities in administrative areas endemic for TBE (occupational risk groups), in particular, carrying out: agricultural, logging, irrigation and reclamation, construction, harvesting, fishing, geological, surveying, expeditionary, deratization, disinfestation works; excavation and relocation of soil, clearing and improvement of forests; 6. Persons whose activities are related to the use of the TBEV; 7. Persons carrying out other types of work associated with the threat of TBE contamination. A person who has received a completed course of vaccination and 1 (or more) revaccination is considered to be vaccinated against TBE. *(Source: Sanitary regulations "Prevention of tick-borne encephalitis" 3.3686-21)*
Name, address/ website of TBE NRC	Irkutsk Anti-Plague Research Institute of Rospotrebnadzor, Irkutsk, Russian Federation *(Source: http://irknipchi.ru)*

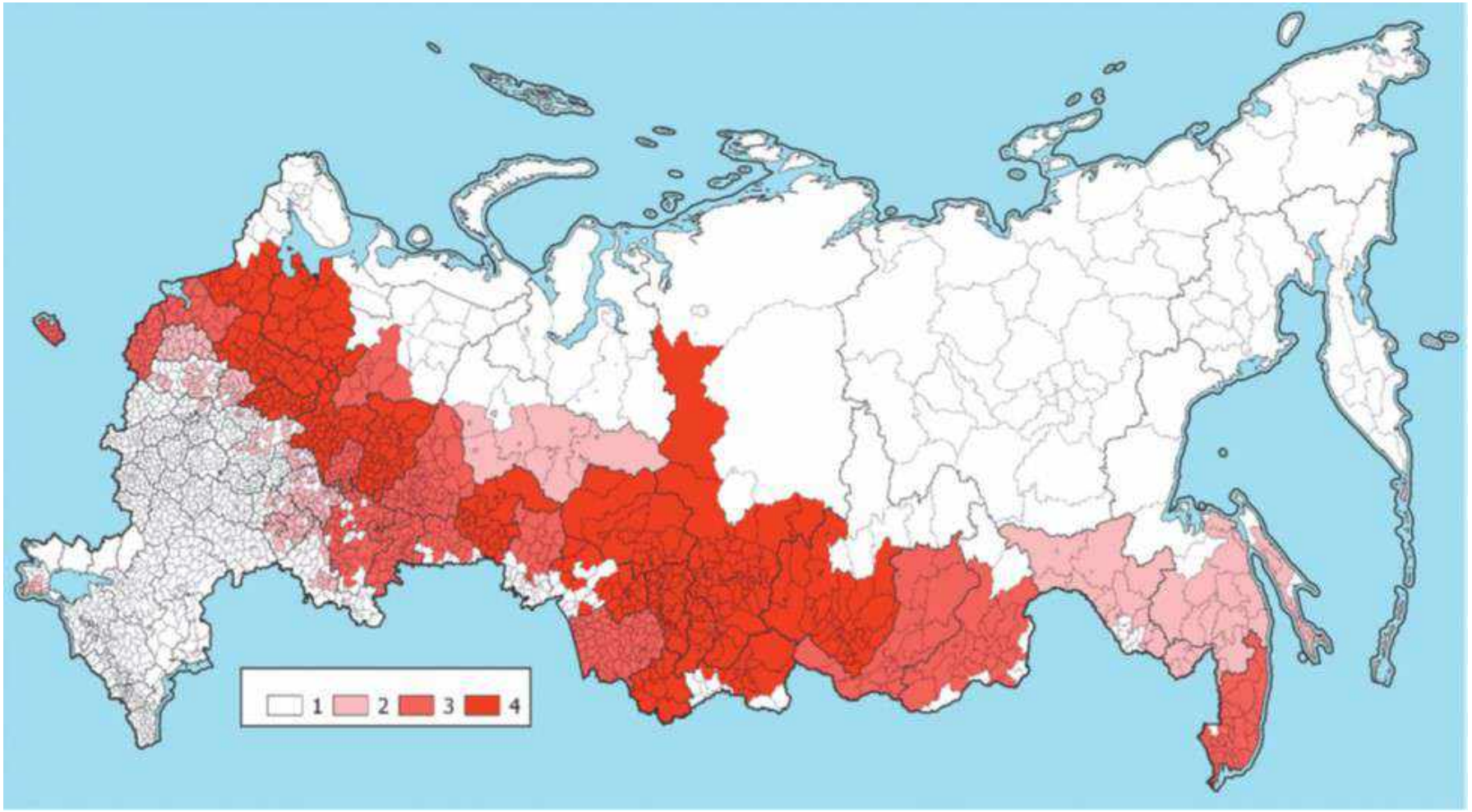
Figure 1: TBE incidence in Russia (all regions, endemic and non-endemic) in 1944–2022 per 100,000 population



*Incidence for 2023 taken from: Nikitin YA et al (2024), https://doi.org/10.21055/0370-1069-2024-1-48-58

Source Data: Appendix—Figure 1

Figure 2: Ranking of territories of constituent entities of Russia according to the long-term average annual values of TBEV-incidence per 100 thousand population (2012-2021)



1 – non-endemic territories; *2* – low level of epidemic risk (up to 0.79%);

3 – medium level (from 0.8 to 3.37%); *4* – high level of risk (more than 3.37%)

Figure 3: Dynamics of ticks infection rate (%), removed from people (A) and environmental objects (B); studied by ELISA and RT-PCR methods in 2015–2022: the left axis of ordinates, as studied by ELISA, corresponds to the red line; the right one, as studied by the PCR method, corresponds to the blue line

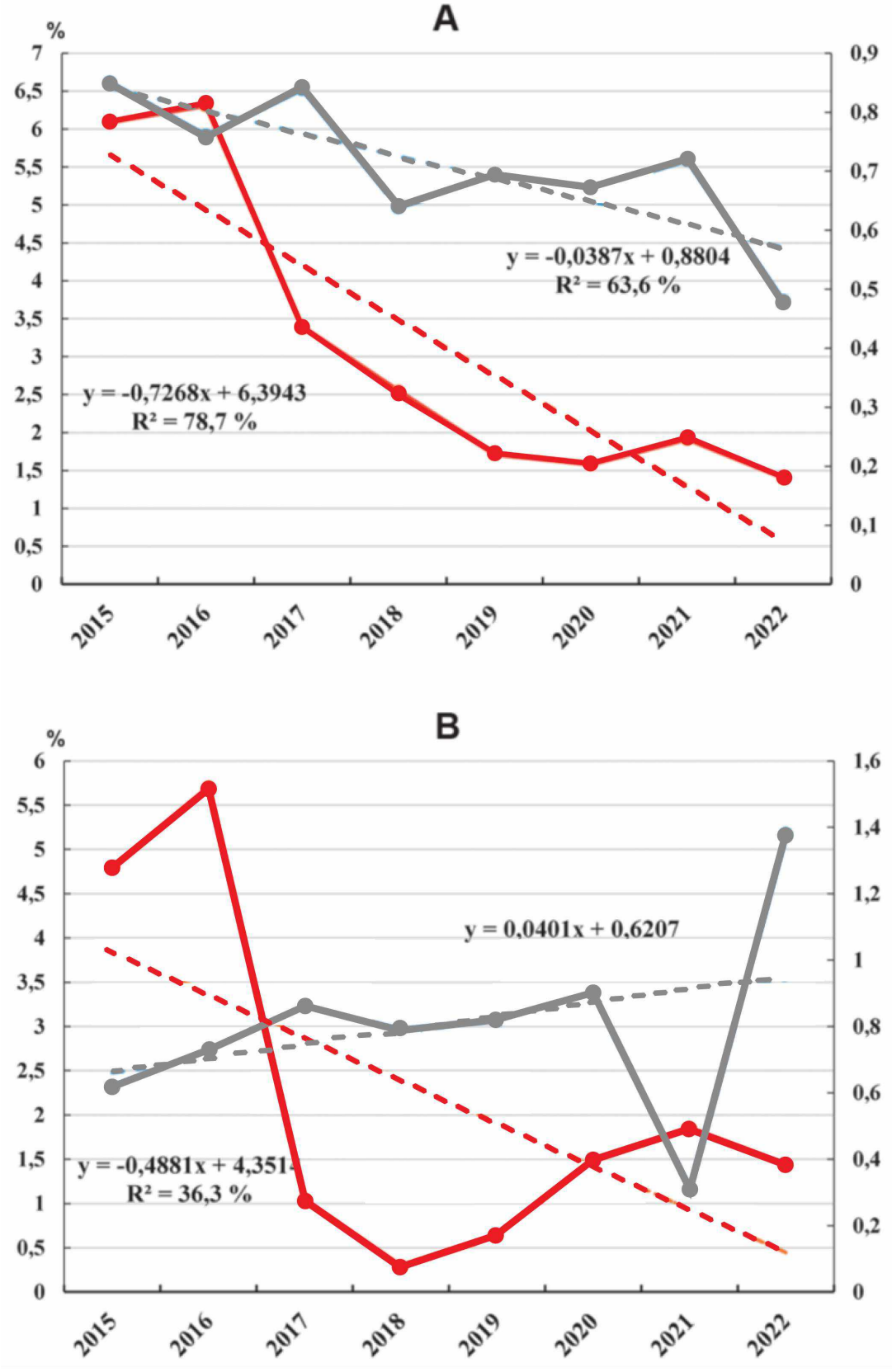
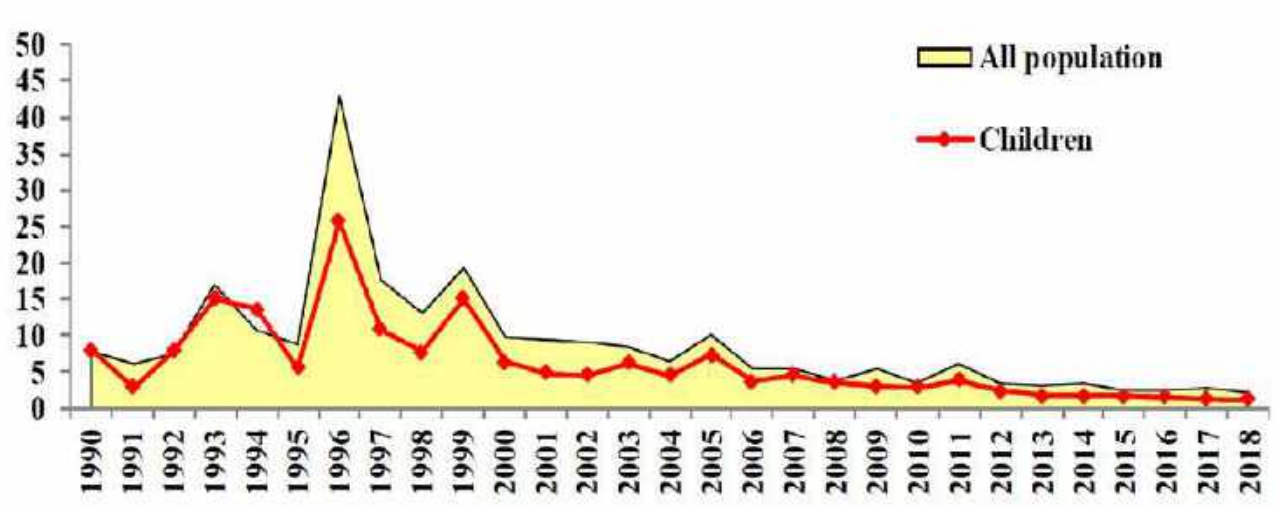
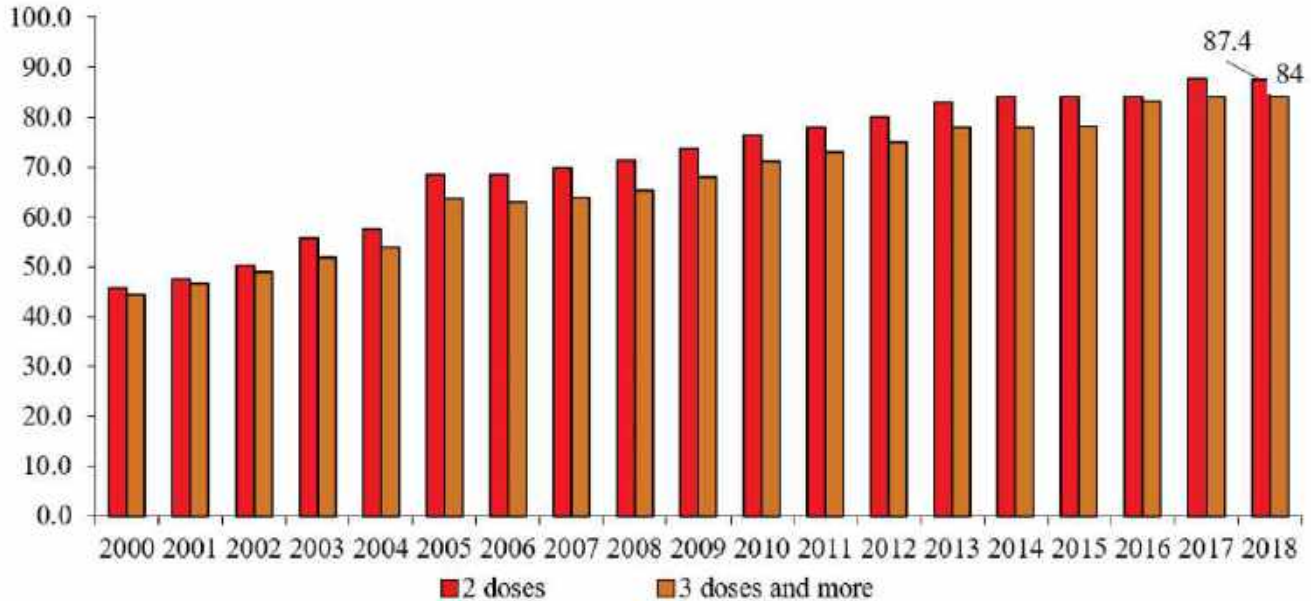


Figure 4: TBE Incidence in Sverdlovsk region by preventive tactics period in 1990–2018 (per 100,000 population, children under 14 years old)



1990-1996	1997-2001	2002-2008	2008 to present
Selective specific TBE vaccination - immunization of the occupational risk groups	Adult population mass TBE vaccination	Routine children ≥ 7 years of age vaccination and mass immunization of adults	Universal routine vaccination of children from 15 months of age and mass immunization of adults
Uptake 30%	**Uptake 55%**	**Uptake 76%**	**Uptake 87%**

Figure 5: Annual TBE vaccine uptake by the number of doses in Sverdlovsk region, Russia (%)



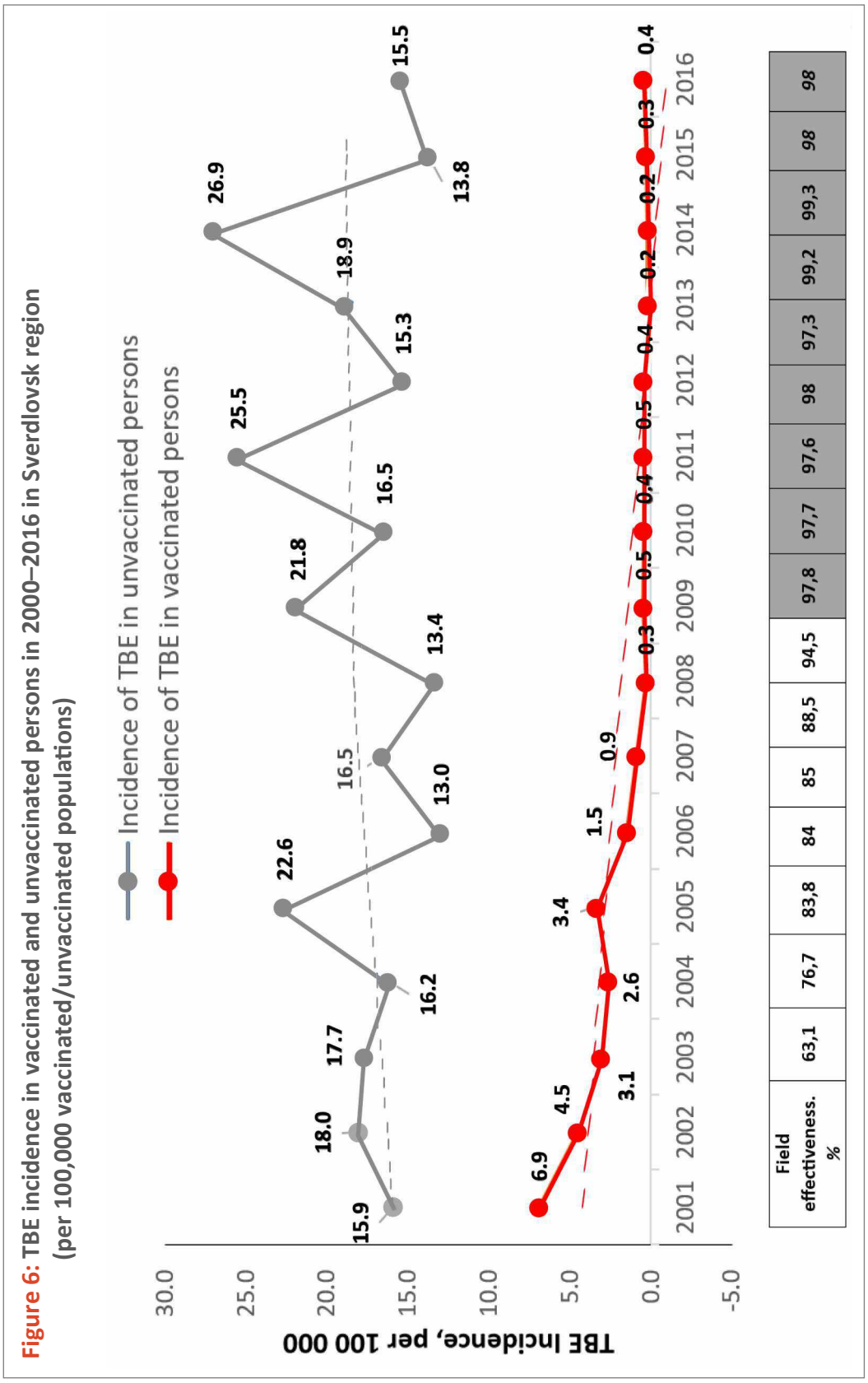


Figure 6: TBE incidence in vaccinated and unvaccinated persons in 2000–2016 in Sverdlovsk region (per 100,000 vaccinated/unvaccinated populations)

Contact: sergey.e.tkachev@gmail.com

Citation:

Tkachev S, Esyunina M, Syrochkina M. TBE in Russia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-27-7

Appendix

Source data: Figure 1

Year	Number of cases	Incidence / 10^5
1944	n/a	0.2
1945	n/a	0.2
1946	n/a	0.2
1947	n/a	0.2
1948	n/a	0.5
1949	n/a	0.6
1950	n/a	0.7
1951	n/a	0.6
1952	n/a	1
1953	n/a	2
1954	n/a	2.1
1955	n/a	3.2
1956	n/a	4.5
1957	n/a	3.5
1958	n/a	2.7
1959	3516	3
1960	n/a	3.1
1961	n/a	2.8
1962	n/a	2.6
1963	n/a	2.7
1964	n/a	4.1
1965	n/a	2.9
1966	n/a	2.6
1967	n/a	2.2
1968	n/a	1.6
1969	n/a	1.8
1970	1169	0.9
1971	1175	0.9
1972	1707	1.3
1973	1189	0.9
1974	1062	0.8
1975	1336	1
1976	1883	1.4
1977	1220	0.9
1978	2184	1.6
1979	1649	1.2
1980	2072	1.5
1981	2221	1.6
1982	2513	1.8
1983	2248	1.6
1984	3115	2.2
1985	2423	1.7
1986	2728	1.9
1987	3620	2.5
1988	2774	1.9
1989	3528	2.4
1990	5475	3.7
1991	5194	3.5
1992	6239	4.2
1993	7571	5.1
1994	5640	3.8
1995	5935	4
1996	10371	7
1997	6804	4.6
1998	7531	5.1
1999	10011	6.8
2000	6010	4.1
2001	6569	4.5
2002	5231	3.6
2003	4773	3.3
2004	4178	2.9
2005	4593	3.2
2006	3433	2.4
2007	3142	2.2
2008	3140	2.2
2009	3141	2.2
2010	3094	2.18
2011	3533	2.47
2012	2716	1.9
2013	2236	1.57
2014	1978	1.36
2015	2304	1.58
2016	2035	1.39
2017*	1934	1.3
2018**	1727	1.18
2019***	1775	1.21
2020	989	0.67
2021	1015	0.69
2022	1957	1.34
2023	1778	1.22

**State Report "About the sanitary-hygiene wellbeing of the population of the Russian Federation in 2017"*
http://rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=10145

***State Report "About the sanitary-hygiene wellbeing of the population of the Russian Federation in 2018"*
https://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=12053

****State Report "About the sanitary-hygiene wellbeing of the population of the Russian Federation in 2019"*
https://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=14933

References

1. Chumakov MP. Tick-transferred spring-summer encephalitis in the European part of the USSR and Western Siberia. *Zoolog J*. 1940;19(2):335
2. Zilber LA. [To the history of Far-East encephalitis investigations]. *Vopr Virusol.* 1957;(6):323-331
3. Pogodina VV. [A path to true history]. *Vopr Virusol*. 2007;52(5):5-8
4. Zlobin VI. [TBE: etiology, epidemiology and prevention problems in the beginning of the XXI century]. Ural Medical Journal. 2006; 6-11
5. Pogodina VV. [Memories of Elizabeth Nikolaevna Levkovich]. Moscow. 2001
6. Volkova LI. [Pathomorphosis of acute TBE in the Sverdlovsk region]. PhD thesis. Perm. 2001;34
7. Volkova LI, Romanenko VV, Struin NL, et al. [Epidemiology, etiology, clinical presentation, diagnosis, treatment and prevention of TBE. Methodological instructions]. Yekaterinburg. 2004
8. Smorodintsev AA, Dubov AV. [TBE and its prevention by vaccination]. Leningrad: Medicine]. 1986
9. Smorodintsev AA, Levkovich EN, Dankovskiy NL. [Epidemiological effectiveness of active immunization against TBE]. *JMEI*. 1941;4:12
10. Levkovich EN, Zasukhina GD, Chumakov, et al. [Tissue culture vaccine against TBE]. *Vopr Virusol*. 1960;2:233-236
11. Lvov DK. [Immunoprophylaxis of TBE]. Doctoral dissertation. Moscow. 1965
12. Bilalova GP, Bystritsky LD, Vorobyova MS, Krasilnikov IV. [The history of vaccine production for the prevention of tick-borne encephalitis in the city of Tomsk: from brain vaccine to EnceVir vaccine]. B*ull. Sib. Dep. RAMS*. 2007;4:105-110
13. Vorovich MF, Hapchaev Yu Kh, Prilukova NS, Nagirieva LI, Grachev VP. [Russian inactivated dry vaccine against tick-borne encephalitis]. *Biomedicines*. 2004;2(14):17-20
14. Gerasimov SG, Druzhinina TA, Karan LS, et al. [Features of TBE in the Yaroslavl region at the present stage. The problem of the evolution of infection]. *Epidemiol Infect Dis*. 2014;19(4):37-44
15. Glinsky NP, Kokorev VS, Patsuk NV, Kuchkova EV, Gogoleva OYu. [Tick-borne encephalitis: epidemiology, clinic, diagnosis, prevention]. Yekaterinburg. *AMB Publishing House*. 2006
16. Danchinova GA, Khasnatinov MA, Zlobin VI. [Ixodid ticks in the south of Eastern Siberia and Mongolia and their spontaneous infection with pathogens of natural focal transmissible infections]. *Bulletin of Siberian Medicine*. 2006; 5(1):137-143
17. Alekseev AN, Dubinina EV, Yushkova OV. [*Functioning of "tick-causative agents" parasitic system in conditions of increasing anthropogenous pressure*]. S-Peterburg: Insanta. 2008
18. Korenberg EI, Pomelova VG, Osin NS. [*Natural focal infections transferred by Ixodes ticks*]. Moscow. 2013
19. Korenberg E, Likhacheva T. Analysis of the long-term dynamics of tick-borne encephalitis (TBE) and ixodid tick-borne borrelioses (ITBB) morbidity in Russia. *Zentralbl Bakteriol*. 2006;296(S1):54–58. doi:10.1016/j.ijmm.2006.02.001
20. Shchuchinova LD, Kozlova IV, Zlobin VI. Influence of altitude on tick-borne encephalitis infection risk in the natural foci of the Altai Republic, Southern Siberia. *Ticks Tick Borne Dis.* 2015;6(3):322-329. doi:10.1016/j.ttbdis.2015.02.005
21. Kholodilov I, Belova O, Burenkova L, et al. Ixodid ticks and tick-borne encephalitis virus prevalence in the South Asian part of Russia (Republic of Tuva). *Ticks Tick Borne Dis*. 2019;10(5):959-969. doi:10.1016/j.ttbdis.2019.04.019
22. Morozov VG, Krasnobaev YP, Burenkova LA, et al. [Epidemiologic characteristic of the natural focuses of tick-borne encephalitis and borreliosis in the Zhiguli reserve territory]. *Samar Luka Probl Reg Glob Ekol*. 2009;18:106-112
23. Rar V, Livanova N, Tkachev S, et al. Detection and genetic characterization of a wide range of infectious agents in Ixodes pavlovskyi ticks in Western Siberia, Russia. *Parasit Vectors.* 2017;10(1):258. doi:10.1186/s13071-017-2186-5
24. Rar V, Livanova N, Sabitova Yu, et al. Ixodes persulcatus/pavlovskyi natural hybrids in Siberia: Occurrence in sympatric areas and infection by a wide range of tick-transmitted agents. *Ticks Tick Borne Dis*. 2019;10(6):101254. doi:10.1016/j.ttbdis.2019.05.020
25. Demina TV, Dzhioev YP, Verkhozina MM, et al. Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. *J Med Virol*. 2010;82 (6):965-976. doi:10.1002/jmv.21765
26. Zlobin VI, Verkhozina MM, Demina TV, et al. [Molecular epidemiology of tick-borne encephalitis]. *Vopr Virusol*. 2007;52(6):4-9
27. Zlobin VI, Demina TM, Mamaev LV, et al. [Analysis of TBEV genetic variability by the primary structure of the surface E-protein gene]. *Vopr Virusol*. 2001;1:13-16
28. Tkachev SE, Fomenko NV, Rar VA, Igolkina YP, Kazakova YV, Chernousova NY. PCR-detection and molecular-genetic analysis of tick-transmitted pathogens in patients of Novosibirsk region, Russia. *Int J Med Microbiol.* 2008;298:365-367. doi:10.1016/j.ijmm.2007.12.010

29. Demina TV, Tkachev SE, Kozlova IV, et al. Comparative analysis of complete genome sequences of European subtype tick-borne encephalitis virus strains isolated from Ixodes persulcatus ticks, long-tailed ground squirrel (Spermophilus undulatus), and human blood in the Asian part of Russia. *Ticks Tick Borne Dis*. 2017;8 (4):547-553. doi:10.1016/j.ttbdis.2017.03.002

30. Kozlova IV, Demina TV, Tkachev SE, et al. Characteristics of the Baikal subtype of tick-borne encephalitis virus circulating in Eastern Siberia. *Acta Biomedica Scientifica*. 2018;3(4):53–60. doi:10.29413/ABS.2018-3.4.9

31. Ruzek D, Avšič Županc T, Borde J, et al. Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. *Antiviral Res.* 2019;164:23-51. doi:10.1016/j.antiviral.2019.01.014

32. Andaev EI, Nikitin AY, Tolmacheva MI, et al. [Epidemiological Situation on Tick-Borne Viral Encephalitis in the Russian Federation in 2022 and Forecast of its Development for 2023]. *Problemy Osobo Opasnykh Infektsii*. 2023;1:6–16. doi:10.21055/0370-1069-2023-1-6-16

33. Rospotrebnadzor. [On the state of sanitary and epidemiological well-being of the population of the Russian Federation the in 2022]. Accessed March 29, 2024. https://rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=25076

34. Rospotrebnadzor. [About the sanitary-hygiene wellbeing of the population of the Russian Federation in 2017]. Accessed March 29, 2024. http://rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=10145

35. Rospotrebnadzor. [About the sanitary-hygiene wellbeing of the population of the Russian Federation in 2018]. Accessed March 29, 2024. https://www.rospotrebnadzor.ru/bitrix/redirect.php?event1=file&event2=download&event3=gosudarstvennyy-doklad-zashchita-prav-potrebiteley-v-rossiyskoy-federatsii-v-2018-godu.pdf&goto=/upload/iblock/332/gosudarstvennyy-doklad-zashchita-prav-potrebiteley-v-rossiyskoy-federatsii-v-2018-godu.pdf

36. Rospotrebnadzor. [On the state of sanitary and epidemiological well-being of the population of the Russian Federation the in 2019]. Accessed March 29, 2024. https://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=14933

37. Rospotrebnadzor. [About the sanitary-hygiene wellbeing of the population of the Russian Federation in 2016]. Accessed March 29, 2024. http://www.rospotrebnadzor.ru/bitrix/redirect.php?event1=file&event2=download&event3=gosudarstvennyy-doklad-2016.pdf&goto=/upload/iblock/0b3/gosudarstvennyy-doklad-2016.pdf

38. Esyunina MS. [Current trends in the incidence of tick-borne encephalitis in various tactics of vaccination and the improvement of epidemiological surveillance and control]. PhD dissertation. Perm. 2015

39. Rospotrebnadzor. [On the state of sanitary and epidemiological well-being population in the Sverdlovsk region in 2017]. Accessed March 29, 2024. http://www.66.rospotrebnadzor.ru/c/document_library/get_file?uuid=0091fbe2-bfaa-4cc0-9eaa-1e8a91053b58&groupId=10156

40. Rospotrebnadzor. [On the state of sanitary and epidemiological well-being population in the Sverdlovsk region in 2018]. Accessed March 29, 2024. http://www.66.rospotrebnadzor.ru/c/document_library/get_file?uuid=cffb1abc-98c1-48aa-8a30-044b2b04c2c1&groupId=10156

Chapter 13

TBE in Serbia

Pavle Banović

E-CDC risk status: endemic (last edited: date 24.03.2024, data from 2023)

History and current situation

Tick-borne encephalitis virus (*Orthoflavivirus encephalitis*; TBEV) was reported in Serbia for the first time in 1972 when 2 TBEV strains were isolated from questing *Ixodes ricinus* and *Ixodes persulcatus* collected in the Pešter plateau (Western Serbia).[1,2] Since then, there were no reports about TBEV in questing ticks until 2017, when Potkonjak et al. reported presence of TBEV-Eu in *I. ricinus* ticks collected at Fruška Gora Mountain (North Serbia) and suburban parts of Belgrade.[3] Regardless of occasional TBEV findings in ticks from Serbia, there is still no evidence of active TBEV foci in any part of the country, as data from reservoir and sentinel animals are lacking.

Serosurveys conducted in the period of 1962-1969 via hemagglutination inhibition test found great variation in prevalence of TBEV-reactive antibodies in populations across Serbian regions, with highest seroprevalence rate in the Sandžak-Raška region (52.6%), followed by Kosovo Autonomous Province (37.8%), Western Serbia (19.4%), Banat (8%) and Belgrade region (7.3%). Regions with lowest seroprevalence were Southeastern Serbia (3.6%) and Srem (1.1%). Nevertheless, these results should be interpreted with caution, since hemagglutination inhibition tests can't distinguish TBEV-neutralizing antibodies from antibodies generated against West Nile virus (*Orthoflavivirus nilense*; WNV),[4] that was most probably circulating within Yugoslavia in the same period.[2]

Clinicians in Serbia were facing an obstacle in TBE diagnosis until TBEV-neutralization assay was developed by Pasteur Institute Novi Sad in 2022.[5] More precisely, due to antibody cross-reactivity, there is a high probability that cases of Tick-Borne Encephalitis (TBE) will be misdiagnosed as West Nile encephalitis if ELISA is the only assay used for indirect diagnostics in patients with viral inflammation of the central nervous system.[6] In the same year (2022), a fatal case of imported TBE was described in South Serbia, where neutralization assay was used to confirm the suspected etiology in a patient returning from Switzerland.[5]

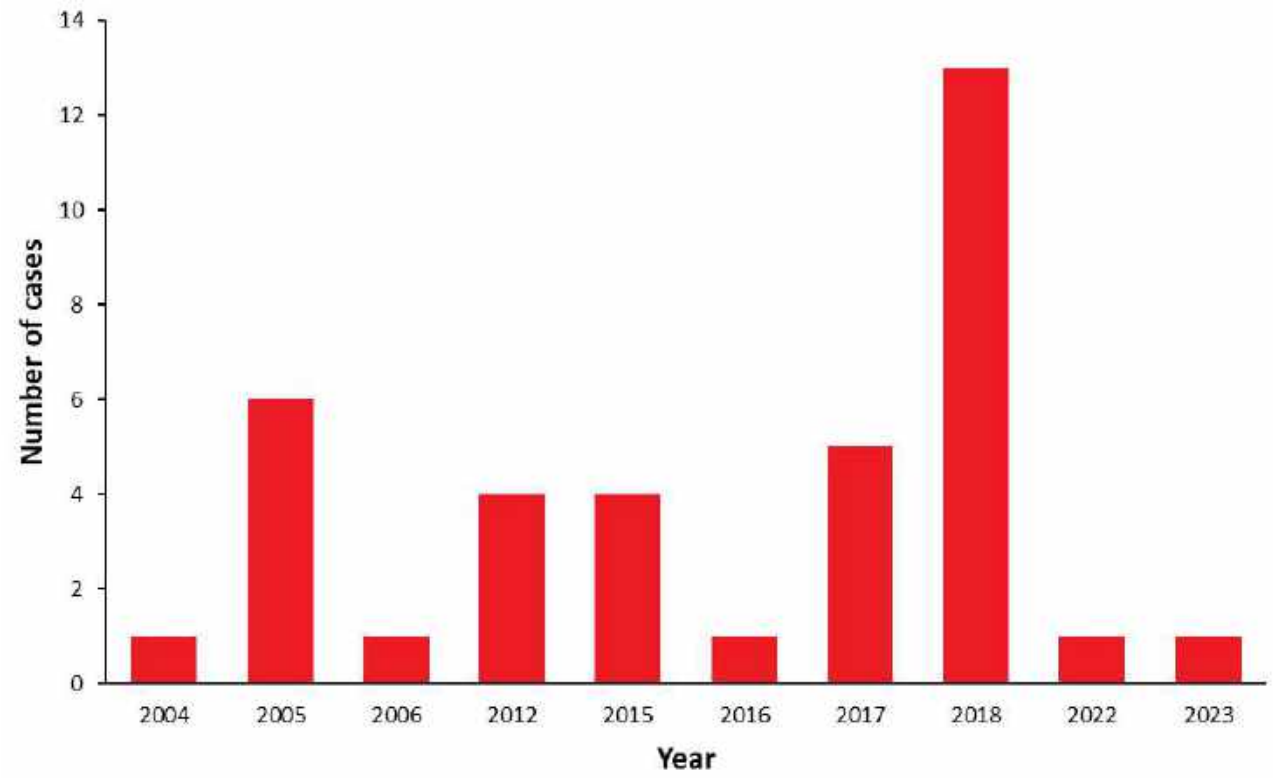
In a serosurvey comparing TBEV-exposure in tick-infested individuals from two Balkan cities (Novi Sad in Serbia and Skopje in North Macedonia), TBEV-neutralizing antibodies were found in one subject from Skopje (1/45; 2.22%) and in none of the enrolled persons from Novi Sad (0/51;0%).[7] Nevertheless, a larger-scale study focused on tick-infested individuals from North Serbia revealed the presence of TBEV-neutralizing antibodies in three individuals (3/450; 0.66%).[8]

Overview of TBE in Serbia

Table 1: TBE in Serbia

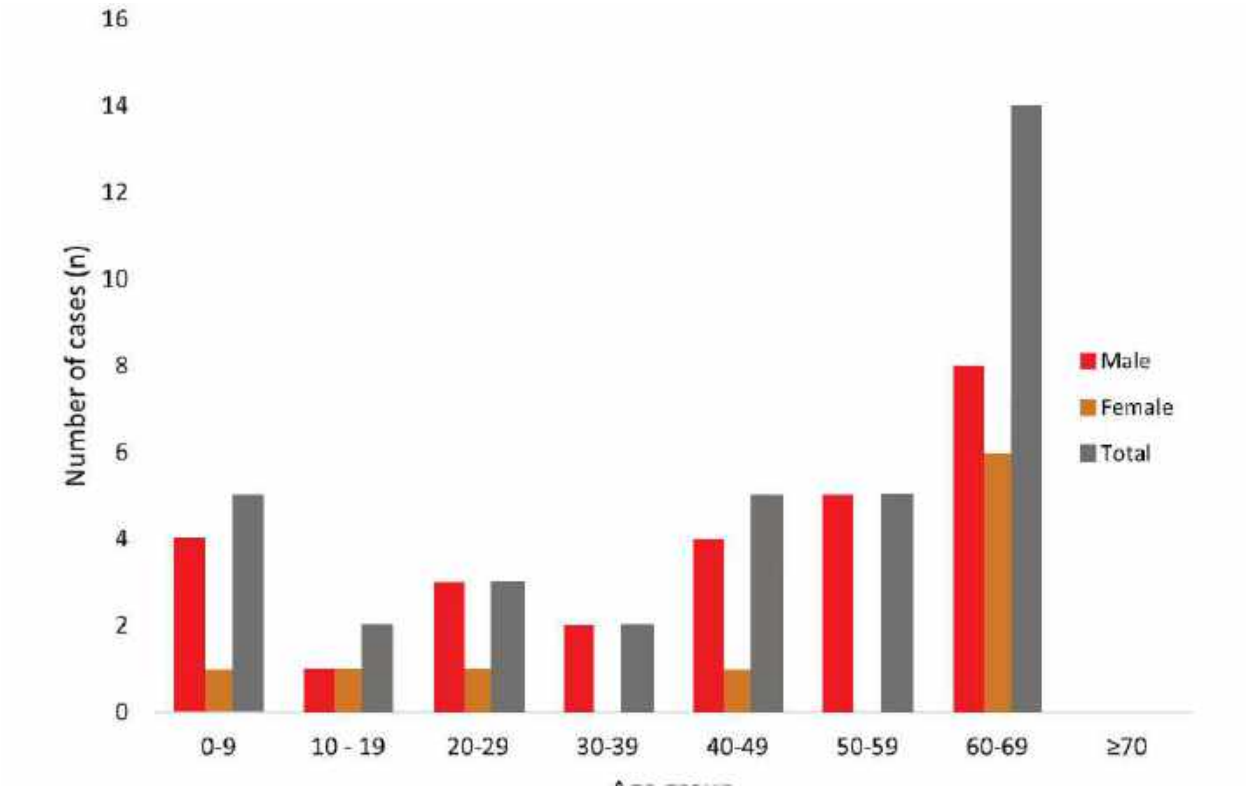
Virus subtypes isolated	TBEV-Eu[3]
Reservoir animals	N/A, no surveillance is established[9]
Percentage infected ticks	0%[10], no surveillance is established[9]
Dairy product transmission	N/A
Case definition used by authorities	There is no nationally regulated TBE case definition. Clinical center of Serbia uses following case definition: Characteristic clinical picture with TBEV-reactive IgM and IgG in the serum and cerebrospinal fluid, done by ELISA with negative serological finding for West Nile-Virus, Herpes simplex virus 1, varicella zoster virus, *B. burgdorferi*, *Leptospira* sp. and *Brucella* sp.[11]
Completeness of case detection and reporting	N/A
Type of reporting	Mandatory
Other TBE surveillance	Since January 2020, surveillance according to the EU Clinical Case Definition has been introduced in all hospitals in Autonomous Province of Vojvodina, as a part of Special Public Health Program. Program is based on software application for Case Definition detection in all departments for infectious diseases.
Special clinical features	N/A
Licensed vaccines	No TBE vaccine is licensed in Serbia
Vaccine recommendations	While no TBE-vaccine is licensed in the country, immunization is recommended for the population living in TBE endemic areas, as well as for professionals and recreational individuals entering TBEV hotspots[12]
National Reference center for TBE	National reference center for TBE: Institute of Virology, Vaccines and Sera "Torlak" Vojvode Stepe 458, Belgrade. Laboratory with TBEV-neutralization assay: Pasteur Institute Novi Sad, Hajduk Veljkova 1, Novi Sad.

Figure 1: TBE cases 2004-2023; NOTE: The TBE case definition is different from the E-CDC definition, see Table 1 above.



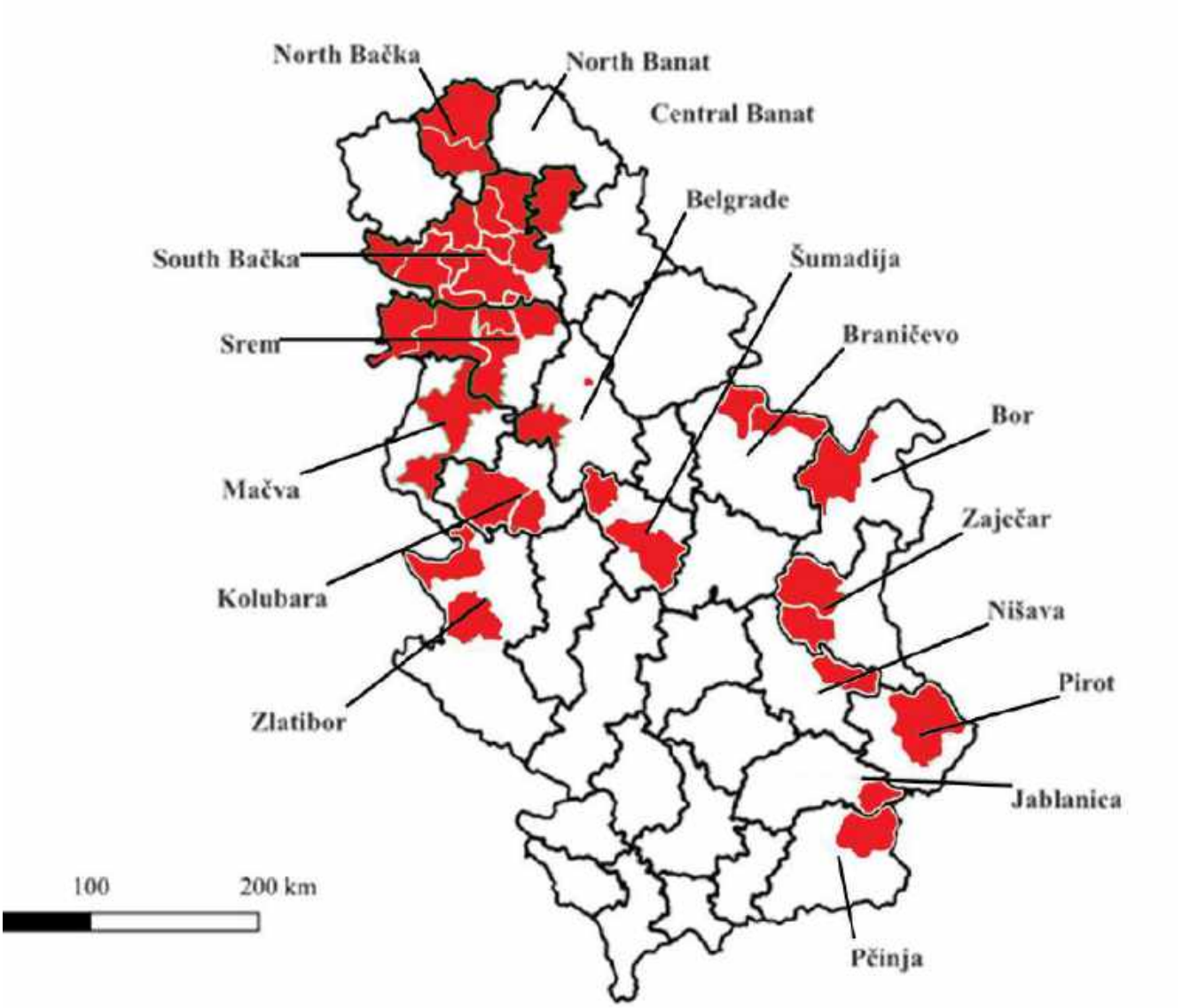
Source Data: Appendix-Figure 1

Figure 2: Age and Gender Distribution TBE case numbers 2004-2023; NOTE: The TBE case definition is different from the E-CDC definition.



Source Data: Appendix-Figure 2

Figure 3: Map of Serbia where red marks municipalities where tick infestation occurred for each person tested for TBEV-neutralizing antibodies in the most recent serosurvey[8]. Persons with TBEV-neutralising antibodies were exposed to tick bites in the regions of Šumadija (Mountain Bukulja), Srem (Mountain Fruška Gora) and Mačva (Mountain Divčibare).



Acknowledgments

TBE epidemiology data for the period 2004-2018 was provided by Institute of Public Health of Serbia "Dr. Milan Jovanović Batut" and Institute of Public Health of Vojvodina

Authors and affiliations

Assist. Dr. sci. med. Pavle Banović, Department of Microbiology with Parasitology and Immunology, Faculty of Medicine in Novi Sad, University of Novi Sad, 21000 Novi Sad, Serbia and Diagnostics and Laboratory Research Task Force, Balkan Association for Vector-Borne Diseases, 21000 Novi Sad, Serbia and Pasteur Institute Novi Sad, 21000 Novi Sad, Serbia

Appendix

Source data: Figure 1

Year	Number of TBE cases*
2004	1
2005	6
2006	1
2012	4
2015	4
2016	1
2017	5
2018	13
2022	1**
2023	1***

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	4	1	5
10-19	1	1	2
20-29	3	1	3
30-39	2	0	2
40-49	4	1	5
50-59	5	0	5
60-69	8	6	14
>70	0	0	0

Contact: Pavle.banovic@mf.uns.ac.rs

Citation:
Banović P, TBE in Serbia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. The TBE Book. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-28-7

References

1. Bordoski M, Gligić A, Bosković R. Arbovirus infections in Serbia. Vojnosanit Pregl. 1972;29(4):173-175.
2. Vesenjak-Hirjan J, Punda-Polić V, Dobe M. Geographical distribution of arboviruses in Yugoslavia. *J Hyg Epidemiol Microbiol Immunol.* 1991;35(2):129-140.
3. Potkonjak A, Petrović T, Ristanović E, et al. Molecular Detection and Serological Evidence of Tick-Borne Encephalitis Virus in Serbia. *Vector-Borne Zoonotic Dis.* 2017;17(12):813-820. doi:10.1089/vbz.2017.2167
4. Holzmann H. Diagnosis of tick-borne encephalitis. *Vaccine*. 2003;21 Suppl 1:S36-40.
5. Popović Dragonjić L, Vrbić M, Tasić A, et al. Fatal Case of Imported Tick-Borne Encephalitis in South Serbia. *Tropical Medicine and Infectious Disease.* 2022;7(12):434. doi:10.3390/tropicalmed7120434
6. Kunze M, Banović P, Bogovič P, et al. Recommendations to Improve Tick-Borne Encephalitis Surveillance and Vaccine Uptake in Europe. *Microorganisms.* 2022;10(7):1283. doi:10.3390/microorganisms10071283
7. Jakimovski D, Mateska S, Dimitrova E, et al. Tick-Borne Encephalitis Virus and *Borrelia burgdorferi* Seroprevalence in Balkan Tick-Infested Individuals: A Two-Centre Study. *Pathogens.* 2023;12(7):922. doi:10.3390/pathogens12070922
8. Banović P, Mijatović D, Bogdan I, et al. Evidence of tick-borne encephalitis virus neutralizing antibodies in Serbian individuals exposed to tick bites. *Front Microbiol*. 2023;14:1314538. doi:10.3389/fmicb.2023.1314538
9. Jovanović V. Report on Infectious Diseases in the Republic of Serbia for the Year 2022. Institute of Public Health of Serbia "Dr. Milan Jovanović Batut"; 2023. Accessed 24 March, 2024. https://www.batut.org.rs/download/izvestaji/GodisnjiIzvestajZarazneBolestiSrbija2022.pdf
10. Pustahija T. *Seroprevalence and epidemiological characteristics of tick-borne encephalitis in Vojvodina*. University of Novi Sad; 2023. Accessed 24 March, 2024. https://nardus.mpn.gov.rs/bitstream/handle/123456789/21781/Disertacija_14132.pdf?sequence=1&isAllowed=y
11. Poluga J, Barac A, Katanic N, et al. Tick-borne encephalitis in Serbia: A case series. *The Journal of Infection in Developing Countries.* 2019;13(06):510-515. doi:10.3855/jidc.11516
12. Lončarević G. Stručno-metodološko uputstvo za sprovođenje obavezne i preporučene imunizacije stanovništva za 2017. Accessed 24 March, 2024. https://www.batut.org.rs/download/SMUzaRedovnuImunizaciju2017.pdf

Chapter 13

TBE in Slovakia

Jana Kerlik

E-CDC risk status: endemic (data as of end 2023)

History and current situation

The former Czechoslovak Republic was one of the first countries in Europe where the tick-borne encephalitis (TBE) virus was identified. This discovery was made in 1947, when Rampas and Gallia observed a high incidence of disease identified as "Czechoslovakia encephalitis", and TBE virus was isolated from *Ixodes ricinus*.[1]

In 1951, for the first time ever, and again in Czechoslovakia, the alimentary transmission of TBE virus from infected animals to humans was confirmed during a large outbreak in Rožňava. There were 271 hospitalized and serologically confirmed TBE patients. Blaškovič et al. found that most patients had drunk milk from the local dairy, which did not comply with basic sanitary requirements. The milk had not been pasteurized, but only stirred, equalized, and distributed. In addition, the goat milk that had been supplied to the dairy was also possibly infected.[2] During the examination of the TBE focus in Rožňava, the goats were found with high anti-TBE virus titers.[3]

A list of natural foci of TBE in Slovakia was developed by the Public Health Authority of Slovakia in 2002 directly on the basis of virus isolation data from ticks and reservoir animals in the years 1964–1997 from the Institute of Virology, Slovak Academy of Sciences in Bratislava as well as indirectly according to the site of infection in patients with TBE as reported during 1972–2002.[8] In recent years there has been a shift of natural TBE foci from the southern to the northern and central areas of the country.[9] The reason is attributed to several factors including climate change.[4]

There is a long-term increasing trend of TBE cases in Slovakia. In 2022 we observed the highest number of TBE cases over the last 60 years[31].

Slovakia is well known in Europe for TBE alimentary outbreaks that are reported almost every year.[10] Over the last few years, there has been a growing trend in the number of food-borne TBE outbreaks. The percentage of TBE virus infections through consumption of unpasteurized milk and its products is quite high compared with other countries, e. g. in 2023, 34% of alimentary TBE cases were reported[31]. Slovaks like to consume traditional products made from raw goat and sheep milk, especially sheep cheese. Moreover, raw goat milk has been recently promoted as a product to improve health and immunity in humans.

In 2020 we reported a case of probable transmission of TBE virus from an unvaccinated mother to an infant through breast-feeding.[5]

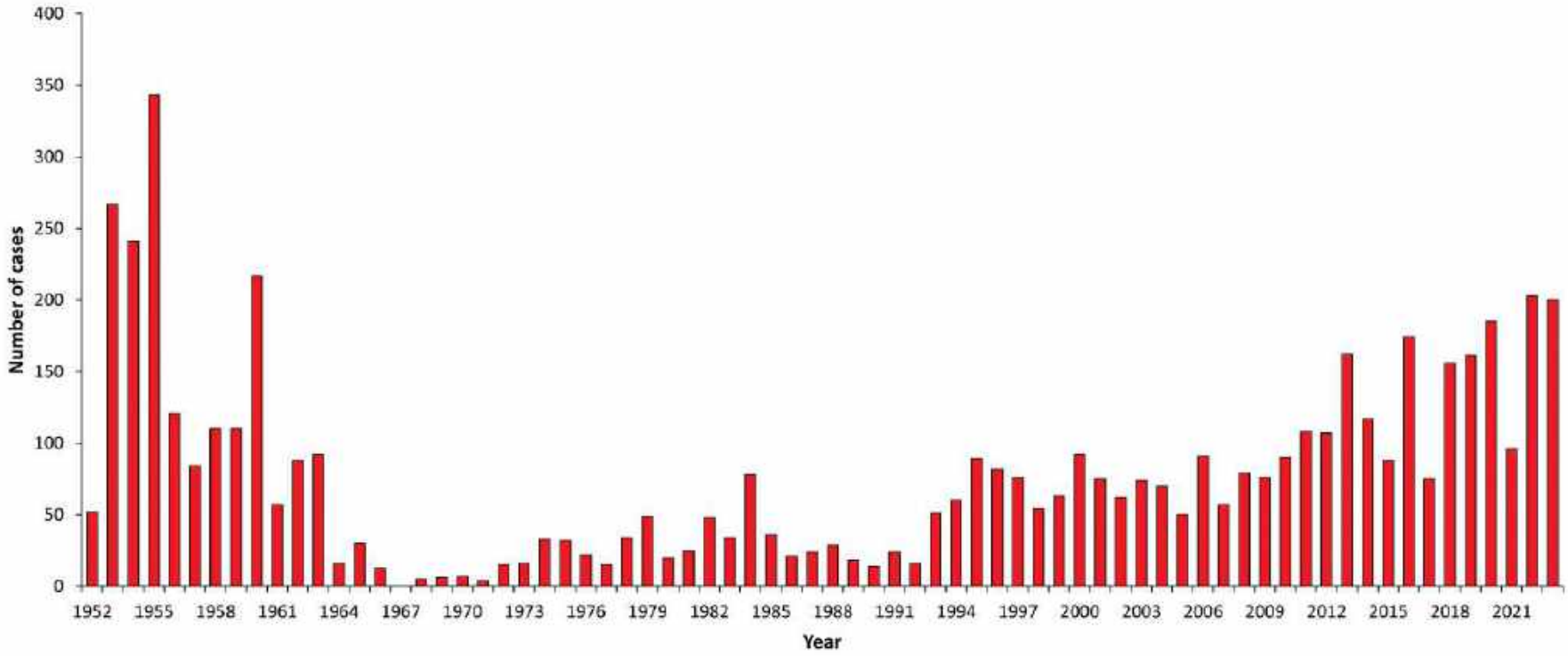
Overview of TBE in Slovakia

Table 1: TBE in Slovakia

Virus subtypes isolated	European subtype[1]
Reservoir animals	Tribeč region (Jarok pri Nitre, Jelenec, Topoľčianky), **1965**: Out of 46 blood and brain samples taken from moles (Talpa europaea), 7 positive isolations of TBE VIRUS were obtained. Therefore, moles can represent not only an important host animal, but may also be considered a reservoir of TBE VIRUS in elementary foci[11] Tribeč region, **1967**: Isolation of virus from the blood of *Apodemus flavicollis*, *Clethrionomys glareolus*, and *Erinaceus roumanicus*[12] Tribeč region, **1967**: 2 TBE VIRUS strains were isolated from *Ixodes ricinus* collected on 2 Turdus merula[13] Lúky pod Makytou, **1981**: 5 strains of TBE VIRUS isolated from ticks and organs of *Apodemus flavicollis* (in 15% infected)[14] Western Slovakia (6 localities), **1981–1986**: 6 TBE VIRUS strains isolated from organs of *C. glareolus* (4), *Apodemus flavicollis* (1), *Sorex araneus* (1)[15] Záhorská Ves, **1990–1992**: 8 TBE virus isolates from organs of *C. glareolus* (6), *Apodemus flavicollis* (1), *Apodemus sylvaticus* (1)[16] Košická Belá, 2013: TBE virus from the brain sample of *Buteo buteo*[17] The Drienovská wetland, **2019-2020**: 9.8% seropositivity in the birds (n = 37) of 376 tested sera[28]
Percentage infected ticks	The number of infected ticks in endemic areas varies widely from 0.1% to 5% depending on the season and habitat[18] Tribeč, **1964**: On average, 0.2% of ticks were infected by TBE virus in the entire Tribeč region. When only elementary foci were taken into account, this proportion increased to 0.4% (Topoľčianky) and 0.8% (Jelenec)[18] Záhorská Bystrica, **1965**: 1.7% of female ticks infected by TBE virus[19] Devín, **1973**: 0.1% of nymphs and 1.1% of female ticks infected by TBE virus[20] Slovakia, **1981**: In Slovakia there are 2 types of TBE VIRUS natural foci – Carpathian and Pannonian. In Carpathian natural TBE virus foci, there were 2.6% of ticks infected by TBE virus. In the Pannonian natural TBE virus foci, there were 0.1% of ticks infected by TBE virus[21] Kurínec, **1982**: 0.8% of nymphs and 6% of male ticks (*I. ricinus*) in south-central Slovakia[22] Carpathian and Pannonian types of TBE natural foci, **1972–1982**: The proportion of infected ticks in both types of natural foci was 1.7% in total. In Carpathian elementary foci (ranging from 0.4% to 4.1%; average of 2.5% of ticks were infected). In Pannonian elementary foci (ranging from 0.07% to 6.0%; average of 0.9% of ticks were infected)[23] Western and Central Slovakia, **1980–1984**: Western Slovakia, April–July 1980 (0.7%), May 1984 (0.1%), Central Slovakia April–May 1982 (0.2%)[24] Western Slovakia, **1985–1990**: In Slovakia surveillance of TBE virus in ticks, carried out during 1985–1990 by the Virology Institute of the Slovak Academy of Sciences in Bratislava, showed that the TBE virus distribution rates among ticks ranged from 0.30% (Jarok, Bardoňovo in 1987) to 0.38% (Malacky in 1990) in the 25 sites in the western region (data not published) Žiar nad Hronom, Banská Štiavnica a Žarnovica, **2002–2007**: In the small sample of 142 ticks tested, there were 4.98% infected with TBE virus[25]

Table 1: TBE in Slovakia (continued)	
Dairy product transmission	Slovakia is well known in Europe for TBE alimentary outbreaks that are reported almost every year.[10] Over the few last years, there has been a growing trend in the number of food-borne TBE outbreaks. The percentage of TBE virus infection through consumption of unpasteurized milk and its products is quite high compared with other countries, e. g. in 2023, 34% of alimentary TBE cases were reported[31]. During **2007–2016** a total of 26 TBE alimentary outbreaks (2 or more cases/outbreak) with 142 TBE cases have been observed (13.9% of all TBE cases). Larger outbreaks with 3 or more cases have been recorded 13 times. The most common transmission factor of TBE virus during outbreaks has been goat milk and its products (61.5%, 16 outbreaks). Sheep's milk and products have caused probably 7 outbreaks (26.9%) and cow's milk was the probable cause of 2 TBE outbreaks (7.7%). In one TBE outbreak, the probable TBE transmission factor was reported to be mixed goat and sheep milk.[10] In the majority of outbreaks (22) the probable transmission factor of TBE virus was identified epidemiologically. In 2016 a TBE outbreak with the highest number of TBE cases (44) over the past 30 years was reported in Eastern Slovakia, sheep cheese was considered as TBE virus transmission factor by retrospective case control study.[26] In 2023 a TBE outbreak with 28 cases was reported in Central Slovakia. Sheep cheese was considered as the probable TBE virus transmission factor[31].
Case definition used by authorities	Based on ECDC, 2018.[27]
Completeness of case detection	No valid data to estimate the percentage of undetected and underreported cases.
Type of reporting	Mandatory
Other TBE surveillance	No
Special clinical features	Sequelae 52% (after 3 years)[30]
Licensed vaccines	FSME-Immun since 1995; FSME-Immun Junior since 2005
Vaccination recommendations	According to Decree No 585/2008 Coll. of the Ministry of Health of the Slovak Republic, which defines details on prevention and control of communicable diseases, TBE vaccination is compulsory for employees of virological laboratories working with TBE virus and TBE vaccination is recommended for occupationally exposed persons (forest workers, students of forestry schools, agriculture workers, etc.). Insurance companies partially reimburse TBE vaccine in Slovakia.[6,7]
Vaccine uptake	20% for 2 or more TBE vaccine doses (general population, survey)[29]
National Reference center for TBE	NRC for arboviruses and hemorrhagic fevers Public Health Authority of Slovakia Trnavská cesta 52 826 45 Bratislava, Slovakia

Figure 1: Burden of TBE in Slovakia over time; trend of TBE in Slovakia, 2014-2023 [1]



References

1952–1996: Grešíková M. [Tick-borne encephalitis – permanent public health problem]. VEDA Bratislava 1999, p 62. [Monograph in Slovak].

1997–2022: [Epidemiologický informačný systém] [Internet] Epidemiological Information System; 2022 [Cited 2023 Feb 20]. Data export 2006–2022. Available at: www.epis.sk [In Slovak]

**According to ECDC classification Slovakia is in 2/3 areas endemic. There were 161 TBE cases in 2019.*
There were 4 alimentary outbreaks: 2 cases - goat milk; 2 cases - goat milk (cheese); 3 cases - goat milk; 7 cases - goat milk (cheese)
There were 9 sporadic cases, where the transmission mechanism was ingestion of milk/cheese of goat and sheep origin.

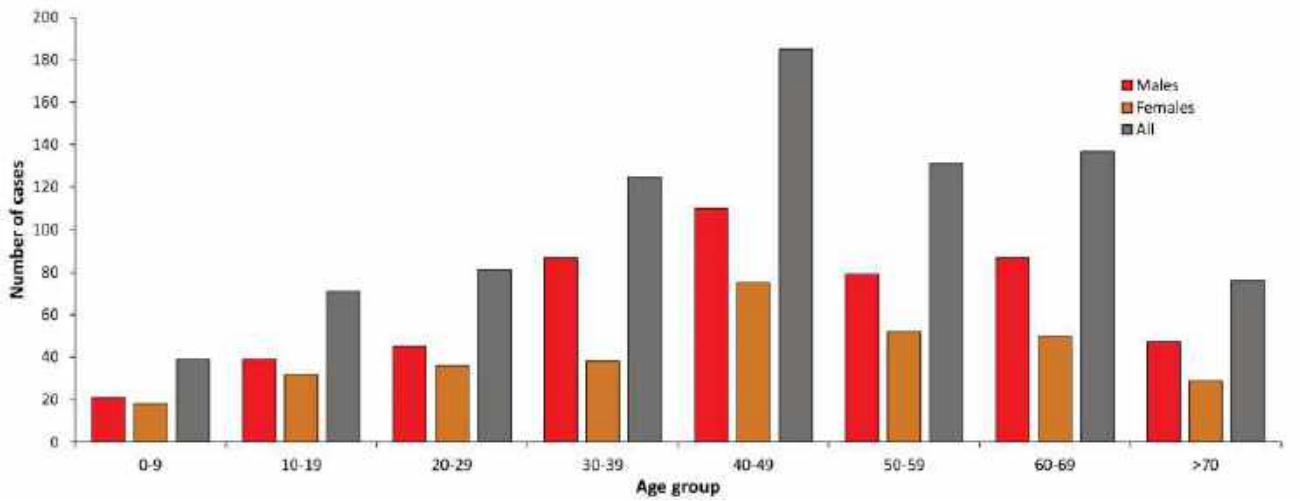
***There were 5 family alimentary TBE outbreaks (4 - milk and products of sheep origin, 1 - milk and products of goat origin; 11 cases)*

****There was 1 alimentary TBE outbreak (1- milk and products of goat origin; 5 cases)*

*****Total of 207 TBE cases reported of which 4 were imported.*

Source Data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE in Slovakia, 2019-2023



References

[Epidemiologický informačný systém] [Internet] Epidemiological Information System; 2017 [Cited 2017 Jan 5]. Data export 2015. Available at: www.epis.sk [In Slovak].

Source Data: Appendix—Figure 2

Figure 3: Sites of TBE virus infection in Slovakia, 2019 - 2023

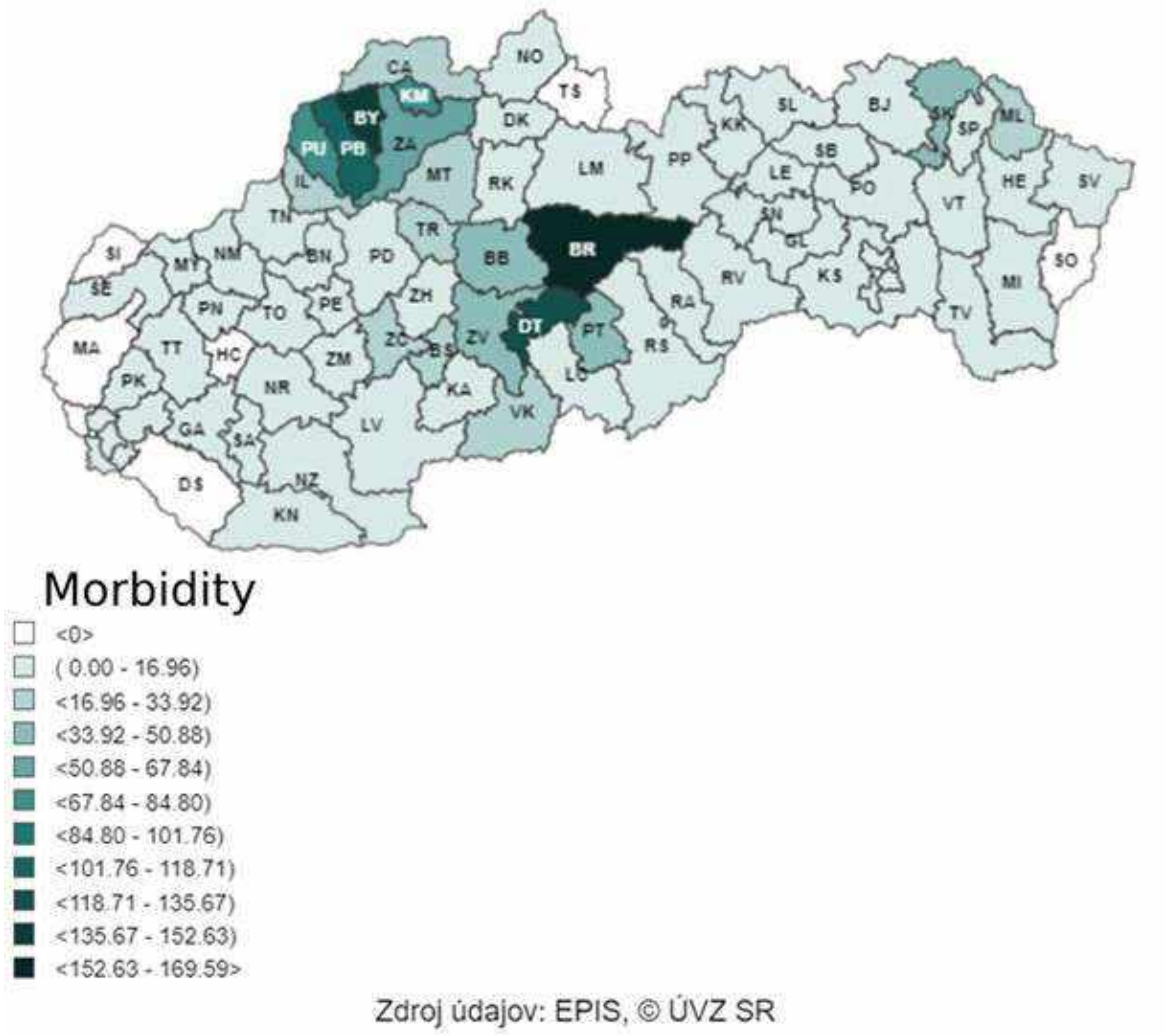
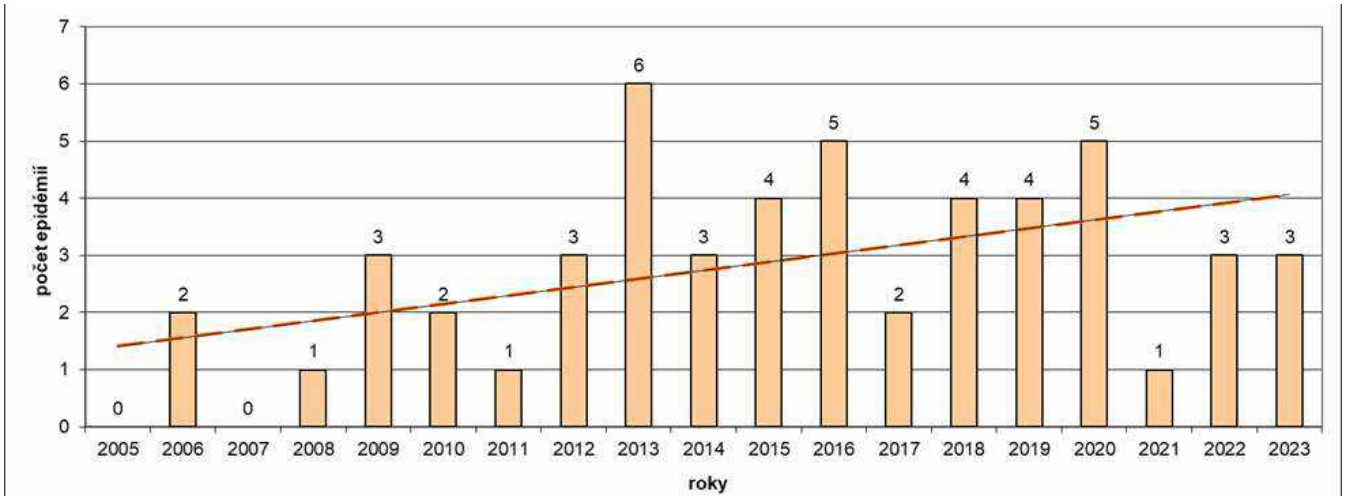


Figure 4: Number and trend of TBE alimentary outbreaks in Slovakia, 2005–2023



Appendix

Source data: Figure 1

Year	Number of cases	Incidence / 10^5
1952	52	1.5
1953	267	7.4
1954	241	6.6
1955	343	92
1956	121	3.2
1957	84	2.2
1958	110	2.8
1959	110	2.8
1960	217	5.4
1961	57	1.4
1962	88	2.1
1963	92	2.1
1964	16	0.4
1965	30	0.7
1966	13	0.3
1967	not available	not available
1968	5	0.1
1969	6	0.1
1970	7	0.2
1971	4	0.1
1972	15	0.3
1973	16	0.4
1974	33	0.7
1975	32	0.7

Year	Number of cases	Incidence / 10^5
1976	22	0.5
1977	15	0.3
1978	34	0.7
1979	49	1
1980	20	0.4
1981	25	0.5
1982	48	1
1983	34	0.7
1984	78	1.5
1985	36	0.7
1986	21	0.4
1987	24	0.5
1988	29	0.6
1989	18	0.3
1990	14	0.3
1991	24	0.5
1992	16	0.3
1993	51	1.07
1994	60	1.1
1995	89	1.6
1996	82	1.5
1997	76	1.4
1998	54	1
1999	63	1.17

Year	Number of cases	Incidence / 10^5
2000	92	1.71
2001	75	1.39
2002	62	1.15
2003	74	1.38
2004	70	1.3
2005	50	0.93
2006	91	1.69
2007	57	1.06
2008	79	1.46
2009	76	1.4
2010	90	1.66
2011	108	1.99
2012	107	1.98
2013	162	2.99
2014	117	2.16
2015	88	1.62
2016	174	3.21
2017	75	1.38
2018	156	2.87
2019	161*	2.95
2020	185**	3.39
2021	96***	1.76
2022	203****	3.74
2023	200	3.73

Source data: Figure 2

Age group	Males	Females	All
0-9	21	18	39
10-19	39	32	71
20-29	45	36	81
30-39	87	38	125
40-49	110	75	185
50-59	79	52	131
60-69	87	50	137
>70	47	29	76

Contact: jana.kerlik@vzbb.sk

Citation:
Kerlik J. TBE in Slovakia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi: 10.33442/26613980_13-29-6

References

1. Rampas J, Gallia F. [Isolation of tick-borne encephalitis virus from ticks *Ixodes ricinus*]. *Čas Lék Čes*. 1949;88:1179-80.
2. Blaškovič D. [Outbreak of encephalitis in Rožňava natural focus of infections]. *Bratislava: VEDA SAV*, 1954. 314 p.
3. Bárdoš V, Hanzal F, Havlík O, et al. [Csechoslovak tick-borne encephalitis]. Praha: Státní zdravotnické nakladatelství, 1954. 92 p.
4. Dorko E, Rimárová K, Kizek P, Stebnický M, Zákutná L. Increasing incidence of tick-borne encephalitis and its importance in the Slovak Republic. *Cent Eur J Public Health*. 2014;22(4):277-281. doi:10.21101/cejph.a3939.
5. Kerlik J, Avdičová M, Musilová M, Bérešová J, Mezencev R. Breast Milk as Route of Tick-Borne Encephalitis Virus Transmission from Mother to Infant. *Emerg Infect Dis.* 2022;28(5):1060-1061. doi:10.3201/eid2805.212457
6. [Decree No. 585/2008 Coll. of the Ministry of Health of the Slovak Republic, which defines details on prevention and control of communicable diseases]. Zbierka zákonov SR. 2008 Dec 10;Pt 202:5024-41.
7. [Criteria for submission of the contribution for the payment of the vaccine against TBE]. Dôvera; 2024. Accessed March 5, 2024. https://www.dovera.sk/poistenec/potrebujem-poradit/vyhody-pre-poistencov/kriteria-na-poskytnutie-prispevku-na-uhradu-vakciny-proti-kliestovej-encefalitide.
8. [Natural foci of tick-borne encephalitis in Slovakia] [Internet] Public Health Authority of Slovak Republic; 2017. Accessed 9 January, 2017. https://www.uvzsr.sk/ index.php?option=com_content&view=article&id=395:prirodne-ohniska-klieovej-encefalitidy-na-slovensku&catid=68:epidemiologia&Itemid=76.
9. Lukan M, Bullova E, Petko B. Climate warming and tick-borne encephalitis, Slovakia. *Emerg Infect Dis*. 2010;16(3):524-526. doi:10.3201/eid1603.081364
10. Kerlik J, Avdičová M, Štefkovičová M, et al. Slovakia reports highest occurrence of alimentary tick-borne encephalitis in Europe: Analysis of tick-borne encephalitis outbreaks in Slovakia during 2007-2016. *Travel Med Infect Dis*. 2018;26:37-42. doi:10.1016/j.tmaid.2018.07.001
11. Kozuch O, Grulich I, Nosek J. Serological survey and isolation of tick-borne encephalitis virus from the blood of the mole (Talpa europaea) in a natural focus. *Acta Virol*. 1966;10:557-60.
12. Kozuch O, Gresíková M, Nosek J, Lichard M, Sekeyová M. The role of small rodents and hedgehogs in a natural focus of tick-borne encephalitis. *Bull World Health Organ*. 1967;36 Suppl (Suppl 1):61-66.
13. Ernek E, Kozuch O, Lichard M, Nosek J. The role of birds in the circulation of tick-borne encephalitis virus in the Tribec region. *Acta Virol*. 1968;12:468-70.
14. Nosek J, Kožuch O, Grešíková M, et al. [Uncovering natural foci of tick-borne encephalitis in Central Slovakia]. II. Synecology of tick-borne encephalitis virus in Central river Váh area. Bratisl Lek listy. 1982;77:257-63.
15. Kozuch O, Labuda M, Lysý J, Weismann P, Krippel E. Longitudinal study of natural foci of Central European encephalitis virus in West Slovakia. *Acta Virol*. 1990;34:537-44.
16. Kožuch O, Guryčová D, Lysý J, Labuda M. Mixed natural focus of tick-borne encephalitis, tularemia and haemorrhagic fever with renal syndrome in west Slovakia. Acta Virol. 1995;39:95-8.
17. Csank T, Bhide K, Bencúrová E, et al. Detection of West Nile virus and tick-borne encephalitis virus in birds in lovakia, using a universal primer set. *Arch Virol.* 2016;161:1679-83. doi:10.1007/s00705-016-2828-5
18. Grešíková M, Nosek J. Isolation of Tick-borne Encephalitis Virus from Ixodes ricinus Ticks in the Tribec region. *Bull World Health Organ*. 1967;36(Supp 67-71).
19. Grešíková M, Kožuch O, Nosek J. Die rolle von *Ixodes ricinus* als Vektor des Zeckenencephalitis Virus in verschiedenen mitteleuropäischen Naturherden [The role of *Ixodes ricinus* as vector of tick-borne encephalitis virus in different Central European natural foci]. Zentralbl Bakteriol Orig. 1968;207 (4):423-429.
20. Grešíková M. [Isolation of tick-borne encephalitis strains Bratislava from ticks *Ixodes ricinus* collected by Devin pathway]. *Bratisl Lek Listy*. 1975;64:1-128.
21. Grešíková M. [Tick-borne encephalitis – permanent public health problem]. *VEDA Bratislava*. 1999, p 62.
22. Grešíková M, Palanová A, Pötheová A, Teplan J, Sekezová M, Kohutová V. [Contribution to harnessing natural foci of tick-borne encephalitis in the south central Slovakia]. *Bratisl Lek Listy*. 1983;80:385-512.

23. Gresíková M, Kozuch O, Sekeyová M, Nosek J. Studies on the ecology of tick-borne encephalitis virus in the Carpathian and Pannonian types of natural foci. *Acta Virol*. 1986;30:325-31.

24. Grešíková M, Sláčiková M, Kožuch O. Tick-borne encephalitis in Slovakia during years 1980-1984. *Bratisl Lek Listy*. 1987;88:358-65.

25. Košťanová Z. We searched for one, we discovered more and what's next? Zoonoses – Protection of public and animal health reviewed abstracts from the 5th scientific congress. Slovak Medical University, Bratislava, 2016. 322 p. https://www.sevs.sls.sk/images/public/Reviewed_Abstracts_Zoonoses_2016.pdf

26. Tarkovská V, Seligová J, Molčányi T. [Tick-borne encephalitis outbreak with alimentary transmission in district Košice surrounding in 2016]. Collection of abstracts XXII. Červenka days, 2017;p. 17.

27. Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. Accessed 25 March, 2024. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D0945.

28. Peňazziová, K.; Korytár, Ľ.; Cingeľová Maruščáková, I.; Schusterová, P.; Loziak, A.; Pivka, S.; Ondrejková, A.; Pistl, J.; Csank, T. Serologic Investigation on Tick-Borne Encephalitis Virus, Kemerovo Virus and Tribeč Virus Infections in Wild Birds. *Microorganisms*. 2022;10(12):2397. Published 2022 Dec 2. doi:10.3390/microorganisms10122397

29. Pilz A, Erber W, Schmitt HJ. Vaccine uptake in 20 countries in Europe 2020: Focus on tick-borne encephalitis (TBE). *Ticks Tick Borne Dis*. 2023;14(1):102059. doi:10.1016/j.ttbdis.2022.102059

30. Paraličová Z, Sekula J, Jarčuška P, et al. Outbreak of Alimentary Tick-Borne Encephalitis in Eastern Slovakia: An Analysis of Affected Patients and Long-Term Outcomes. Pathogens. 2022;11(4):433. Published 2022 Apr 2. doi:10.3390/pathogens11040433

31. Public Health Authority of Slovak Republic. Epidemiological Information system.

Chapter 13

TBE in Slovenia

Zoran Simonović, Veronika Učakar and Maja Praprotnik

ECDC risk status: endemic (last edited: date 13.03.2024, data as end of 2023)

History and current situation

TBE is endemic in Slovenia, and the incidence rate is one of the highest in the EU. In Slovenia, TBE virus was confirmed for the first time in 1953 with isolation of the virus from a patient's blood.[1] In 1955, the virus was isolated from a tick *Ixodes Ricinus.*[2]

Notification of TBE cases as well as deaths due to TBE has been mandatory in Slovenia since 1977.[3] In the past, case definition for TBE surveillance was not available. It was at the treating physicians' discretion to establish TBE diagnosis for clinical management purposes and report such cases also for surveillance purposes. In recent years, Slovenia has adopted the EU case definition of TBE for the purposes of epidemiological surveillance.[4] Cases with central nervous system involvement and laboratory confirmation or cases with central nervous system involvement and an epidemiological link (exposure to common source – unpasteurized dairy products) are notified. Surveillance data has been collected within the communicable diseases surveillance system by the National Institute of Public Health of Slovenia (NIPH).[5]

The number of TBE reported cases in Slovenia varies every year. In the period from 1983 to 2023, the number of annually reported TBE cases was between 62 and 532 (incidence rates between 3.0 and 26.6/100,000), which amounts to a mean of 194 cases/year, and a mean annual incidence rate of 9.6/100,000 (Figure 1). In contrast to reports on increasingly higher incidence rates of TBE during the last decade from many EU countries,[6] in Slovenia the reported incidence rates during the last decade (2014 - 2023) have decreased compared to the previous two decades (1994 – 2013) (Figure 1). Diverging long-term trends in the occurrence of TBE fluctuates due to multiple factors: virus evolution, climatic factors influencing changes in tick activity and population, number of small forest mammals, as well as human behavior (e.g., changes in leisure activities) play an important role. In addition, changes in surveillance systems, diagnostic methods and vaccination policies can also have an effect on the observed trend.[6,7]

TBE occurs seasonally in Slovenia, usually from May to October, with a peak in June and July, which is linked to tick activity.[8] In recent years an increase in the number of the cases in the elderly has been observed.[3] Since 2014, TBE incidence rates have been the highest in the 55–64 age group in most years, with males being more frequently affected than females (Figure 2). In men, the 65–74 age group and in women the 45–54 age group followed, with the second highest rates in the period 2014 - 2023. In contrast to the TBE incidence, the disease burden expressed in disability-adjusted life years (DALYs) was higher in children aged 5–14 years than in adults aged 50–74 years.[9]

The endemic area for TBE is most of Slovenia, except for the area along the Adriatic Sea. In the past decade (2014 – 2023) cases of TBE were recorded in all Slovenian statistical regions (Figure 3). Although some regions in Slovenia have a higher 10-year average number of TBE cases than others, TBE occurs throughout the country, with the most affected areas in the north and central regions down to the southwestern part of the country, excluding the coastal region.

People who are staying in the endemic areas (temporarily or permanently) have a higher risk for TBE infection. These are mainly people working in forestry, wood and wood-processing industries and construction. The risk is also higher among farmers, if their farmlands are located near forested areas, which present a natural habitat for ticks. There have also been observations of increased TBE incidence among people who visit forests for recreational purpose or forest fruit-picking. An epidemiological study that included 1,564 cases of TBE in Slovenia showed that 82.3% of cases had a tick bite on one or multiple sites on the body. The estimated duration of tick attachment was less than 6 h in 23.5% of TBE cases. Long attachments (more than 24 h) were reported by 10% of the patients. The tick bite occurred while the TBE patients were engaged in leisure time activities (sports or camping, 32.8%), mushroom or berry picking (30.2%), or farming (23.3%). Almost two-thirds of TBE patients reported that they had practiced at least one of the recommended preventive measures, most frequently self-inspection, and least often repellent use.[10]

Preventive measures against TBE include the use of repellents, appropriate clothing and daily inspection of the skin to remove ticks. The most effective method of preventing TBE is vaccination.[11] Mandatory vaccination against TBE was introduced in Slovenia in 1986 for those at risk of occupational exposure, and in 1990 for students at risk of exposure during curricular training, while the rest of the population needed to pay for the vaccination

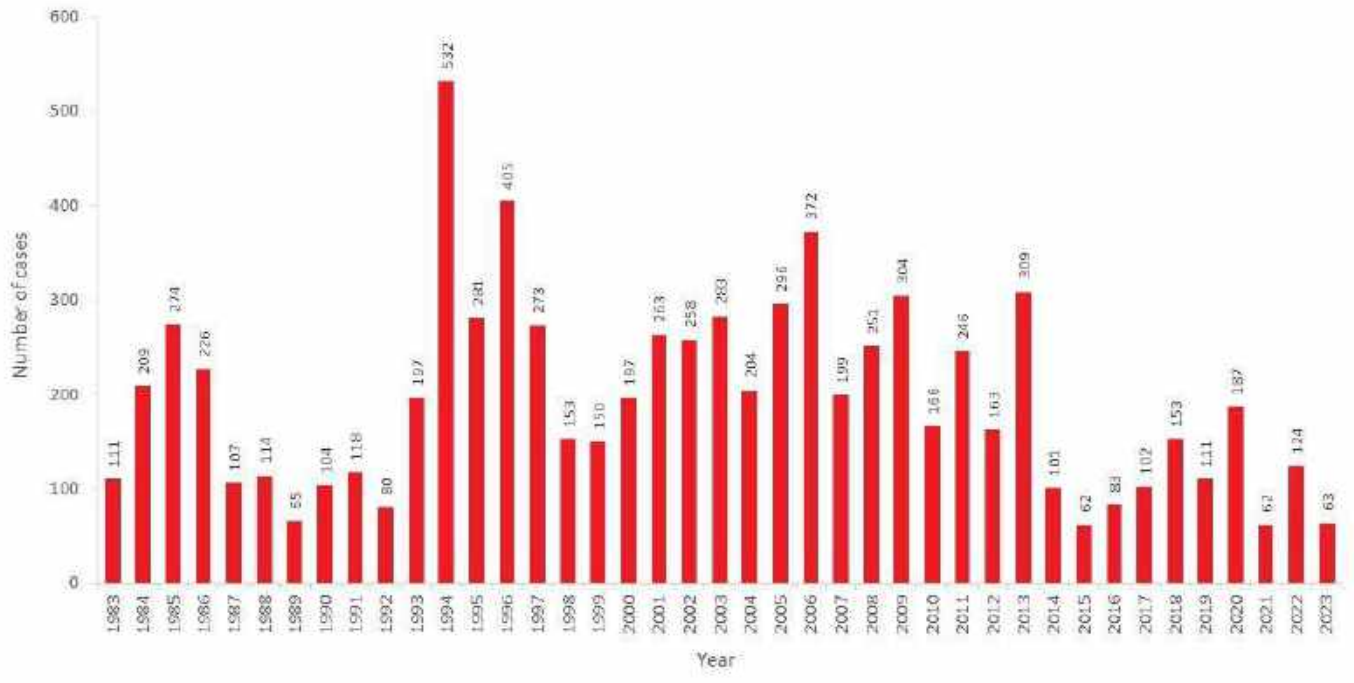
themselves. TBE vaccination coverage in Slovenia remained low: by 2007, the proportion of the general population reporting to ever have been vaccinated against TBE was 12.4%.[12] From 2019, Slovenia introduced TBE vaccination for adults and children in the national vaccination program, for children at first after the age of three years, then later changed to after the age of one year, and for adults who reach 49 years of age in the current year. Vaccination for this group is carried out with three doses of vaccine, paid for from the compulsory health insurance. As a general rule, the three-dose basic vaccination is financed. Those who have previously started vaccination on a "self-pay" basis may be vaccinated with the following three doses at the expense of the mandatory health insurance. Vaccination is also available to people who delayed TBE vaccination (children born in 2016 or later and adults who reached 49 years of age in 2019 or later and have not yet received three doses at the expense of the mandatory health insurance).[13] In Slovenia the vaccination coverage among children with at least one dose of TBE vaccine enrolled in this program born between 2016 and 2019 ranged from 35.2 - 52.2%. The vaccination coverage among adults with at least one dose of TBE vaccine enrolled in this program born between 1970 and 1973 ranged from 14.7 - 21.1%.[14]

Overview of TBE in Slovenia

Table 1: TBE in Slovenia	
Virus subtypes isolated	European subtype of TBE virus (TBEV) present in Slovenia. Relatively high genetic variability of Slovenian TBEV with correlation between geographical and phylogenetic clustering was detected.[15]
Reservoir animals	Rodents; TBEV antibodies were detected in 5.9% of rodent sera. Bank voles had higher rate of infection than mice.[16]
Percentage infected ticks	In Slovenia the main vector is *Ixodes ricinus* and the prevalence of TBEV tick infection is 0.47%.[17]
Dairy product transmission	In previous decades one food-borne outbreak of TBE was reported in Slovenia associated with consumption of raw goat milk (3 cases).[18,19]
Case definition used by authorities	Slovenia adopted the EU case definition for epidemiological surveillance of TBE.[4]
Completeness of case detection and reporting	No data.
Type of reporting	Reporting of TBE cases is mandatory in Slovenia. Cases with central nervous system involvement and laboratory confirmation or cases with central nervous system involvement and epidemiological link (exposure to common source – unpasteurized dairy products) are notified.[5]
Other TBE surveillance	Not established.
Special clinical features	A biphasic course of the illness was reported by 56% of patients. Adults (15 – 60 years old) more often presented with fever, headache, stiff neck and photophobia, whereas seniors (more than 60 years old) more frequently reported fatigue, altered consciousness and decreased muscle strength, these differences indicating a more classic course of TBE in the younger group and a somehow different and more severe acute disease in the older group.[20] Direct comparison of clinical and epidemiological characteristics of TBE in children and adults revealed differences in several clinical and laboratory features and corroborates the previous conclusion that TBE in childhood is a milder illness than TBE in adults.[21]
Licensed vaccines	FSME-IMMUN.[22]

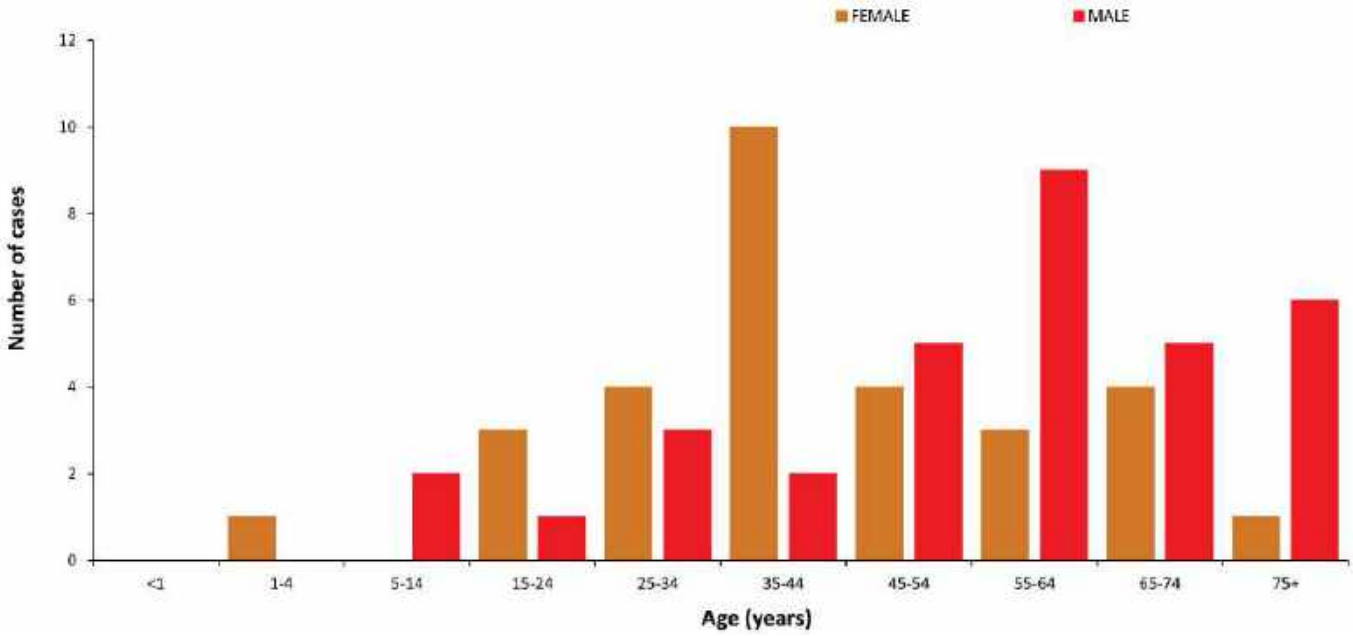
Table 1 continued	
Vaccine recommendations	TBE vaccination for adults and children included in the Slovenian national vaccination program. For children after the age of one year and for adults who reach 49 years of age in the current year. Vaccination is carried out with three doses of vaccine, paid for by the mandatory health insurance. As a general rule, the three-dose basic vaccination is financed. Those who have previously started vaccination on a "self-pay" basis may be vaccinated with the following three doses at the expense of the mandatory health insurance. As a “catch-up”, vaccination is also available to people who have not yet been TBE vaccinated (children born in 2016 or later and adults who have reached 49 years of age in 2019 or later and have not yet received three doses at the expense of the mandatory health insurance).[13]
Vaccine uptake	In Slovenia the vaccination coverage among children with at least one dose of TBE vaccine enrolled in national vaccination program born between 2016 and 2019 ranged from 35.2 - 52.2%. The vaccination coverage among adults with at least one dose of TBE vaccine enrolled in this program born between 1970 and 1973 ranged from 14.7 - 21.1%.[14]
National Reference center for TBE	National Institute of Public Health Trubarjeva cesta 2, 1000 Ljubljana, Slovenia https://nijz.si/

Figure 1: TBE case numbers over time, Slovenia, 1983-2023



Source Data: Appendix-Figure 1

Figure 2: Age and gender distribution of TBE cases, Slovenia, 2014-2023



Source Data: Appendix-Figure 2

Figure 3: Ten-year average incidence of TBE per 100,000 population by statistical region of residence, 2014–2023

Authors and Affiliations

Zoran Simonović, Veronika Učakar, Maja Praprotnik
Nacional Institute of Public Health, Slovenija

Contact: zoran.simonovic@nijz.si

Citation:
Simonović Z, Učakar V, Praprotnik M. TBE in Slovenia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press;2024. doi:10.33442/26613980_13-30-7

Appendix

Source data: Figure 1

Year	Number of cases
1983	111
1984	209
1985	274
1986	226
1987	107
1988	114
1989	65
1990	104
1991	118
1992	80
1993	197
1994	531
1995	157
1996	406
1997	274
1998	137
1999	150
2000	196
2001	260
2002	262
2003	282
2004	199
2005	297
2006	372
2007	199
2008	251
2009	304
2010	166
2011	247
2012	164
2013	309
2014	100
2015	62
2016	83
2017	102
2018	153
2019	111
2020	187
2021	62
2022	124
2023	63

Source data: Figure 2

Age group (years)	Males	Females
<1	0	0
1-4	0	1
5-14	2	0
15-24	1	3
25-34	3	4
35-44	2	10
45-54	5	4
55-64	9	3
65-74	5	4
75+	6	1

References

1. Vesenjak-Zmijanac J, Bedjanič M, Rus S, Kmet J. Virus meningoencephalitis in Slovenia: isolation of the causative agent. *Bull WHO*. 1955;12:513-20.
2. Virus meningo-encephalitis in Slovenia. 4. Isolation of the virus from the ticks *Ixodes ricinus*. *Bull World Health Organ*. 1956;15(1-2):275-279.
3. Grgič-Vitek M, Klavs I. High burden of tick-borne encephalitis in Slovenia – challenge for vaccination policy. *Vaccine*. 2011;29:5178-83. doi:10.1016/j.vaccine.2011.05.033
4. European Centre for Disease Prevention and Control. EU case definitions. Tick-borne encephalitis. Published July 3, 2018. Accessed March 12, 2024. https://www.ecdc.europa.eu/en/all-topics/eu-case-definitions
5. Sočan M, Šubelj M, Grilc E, Frelih T, Grmek Košnik I, Čakš-Jager N. [Definitions of notifiable communicable diseases for the purposes of epidemiological surveillance.]. *Nacionalni inštitut za javno zdravje*. Published January 15, 2024. Accessed March 12, 2024. https://nijz.si/publikacije/definicije-prijavljivih-nalezljivih-bolezni-za-namene-epidemioloskega-spremljanja/
6. Van Heuverswyn J, Hallmaier-Wacker LK., Beauté J, et al. Spatiotemporal spread of tick-borne encephalitis in the EU/EEA, 2012 to 2020. *Euro Surveill*. 2023;28(11):2200543. doi:10.2807/1560-7917.ES.2023.28.11.2200543
7. Pustijanac E, Buršić M, Talapko J, Škrlec I, Meštrović T, Lišnjić D. Tick-Borne Encephalitis Virus: A Comprehensive Review of Transmission, Pathogenesis, Epidemiology, Clinical Manifestations, Diagnosis, and Prevention. *Microorganisms*. 2023; 11(7):1634. Published 2023 Jun 22. doi:10.3390/microorganisms11071634
8. Sočan M, Praprotnik M. [Monitoring of infectious diseases transmitted by arthropods in Slovenia in 2021]. Nacionalni inštitut za javno zdravje. Published September 8, 2023. Accessed March 12, 2024. https://nijz.si/nalezljive-bolezni/spremljanje-nalezljivih-bolezni/spremljanje-nalezljivih-bolezni-ki-jih-prenasajo-clenonozci-v-sloveniji/
9. Fafangel M, Cassini A, Colzani E, et al. Estimating the annual burden of tick-borne encephalitis to inform vaccination policy, Slovenia, 2009 to 2013. *Euro Surveill*. 2017;22:30509. doi:10.2807/1560-7917.ES.2017.22.16.30509
10. Blasko-Markic M, Socan M. Tick-borne encephalitis in Slovenia: data from a questionnaire survey. *Vector Borne Zoonotic Dis*. 2012;12:496-502. doi:10.1089/vbz.2011.0871
11. World Health Organization. Vaccines against tick-borne encephalitis: WHO position paper. *Wkly Epidemiol Rec*. 2011;86(24):241-56.
12. Grgic-Vitek M, Klavs I. Low coverage and predictors of vaccination uptake against tick-borne encephalitis in Slovenia. *Eur J Public Health*. 2012;22(2):182-6. doi:10.1093/eurpub/ckr018
13. Nacionalni inštitut za javno zdravje. Klopni meningoencefalitis. Cepljenje – osnovne informacije. Published 2023. Accessed March 12, 2024. https://nijz.si/wp-content/uploads/2023/04/KME-letak-skupni-mar_2023.pdf
14. Nacionalni inštitut za javno zdravje. Preliminarno poročilo o precepljenosti otrok in odraslih proti klopnemu meningoencefalitisu v Sloveniji. Published May 1, 2023. Accessed March 12, 2024. https://nijz.si/wp-content/uploads/2023/04/preliminarno_porocilo_KME_10052023.pdf
15. Fajs L, Durmiši E, Knap N, Strle F, Avšič-Županc T. Phylogeographic characterization of tick-borne encephalitis virus from patients, rodents and ticks in Slovenia. *PloS One*. 2012;7(11):e48420. doi:10.1371/journal.pone.0048420
16. Knap N, Korva M, Dolinšek V, Sekirnik M, Trilar T, Avšič-Županc T. Patterns of Tick-Borne Encephalitis Virus Infection in Rodents in Slovenia. *Vector Borne Zoonotic Dis*. 2012;12:236-42. doi:10.1089/vbz.2011.0728
17. Durmiši E, Knap N, Saksida A, Trilar T, Duh D, Avšič-Županc T. Prevalence and molecular characterization of tick-borne encephalitis virus in i. ricinus ticks collected in Slovenia. *Vector Borne Zoonotic Dis*. 2011;11:659-64. doi:10.1089/vbz.2010.0054
18. Hudopisk N, Korva M, Janet E, et al. Tick-borne encephalitis associated with consumption of raw goat milk, Slovenia, 2012. *Emerg Infect Dis*. 2013;19:806-8. doi:10.3201/eid1905.121442
19. Elbaz M, Gadoth A, Shepshelovich D, Shasha D, Rudoler N, Paran Y. Systematic Review and Meta-analysis of Foodborne Tick-Borne Encephalitis, Europe, 1980-2021. *Emerg Infect Dis*. 2022;28(10):1945-1954. doi:10.3201/eid2810.220498
20. Logar M, Bogovic P, Cerar D, Avsic-Zupanc T, Strle F. Tick-borne encephalitis in Slovenia from 2000 to 2004: comparison of the course in adult and elderly patients. *Wien Klin Wochenschr*. 2006;118(21-22):702-707. doi:10.1007/s00508-006-0699-6
21. Logar M, Arnez M, Kolbl J, Avsic-Zupanc T, Strle F. Comparison of the epidemiological and clinical features of tick-borne encephalitis in children and adults. *Infection.* 2000;28(2):74-77. doi:10.1007/s150100050050
22. Nacionalni inštitut za javno zdravje. Cepiva proti posameznim boleznim – klopni meningoencefalitis. Published August 27, 2018. Accessed March 13, 2024. https://nijz.si/nalezljive-bolezni/cepljenje/cepiva-proti-posameznim-boleznim-klopni-meningoencefalitis/

Chapter 13

TBE in South Korea

Song Joon Young

E-CDC status: imperiled (Updated April 1, 2024)

History and current situation

Although no human case of tick-borne encephalitis (TBE) has been documented in South Korea to date, 5 surveillance studies have been conducted to evaluate the prevalence of tick-borne encephalitis virus (TBEV) in wild ticks.[1-5] Four studies collected ticks by dragging or flagging in grassland and forest, while 1 study tested wild mammals (boars and rodents) by removing ticks from them. In the wild of South Korea, *Haemaphysalis* spp. were the predominant species found by tick dragging, while *Ixodes nipponensis* became predominant when harvested from small mammals.[6]

According to the results, TBEV was detected in numerous regions (Figure 1)[1-5]:

- Gyeonggi-do (Yangpyeong and Dongducheon), Gangwon-do (Pyeongchang, Jeongseon, Sokcho, and Chuncheon), Jeonllabuk-do (Gunsan and Gurye), Gyeongsangbuk-do (Hapcheon, Dongu, Andong, and Uiseong), Gyeongsangnam-do (Yangsan), and Jeju-do (Jeju).

The first study was conducted in 12 regions of 5 provinces of South Korea in 2005–2006.

TBEV was detected from *Haemaphysalis longicornis* (minimum field detection rate, 0.2%), *H. flava* (0.8%), *H. japonica* (0.9%), and *I. nipponensis* (1.6%), as depicted in Table 1.

The minimum field detection rate ([number of detection positive pools/ total number of examined ticks] × 100) was particularly high in Yangpyeong (5.9%–20.0%), Dongducheon (1.3%–6.7%), Pyeongchang (0.8%–1.3%), and Jeongseon (0.4%–8.3%) with variation by tick species. As usual, 1–30 ticks were included in each pool. Phylogenetic analysis revealed that the TBEV in South Korea belonged to the Western subtype, contrary to neighboring countries including Japan, China, and northeastern Russia, where the Far-Eastern subtype was only isolated (Table 1).

In the second study by the same research team, TBEV was also isolated from ticks feeding on wild rodents (*Apodemus agrarius*) captured in Hapcheon, Gyeongsangnam-do.[2] These TBEV isolates (KrM216, KrM219) caused symptoms of encephalitis in suckling mice and were able to grow from brain preparations in cell culture. In 2007, the third TBEV surveillance was conducted in the southern provinces of South Korea, including Jeju Special Self-Governing Province (Jeju Island), Jeollanam-do, Gyeongsangbuk-do, and Gyeongsangnam-do.[3] Among the 6,788 ticks collected, 4,077 were pooled (649 pools) by collection site. In Jeju Island, the minimum field detection rate was 0.17% in *H. longicornis* and 0.14% in *H. flava*. In accordance with the previous study, the Jeju strains were identified as Western subtype TBEV by phylogenetic analysis.

Later during 2011–2012, the fourth larger-scale surveillance study was carried out in 25 localities of 10 provinces of South Korea.[4] A total of 13,053 ticks were collected with *H. longicornis* as the most abundant species (90.8%, 11,856/13,053), followed by *H. flava* (8.8%, 1,149/13,053), *I. niponensis* (0.3%, 42/13,053), and *Ixodes persulcatus* (0.05%, 6/13,053). The minimum field detection rate for *H. longicornis, H. flava*, and *I. nipponensis* were 0.06%, 0.17%, and 2.38%, respectively, and the TBEV sequences obtained were identified as the Western subtype, consistent with the previous reports.[1-3]

In 2014, the most recent surveillance study was conducted to evaluate the prevalence of TBEV and other tick-transmitted viruses (Powassan virus, Omsk hemorrhagic fever virus, Langat virus, and severe fever with thrombocytopenia virus) among wild ticks.[4] A total of 21,158 ticks were collected by dragging at 139 sites in 6 provinces; *H. longicornis* was the dominant tick species (83.04%, 17,570/21,158), while other tick species, *H. flava* (15.68%, 3317), *I. nipponensis* (1.18%, 249), Amblyomma testudinarium (0.05%, 11), and *H. phasiana* (0.04%, 8), were much less common. TBEV was detected by nested reverse transcriptase-polymerase chain reaction (RT-PCR) in the Andong, Uiseong, Daegu, and Yangsan areas. The maximum likelihood estimation (estimated numbers of viral RNA-positive ticks per 1,000 ticks) for *H. longicornis, H. flava*, and *I. nipponensis* was 0.23%, 0.90%, and 8.02%, respectively. On phylogenetic analysis, the TBEV strains identified in this study belonged to the Western subtype also.

Two serological surveillance studies for TBEV were reported in South Korea.[7,8] The first study was conducted from January 2017 to August 2018; a total 583 sera were obtained from the forest and field workers in South Korea.[7] Seroprevalence of TBEV was 0.9% (5/583) by IgG ELISA, and 0.3% (2/583) by neutralization assay. One forest worker in Jeju had positive anti-TBEV IgG titer (56.1 RU/mL) and

neutralization titer (1:113). This man has been working as a forest worker for 6 years in Jeju. However, he immigrated to Jeju from Jilin (northern China) 8 years ago, so TBEV infection could have occurred when he lived in China. In the other forest worker in Hongcheon, neutralization titer against TBEV was marginally positive (1:10), but anti-TBEV IgG was not detected. In another study, serological surveillance was conducted for healthy farmers in Jeju island during 2015-2018 using TBEV ELISA kits.[8] This study revealed a 1.9 % seroprevalence of TBEV, but not confirmed by neutralization assay.

Even though no confirmed human TBE case was reported in South Korea, TBEV might have been endemic in various localities and *H. longicornis, H. flava*, and *I. nipponensis* would be potential vectors of the Western subtype TBEV.

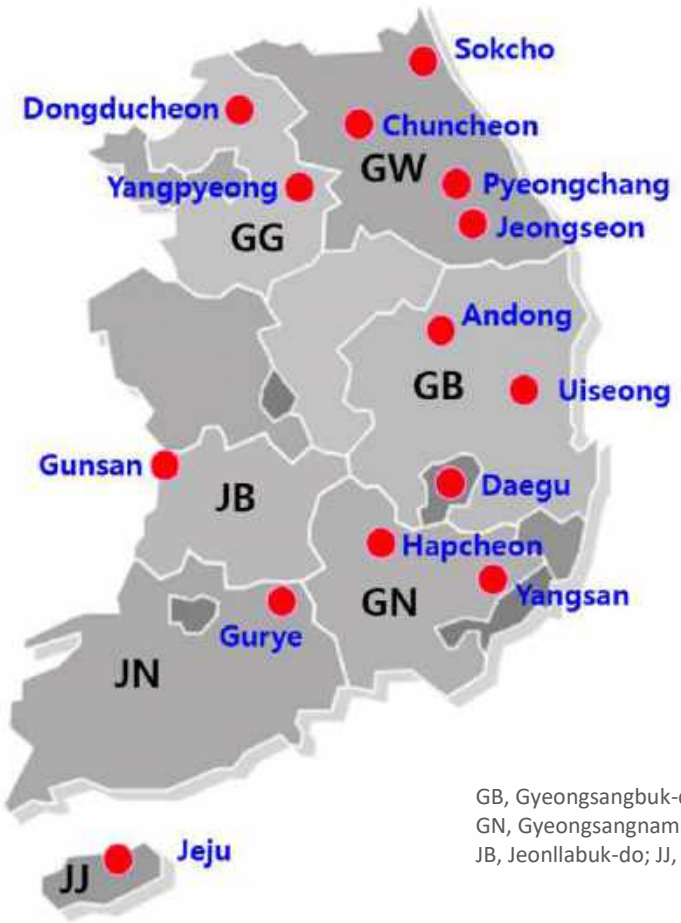
In South Korea, TBE is designated as a group 4 Nationally Notifiable Infectious Disease, requiring immediate reporting for laboratory-confirmed cases.[9] Although no case of TBE has been confirmed in South Korea, human encephalitis cases with unknown causes have been increasingly reported. TBE screening at the Korean Disease Control and Prevention Agency (KDCA) was started in 2006. As for undefined encephalitis cases or suspected TBE cases, blood and cerebrospinal fluid (CSF) samples are required to be sent out to KDCA to perform enzyme-linked immunosorbent assay (ELISA) and RT-PCR for TBEV. However, there are significant limitations of TBEV clinical surveillance in South Korea. First, TBE disease awareness is quite low, and diagnostic practice is limited in clinical settings. Neurologists often take care of undefined meningitis/encephalitis cases, but they are completely unfamiliar with TBE. Second, considering the short duration of TBE viremia, it is not easy to confirm the infection using blood and CSF samples collected at later clinical stages. To better characterize the disease burden of TBE in South Korea, serologic studies are required to evaluate TBE prevalence in high-risk populations such as forest workers and farmers in the endemic areas. At the same time, active surveillance with enhanced awareness would be essential to find missed TBE cases.

As of March 2024, no human cases of TBE have been reported.[10]

Table 1: TBE in South Korea

Viral subtypes, distribution	Western subtype[1-5]
Reservoir animals	Wild rodent *(Apodemus agrarius)*
Infected tick species	*Haemaphysalis longicornis, Haemaphysalis flava,* *Haemaphysalis japonica*, and *Ixodes nipponensis*
Dairy product transmission	Not documented
Mandatory TBE reporting	Yes: TBE is a group 4 Nationally Notifiable Infectious Diseases in South Korea[11] Case definition: laboratory-confirmed patient 1. Clinical criteria: person with symptoms of inflammation of the central nervous system, including meningitis, meningo-encephalitis, encephalomyelitis and etc. 2. Laboratory criteria • Detection of TBE-specific IgM antibody in the serum/CSF (confirmation of TBE-specific antibodies is required by serum neutralization assay) • Sero-conversion or ≥4-fold increase of TBE-specific antibodies in paired serum samples • Detection of TBE viral nucleic acid in clinical specimen
Other TBE-surveillance	None
Special clinical features	No information available
Available vaccines	None
National Reference Center	Korean Disease Control and Prevention Agency (KDCA)

Figure 1: Geographical location where tick-borne encephalitis virus (TBEV) positive ticks or wild rodents were identified in South Korea



GB, Gyeongsangbuk-do; GG, Gyeonggi-do; GN, Gyeongsangnam-do; GW, Gangwon-do; JB, Jeonllabuk-do; JJ, Jeju-do; JN, Jeonllanam-do.

Contact: infection@korea.ac.kr

Citation:
Song JY. TBE in South Korea. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-31-7

References

1. Kim SY, Jeong YE, Yun SM, Lee IY, Han MG, Ju YR. Molecular evidence for tick-borne encephalitis virus in ticks in South Korea. *Med Vet Entomol.* Mar 2009;23(1):15-20. doi:10.1111/j.1365-2915.2008.00755.x

2. Kim SY, Yun SM, Han MG, et al. Isolation of tick-borne encephalitis viruses from wild rodents, South Korea. *Vector Borne Zoonotic Dis.* Spring 2008;8(1):7-13. doi:10.1089/vbz.2006.0634

3. Ko S, Kang JG, Kim SY, et al. Prevalence of tick-borne encephalitis virus in ticks from southern Korea. *J Vet Sci.* Sep 2010;11(3):197-203. doi:10.4142/jvs.2010.11.3.197

4. Yun SM, Lee YJ, Choi W, et al. Molecular detection of severe fever with thrombocytopenia syndrome and tick-borne encephalitis viruses in ixodid ticks collected from vegetation, Republic of Korea, 2014. *Ticks Tick Borne Dis*. Jul 2016;7(5):970-978. doi:10.1016/j.ttbdis.2016.05.003

5. Yun SM, Song BG, Choi W, et al. Prevalence of tick-borne encephalitis virus in ixodid ticks collected from the republic of Korea during 2011-2012. *Osong Public Health Res Perspect*. Dec 2012;3(4):213-21. doi:10.1016/j.phrp.2012.10.004

6. Ree HI. In: Medical Entomology: Medical Arthropodology. 4th ed. Seoul: Komoonsa; 2005:345-90.

7. Noh JY, Song JY, Bae JY, et al. Seroepidemiologic survey of emerging vector-borne infections in South Korean forest/field workers. *PLoS Negl Trop Dis*. Aug 2021;15(8):e0009687. doi:10.1371/journal.pntd.0009687

8. Yoo JR, Oh JH, Lee KH, Song SW. Serological evidence of tick-borne encephalitis virus infection in South Korea, 2015-2018. *Ticks Tick Borne Dis*. May 2020;11(3):101408. doi:10.1016/j.ttbdis.2020.101408

9. Korean Disease Control and Prevention Agency. Case Definitions for National Notifiable Infectious Diseases; 2016.

10. Korea Disease Control and Prevention Agency. Guideline on the Management of Tick- and Rodent-Borne Infections. KDCA. Accessed March 8, 2024. https://www.kdca.go.kr/filepath/boardSyview.es?bid=0019&list_no=722023&seq=1.

11. Yoo SJ, Park JH. Necessity of a Surveillance System for Tick-borne Encephalitis. *Osong Public Health Res Perspect*. Apr 2017;8(2):155. doi:10.24171/j.phrp.2017.8.2.08

Chapter 13

TBE in Sweden

Åke Lundkvist

E-CDC risk status: endemic (data as of end 2023)

History and current situation

Tick-borne encephalitis virus (TBEV) was isolated in Sweden for the first time in 1958 from ticks and from 1 tick-borne encephalitis [TBE] patient.[1] In 2003, Haglund and colleagues reported the isolation, the antigenic and genetic characterization of 14 TBEV strains from Swedish patients based on samples collected 1991–1994.[2] The first serum sample, from which the TBEV was isolated, was obtained 2–10 days after onset of disease and found to be negative for anti-TBEV immunoglobulin M (IgM) by enzyme-linked immunosorbent assay (ELISA), whereas TBEV-specific IgM (and TBEV-specific immuno-globulin G/cerebrospinal fluid [IgG/CSF] activity) was demonstrated in later serum samples taken during the second phase of the disease.

Of 20 patient serum samples inoculated into the brain of suckling mice, 14 induced obvious signs of illness (death or clear physical signs in all cases, 5–7 days after inoculation), and TBEV was isolated from all animals. Three earlier Swedish TBEV patient isolates from 1958,[1] 1959, and 1966, respectively, were included in the same study. Phylogenetic analyses of the partial sequence (domain III) of the E gene revealed that all Swedish TBEV strains grouped together with the previously characterized strains (Neudoerfl, Kumlinge-A52, Hypr, and TBE 263) of the Western or European subtype of TBEV (TBEV-EU).

In 2007, a partial TBEV sequence (approximately one-third of the viral genome) from a small pool of ticks collected in the Stockholm archipelago on the island of Torö was reported.[3]

The sequence was characterized and compared with those of other tick-borne flaviviruses, which again led to classification of the virus as TBEV-EU. The same group reported in 2011 on the first complete genome of a Swedish TBEV strain by completing the earlier partial sequencing (see above).[4] The total RNA was sufficient for the sequencing of a complete TBEV genome (Torö-2003), without conventional enrichment procedures such as cell culture or amplification in suckling mice. Sequence analyses also revealed that Torö-2003 belongs to the TBEV-EU subtype, being most similar to TBE 263 with 97.4% and 98.8% homologies at the nucleotide and amino acid levels, respectively.

In 2014, Veje and co-workers reported 2 cases of TBE in which TBEV RNA could be detected in urine by real-time polymerase chain reaction (PCR) during the encephalitic phase.[5] The TBEV RNA quantities from 1 patient allowed sequencing of 10,432 nucleotides (nt), which confirmed the PCR finding in urine, and phylogenetic analysis showed that the virus belonged to the TBEV-EU clade.

In 2016, Henningsson and associates reported isolation and a complete TBEV sequence from a biting tick.[6] By performing nt sequencing of the virus strain (Tick/SWE/Habo/2011/1) via 2 different strategies (deep sequencing of the A549 isolate and direct sequencing of PCR amplicons of RNA extracted from the tick, respectively), the authors showed that the 2 sequences were identical over 3,382 nt, thereby suggesting that the virus isolation procedure did not introduce a selection bias with regard to the compared nt sequences.

As in other areas of Europe, the number of reported TBE cases has increased during the last 25 years. The mortality of TBE in Sweden is significant (1.4%)[7] and the associated morbidity and long-term sequelae make it a disease of great importance in the endemic regions.[8-10] TBE has been reported in Sweden from diagnostic laboratories on a voluntary basis since the 1970s and notification has been mandatory since 2004. During the years 2007–2019, between 181 and 391 (year 2017) cases of TBE were reported annually in Sweden despite the fact that vaccination has increased in the exposed population. There are 2 TBE vaccines available in Sweden: FSME-Immun (Pfizer) introduced in 1988 and Encepur (Bavarian Nordic) introduced in 2003.

Vaccination against TBE is voluntary in Sweden. The vaccination schedule recommended in Sweden follows the recommendations of the manufacturers, with one exception being that after dose 4 and onwards, a 5-year interval is recommended, irrespective of age (the manufacturers recommend 3-year booster intervals after the age of 50). The change to a 5-year interval after dose 4 and onwards was based on a large study of the serological response in 535 persons in Sweden after TBE vaccination.[11] However, if TBE vaccination is initiated over age 60, the recommended schedule is 1 extra dose 2 months after the second dose, i.e. the initial vaccination includes 4 doses at 0, 1, 3, and 5–12 months.

The number of vaccine doses sold in Sweden has averaged from 500,000 to 600,000 annually since 2006, but increased to 1.2 million doses per year in 2018. The number of

sold doses has continued to increase and was around 1.8 million in 2022. Because TBE vaccination is not included in any official vaccination registry, the actual number of immunized individuals is unknown.

To estimate the TBE vaccination coverage in the greater Stockholm region, a questionnaire was sent to a randomized sample of 8,000 individuals in 2013.[12] Three percent of all respondents reported being vaccinated against TBE at least once. Based on these findings, the estimated TBE incidence in the unvaccinated regional population was 8.5–12/100,000, which is comparable to highly endemic areas in the Baltics and Central Europe.

The protection rate of the vaccine has been estimated to be 96% to 98% according to field studies in Austria. In a study from 2010, data from 27 Swedish patients with clinical symptoms and signs of TBE, together with serological evidence of TBEV infection despite active vaccination, was presented.[13] Vaccination failures were characterized by a slow and initially non-detectable development of TBEV-specific IgM, seen together with a rapid rise of IgG and neutralizing antibodies in serum. The majority (70%) of the 27 patients were above age 50, which indicated the need for a modified immunization strategy in the elderly (as noted above).

Recently, a new tool (TBE suspension multiplex immune-assay, TBEV SMIA) for improved diagnostics of TBEV infections was reported.[17] The TBEV SMIA can accurately differentiate TBEV infections from TBE vaccination and further studies have now been initiated to evaluate the efficiency of the assay for diagnosis of potential vaccine failures.

Recently, the TBEV SMIA was evaluated using samples from 14 previously confirmed Swedish TBEV vaccine failure patients.[18] The conclusion was that detection of antibodies directed to TBEV NS1 antigen is a useful tool to considerably simplify and improve the quality in investigations regarding suspected TBEV infection in vaccinated patients.

In January 2024, a study on the prevalence of TBEV infections as well as the prevalence of TBE vaccinations in nine geographical regions of Sweden was published.[19] The results correlated well to the reported number of TBE cases in the various regions, and to the expected vaccine coverage. However, the results indicated that the proportion of TBEV infections resulting in a notified clinical TBE is much lower than previously believed, only between 0.4-8.7 % in the different regions. The study was based on the TBEV SMIA[17,18] and the blood donor samples were collected 2018-2019. A similar study is now planned on new blood donor samples covering all the 21 regions of the whole country. Similar studies are also planned in several European countries.

In northern Europe, including Sweden, TBEV-EU is usually transmitted to humans by the common tick, *Ixodes ricinus*. Pettersson and colleagues investigated the prevalence in host-seeking *I. ricinus* southern and central Sweden and reviewed all relevant published records on the prevalence of TBEV in ticks in northern Europe.[14] Estimated mean minimum infection rate (MIR) of TBEV in nymphal and adult *I. ricinus* for northern Europe (i.e. Denmark, Norway, Sweden, and Finland) was 0.28% and 0.23% for southern Sweden. Also, the infection prevalence of TBEV was significantly lower for nymphs (0.10%) than for adult ticks (0.55%). In a well-known TBEV-endemic region, Torö island, southeast of Stockholm, the TBEV prevalence was 0.51% in nymphs and 4.48% in adult ticks.

In a review of the ecology and epidemiology of TBE in Sweden, Jaenson and colleagues analyzed the possible reasons behind the gradually increasing incidence of human TBE during the last 20 years.[15] The authors made the following conclusions:

i. Due to a large roe deer population during the 1980s and 1990s, the Swedish tick population gradually increased. At the turn of the century, the tick population in Sweden was probably larger than ever.

ii. The roe deer population gradually declined after its peak in the late 1980s and early 1990s.

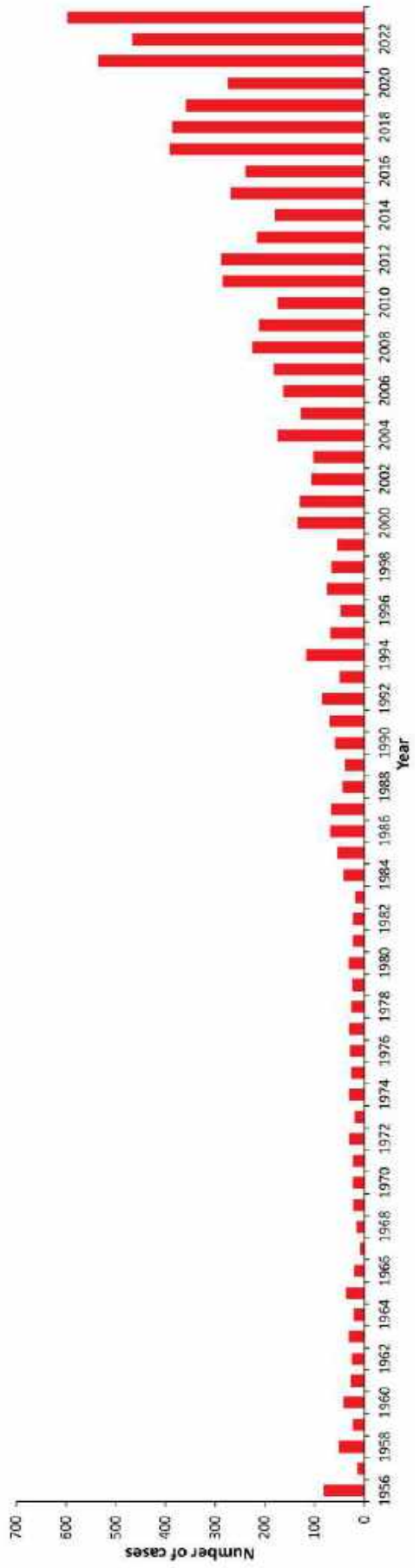
iii. During the decline of the roe deer population, a gradually larger proportion of the tick larvae and nymphs probably fed on small mammals, which are reservoir-competent hosts for TBEV. Consequently, since the mid-1990s, a larger proportion of the tick population became infected with TBEV.

iv. Climate change and weather events associated with higher temperatures further influenced the infection prevalence in the tick population and therefore also the annual incidence in humans.

Overview of TBE in Sweden

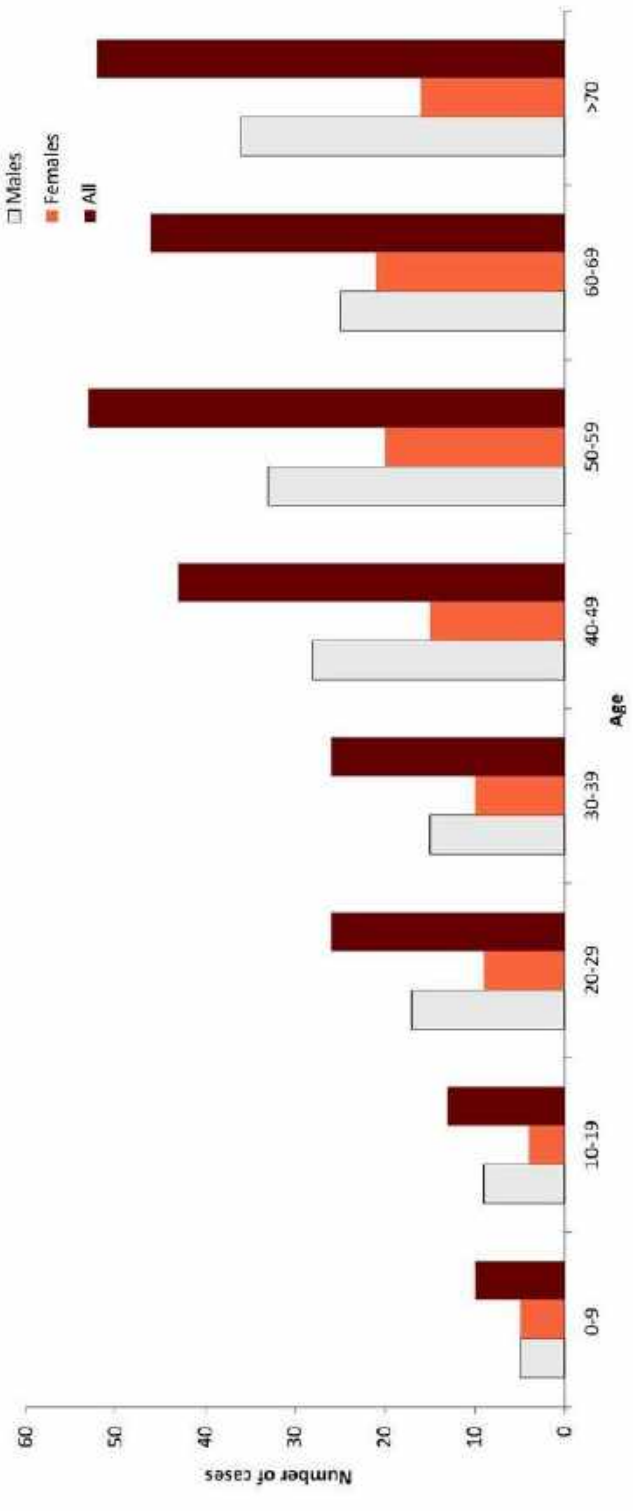
Table 1: TBE in Sweden	
Viral subtypes, distribution	Only western/European TBEV (TBEV-EU), southern part of the country[1-6]
Reservoir animals	Not documented
Infected tick species (%)	*I. ricinus*, 0.23% to 4.48%[14]
Dairy product transmission	Not documented
Mandatory TBE reporting	Each diagnostic laboratory plus the responsible physician report to the Public Health Agency of Sweden **Case definition**: **TBEV-infection (viral TBE)** **Suspected case:** - Epidemiological link - Clinical symptoms consistent with TBE - Pleocytosis (CSF) and/or neurological symptoms of encephalitis - Detection of TBEV-specific serum IgM **Confirmed case:** **At least one of the following:** - Detection of TBE-specific IgM and IgG in serum - Detection of TBE-specific IgM in CSF - Seroconversion or significant titer rise in paired serum samples - Detection of TBEV RNA in CSF (or post-mortem in brain tissue) - Detection of TBEV RNA in serum **Note**: Previous TBE vaccination and/or immunosuppression influence the patients' antibody responses and thus repeated sampling may be necessary for an accurate diagnosis. Also earlier infections, or vaccinations, against other flaviviruses may complicate the diagnostics due to cross-reactive antibodies. *Source: The Public Health Agency of Sweden (see below)*
Other TBE surveillance	No
Clinical characteristics	36%–40% with sequelae (after 1 year); mortality: 1.4%[7-8]
Available vaccines	FSME-Immun (Pfizer) introduced in 1988 and Encepur (Bavarian Nordic) introduced in 2003. 500,000–600,000 doses/year;[13,16] 1,200,000 doses/year in 2018 (unpublished data)
Vaccination recommendations and reimbursement	Revised each year No reimbursement
Vaccine uptake by age group/risk group/ general population	No data available
Name, address/ website of TBE NRC	The Public Health Agency of Sweden SE-171 82 Solna , Sweden www.folkhalsomyndigheten.se

Figure 1: Burden of TBE in Sweden over time



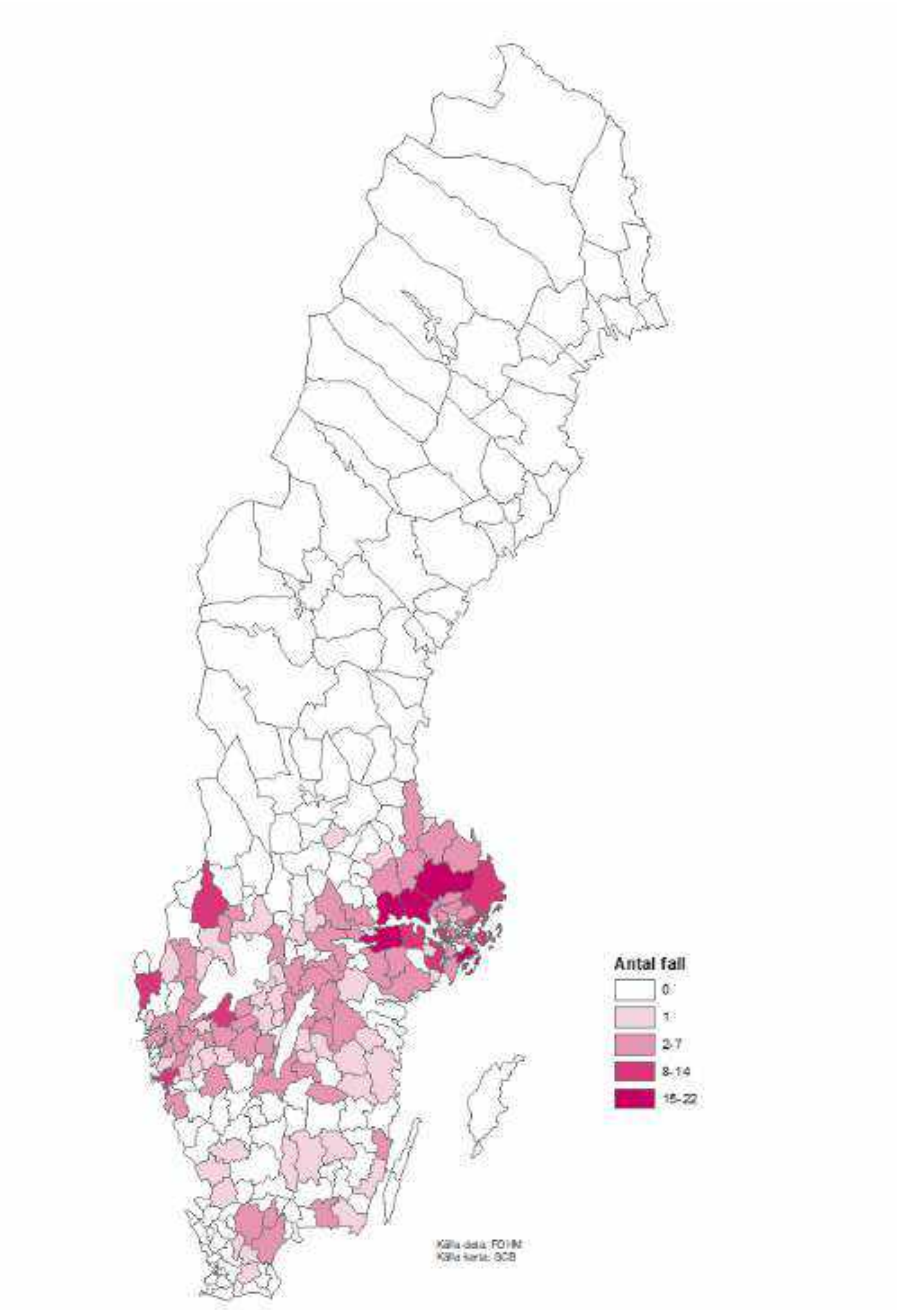
Source Data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE cases in Sweden (2018)



Source Data: Appendix Figure 2

Figure 3: TBE cases per municipality in 2021



Source Data: PHA Sweden. Available online here: https://www.folkhalsomyndigheten.se/nyheter-och-press/nyhetsarkiv/2022/april/sasongen-for-tbe-narmar-sig/)

Appendix

Source data : Figure 1

Year	Number of cases	Incidence / 10^5
1956	82	1.1
1957	12	0.16
1958	50	0.67
1959	22	0.29
1960	41	0.55
1961	26	0.34
1962	24	0.32
1963	30	0.39
1964	20	0.26
1965	35	0.45
1966	19	0.24
1967	8	0.1
1968	14	0.18
1969	21	0.26
1970	22	0.27
1971	22	0.27
1972	29	0.036
1973	18	0.22
1974	29	0.036
1975	25	0.3
1976	27	0.33
1977	29	0.35
1978	25	0.3
1979	23	0.28
1980	30	0.36
1981	22	0.26
1982	22	0.26
1983	17	0.2
1984	41	0.49
1985	52	0.62
1986	67	0.8
1987	66	0.78
1988	43	0.51
1989	37	0.43
1990	58	0.68
1991	68	0.79
1992	84	0.97
1993	48	0.55
1994	116	1.3
1995	67	0.76
1996	45	0.51
1997	74	0.84
1998	65	0.73
1999	53	0.6
2000	133	1.5
2001	128	1.4
2002	104	1.2
2003	101	1.1
2004	174	1.9
2005	126	1.4
2006	161	1.8
2007	181	2
2008	224	2.4
2009	210	2.2
2010	174	1.8
2011	284	3
2012	287	3
2013	209	2.17
2014	178	1.83
2015	268	2.72
2016	238	2.38
2017	391	3.86
2018	385	3.76
2019	358	3.47
2020	274	2.64
2021	534	5.11
2022	465	4.42
2023	596	5.61

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	5	5	10
10-19	9	4	13
20-29	17	9	26
30-39	15	10	26
40-49	28	15	43
50-59	33	20	53
60-69	25	21	46
>70 341	36	16	52

Contact: ake.lundkvist@imbim.uu.se

Citation:
Lundkvist A. TBE in Sweden. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-32-7

References

1. Von Zeipel G. Isolation of viruses of the Russian Spring-Summer Encephalitis-Louping ill group from Swedish ticks and from a human case of meningo-encephalitis. *Arch Gesamte Virusforsch.* 1959;9:460-9.
2. Haglund M, Vene S, Forsgren M, et al. Characterization of human tick-borne encephalitis virus from Sweden. *J Med Virol.* 2003;71:610-21.
3. Melik W, Nilsson AS, Johansson M. Detection strategies of tick -borne encephalitis virus in Swedish *Ixodes ricinus* reveal evolutionary characteristics of emerging tick-borne flaviviruses. *Arch Virol.* 2007;152:1027-34.
4. Elväng A, Melik W, Bertrand Y, Lönn M, Johansson M. Sequencing of a tick-borne encephalitis virus from Ixodes ricinus reveals a thermosensitive RNA switch significant for virus propagation in ectothermic arthropods. *Vector Borne Zoonotic Dis.* 2011;11:649-58.
5. Veje M, Studahl M, Norberg P, et al. Detection of tick-borne encephalitis virus RNA in urine. *J Clin Microbiol.* 2014;52:4111-2.
6. Henningsson AJ, Lindqvist R, Norberg P, et al. Human tick-borne encephalitis and characterization of virus from biting tick. *Emerg Infect Dis.* 2016;22:1485-7.
7. Haglund M, Forsgren M, Lindh G, Lindquist L. A 10-year follow -up study of tick-borne encephalitis in the Stockholm area and a review of the literature: need for a vaccination strategy. *Scand J Infect Dis.* 1996;28:217-24.
8. Günther G, Haglund M, Lindquist L, Forsgren M, Sköldenberg B. Tick-borne encephalitis in Sweden in relation to aseptic meningoencephalitis of other etiology: a prospective study of clinical course and outcome. *J Neurol.* 1997;244:230-38.
9. Haglund M, Günther G. Tick-borne encephalitis – pathogenesis, clinical course and long-term follow- up. *Vaccine.* 2003;21 Suppl 1:11-8.
10. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet.* 2008;371(9627):1861-71.
11. Vene S, Haglund M, Lundkvist Å, Lindquist L, Forsgren M. Study of the serological response after vaccination against tick -borne encephalitis in Sweden. *Vaccine.* 2007;25(2):366-72.
12. Askling HH, Insulander M, Hergens MP, Leval A. Tick-borne encephalitis (TBE)-vaccination coverage and analysis of variables associated with vaccination, Sweden. *Vaccine.* 2015;33(38):4962-8.
13. Andersson C, Vene S, Insulander M, et al. Vaccine failures after active immunization against tick-borne encephalitis. *Vaccine.* 2010;28(16):2827-31.
14. Pettersson JH, Golovljova I, Vene S, Jaenson TG. Prevalence of tick-borne encephalitis virus in *I. ricinus ticks in northern Europe with particular reference to Southern Sweden. Parasit Vectors.* 2014;7:102.
15. Jaensson TG, Hjertqvist M, Bergström T, Lundkvist Å. Why is tick-borne encephalitis increasing? A review of the key factors causing the increasing incidence of human TBE in Sweden. *Parasit Vectors.* 2012;5:184.
16. Slunge D. The willingness to pay for vaccination against tick-borne encephalitis and implications for public health policy: Evidence from Sweden. *PLoS One.* 2015;10:e0143875.
17. Albinsson B, Vene S, Rombo L, Blomberg J, Lundkvist Å, Rönnberg B Distinction between serological responses following tick-borne encephalitis virus (TBEV) infection vs vaccination, Sweden 2017. *Euro Surveill.* 2018;23(3).
18. Albinsson B, Rönnberg B, Vene S, Lundkvist Å. Antibody responses to tick-borne encephalitis virus non-structural protein 1 and whole virus antigen-a new tool in the assessment of suspected vaccine failure patients. *Infect Ecol Epidemiol.* 2019;9(1):1696132.
19. Albinsson B, Hoffman T, Kolstad L, Bergström T, Bogdanovic G, Heydecke A, Hägg M, Kjerstadius T, Lindroth Y, Petersson A, Stenberg M, Vene S, Ellström P, Rönnberg B, Lundkvist Å. Seroprevalence of tick-borne encephalitis virus and vaccination coverage of tick-borne encephalitis, Sweden, 2018 to 2019. *Euro Surveill.* 2024 Jan;29(2):2300221. doi: 10.2807/1560-7917.ES.2024.29.2.2300221. PMID: 38214080; PMCID: PMC10785208

Chapter 13

TBE in Switzerland and Liechtenstein

Kyra Zens

E-CDC risk status: endemic (data as of end 2023)

History and current situation

Tickborne Encephalitis (TBE) was first reported in Switzerland in 1969.[1] From the 1970s through the 1990s the causative agent, the tickborne encephalitis virus (TBEV), was found to be endemic in geographically localized areas within the northeastern part of the country.[2-4] A formal case definition and surveillance activities were introduced in 1984 and TBE was made a mandatory notifiable disease in 1988.[5] Currently, all suspected TBE cases are reported to the Swiss Federal Office of Public Health (FOPH) using a two -tiered system. First, all laboratory tests indicative of acute TBEV infection are reported to the FOPH. Then, attending physicians are requested to complete a notification form providing specific clinical information, which is forwarded to the cantonal physician for review and then returned to the FOPH (Table 1). Both laboratory and completed clinical reporting forms are registered and maintained by the FOPH5. The TBE case definition used in Switzerland is based on a combination of clinical and laboratory criteria and is similar to, but differs slightly from, that used by the ECDC in that "possible" cases, in addition to "probable" and "confirmed" cases, are included (Table 1).[6-9]

The majority of TBE cases in Switzerland are reported between April and October10 (Figure 1). Cases are more commonly reported in men, compared to women, and individuals aged 50-69 are most affected, though a bimodal trend with a smaller peak in cases among children aged 5-9 is also observed (Figure 2).[10] Recent work has demonstrated that approximately 5% of unvaccinated individuals throughout the country are seropositive, suggesting that exposures far outnumber clinically confirmed cases of disease.[11] Among clinical TBE cases, approximately 75% recalled a tick bite within the 4 weeks prior to disease onset.[6,8] Approximately 75% result in hospitalization. Meningitis is observed in 19-49% of cases,[6,12,13] meningoencephalitis in 43-59% of cases,[6,12,13] and meningoencephalomyelitis and/or radiculitis in 5-7%.[6,12,13] Just under 1% of cases are fatal (Table 1).[6,8,13]

Over the last two decades, both the geographic range and total incidence of TBE cases have increased dramatically throughout Switzerland.[10,14,15] From an initial localization to the northeastern part of the country, TBE cases have increasingly been reported further west- and southward. This has been paralleled by increases in the range of TBEV-infected ticks[16-23] and small and large mammal populations with positive anti-TBEV serology (Table 1).[24-28] Currently, TBEV has been identified in ticks from most regions of Switzerland and in Liechtenstein, and, accordingly, human cases are now found in most areas of the country.[29] In 2020, the nationwide average disease incidence exceeded the WHO's definition of "highly endemic", with greater than 5.0 cases/100,000 individuals reported.[10]

Official recommendations for vaccination against TBE have been in place in Switzerland and Liechtenstein since 2006; initially for all individuals aged 6 and older living or spending significant time in 71 "high risk" areas throughout both countries (Table 1).[30] These risk areas, based on reported cases and viral surveillance in the environment, were updated and expanded annually to reflect the changing epidemiology of the disease.[29,31] The resulting risk area map (Figure 3b) was used until 2018 to define TBE vaccination recommendations throughout the country.[29,31] However, in 2019, in view of the continuing increases in incidence and geographic range of disease, health authorities in Switzerland and Liechtenstein expanded the risk area and simplified the vaccination recommendation to cover the entirety of both countries – with the exceptions of the Swiss cantons of Geneva and Ticino (Figure 3a – 3c).[14,29] In 2024 the recommendation was further revised to include the canton of Geneva (from summer 2024) as well as to recommend vaccination beginning at 3 years of age.[32]

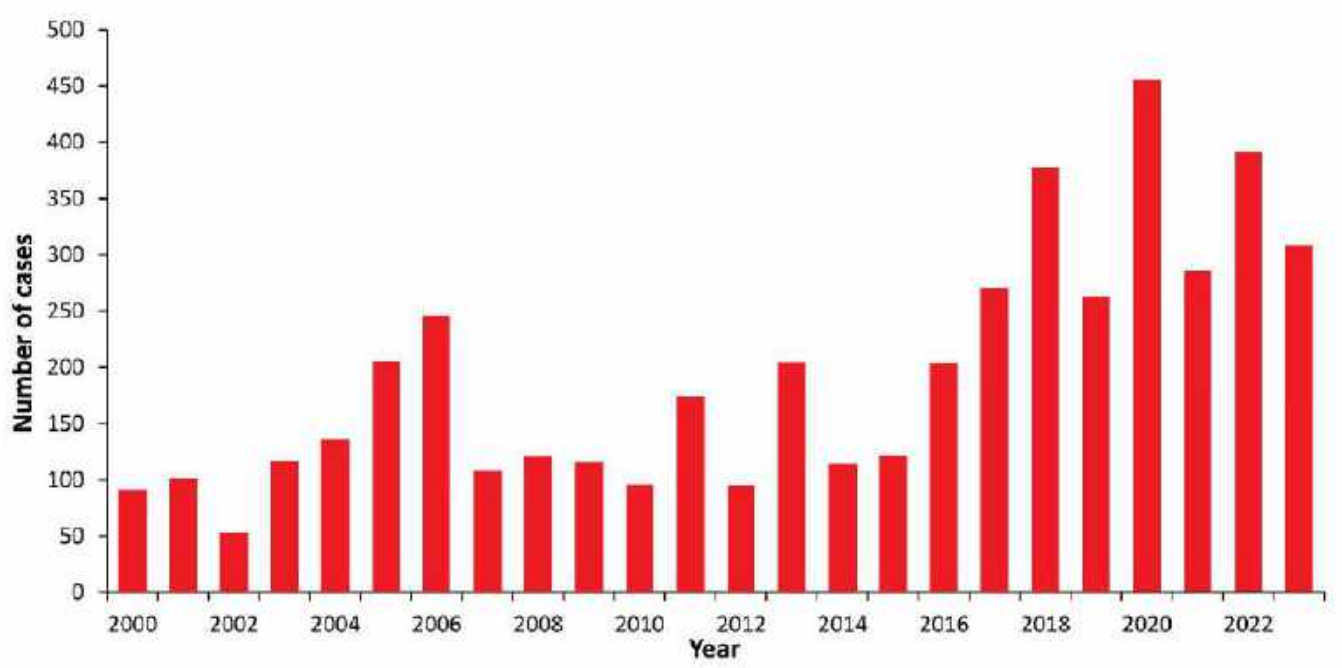
Vaccination is reimbursed by compulsory health insurance for individuals to which the recommendation applies; namely those 3 years of age and older living or spending significant time in risk area.[14,32] In children 1–2 years of age, vaccination is considered and reimbursed on a case-by-case basis.[14,32] Considerations are also made for those with "high risk" occupations, though the cost of vaccination is to be reimbursed by the employer (Table 1).[14,32] Nationwide, between 2020 and 2022, just 2% of 2-year-olds were vaccinated, increasing to 50% coverage among 8- and 16-year-olds. Among adults, from the most recent data in 2018, 42% had received at least one TBE vaccine dose while 33% had completed at least the three dose primary series (Table 1).[33] Following completion of primary immunization, Switzerland has a unique recommendation for administration of booster vaccine doses every 10 years,[30,34] unlike most other European countries and in contrast to the label. However, recent epidemiologic studies in the country have demonstrated that vaccine effectiveness (VE) remains high in both children[35] and adults[36] over this interval, with sustained protection for at least 10 years after the last vaccine dose was received.

Overview of TBE in Switzerland

Table 1: TBE in Switzerland	
Virus subtypes isolated	Only the European subtype has been described[17,20,22,23]
Reservoir animals	Small mammals, generally rodents (*Apodemus flavicollis*, *A. sylvaticus*, *Myodes glareolus*), are the primary reservoir hosts for TBEV observed in Switzerland.[24] TBEV-infected ticks have also been found on migrating birds[21]
Percentage infected ticks	Only *Ixodes ricinus* ticks described; Prevalence in ticks is focal and ranges widely, generally less than 1% of questing ticks but as high as 14.3%[16-23,25,26,37]
Dairy product transmission	Not documented, risk estimated to be low[38]
Case definition used by authorities	Possible Case: positive IgM serology with influenza-like illness (ILI) or non-specific neurological signs & symptoms, **OR**, positive IgM + positive IgG serology without specific clinical symptoms Probable Case: positive IgM serology with meningitis, meningoencephalitis, encephalomyelitis or radiculitis, **OR**, positive IgM + positive IgG serology with influenza-like illness (ILI) or non-specific neurological signs & symptoms Confirmed Case: positive IgM + positive IgG serology with meningitis, meningoencephalitis, encephalomyelitis or radiculitis, **OR**, TBE-RNA detection by PCR with meningitis, meningoencephalitis, encephalomyelitis or radiculitis
Completeness of case detection and reporting	Case reporting assumed to be complete or near complete due to two-tiered system[5-8], though no specific studies have evaluated this
Type of reporting	A mandatory notifiable disease since 1988 with reporting to the Swiss FOPH following a two-tiered system[5-8]: -First, all laboratory tests positive for evidence of acute TBE are reported -Afterwards, attending physicians are requested to complete a specific notification form providing specific clinical information
Other TBE surveillance	Not routine Studies assessing TBEV in ticks[16-23,25,26,37] Studies assessing seropositive blood donors[11]
Special clinical features	In children:[35] • No neurologic involvement reported in 13% of cases • Meningeal irritation, meningitis observed in 35% of cases • Meningoencephalitis in 49% of cases • Encephalitis, encephalomyelitis, radiculitis, paresis reported in 3% of cases In adults: • Hospitalization observed in 71-75% of reported cases[6,8,13] • Meningitis in 19-49% of cases[6,12,13] • Meningoencephalitis in 43-59% of cases[6,12,13] • Meningoencephalomyelitis/Radiculitis in 5-7% of cases[6,12,13] • Slightly under 1% of cases are fatal[6,8,13]

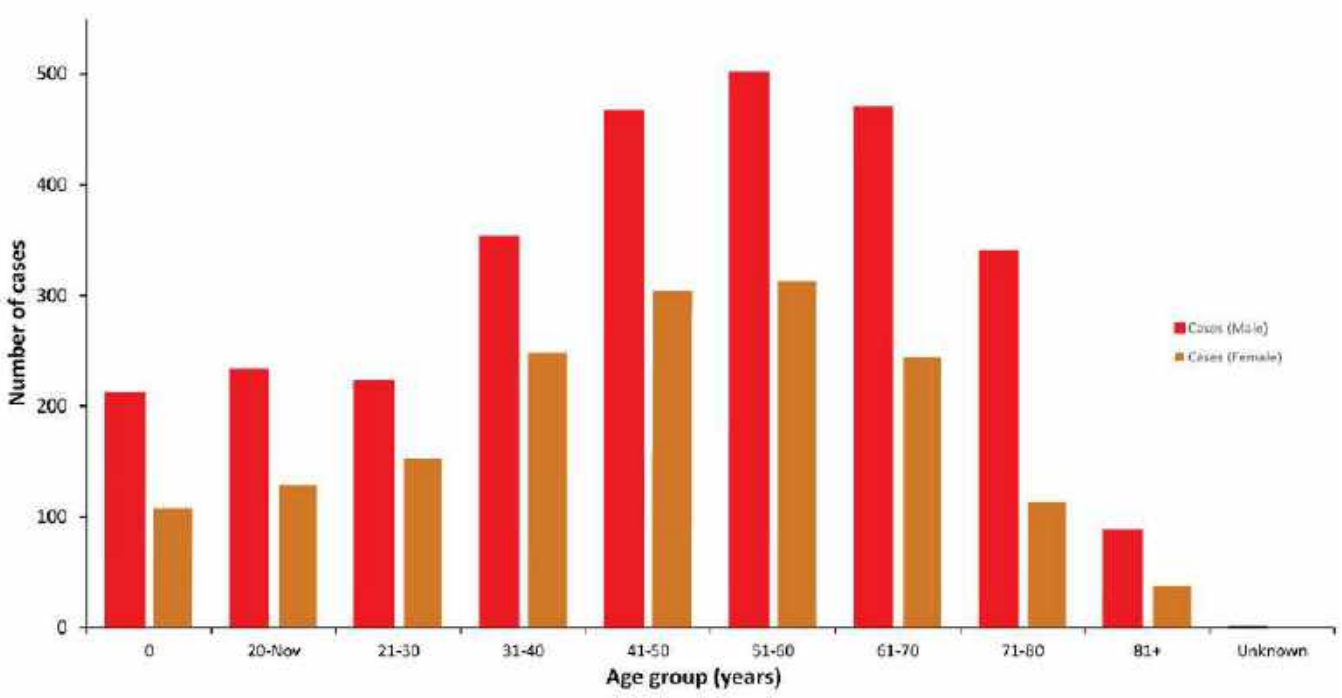
Licensed vaccines	Encepur N® (Bavarian Nordic) Adult Formulation[39] Encepur N® Kinder (Bavarian Nordic) Pediatric Formulation[39] FSME-Immun® (Pfizer) Adult Formulation[40] FSME-Immune® Junior (Pfizer) Pediatric Formulation[41]
Vaccination recommendations	Localized recommendations based primarily on area of residence since 2006[30]; in 2019 and 2024 the recommendation was expanded to cover all of Switzerland and Liechtenstein with the exceptions of Geneva and Ticino[14,29,32] Vaccination is reimbursed by compulsory health insurance for individuals covered by the recommendation: • Individuals 3 years of age and older living or spending significant time in risk areas[14,32,33] • In children 1–2 years of age vaccination is considered and reimbursed on a case-by-case basis[14,32,33] • For individuals with “high risk” occupations, costs of vaccination are covered by the employer[14,32,33]
Vaccine uptake	In children[34,43] - Average national vaccination uptake (3+ doses) 2019-2022: • 2 years old: 2.3% (1.8-2.9) • 8 years old: 48.7% (46.9-50.6) • 16 years old: 50.1% (48.3-52.0) In adults[35] - Average national vaccination uptake (3+ doses) 2018: -18-39 years old: 34.7% (31.5–38.0%) -40-59 years old: 31.3% (29.0–33.8%) -60-79 years old: 32.4% (30.1–34.8%)
National Reference center for TBE	Nationales Referenzzentrum für durch Zecken übertragene Krankheiten (NRZK; National Reference Centre for Tick-borne Diseases) Website: www.swissticks.ch The reference center consists of two partners: Institut für Mikrobiologie des Centre Hospitalier Universitaire Vaudois (CHUV) Rue du Bugnon 48 1011 Lausanne Tél. +41 21 314 46 48 / +41 21 314 40 56 (secrétariat) Tél. +41 21 314 49 79 (Prof. G. Greub) Mail: gilbert.greub@chuv.ch ADMED Microbiologie Boucle de Cydalise 16+2300 La Chaux-de-Fonds Tél. +41 32 967 21 01 Mail: admed.microbiologie@ne.ch

Figure 1: Number of reported TBE cases in Switzerland, 2000-2023



Source Data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE cases in Switzerland 2000–2023



Source Data: Appendix—Figure 2

Figure 3a: TBE cases - Reported exposure sites, 2012-2023.
Latest update available at: https://s.geo.admin.ch/727304e0f5

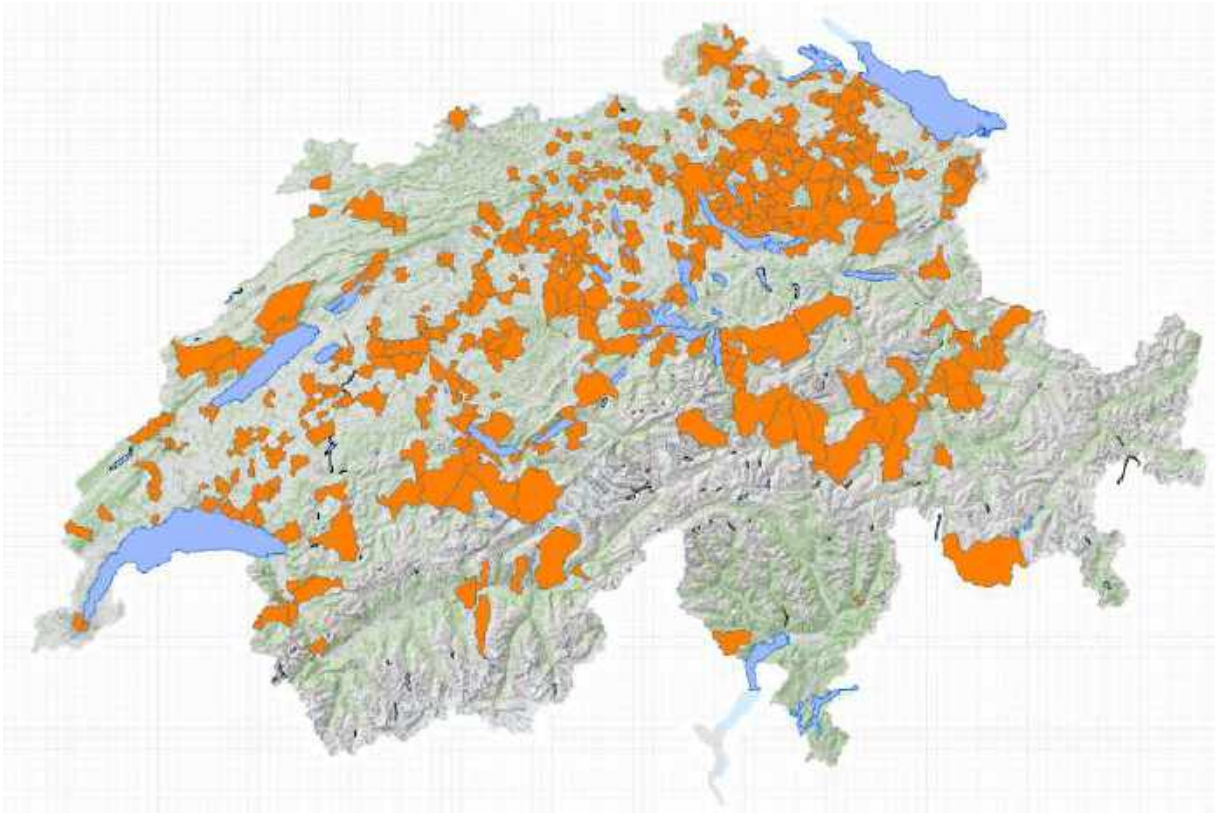


Figure 3b: Risk areas in Switzerland where TBE vaccination was recommended until the end of 2018

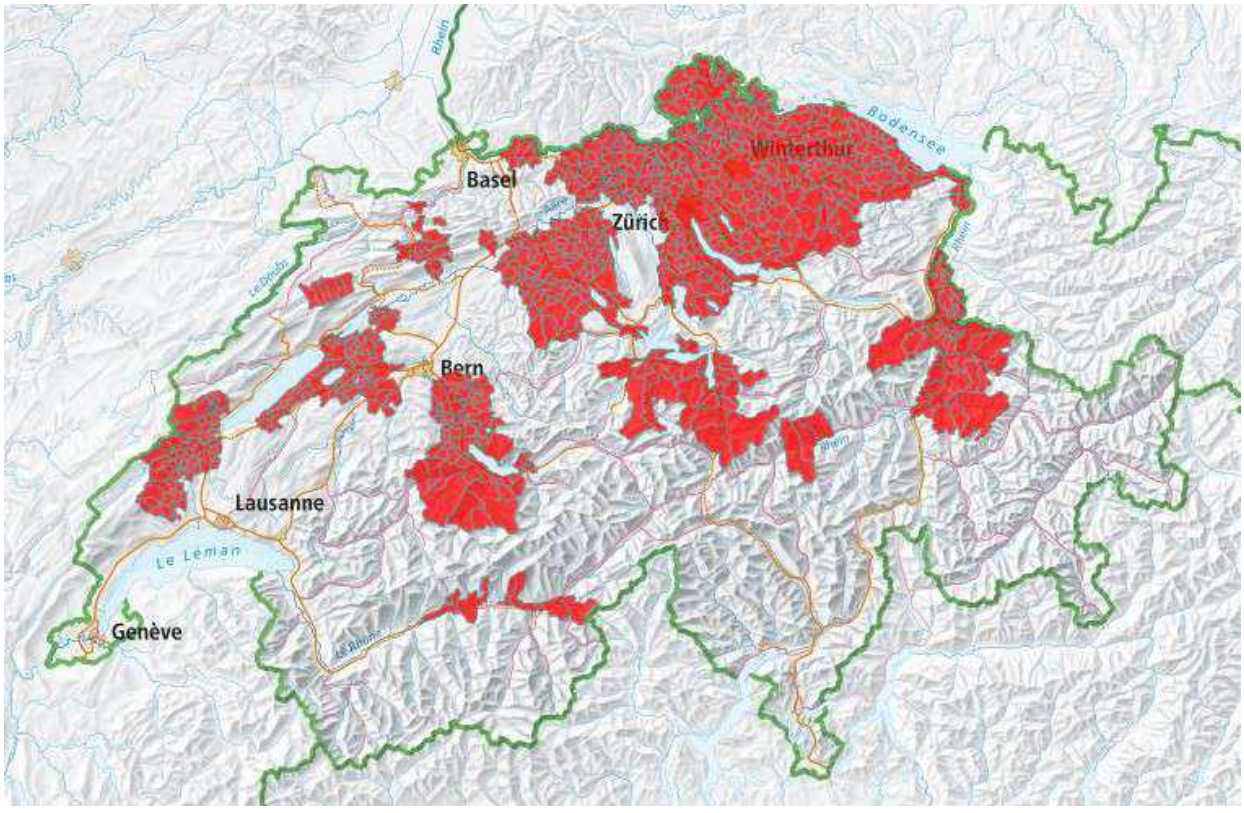
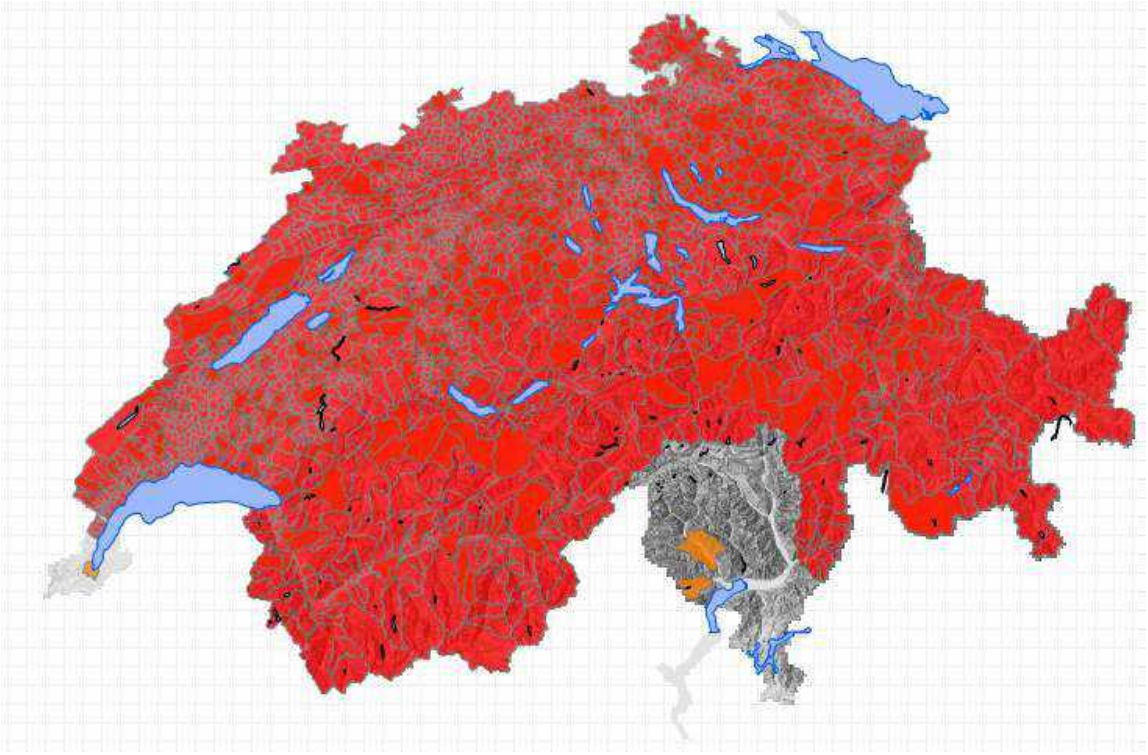


Figure 3c: **Extended risk areas where TBE vaccination was recommended, 2019-2023. Risk areas were further extended for all individuals (residents and visitors aged 3+ years) with the exception of canton Ticino, from April 2024. Official update of map not yet available by April 30, 2024 but can be found afterward with latest update at: https://s.geo.admin.ch/727304e0f5**



Acknowledgments

Unpublished data were kindly provided by Annora Mack and Ekkehardt Altpeter, Federal Office of Public Health FOPH, Division of Communicable Diseases, Bern, Switzerland.

Authors and Affiliations

Kyra Zens
Department of Viral Immunobiology, Institute for Experimental Immunology, University of Zurich, Zurich Switzerland
Department of Public and Global Health, Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich Switzerland

Contact: zens@immunology.uzh.ch

Citation:

Zens K. TBE in Switzerland and Liechtenstein. Chapter 13 In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-33-7

References

1. Krech, T. [Tick-borne Encephalitis (TBE) in Switzerland] *Doctoral thesis, University of Bern.* 1980.
2. Baumberger P, Krech T, Frauchiger B. Entwicklung der Frühsommer-Meningoenzephalitis (FSME) in der Region Thurgau 1990-1995--ein neues Endemiegebiet? [Development of early-summer meningoencephalitis (FSME) in the Thurgau region 1990-1995--a new endemic area?]. *Schweiz Med Wochenschr*. 1996;126(48):2072-2077.
3. Aberham C, Radda A, Holzmann H, Krech T. Detection of tick-borne encephalitis (TBE) virus in Liechtenstein. *Zentralbl Bakteriol*. 1992;277(4):554-560. doi:10.1016/s0934-8840(11)80481-0
4. Schwanda M, Oertli S, Frauchiger B, Krause M. Die Frühsommer-Meningoenzephalitis im Kanton Thurgau: eine klinisch-epidemiologische Analyse [Tick-borne meningoencephalitis in Thurgau Canton: a clinical and epidemiological analysis]. *Schweiz Med Wochenschr*. 2000;130(41):1447-1455.
5. Bundesamt fuer Gesundheit (BAG). [Infectious Disease Reporting - Tick-borne Encephalitis - TBE]. 2023. Accessed 30 March, 2024. https://www.bag.admin.ch/bag/de/home/krankheiten/infektionskrankheiten-bekaempfen/meldesysteme-infektionskrankheiten/meldepflichtige-ik/meldeformulare.html#-1611150545.

Appendix

Source data: Figure 1

Year	Number of cases	Incidence/10^5
2000	90	1.24
2001	100	1.37
2002	52	0.70
2003	116	1.56
2004	135	1.81
2005	204	2.72
2006	245	3.24
2007	107	1.40
2008	120	1.55
2009	115	1.44
2010	95	1.20
2011	173	2.17
2012	94	1.16
2013	203	2.48
2014	113	1.37
2015	121	1.42
2016	202	2.39
2017	269	3.16
2018	376	4.38
2019	262	3.03
2020	454	5.11
2021	285	3.25
2022	388	4.38
2023	307	3.47

Source data: Figure 2

Age group (years)	Cases (Male)	Cases (Female)	Unknown
0-10	212	108	0
11-20	233	128	2
21-30	222	153	0
31-40	353	248	0
41-50	466	304	2
51-60	501	313	1
61-70	470	244	1
71-80	340	113	0
81+	88	38	0
Unknown	1	0	0

Data Include all possible, probable, and confirmed cases according to Swiss TBE case definitions

6. Altpeter E, Zimmermann H, Oberreich J, Péter O, Dvořák C; Swiss Sentinel Surveillance Network. Tick related diseases in Switzerland, 2008 to 2011. *Swiss Med Wkly*. 2013;143:w13725. Published 2013 Jan 8. doi:10.4414/smw.2013.13725

7. Schmidt AJ, Altpeter E, Graf S, Steffen R. Tick-borne encephalitis (TBE) in Switzerland: does the prolongation of vaccine booster intervals result in an increased risk of breakthroughs?. *J Travel Med*. 2022;29(2):taab158. doi:10.1093/jtm/taab158

8. Schuler M, Zimmermann H, Altpeter E, Heininger U. Epidemiology of tick-borne encephalitis in Switzerland, 2005 to 2011. 2014;19(14):pii/20765]. *Euro Surveill*. doi:10.2807/1560-7917.es2014.19.13.20756

9. Euro-Lex. Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. *Official Journal of the European Union*. Accessed 30 March, 2024. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2018.170.01.0001.01.ENG.

10. Bundesamt für Gesundheit BAG. [Numbers for Infectious Illnesses - Tick-borne Encephalitis - TBE]. Accessed 30 March, 2024. https://www.bag.admin.ch/bag/de/home/zahlen-und-statistiken/zahlen-zu-unfektionskrankheiten.exturl.html/aHR0cHM6Ly9tZWxkZXN5c3RlbWUuYmFnYXBwcy5jaC9pbmZyZX/BvcnRpbmcvZGF0ZW5kZXRhaWxzL2QvZnNtZS5odG1sP3dlYmdy/YWI9aWdub3Jl.html

11. Ackermann-Gäumann R, Eyer C, Vock M, et al. Prevalence of anti-tick-borne encephalitis virus (TBEV) antibodies in Swiss blood donors in 2014-2015. *Blood Transfus*. 2023;21(2):100-109. doi:10.2450/2022.0099-22

12. Lammli, B., Muller, A. & Ballmer, P. E. [Late sequelae of early summer meningoencephalitis]. *Schweiz Med Wochenschr* 2000;130:909-915.

13. Zimmermann, H. & Koch, D. [Epidemiology of tick-borne encephalitis (TBE) in Switzerland 1984 to 2004]. *Ther Umsch.* 2005;62:719-25. doi:10.1024/0040-5930.62.11.719

14. Bundesamt für Gesundheit BAG. [Tick-borne encephalitis (TBE): expansion of risk areas]. Accessed 30 March, 2024. https://www.bag.admin.ch/dam/bag/de/dokumente/mt/i-und-b/richtlinien-empfehlungen/empfehlungen-spezifische-erreger-krankheiten/zeckenenzephalitis/zeckenenzephalitis-impfung-risikogebiet.pdf.download.pdf/zeckenenzephalitis-impfung-risikogebiet-de.pdf

15. Bundesamt für Gesundheit BAG. [Tick-borne Encephalitis (TBE): continuted increase in reported cases in 2006]. Accessed 30 March, 2024. https://www.bag.admin.ch/dam/bag/de/dokumente/nat-gesundheitspolitik/klimawandel/hitzewelle/hintergrundinfos/info-zecken/zeckenenzephalitis-2006.pdf.download.pdf/zeckenenzephalitis-2006.pdf

16. Wicki R, Sauter P, Mettler C, et al. Swiss Army Survey in Switzerland to determine the prevalence of Francisella tularensis, members of the Ehrlichia phagocytophila genogroup, Borrelia burgdorferi sensu lato, and tick-borne encephalitis virus in ticks. *Eur J Clin Microbiol Infect Dis*. 2000;19(6):427-432. doi:10.1007/s100960000283

17. Casati S, Gern L, Piffaretti JC. Diversity of the population of Tick-borne encephalitis virus infecting Ixodes ricinus ticks in an endemic area of central Switzerland (Canton Bern). *J Gen Virol*. 2006;87(Pt 8):2235-2241. doi:10.1099/vir.0.81783-0

18. Gäumann R, Mühlemann K, Strasser M, Beuret CM. High-throughput procedure for tick surveys of tick-borne encephalitis virus and its application in a national surveillance study in Switzerland. *Appl Environ Microbiol*. 2010;76 (13):4241-4249. doi:10.1128/AEM.00391-10

19. Burri C, Bastic V, Maeder G, Patalas E, Gern L. Microclimate and the zoonotic cycle of tick-borne encephalitis virus in Switzerland. *J Med Entomol*. 2011;48(3):615-627. doi:10.1603/me10180

20. Lommano E, Burri C, Maeder G, et al. Prevalence and genotyping of tick-borne encephalitis virus in questing Ixodes ricinus ticks in a new endemic area in western Switzerland. *J Med Entomol*. 2012;49(1):156-164. doi:10.1603/me11044

21. Lommano E, Dvořák C, Vallotton L, Jenni L, Gern L. Tick-borne pathogens in ticks collected from breeding and migratory birds in Switzerland. *Ticks Tick Borne Dis*. 2014;5(6):871-882. doi:10.1016/j.ttbdis.2014.07.001

22. Rieille N, Bressanelli S, Freire CC, et al. Prevalence and phylogenetic analysis of tick-borne encephalitis virus (TBEV) in field-collected ticks (Ixodes ricinus) in southern Switzerland. *Parasit Vectors*. 2014;7:443. Published 2014 Sep 22. doi:10.1186/1756-3305-7-443

23. Stegmüller S, Qi W, Torgerson PR, Fraefel C, Kubacki J. Hazard potential of Swiss Ixodes ricinus ticks: Virome composition and presence of selected bacterial and protozoan pathogens. *PLoS One*. 2023;18(11):e0290942. Published 2023 Nov 13. doi:10.1371/journal.pone.0290942

24. Burri C, Korva M, Bastic V, Knap N, Avsic-Zupanc T, Gern L. Serological evidence of tick-borne encephalitis virus infection in rodents captured at four sites in Switzerland. *J Med Entomol*. 2012;49(2):436-439. doi:10.1603/me11084

25. Casati Pagani S, Frigerio Malossa S, Klaus C, et al. First detection of TBE virus in ticks and sero-reactivity in goats in a non-endemic region in the southern part of Switzerland (Canton of Ticino). *Ticks Tick Borne Dis*. 2019;10(4):868-874. doi:10.1016/j.ttbdis.2019.04.006

26. Rieille N, Klaus C, Hoffmann D, Péter O, Voordouw MJ. Goats as sentinel hosts for the detection of tick-borne encephalitis risk areas in the Canton of Valais, Switzerland. *BMC Vet Res*. 2017;13(1):217. Published 2017 Jul 11. doi:10.1186/s12917-017-1136-y

27. Fouché N, Oesch S, Ziegler U, Gerber V. Clinical Presentation and Laboratory Diagnostic Work-Up of a Horse with Tick-Borne Encephalitis in Switzerland. *Viruses*. 2021;13(8):1474. Published 2021 Jul 28. doi:10.3390/v13081474

28. Magouras I, Schoster A, Fouché N, et al. Neurological disease suspected to be caused by tick-borne encephalitis virus infection in 6 horses in Switzerland. *J Vet Intern Med*. 2022;36(6):2254-2262. doi:10.1111/jvim.16533

29. Bundesamt für Gesundheit BAG. Tick-borne Encephalitis (TBE). Accessed 27 March, 2024. https://www.bag.admin.ch/bag/de/home/krankheiten/krankheiten-im-ueberblick/fsme.html

30. Bundesamt für Gesundheit BAG. Recommendation for Vaccination against Tick-borne Encephalitis. Accessed 27 March, 2024. https://www.bag.admin.ch/dam/bag/de/dokumente/nat-gesundheitspolitik/klimawandel/hitzewelle/hintergrundinfos/info-zecken/impfempfehlungen.pdf.download.pdf/impfempfehlungen.pdf

31. Bundesamt für Gesundheit BAG. Update and new presentation of the map with vaccination recommendation for tick-borne encephalitis as of April 2013. Accessed 27 March, 2024. https://www.bag.admin.ch/dam/bag/de/dokumente/mt/i-und-b/richtlinien-empfehlungen/empfehlungen-spezifische-erreger-krankheiten/zeckenenzephalitis/zeckenenzephalitis-impfung-karte.pdf.download.pdf/zeckenenzephalitis-impfung-karte-de.pdf

32. *Empfehlungen zur Impfung gegen Frühsommer-Meningoenzephalitis (FSME)*. Accessed 27 March, 2024. https://www.bag.admin.ch/dam/bag/de/dokumente/mt/i-und-b/richtlinien-empfehlungen/empfehlungen-spezifische-erreger-krankheiten/zeckenenzephalitis/empfehlungen-zur-impfung-gegen-fsmeweb.pdf.download.pdf/240424_Empfehlungen%20zur%20Impfung%20gegen%20FSME_WEB_d.pdf

33. Bundesamt für Gesundheit BAG. Swiss Immunization Schedule. Accessed 27 March, 2024. https://www.bag.admin.ch/bag/de/home/gesund-leben/gesundheitsfoerderung-und-praevention/impfungen-prophylaxe/schweizerischer-impfplan.html

34. Zens KD, Altpeter E, Wymann MN, et al. A combined cross-sectional analysis and case-control study evaluating tick-borne encephalitis vaccination coverage, disease and vaccine effectiveness in children and adolescents, Switzerland, 2005 to 2022. Euro Surveill. 2024;29(18):2300558. doi:10.2807/1560-7917.ES.2024.29.18.2300558

35. Baroutsou V, Zens KD, Sinniger P, Fehr J, Lang P. Analysis of Tick-borne Encephalitis vaccination coverage and compliance in adults in Switzerland, 2018. *Vaccine*. 2020;38(49):7825-7833. doi:10.1016/j.vaccine.2020.10.022

36. Compendium.ch. FSME-IMMUN CC Inj Susp. Accessed 27 March, 2024. https://compendium.ch/product/1364944-fsme-immun-cc-inj-susp-sep-nadel.

37. Zens KD, Haile SR, Schmidt AJ, et al. Retrospective, matched case-control analysis of tickborne encephalitis vaccine effectiveness by booster interval, Switzerland 2006-2020. *BMJ Open* 2022;12:e061228. doi:10.1136/bmjopen-2022-061228

38. Oechslin CP, Heutschi D, Lenz N, et al. Prevalence of tick-borne pathogens in questing Ixodes ricinus ticks in urban and suburban areas of Switzerland. *Parasit Vectors*. 2017;10 (1):558. Published 2017 Nov 9. doi:10.1186/s13071-017-2500-2

39. Berger T, MM, Ackermann-Gäumann R, Moor D, Ingenhoff JE. in *Swiss Society for Microbiology*. 2023.

40. Compendium.ch. [Technical Information]. Accessed 27 March, 2024. https://compendium.ch/product/105785-encepur-n-inj-susp/mpro

41. Compendium.ch. [Technical Information]. Accessed 27 March, 2024. https://compendium.ch/product/1364944-fsme-immun-cc-inj-susp-sep-nadel/mpro

42. Compendium.ch. [Technical Information]. Accessed 27 March, 2024. https://compendium.ch/product/1269220-fsme-immun-junior-inj-susp-o-na/mpro.

43. Bundesamt für Gesundheit BAG. [Cantonal vaccination coverage surveillance Switzerland]. Accessed 27 March, 2024. https://www.bag.admin.ch/bag/de/home/gesund-leben/gesundheitsfoerderung-und-praevention/impfungen-prophylaxe/informationen-fachleute-gesundheitspersonal/durchimpfung.html. 2020.

Chapter 13

TBE in Tunisia

Elyes Zhioua

E-CDC risk status: imperiled country (data as of end 2023)

History and current situation

Ixodes ricinus is principally located in oak forests, in humid to semi-humid microclimatic zones in Northwestern Tunisia.[1] While *I. ricinus* is considered the main vector of tick-borne encephalitis virus (TBEV) in Europe, no reports concerning this arbovirus have been reported from North African countries. To date no human cases of tick-borne encephalitis (TBE) have been reported in Tunisia. Ticks were collected from the oak forest of EL Jouza, located in Northwestern Tunisia, by flagging and from grazing cattle during the period from November 2015 through February 2016, a period corresponding to the peak activity of only adult *I. ricinus* in Tunisia. *I. ricinus* was the most dominant tick species during winter. TBEV was detected in a pool of engorged *I. ricinus* collected from grazing cattle yielding a minimum field detection rate of 0.1%.[2] The European subtype (TBE-EU) was detected. A serological survey was performed on grazing cattle where ticks were collected. Of a total of 96 sera tested by ELISA, no positive sera were detected. Recently, a cross-sectional study performed on sheep (N = 289) from Northern Tunisia showed that one sera was tested positive by sero-neutralization test, leading to an overall antibody prevalence of 0.38%.[3] Despite the fact that no human TBE cases have been reported in Tunisia, the aforementioned results provide strong evidence that TBE is endemic in Northwestern Tunisia. To assess the risk of TBE, serological studies on Tunisian populations at high-risk such as farmers and forestry workers and active surveillance in Northwestern Tunisia are urgently needed.

Overview of TBE in Tunisia

Table 1: Virus, vector, transmission of TBE in Tunisia

Viral subtypes, distribution	European subtype
Reservoir animals	Information not available
Infected tick species (%)	*I. ricinus*
Dairy product transmission	Not documented

Burden of TBE in Tunisia over time: no data available

Age and gender distribution of TBE in Tunisia: no data available

TBEV-isolation and TBE cases in Tunisia:
no reported cases of TBE in the country

Table 2: TBE reporting and vaccine prevention in Tunisia

Mandatory TBE reporting	Not applicable
Other TBE surveillance	Not applicable
Special clinical features	Information not available
Available vaccines	Not applicable
Vaccination recommendations and reimbursement	No recommendations
Vaccine uptake by age group/risk group/general population	Data not available
Name, address/website of TBE NRC	Not available

Contact: elyes.zhioua@gmail.com

Affiliation: Institut Pasteur de Tunis, Unit of Vector Ecology, 13 Place Pasteur BP 74, 1002 Tunis, Tunisia

Citation:
Zhioua E. TBE in Tunisia. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-34-7

References

1. Zhioua E, Bouattour A, Hu CM, et al. Infection of *Ixodes ricinus* (Acari: Ixodidae) by *Borrelia burgdorferi* sensu lato in North Africa. *J Med Entomol.* 1999;36(2):216-218.
2. Fares W, Dachraoui K, Cherni S, et al. Tick-borne encephalitis virus in *Ixodes ricinus* (Acari: Ixodidae) ticks, Tunisia. *Ticks Tick Borne Dis.* 2021;12(1):101606.
3. Khamassi Khbou M, Romdhane R, Foughali AA, et al. Presence of antibodies against tick-borne encephalitis virus in sheep in Tunisia, North Africa. *BMC Vet Res.* 2020;16(1):441.

Chapter 13

TBE in Ukraine

Iryna Kolesnikova; Khrystyna Hrynkevych

E-CDC risk status: endemic (last edited on 16.03.2024, data as of end December, 2023)

History and current situation

The Ukrainian Scientific and Methodological Center for Tick-borne Viral Encephalitis and Natural Focal Diseases of Arboviral Etiology founded in the year 2005 was established in the Laboratory of Vector-borne Viral Infections of the Lviv Research Institute of Epidemiology and Hygiene. Prevention of TBE is based on the Guidelines "Nonspecific prevention of vector-borne natural focal infections transmitted by ixodid ticks[5] (Table 1).

The presence of active natural foci of TBE infection in the Ukraine was determined by regions, where single cases or outbreaks of human diseases were registered (Figure 1).The main vector of TBE virus in the Ukraine is the European forest tick *I. ricinus*, from which 68.4% of domestic strains were isolated. TBE virus has also been isolated from *D. reticulatus* and *H. plumbeum* (plumbeum) ticks. Potential vectors of TBE virus in Ukraine include *I. crenulatus*, *I. hexagonus*, *I. lividus*, *I. trianguliceps*, *D.marginatus*. (https://ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/tick-maps)

In the Ukraine, vaccination against TBE is recommended for individuals visiting endemic areas during the period of highest tick activity (April to November) (Table 1). It is recommended to start vaccination in the fall (September - November), when there is enough time to develop vaccine-protection before potential exposures to the TBEV.

Between 1955 and 2013, a total of 596 cases of TBE (all encephalitis) were registered in the Ukraine (population about 41 million), including 74 (12.5%) imported cases and 522 (87.5%) local cases. The highest number of the 522 autochthonous cases was reported from Crimea (265 cases; 50.7%), followed by Volyn (196 cases; 37.5%), Zakarpattia (24 cases, 4.6%), Dnipro and Ivano-Frankivsk (8 cases each (1.5%), Lviv (4 cases, 0.76%), Vinnytsia, Donetsk, Kharkiv (3 cases, 0,75% each), Mykolaiv and Khmelnytsky (2 cases each, 0,3%) and from Kyiv, Sevastopol, Odesa, and Sumy (1 case each, 0.19%).[3]

From 2011 to 2019 only 2 cases of TBE-encephalitis were detected, 1 in the Kharkiv region and another in the Chernihiv region.[4]

According to the Public Health Centre of the Ministry of Health of Ukraine,[5] 2 cases of viral encephalitis were recorded in Ukraine in 2020.

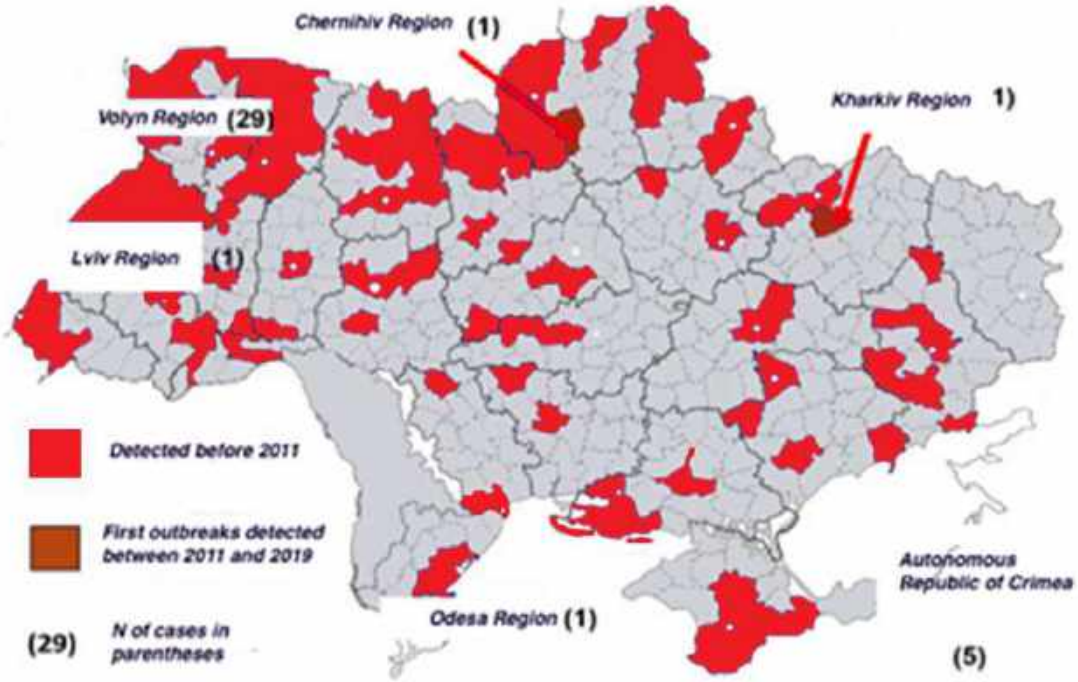
Table 2: Reported cases of TBE encephalitis in the Ukraine by period 1955-2020[3,4,5]

Time period	Case (TBE encephalitis)
1955-2013	522 autochthonous cases 74 imported cases
2011 – 2019	2 reported cases
2020	2 reported cases

Overview of TBE in Ukraine

Table 1: TBE in Ukraine	
Virus subtypes isolated	All 3 major TBEV subtypes are circulating in the Ukraine.[7]
Reservoir animals	Cows, buffaloes and goats[4]
Percentage infected ticks	Unknown
Dairy product transmission	raw milk and milk products from cows and goats[4]
Case definition used by authorities	**Clinical criteria** Any person with symptoms of CNS inflammation (e.g. meningitis, meningoencephalitis, encephalomyelitis, encephalo-radiculitis). **Plus** **Laboratory criteria** Serologic results should be interpreted according to vaccination and previous exposure to other flavivirus infections. Confirmed cases in such situations should be confirmed by neutralization reaction or other equivalent tests.[2]
Completeness of case detection and reporting	Incomplete
Type of reporting	Mandatory
Other TBE surveillance	Tick infection with various pathogens is monitored by the regional Centers for Disease Control and Prevention. Regional Centers for Disease Control and Prevention annually conduct a study of tick populations – to identify species found in a given territory.[6]
Special clinical features	Risk groups: military, foresters, tourists, fishermen, shepherds[1]
Licensed vaccines	TicoVac vaccine (0.5 ml) is indicated for active (prophylactic) immunization of persons aged 16 years and older against TBE. The TicoVac Junior vaccine (0.25 ml) is indicated for active (prophylactic) immunization of children aged 1 to 15 years[5]
Vaccine recommendations	Vaccination is indicated in TBEV-endemic areas: Crimea (Simferopol, Sudatsky, Biloghirsky, Bakhchysaray, Alushty, Kirovsky, Krasnogvardiysky districts; Great Yalta, Laspi Bay of the Sevastopol district), Volhynia (Ratnivskyi, Rozhishchenskyi, Kovelskyi, Kivertsivskyi, Starovyzhivskyi, Kamin-Kashirskyi districts, Lutsk city, Kovel city), Lviv (Yavorivskyi district), Odessa (Balta city) oblasts.[3]
Vaccine uptake	Vaccination is not mandatory
National Reference center for TBE	Ukrainian Scientific and Methodological Center for Tick-borne Viral Encephalitis and Natural Focal Diseases of Arboviral Etiology located at the Laboratory of Vector-borne Viral Infections of the Lviv Research Institute of Epidemiology and Hygiene.[5]
Additional relevant information	The full course with 3 vaccine doses should be started in the fall (September - November), to give enough time to develop immune protection against TBEV. The second dose is administered in spring (in March - April), the third dose one year after the second dose. Further revaccinations are carried out 3 years later and then every 5 years (every 3 years for individuals above age > 65 years).[3]

Figure 1: Enzootic territories (natural foci) for tick-borne viral encephalitis as of 01.01.2020 in Ukraine (https://phc.org.ua/sites/default/files/users/user90/risk_2020_38.pdf).



Acknowledgements

The author acknowledges the kind support from Dr. Khrystyna Hrynkevych for translation and editing.

Authors and affiliations

Prof. Dr. Iryna Kolesnikova. Professor of Epidemiology, Bogomolets National Medical University

Contact: liltairyna@gmail.com

Citation:
Kolesnikova I, Hrynkevych K. TBE in Ukraine. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. The TBE Book. 7th ed. Singapore: Global Health Press; 2024. doi:10.33442/26613980_13-35-7

References

1. Ministry of Health of Ukraine. [How not to get sick if bitten by a tick?]. 2023. Accessed 29 March, 2024. https://moz.gov.ua/article/health/jak-ne-zahvoriti-jakscho-vkusiv-klisch
2. Ministry of Health of Ukraine. [Criteria for determining cases of infectious and parasitic diseases subject to registration]. 2015. Accessed 29 March, 2024. https://moz.gov.ua/uploads/3/15840-pro_20200115_3_dod_1.pdf
3. Ministry of Health of Ukraine. [On the approval of Methodological recommendations "Non-specific prevention of transmissible natural foci infections transmitted by ixodid ticks"]. 2013;369. Accessed 29 March, 2024. https://zakononline.com.ua/documents/show/65573___65573
4. Publications of the Department of Epidemiology. [Functioning of the combined natural center of especially dangerous infections in western Ukraine. Collection of scientific papers]. Accessed 29 March, 2024. https://new.meduniv.lviv.ua/uploads/repository/kaf/kaf_epidemiology/05.%D0%92%D0%B8%D0%B4%D0%B0%D0%B2%D0%BD%D0%B8%D1%87%D0%B0_%D0%B4%D1%96%D1%8F%D0%BB%D1%8C%D0%BD%D1%96%D1%81%D1%82%D1%8C/Publikacii.pdf
5. Ministry of Health of Ukraine. [On improvement of prevention measures tick-borne viral encephalitis in Ukraine]. 2005;431. Accessed 29 March, 2024. https://zakononline.com.ua/documents/show/53697___53697.
6. State Institution "Kharkiv Regional Center for Disease Control and Prevention of the Ministry of Health of Ukraine". Accessed 29 March, 2024. https://labcenter.kh.ua/?page=68&paged=64
7. Yurchenko OO, Dubina DO, Vynograd NO, Gonzalez JP. Partial Characterization of Tick-Borne Encephalitis Virus Isolates from Ticks of Southern Ukraine. *Vector Borne Zoonotic Dis.* 2017;17 (8):550-557. doi: 10.1089/vbz.2016.2094.

Chapter 13

TBE in United Kingdom

Maya Holding and Gillian Ellsbury

E-CDC risk status: affected, unknown if endemic (data as of end 2023)

History and current situation

Until 2019, TBE was considered only to be an imported disease to the United Kingdom. In that year, evidence became available that the TBEV is likely circulating in the country[1,2] and a first "probable case" of TBE originating in the UK was reported.[3] In addition to TBEV, louping ill virus (LIV), a member of the TBEV-serocomplex, is also endemic in parts of the UK. Reports of clinical disease in livestock, caused by LIV are mainly from Scotland, parts of North and South-West England and Wales.[4]

National deer sentinel surveillance was conducted between 2018 and 2021, initially to establish whether TBEV might be present in Great Britain, but undetected. Following confirmation of presence this was continued, to then detect the geographic extent of TBEV and any spread. In the initial samples collected between February 2018 and January 2019,[1] four percent of sera from 1,309 deer culled across England and Scotland were ELISA-positive for TBEV serocomplex. Due to the close homology between LIV and TBEV, it was not possible to differentiate between the two viruses serologically, with 73.1% of ELISA positive samples also tested by LIV hemagglutination inhibition (HAI) test being positive by both methods. Many of the seropositive samples were in areas where LIV has been reported in livestock; however, a focus of the highest seropositivity rate (47.7% by ELISA) was identified in the Thetford Forest area (South-East England), which has no previously published reports of LIV in livestock. Additionally, also seropositivity of 14.3% was detected in Hampshire (Southern England), also a county with no previous LIV reports. Five from 2,041 *I. ricinus* ticks from culled deer in ELISA-positive regions tested positive by LIV/TBE PCR[5] , all five were from the Thetford Forest area. Of the ticks removed from deer in the Thetford Forest area, 2.6% were positive by RT-PCR. A full-length genome sequence was obtained from one positive tick (figure 2). TBEV-UK Thetford was identified to be a TBEV -Eu strain, sharing 99% sequence identity with the Norwegian Mandal strain isolated from ticks in 2009.[6]

Annual tick surveys have been conducted since 2018 in areas where seropositivity in deer have been detected, additionally surveys have been conducted in localities identified through follow up of probable or confirmed TBE cases. TBEV has been confirmed over multiple years in questing ticks in parts of Thetford Forest, the New Forest/ Hampshire Dorset boarder and the North York Moors. The minimum infection rate detected tends to be below 1%, to illustrate, follow-up questing tick surveys were conducted in Hampshire during July and August 2018 and June 2019. Of 915 *Ixodes ricinus* ticks collected and tested in 2018 and 2,155 in 2019, one RT-PCR positive pool was identified from five adult female ticks collected from a site on the Hampshire/Dorset border[2]. Minimum infection rate (MIR) of ticks collected from this site was estimated to be 0.17%. Sequence analysis indicates that TBEV-UK Hampshire was most closely related to TBEV-NL (LC171402.1) detected in ticks in 2017.[7] The diversity of the Thetford and Hampshire TBEV-EU strains (Figure 2) indicates that these were a result of at least two separate importation events into the UK.[1,2]

The first "probable TBE case" originating in the UK was in a 3-month old German infant returning from a family summer vacation in South East England in July 2019.[3] Based on the timing of travel and incubation period, it is not possible that the child was infected in Germany and probable exposure was thought to be in the New Forest National Park, England, following a tick bite there. A second probable case was reported in July 2020 with exposure thought to be in in the Test Valley District of Hampshire, England, less than 20km from the first probable case[15]. In September 2022, a third case tested positive for TBEV by PCR was reported in England, who was likely to have acquired infection in Scotland in June 2022. In October 2022 an additional case, also confirmed by TBEV PCR, was reported in England with probable exposure in the North York Moors.[8] To summarize, overall serological evidence supported by PCR detection and sequence analysis of TBEV-EU RNA indicates that TBEV circulates within the Thetford Forest and the Hampshire/ Dorset border and the North York Moors areas. There have been four probable or confirmed autochthonous TBE cases, three within these areas and one in Scotland. Sequence analysis on these cases has not been possible, therefore it is not known which TBEV strain was the cause of disease in these instances. Work is ongoing to understand the risk of TBEV to the UK human population.

Table 1: Virus, vector, transmission of TBE in United Kingdom	
Viral subtypes, distribution	TBEV-EU
Reservoir animals	Ticks, to be confirmed, but likely rodents?
Infected tick species (%)	*I. ricinus*
Dairy product transmission	Not reported
Case definition used here	Compatible clinical signs plus serological or PCR confirmation
Completeness of case detection and reporting	Unknown
Type of reporting	Acute encephalitis is a notifiable disease.[9] TBEV is now a notifiable organism (from August 2019)[10]
Other TBE surveillance	Ongoing surveillance for possible TBE cases. Ecological studies, in addition to both sentinel and human serosurveillance studies
Special clinical features	None
Licenced vaccines	TicoVac® and TicoVac Junior®[11]
Vaccine recommendations	The UK Joint Committee on Vaccination and Immunisation last reviewed the situation in October 2023. It was agreed that the Green Book wording could be adjusted to highlight accessibility to vaccination for those at risk in the UK context.[12]
Vaccine uptake	Uptake of vaccine not known
Name, address/website of TBE National Reference Center	Rare and Imported Pathogens Laboratory (RIPL) UK Health Security Agency Manor Farm Road Porton Down Wiltshire SP4 0JG www.gov.uk/guidance/tick-borne-encephalitis-epidemiology-diagnosis-and-prevention

Figure 1: Seropositive sentinel deer serum samples tested by both TBEV ELISA and LIV HAI and geographical distribution with density of samples (figure and accompanying legend are adapted and reprinted from reference)[1]

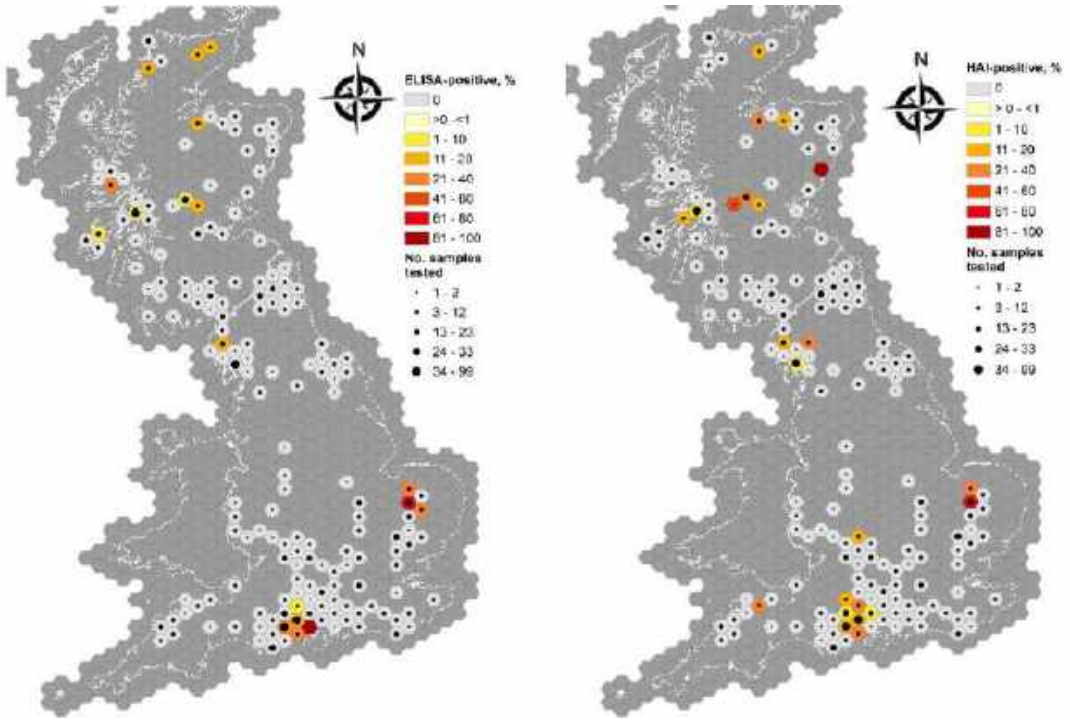
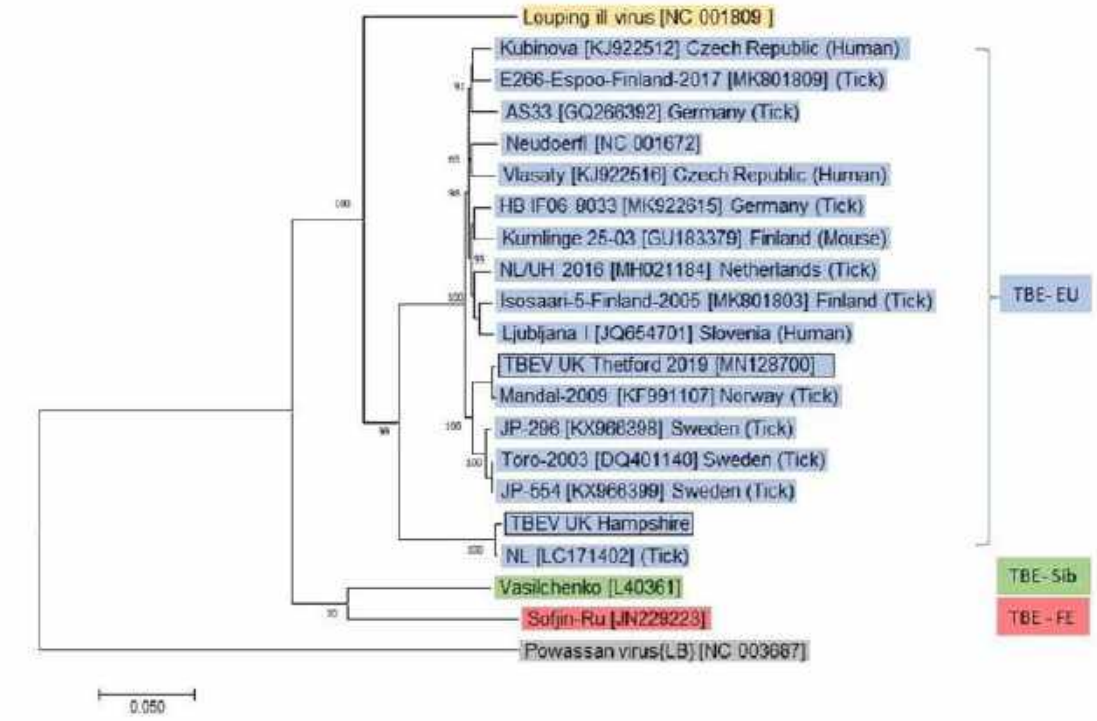
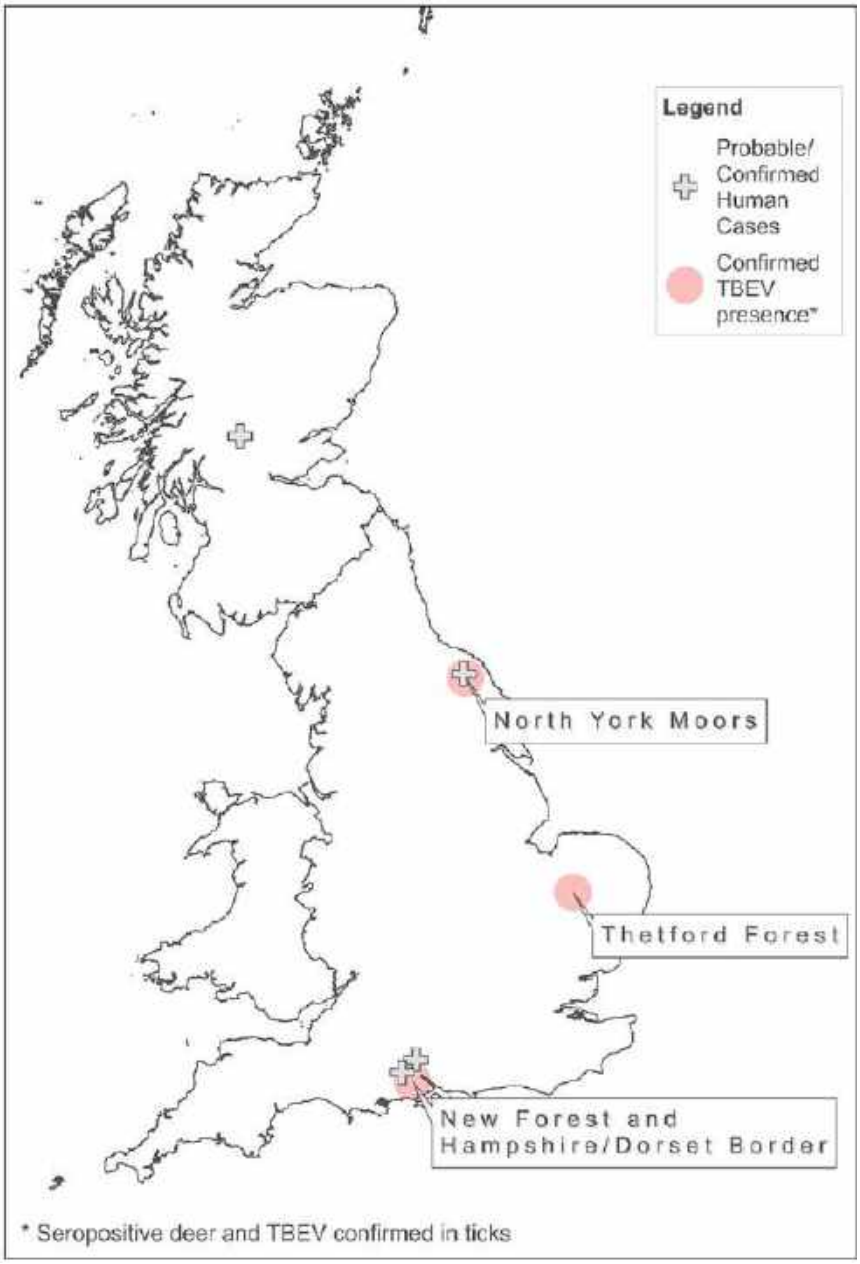


Figure 2: Phylogenetic tree highlighting the TBEV UK-Thetford and TBEV-UK Hampshire strains (figure and accompanying legend are adapted and reprinted from reference)[2]



The boxes highlight the TBEV strains from a tick removed from deer in Thetford 2018 and questing ticks collected in Hampshire in 2019. The tree was constructed with a maximum-likelihood analysis of full length genomes and is rooted with the tick-borne Powassan virus. European TBEV strains are highlighted in blue, Siberian TBEV in green, Far Eastern in pink, and louping ill virus in yellow. Strains are identified with the name, GenBank accession numbers, country location and host.

Figure 3: **Locations where TBEV has been detected in ticks with serological evidence in deer and suspected locations of exposure of probable/confirmed autochthonous TBE cases**



Contact: maya.holding@phe.gov.uk

Citation:

Holding M, Ellsbury G. TBE in United Kingdom. Chapter 13. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Global Health Press;2024. doi:10.33442/26613980_13-36-7

References

1. Holding M, Dowall SD, Medlock JM, Carter DP, Pullan ST, Lewis J, Vipond R, Rocchi MS, Baylis M, Hewson R: Tick-Borne Encephalitis Virus, United Kingdom. *Emerg Infect Dis*. 2020;26(1):90-96.
2. Holding M, Dowall SD, Medlock JM, Carter DP, McGinley L, Curran-French M, Pullan ST, Chamberlain J, Hansford KM, Baylis M et al: Detection of new endemic focus of tick-borne encephalitis virus (TBEV), Hampshire/Dorset border, England, September 2019 . *Euro Surveill*. 2019;24(47).
3. Kreusch TM, Holding M, Hewson R, Harder T, Medlock JM, Hansford KM, et al. A probable case of tick-borne encephalitis (TBE) acquired in England, July 2019. *Euro Surveill*. 2019;24(47).
4. Jeffries CL, Mansfield KL, Phipps LP, Wakeley PR, Mearns R, Schock A, et al. Louping ill virus: An endemic tick-borne disease of Great Britain. *J Gen Virol*. 2014;95:1005–14. doi:10.1099/vir.0.062356-0.
5. Schwaiger M, Cassinotti P. Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA. *J Clin Virol.* 2003;27:136–45. doi:10.1016/S1386-6532(02)00168-3.
6. Asghar N, Lindblom P, Melik W, Lindqvist R, Haglund M. Tick-Borne Encephalitis Virus Sequenced Directly from Questing and Blood-Feeding Ticks Reveals Quasispecies Variance . *PLoS One.* 2014;9:103264. doi:10.1371/journal.pone.0103264.
7. Jahfari S, De Vries A, Rijks JM, Van Gucht S, Vennema H, Sprong H, et al. Tick-Borne Encephalitis Virus in Ticks and Roe Deer, the Netherlands*. Emerg Infect Dis*. 2017;23:1028–30. doi:10.3201/eid2306.161247.
8. Human Animal Infections Risk Surveillance group. HAIRS risk assessment: tick-borne encephalitis. 2023. Available at: https://www.gov.uk/government/publications/hairs-risk-assessment-tick-borne-encephalitis.
9. The Health Protection (Notification) Regulations 2010 n.d. Available at: http://www.legislation.gov.uk/uksi/2010/659/schedule/1/made.
10. Public Health England. Tick-borne encephalitis: epidemiology, diagnosis and prevention. n.d. Available at: https://www.gov.uk/guidance/tick-borne-encephalitis-epidemiology-diagnosis-and-prevention.
11. Immunisation Against Infectious Disease: Chapter 31, Tick Borne Encephalitis. Green B., n.d., p. 385–90. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/569289/2905811_Green_Book_Chapter_31_v3_0.pdf.
12. Joint Committee on Vaccination and Immunisation. Joint Committee on Vaccination and Immunisation October 2023 Minutes. 2023.
13. Holding M, Dowall SD, Medlock JM, Carter DP, et al. Detection of new endemic focus of tick-borne encephalitis virus (TBEV), Hampshire/Dorset border, England, September 2019. Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin. 2019;24(47).
14. Holding M, Dowall SD, Medlock JM, et al. Tick-Borne Encephalitis Virus, United Kingdom. *Emerg Infect Dis*. 2020;26(1):90-96 .
15. Mansbridge CT, Osborne J, Holding M, Dryden M, Aram M, Brown K, Sutton J. Autochthonous tick-borne encephalitis in the United Kingdom: A second probable human case and local eco-epidemiological findings. Ticks *Tick Borne Dis*. 2022; 13(1):101853. doi: 10.1016/j.ttbdis.2021.101853

Chapter 14

Public health aspects of TBE

Phung Lang, Rahel Ackermann-Gäumann

Key Points

- The identification of TBE endemic areas is crucial to inform national and international TBE risk management programs. However, identification of TBE endemic areas remains incomplete.
- The risk of tick-borne disease is predicted to increase with climate change through several mechanisms, but the relationship between climate and tick-borne disease is complex and influenced by both environmental and human factors.
- Uptake and compliance with TBE vaccination in Europe vary greatly, with overall low rates.
- Disparities in TBE awareness and vaccine uptake exist between endemic and non-endemic countries. Targeted education, involvement of healthcare professionals, and accessible vaccination strategies are needed to address barriers and improve prevention for those living in or travelling to TBE endemic areas.
- Because children also suffer from long-term cognitive impairment and because TBE cases in children are likely to be underreported, TBE vaccination is important for this age group.
- Increasing vaccination rates across all age groups is the most effective and efficient strategy to reduce the burden of TBE and protect the overall population's health.
- To effectively manage and prevent the spread of TBE, a comprehensive One Health approach must consider the complex interactions between humans, animals, ticks, and the environment.

Introduction

Public health measures are a key strategy for reducing the transmission of pathogens with epidemic potential. These measures encompass vaccination programs and non-pharmaceutical interventions that can be implemented by individuals, institutions, communities, local and national governments, and international bodies to slow or stop the spread of an infectious disease. TBE requires significant public health attention due to its potential to harm individuals residing in or travelling to TBE endemic areas. The disease can lead to long-term disability and even death. It is important to inform the public about the risks associated with TBE and provide an appropriate public health response.

Reporting and surveillance

TBEV is found in natural foci, which are areas where the virus circulates among ticks and reservoir hosts. As a result, TBE is limited to specific geographical regions, resulting in TBE endemic areas.[1–3] More than 25 countries in Northern, Central, and Eastern Europe have one or more areas where TBE is endemic,[4] with the highest reporting rates in the Baltic States, Slovenia, and the Czech Republic.[5] Together with Russia and part of eastern Asia, these countries form what is known as the "TBE belt".[6] The incidence of TBE has increased over the past 25 years,[7,8] with a northwestward spread in continental Europe, including to regions and altitudes previously believed to be free of the virus.[1,9–11] The number of reported TBE cases in Europe in 2020 was twice that of 2015;[9] nearly 30,000 cases were reported in the EU/EEA countries between 2012 and 2020[11]. However, annual case reporting fluctuates widely due to various factors.[1]

Since 2012, the European Centre for Disease Prevention and Control (ECDC) has required all European Union member states, as well as Iceland and Norway, to report their TBE data annually to the European Surveillance System (TESSy).[12] In 2022, 43% of European countries[13] used the latest diagnostic criteria introduced by the ECDC in 2018.[14] In some countries that do not use the ECDC criteria, such as Italy, national diagnostic criteria are largely similar to the ECDC criteria. Therefore, reported TBE case numbers may not differ significantly.[1,14] However, this may not be the case in countries that have key differences in requirements for the confirmation of a TBE case, such as in Germany, where clinical signs may be limited to non-specific symptoms (i.e., without CNS symptoms).[1,15–17] Country data on TBE prevalence is difficult to compare due to differences in case definitions between countries, resulting in varying degrees of accuracy.[1,9]

TBE is typically an acute disease, and progression may terminate after the first phase, which is called the "abortive" clinical pattern. This form of TBE may be

asymptomatic or manifest as a mild febrile illness, including symptoms such as headache, fever, fatigue, myalgia, anorexia, nausea, and vomiting, without progression to any form of encephalitis.[18,19] However, only a few countries, namely Austria, Latvia, Germany, and Slovenia, collect data on nonspecific non-CNS symptoms.[1,15,20–23] Additionally, mild CNS symptoms may go unreported since they do not fulfill the ECDC criteria, leading to underreporting of TBE. This is particularly noteworthy in pediatric patients, where symptoms are often mild and can be misdiagnosed.[1] Cases of TBE in children are very likely to be underreported compared to adults, as up to two-thirds of pediatric TBE cases are missed.[9,24,25]

Clinicians who do not test for TBEV infection due to a lack of recognition of the possibility of CNS inflammation may impact the number of reported TBE cases. Furthermore, if they suspect CNS inflammation, they may be less inclined to perform a CSF examination that supports a TBE diagnosis.[1]

Access to diagnostic tests for TBE is limited, as is knowledge on their appropriate use.[1] Serological assays are the preferred method for TBE diagnosis.[26] However, interpreting serologic test results is challenging due to the high cross-reactivity of the antigenic structure among orthoflaviviruses, particularly in areas where other orthoflaviviruses co-circulate or where vaccination against other orthoflaviviruses is common.[27] Improved laboratory capacities and implementation of neutralization assays in these countries could improve identification of TBE by distinguishing it from other orthoflaviviral infections.[1,28] Due to strict biosafety regulations in a number of Western countries, the performance of neutralization assays is restricted to laboratories equipped with a biosafety level 3 facility (biosafety level 4 in the United States). Therefore, alternative assays not requiring the work with infectious viruses could also be of value.[29,30]

Accurately determining the tick populations infected with TBEV and the number of human TBE cases is crucial for defining TBE risk areas. Endemic areas, which are risk areas where recurrent transmission of TBEV to humans occurs over several seasonal cycles,[31] must be documented in most countries to make targeted vaccination recommendations.[1,32,33]

The geographic restriction of TBE allows for targeted surveillance in high-risk areas. However, incomplete surveillance can lead to a poor understanding of TBE endemic areas and potentially inadequate vaccine recommendations.[1] This was demonstrated in Poland, where numerous new endemic districts were identified, including foci far away from previously known endemic districts, during an enhanced surveillance project.[34] Restricted surveillance may hinder the early identification of new TBE endemic areas, thereby increasing the risk of TBEV infection for the public. Moreover, designating areas as endemic or high-risk may limit awareness and diagnosis of TBE in non-endemic areas, despite a national obligation to report TBE cases. This may lead to a decrease in the ability to detect cases of TBE in areas where the disease was not previously present, as well as in the diagnosis of imported cases of TBE.[1]

Overall, the identification of TBE endemic areas is crucial to inform national and international TBE risk management programs.[1] However, identification of TBE endemic areas remains incomplete, and TBE surveillance in Europe is generally sporadic rather than systematic.[9] TBE cases are likely to be underreported, and the true burden of TBE disease is significantly underestimated.[9]

Impact of climate change on tick-borne encephalitis

Infection transmission occurs when the activities of reservoirs, vectors, and humans overlap, with variations depending on the pathogen and location. Climate change has the potential to affect all of these stages and their interactions.[35] Climate change is expected to increase the risk of ticks and tick-borne diseases in a number of ways.[36–38] However, the relationship between tick-borne diseases and climate is not linear. Rather, it is influenced by other environmental and human factors.[36–41]

Ixodes ricinus, the primary vector of TBEV in Europe, is particularly sensitive to environmental conditions, as this tick species requires a microclimatic relative humidity of at least 80% during its extended non-parasitic periods to avoid lethal dehydration. While changes in climate and the duration of different seasons will affect tick survival, activity, and development, there is insufficient evidence to support the concept that an increase in temperatures will directly lead to a higher tick abundance simply by accelerating developmental rates. Instead, shifts in development rates will alter patterns of seasonal activity.[35,42]

Indirect effects of climate change will affect the number of infected ticks by affecting vegetation.[35] For example, there is a link between tree mast, rodent population dynamics, nymphal tick density, and the incidence of human TBE two years later.[43–46] While climate warming has increased seed production in certain trees, mast seeding events have decreased.[47] A warming climate in central Europe is expected to lead to shifts in dominant tree species, resulting in a favorable microclimate for the survival of the free-living tick stages.[35]

Climate change will indirectly affect the transmission of tick-borne pathogens by affecting the survival and abundance of tick maintenance hosts, such as deer, and pathogen reservoir hosts, such as rodents and birds.[35,48,49] Increasing

temperatures will expand the distribution range of both reservoir and tick maintenance hosts[50,51] as well as their abundance and activity.[51,52]

Climate change may affect disease risk by influencing long-term land use (e.g., farming, tourism).[35] Human behavior is also expected to adapt as the climate changes. People may resume outdoor activities earlier in the spring and maintain them longer in the fall, thereby increasing the duration of annual tick contact for both animal hosts and humans. The risk of climate change to human exposure is more likely to be associated with shorter winters than with extreme summer heat.[36–38]

The influence of climatic factors on virus replication has not been elucidated. However, there is evidence that certain TBEV strains can adapt to different environmental temperatures within the tick.[53] The spread of TBEV infection locations is significantly more frequent where precipitation and temperature are high in summer and frost days are low in winter.[54] With projected climate change, the range of *I. ricinus* can expand to higher latitudes, particularly in northern and eastern Europe, and to higher altitudes.[10,55–58]

While *I. ricinus* is the primary vector of TBEV, the virus has also been isolated from other tick species. Therefore, changes in the range of these species may also affect the risk of contracting TBE. Statistical habitat models predict a further distribution and a potential long-term establishment of the tick species *Dermacentor reticulatus* and *Hyalomma marginatum.*[35,59]

Taken together, climate change can affect the transmission of tick-borne diseases by influencing the survival, abundance, and activity of ticks, as well as their hosts. The relationship between tick-borne diseases and climate is complex. Changes in temperature, precipitation, and vegetation are expected to shift the geographical distribution and incidence of diseases like TBE. This is due to factors such as changes in tick activity patterns and the expansion of tick habitats, which increase the risk of TBE in certain regions.

TBE and tick awareness and risk subjects and general protective measures

As there is currently no specific treatment available for TBE infection, prevention is strongly recommended. Vaccination is the most effective mechanism of protection against the development of TBE, in addition to the elimination of all possible exposures. General protective measures and behaviors are recommended as primary and secondary preventive measures, as summarized in Table 1.[60] The best way to reduce the risk of exposure is to avoid tick-infested areas, especially during the peak tick season in spring and late summer. However, it is not always possible to avoid exposure to ticks, especially for residents of endemic areas. Therefore, it is recommended to wear protective clothing with long sleeves and long trousers tucked into socks or boots, to use repellents on exposed skin, and to impregnate clothing with an acaricide (such as permethrin or pyrethroids). After a tick bite, TBEV is immediately transmitted to the host through the tick's saliva. It is recommended to remove the tick as soon as possible, even if it is already firmly attached to the skin, to prevent other potential infections. In the event of a tick bite, the tick should be removed using fine-tipped tweezers/forceps or a specially designed tick card/removal tool by pulling straight out without squeezing or twisting the tick. Unpasteurized dairy products in tick-infested areas may also contain TBE;[61–63] avoid eating or drinking unpasteurized milk and cheese from goats, sheep or cows from these areas.

The main individual-level risk factors for TBE can be divided into two categories: behavioral and occupational risks, and biological risks. Behavioral and occupational risks include factors that increase the likelihood of exposure to ticks and contracting TBE. Forestry workers, farmers and hunters are at higher risk of contracting TBE, due to the nature of their work. Additionally, leisure activities in the countryside also increase the risk of exposure to TBE, which are more common among older individuals with more leisure time. Studies of clinical TBE cases in Switzerland found that around 80-90% of patients with TBE or Lyme borreliosis contracted the disease during leisure activities.[64–66] Another related risk is the geographic region in which an individual lives, works, or spends leisure time.[64–68]

Biological risks for TBE disease include gender and age.[12,65,66,69,70] Cases are more common in men, but this may be due to an increased risk of exposure rather than a different immune response to TBE in men and women. Both the incidence and severity of the disease increase with age.[70,71] Existing comorbidities, immunosuppression and certain genetic predispositions also increase the risk of severe disease following exposure but not of the risk of exposure itself. Adults over the age of 50 not only have an increased incidence of TBE, but they also tend to experience more severe disease and have a higher risk of lasting neurological sequelae.[70–72] Immunocompromised individuals, such as immunosuppressed patients, organ or hematopoietic stem cell transplant recipients, and HIV-infected individuals, are particularly susceptible to TBE and often experience severe or fatal disease.[70,73–76]

Published research has identified several factors associated with awareness of TBE and uptake of TBE vaccines. A recent study assessed TBE awareness and vaccination rates in 2020 in 20 European countries.[67] Of these, 14 countries were identified as TBE endemic and 6 as non-endemic. The results showed that there was a difference in TBE awareness (74% vs. 30%) and TBE vaccine awareness (56%

Table 1: General primary and secondary preventive measures[60]

	Measure	Comment
Behavior	Avoid tick-infested areas Avoid unpasteurized dairy products Adhere to personal protection measures when working with viable TBEV	Whenever possible
Clothing	Light-colored clothing that covers arm and legs (long-sleeved shirts – tight at the wrists, long pants – tight at the ankles and tucked into the socks); shoes covering the entire foot	Dark clothing is proven to be more attractive for ticks (which in addition are more difficult to identify on a dark background)
Use of repellents	Apply adequate repellent (with proven action against ticks) to clothing and skin	e.g. DEET in higher concentrations, (p)icaridine as well as permethrin / pyrethroids are proven to act against ticks; allow clothing to dry up before wearing
Early detection	Adults should be checked daily; children should be checked more frequently, i.e. after some hours of exposure (could result in 2 to 3 checks per day)	The checks should especially focus on waist bands, sock tops, under arms, other moist areas (for children: head and especially behind the ears); even adults may need the assistance of a second person to check the whole body
Early removal of ticks	Remove tick as soon as possible using fine-tipped tweezers or special cards (resembling carved credit cards); grasp the tick firmly as close to the skin as possible and simply tear it out without squeezing or rotating the tick	Don´t suffocate the tick (oil, cream, nail polish, water); don't burn the tick; don´t apply "home remedies"; don´t wait for medical services if not promptly available

vs. 12%) between endemic and non-endemic countries.[67] Motivating predictors of TBE vaccination include recommendation from a physician (in both endemic and non-endemic countries), personal or occupational risk exposure, fear of TBE, dog ownership, experience with tick-related health problems, desire to avoid contracting the disease, trust in vaccine recommendations, frequent outdoor activities, gardening and travel to an endemic area.[67,68,77–80] While those who were vaccinated against TBE were better informed about TBE disease than non-vaccinated individuals in a non-endemic TBE area, getting a TBE vaccination was not associated with a reduced uptake of general protective measures.[81] Barriers to TBE vaccination include not living in or visiting risk areas, low risk perception, fear of adverse events following vaccination, lack of information about TBE and the vaccine, unavailability of the TBE vaccine, and the belief that vaccination is unnecessary.[67,68,78,79]

Individual-level risk factors for TBE include higher exposure risks for forestry workers and individuals engaging in outdoor activities in endemic areas. Additionally, age, gender and comorbidities can contribute to the degree of susceptibility to TBE. The recognition of differences in TBE awareness and vaccine uptake between endemic and non-endemic countries underlines the need for targeted education, involvement of health professionals, and accessible vaccination strategies to eliminate barriers and enhance prevention.

Vaccination schedules and recommendations

There are six licensed vaccines available, all of which use inactivated whole virus strains. These vaccines can be grouped into European, Russian, and Chinese vaccines.[82] Currently, two European vaccines are available in many European countries and Canada, and one is available in the United States. They are based on the Austrian isolate Neudoerfl (FSME-IMMUN) and the German isolate K23 (Encepur), both TBEV-Eu strains. Additionally, licensed vaccines in Russia and some neighboring countries are based on the Russian TBEV-FE isolate Sofjin (TBE vaccine Moscow and Tick-E-Vac/Klesch-E-Vac) and TBEV-FE strain 205 (EnceVir). In China, SenTaiBao, which is based on the Chinese TBEV-FE strain Sen-Zhang, has been approved as a TBEV vaccine (reviewed in[17,19,82–85]). Pediatric formulations are available for FSME-IMMUN, Encepur, TBE vaccine Moscow, Tick-E-Vac, and EnceVir vaccines.[19] The standard immunization schedule for all vaccines, except for Sen Tai Bao which has only two doses, consists of three doses. The initial vaccination is followed by a second injection 4-12 weeks later, and a third injection is given 5-12 months later, with variations in the specific intervals between vaccine brands. Vaccine manufacturers prescribe booster doses to maintain protection: the first three years after primary

Table 2: Booster dosing schedules in adults in Switzerland, Finland, and Belgium. Adapted from Schelling et al, 2024[91]

Country	First booster	Subsequent boosters
Switzerland[86] (64)	after 10 years	every 10 years
Finland[87] (65)	after 3 years	age <50: every 10 years age 50-60: every 5 years age >60: every 3 years
Belgium[88] (66)	after 3 years	age <60: every 5-10 years age ≥60: every 3 years
Latvia[89,90] (ref)	after 3 years	every 10 years

immunization and subsequent boosters every three to five years. Sen Tai Bao is an exception, requiring an annual booster dose.[17,19,85] In addition to conventional schemes, rapid vaccination schedules are available for most of these vaccines. If necessary, European vaccines can be used interchangeably.[19]

Although TBE vaccination is common in Europe, recommendations for TBE vaccination vary even among countries where TBE is endemic.[1,9,67] At present, only Austria and Switzerland have national universal vaccination programs.[1] In the Czech Republic, Estonia, Germany, Hungary, Latvia, Lithuania, and Slovenia, vaccination is generally recommended. Other European countries link their vaccine recommendations to specific factors, such as predefined risk areas. For example, in Croatia, Poland, and Serbia, vaccination is recommended for people living in or travelling to endemic areas. In Belarus, Italy, Kazakhstan, Mongolia, Slovakia, Sweden, Russia, and Ukraine, vaccination is recommended for those with possible occupational exposure. Several countries, including Belgium, Bulgaria, Finland, France, Greece, Ireland, Israel, Netherlands, Spain, UK, and Turkey, provide recommendations for individuals travelling to endemic regions[9]. Simplifying vaccine recommendations could aid the public in understanding local guidelines.[1]

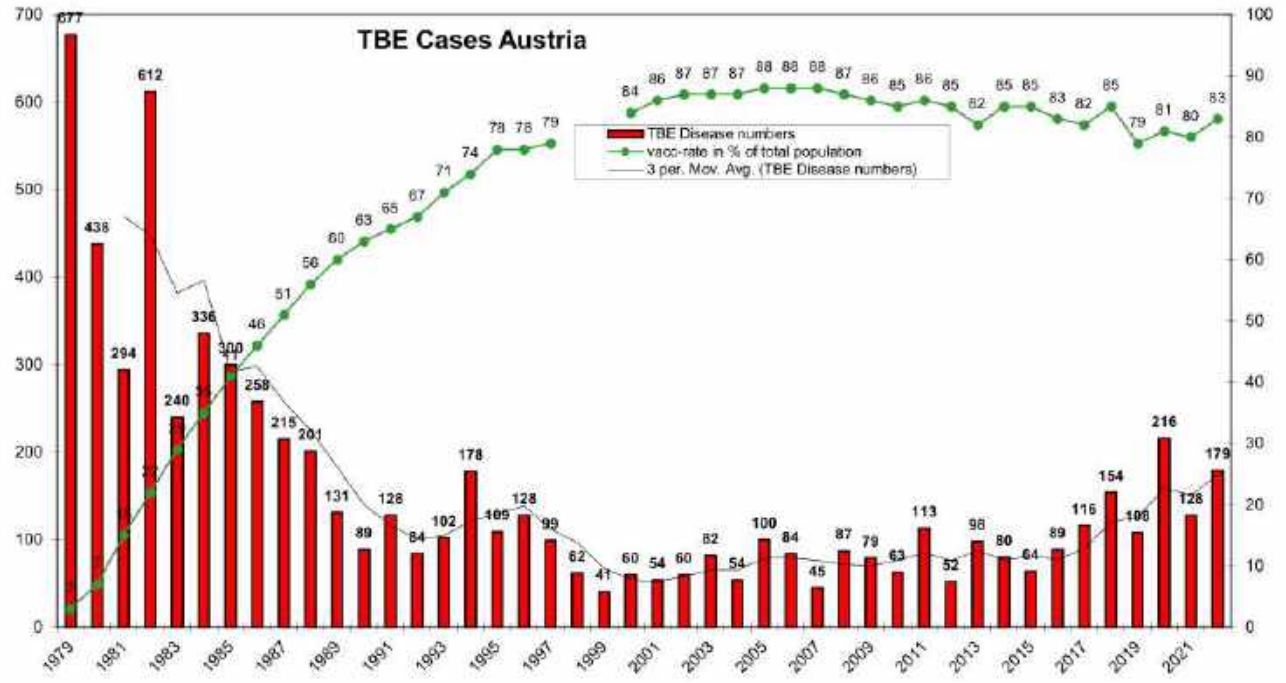
Although most countries require documentation of TBE-endemic areas in order to make targeted vaccination recommendations,[1,32,33] it is unclear how national vaccination recommendations relate to observed TBE incidence, as incidence surveillance systems may underreport cases.[9] The unpredictability of TBEV microfoci and the difficulty in identifying TBE-endemic areas raise questions about the suitability of vaccine recommendations that focus solely on these areas. Therefore, it may be advisable to expand TBE vaccine recommendations to cover the entire population, rather than just those residing in or travelling to currently identified endemic areas.[1]

Regarding booster vaccinations, some countries, such as Switzerland, Finland, Belgium, and Latvia, have extended the recommended interval from every 3-5 years to up to 10 years, as approved locally (Table 2).[86–90]

In 2006, the Federal Office for Public Health in Switzerland recommended extending the booster intervals for TBE vaccine from 3 to 10 years. TBE vaccine reluctance was associated with the need for frequent boosters.[92] After adjusting the vaccination schedule, the sales of the annual TBE vaccine increased more than four times,[93] and vaccination coverage (1 dose) among children aged 16 increased from 10% (95% CI: 8.8-11.2%) in 2005-07 to 55% (95% CI: 53.0-56.6%) in 2020-22.[94] In adults, the vaccination coverage reached 42% in 2018 (up to 50% in endemic regions).[68] The Swiss strategy has not only been more cost-effective but has also led to a significant increase in the number of people accepting TBE vaccination without an increased rate of vaccine breakthrough infections in any age group, which is a substantial benefit for public health.[95]

TBE vaccination is fully or partially reimbursed in only a few countries. Typically, reimbursement is linked to specific factors.[1,9] For example, Switzerland and Germany provide reimbursement for individuals who are traveling to, living in, or working in risk areas. Hungary provides reimbursement for residents of highly endemic areas, and Latvia provides partial reimbursement for children and adolescents living in endemic areas. In Austria, designated risk groups receive full coverage for vaccination costs. In Estonia, Latvia, and Poland, employers fully reimburse vaccination expenses for their employees falling into high-risk categories. In Slovenia, compulsory insurance schemes facilitate reimbursement for high-risk workers. In the Czech Republic, there are contributions from preventive funds from health insurance companies. TBE endemic countries that do not offer reimbursement for the TBE vaccine include

Figure 1: TBE – annual disease numbers and vaccination rate in Austria[60]



TBE vaccination status data were collected annually by surveys conducted by GfK Austria Health Care (Vienna, Austria). TBE case data are collected by the Center for Virology, Medical University of Vienna, Austria, serving as a national reference laboratory for TBEV

Sweden and Romania.[9] The absence of a broad reimbursement policy may be a significant factor in low vaccine uptake,[96] as discussed below.

Vaccine effectiveness and vaccine uptake

TBE vaccines are highly effective in preventing infection, disease, and other outcomes, including serious outcomes, regardless of age.[20,21,68,79,96–104] Vaccine effectiveness ranges from at least 91.5% for receipt of three or more doses[68] to at least 95.4%[20] for receipt of four or more doses.[96] Studies have reported minimal differences in vaccine effectiveness estimates between individuals who received their last dose ≤10 years ago and those who received it more than 10 years ago.[79,96,102,104]

The impact of vaccination on disease incidence was well-documented in Austria. Austria is unique among European countries in having implemented an annual, nationwide TBE awareness and vaccination campaign as early as 1981, targeting the entire population. The implementation of vaccination programs has led to a substantial reduction in the incidence of TBE cases. In Austria, the number of TBE cases has decreased by approximately 90% compared to the time before vaccination programs were introduced and when vaccination coverage was low[98] (Figure 1).

Between 2000 and 2011, TBE vaccination in Austria prevented approximately 333 cases annually within a population of 8.2 million.[97] In Switzerland, TBE vaccination of adults was estimated to prevent 112-162 cases in 2018 among a population of 6.6 million adults.[68] During the three -year study period in Latvia, vaccination was estimated to have prevented 897 hospitalizations, 26 intensive care admissions, 34 patients discharged from the hospital with paresis, and 20 deaths. Additionally, in the Czech Republic, TBE vaccination was estimated to prevent approximately 204 cases per year from 2018 to 2022 among a population of 10.4 million.[105] Vaccination prevented over 1,000 cases of TBE and hundreds of hospitalizations annually in the four countries studied, highlighting the significant public health impact of TBE vaccines. These vaccines are widely used in over 25 European countries with TBE-endemic areas, suggesting that thousands of TBE cases are likely prevented each year through vaccination. However, even though TBE vaccines are effective, the incidence of TBE remains high in the endemic areas of many countries due to the high number of unvaccinated individuals.[96]

Uptake and compliance with TBE vaccination in Europe vary greatly, with overall low rates.[1,67,106] The average TBE vaccine uptake in European countries was only 22% in endemic countries and 5% in non-endemic countries in 2020.[67] At the country level, TBE vaccine coverage varies widely in endemic countries. Austria has the highest coverage at 81%, followed by Latvia at 62%. In contrast, Finland and Hungary have coverage of just under one-third of the population, while Slovakia, Poland, and Romania have the lowest coverage at 12%, 11%, and 7%, respectively (Table 3). In non-endemic countries, TBE vaccine coverage is

Table 3: Vaccine uptake in endemic European countries[67]. "Vaccine uptake" was defined as the percentage of subjects with at least 1 TBE vaccination at any time. "Dose 3 compliance" measured the percentage of subjects who completed the primary series on time according to the licensed vaccine regimen after receiving their first dose of vaccine. "Protection share" measured the percentage of subjects who were within the licensed vaccination regimen after receiving at least 3 prior TBE vaccinations.

Country	Vaccine uptake (%)	Dose 3 compliance (%)	Protection share (%)
All endemic	36	46	26
Austria	81	74	47
Latvia	62	70	44
Germany	48	43	25
Sweden	47	57	41
Estonia	45	59	41
Switzerland	43	40	24
Slovenia	39	63	41
Lithuania	37	67	43
Czech Republic	33	48	31
Hungary	30	29	15
Finland	26	41	26
Slovakia	12	35	16
Poland	11	28	12
Romania	7	no data	no data

very low, with only 1% in France, 5% in Belgium, 6% in the Netherlands, 7% in Norway, and 8% in Denmark and the United Kingdom.[67]

In Russia, vaccination coverage varies greatly between regions, as reviewed in.[19] The Rospotrebnadzor regulations prescribe mandatory vaccination of adolescents (at school) and high-risk groups in endemic territories, which is funded from the regional budget. In certain endemic areas, vaccination coverage can be high (e.g., 88% in the Sverdlovsk region). However, in other endemic regions, less than 10% of the population is vaccinated. The differences arise because vaccination is administered in endemic districts, while the level of vaccine coverage is calculated for the entire region. In non-endemic areas, vaccination is not compulsory, making it challenging to assess the impact of vaccination.[19]

In certain countries, high levels of disease and vaccine awareness may result in high vaccination rates, as seen in Austria. However, in other countries like the Czech Republic, despite high levels of awareness, vaccination rates remain low.[67] In fact, vaccine uptake is a multifaceted issue that does not always correspond with vaccine awareness.[1,67] The low vaccination rates across most of Europe can be attributed to various factors, including the

complexity of the TBE vaccination schedule, low awareness of the potential consequences of TBE, and limited vaccine accessibility and reimbursement.[106]

The limited reimbursement of vaccine costs may reduce vaccine uptake due to economic constraints. For example, in Slovakia and Poland, the proportion of individuals who receive the vaccine is approximately five times smaller than the proportion of individuals who are aware of the availability of a TBE vaccine (12% vs. 63% in Slovakia, and 11% vs. 47% in Poland, respectively).[67] In these countries, vaccination is (partially) reimbursed for high-risk occupational groups only.[9] In contrast, in countries like Switzerland and Germany where the TBE vaccine is fully reimbursed for individuals staying in endemic areas, a high proportion of people who are aware of the vaccine's availability actually get vaccinated[9] (43% vs 59% in Switzerland, and 48% vs. 55% in Germany, respectively).[67] Reimbursement can therefore be an important motivator for individuals to be vaccinated, and the introduction of a broad reimbursement policy can support better vaccine uptake. It is noteworthy, however, that despite the availability of low-cost TBE vaccines, their uptake remains low in some endemic countries due to limited awareness of the burden of the disease and the risk it poses.[96] Thus, the relationship between vaccine uptake and reimbursement is not linear. It is influenced by other factors, as described above.

Necessity of pediatric vaccination

TBE vaccination is safe and effective and is currently recommended by the WHO for children one year of age and older.[20,96,107–109] Seroconversion rates in children (up to 15 years of age based on data from clinical development programs) are similar to those in adults, approaching 100% even in children as young as 1 year of age.[110–112] Studies have also shown high levels of protection and antibody persistence (94-100% seropositivity), with protection lasting up to 5 years following primary vaccination with three doses.[113,114] A recent case-control study showed that TBE vaccination is highly effective (>90%) in fully vaccinated children 0-17 years in Switzerland and remains high for up to 10+ years post completion of primary vaccination.[107]

Despite evidence that the TBE vaccines used in Europe are both effective and safe, they are administered conservatively in children. Disease incidence is lower in children than in adults.[14,24,70,71,115] However, infection in children may be underreported because symptoms are non-specific and vague, and children may not be able to describe their symptoms.[25,72] About 40-80% of the children can recall tick-bites.[25,116–118] In a study of asymptomatic TBE infections in a highly endemic area of northern Poland, only 2% of 180 unvaccinated children were seropositive for TBE, compared with 5% of adults, suggesting that TBE infections may be undiagnosed.[119]

The clinical course of TBE infection in children is similar to that in adults, albeit less severe. Although the frequency of occurrence varies, non-specific symptoms usually include fever, fatigue/malaise, behavioral changes, photophobia, myalgias.[120] The most common clinical manifestation of the disease in children is meningitis in 60-80% of the cases, followed by 20-40% meningoencephalitis and 0-4% meningoencephalomyelitis.[121,122] Disease severity is lower in children than in adults, but this discrepancy varies across the different age groups (0-5, 6-11 and 12-17 years).[107] The biphasic clinical course typical of TBE infection is less common in pre-school children than in older children and adults.[25] Consistent with the reduced overall incidence and severity of disease, permanent neurological sequelae of TBE infection are less common in children (0-2%)[115,123–126] than in adults (30-50%).[127–132] In a study of 523 TBE patients in Germany, overall 95% of 59 children and 64% of 464 adults recovered completely; compared with adults aged 18-39 years, the recovery rate in children was 79% higher.[72] Post-encephalitic syndrome is reported 3-10 times more frequently in adults than in children, regardless of the severity of TBE and the time point during the 18-month follow-up.[72]

A comprehensive systematic review focusing on the epidemiology, clinical characteristics, and outcomes of TBE in the pediatric population confirmed that the disease is less severe in children. However, recent follow-up cases have shown that a significant proportion of children suffered from long-term cognitive impairment.[24] These recent studies evaluating cognitive function in recovered pediatric TBE patients found abnormal EEG and MRI findings, a higher incidence of headache, fatigue, cognitive impairment, and reduced motor function compared to controls.[118,125,133–135] Thus, although mild in the early stages, infections can lead to long-term neurological sequelae and increased morbidity in children, which can affect their performances in school and everyday life.[72,118,122]

A recent study in Switzerland evaluated 463 TBE cases in children aged 0-17 years.[107] The study found that diagnoses of disease severity in young children aged 0-6 years are not different from those in older children. More severe disease, such as meningoencephalomyelitis, encephalomyelitis, and radiculitis, occurred in 1-5% of children across all three age groups (0-5, 6-11 and 12-17 years). The study also found that unvaccinated children were 6.7 times more likely than vaccinated children (1 or more doses) to develop neurological disease symptoms. Incompletely vaccinated children (2 doses or less) and completely vaccinated children (3 or more doses) were less likely to experience mild neurological disease compared to unvaccinated children.

Given the recent increase in incidence and severity of TBE, it is important to improve vaccination rates among children and adolescents. As they are more likely to engage in outdoor activities, children are at high risk, particularly those between 5 and 14 years.[12,64,66] Among the factors associated with uptake of TBE vaccination, having had a recent tick bite was the only predictor of having had a child vaccinated against TBE.[119] As TBE cases in children may be underreported, and mild symptoms may develop into long-term cognitive impairment, vaccination should be encouraged for children, especially those living in or travelling to TBE-endemic area.

TBE vaccination and travel

Global incidence estimates of TBE range from 10,000 to 12,000 cases per year,[109] with many cases remaining unreported or misdiagnosed.[1] According to the United Nations World Tourism Organization, there were almost 1.3 billion international tourist arrivals in 2023, which represents an increase of 34% from 2022.[136] More than half of these arrivals occurred in Europe.[136] The increase in international tourism, particularly in Europe, increases the risk of individuals travelling from non-endemic to endemic TBE areas.[137,138]

While the risk of mortality due to TBE is relatively low (ranging from 1% in central Europe up to 40% in the Far East),[139] the burden of long-term morbidity can be significant, lasting from months to years and ranging from post-encephalitic syndrome to permanent paralysis and seizures.[140] As there is currently no specific treatment for TBE, prevention is recommended. This includes preventing tick-bites, as described earlier, and vaccination. TBE vaccines may be administered in an accelerated schedule shortly before travel.[96,108] Vaccination is recommended for travellers from non-endemic countries with a high risk of tick exposure during travel between April and November.[138,141] Therefore, it is important to assess the risk of acquiring TBE for travellers from non-endemic countries visiting endemic countries before deciding whether to get vaccinated. This assessment should consider both environmental and personal factors. Environmental concerns relate to the choice of destination, including whether the area is endemic for TBE, the season, and altitude. Surveillance data have shown that tick activity is highest between April and November in endemic areas,[12,142] and TBEV foci have been found in places as high as 2100 meters above sea level.[143] When assessing the risk of exposure, it is important to consider individual behavior, the type of outdoor activity, duration of stay, and demographic variables such as age, gender, and personal health status.[140]

Several studies have assessed awareness of TBE and the TBE vaccine among travellers.[78,144,145] One study assessed perceptions of TBE risks among travellers from Canada, Germany, Sweden and the United Kingdom who were travelling to a TBE-endemic country.[144] The study found that 69% of travellers were aware of the disease, and 26% prepared for their trip by searching for information online. While 14% were aware that TBE vaccines were offered by travel clinics, 52% were not aware of the existence of travel clinics. Furthermore, while 14% of participants reported feeling at high risk when travelling to an endemic region, 26% never felt at risk. Among those who engaged in pre-defined at-risk activities, such as camping or hiking in the forests, 79% were aware of at least one correct TBE prevention measure. However, only 15% had been vaccinated within the last 3 years and 11% had been vaccinated following a clinic recommendation. Only 35% of the participants had heard of a TBE vaccine. Health professionals working in travel clinics recommended TBE vaccination to 61% of their travellers going to endemic areas.[144] Another study that surveyed international travellers residing in the United States found that the likelihood of travellers choosing the TBE vaccine depends on the level of endemic risk in the destination country, provided that the vaccine is available at no cost.[145] Almost all travellers (94%) would choose to be vaccinated should the risk be at the highest level, whereas 6% would remain unvaccinated regardless of the risk level. Respondents who participated in outdoor activities were more likely to choose vaccination than the average respondent.

While TBE awareness may have increased among travellers and travel clinics, vaccination may not be available in the country of origin where TBE is not endemic. Additionally, the subsequent costs of vaccination, diagnosis, and medical care may not be covered. If symptoms of infection occur upon returning home, they may not be recognized, leading to a misdiagnosis or no diagnosis at all, especially if adequate diagnostic testing tools are not available.[146]

In conclusion, it is important for both travellers and health professionals in travel clinics to be well-informed about the risks, preventive measures and symptoms of TBE when travelling from a TBE non-endemic country to an endemic destination. Lack of awareness or failure to take the necessary precautions could increase the likelihood of infection. These concerns highlight the need for international guidelines on TBE for travellers.

Economic impact

Health economic evaluations inform medical procurement and reimbursement decisions by public and private healthcare providers. The most common form of health economic evaluation is cost-effectiveness analysis, which presents the ratio of the incremental cost of an intervention to the incremental health benefits of an intervention.[147] However, there are only a few cost-effectiveness evaluations of the TBE vaccine.

In 1981, Austria introduced an overall TBE vaccination campaign[97] that led to a significant reduction in TBE cases.[99] The economic benefit of the campaign, which included reducing costs for inpatient care, loss of productivity, and premature retirement, was evaluated to be EUR 24 million for the years 1981 to 1990[148] and EUR 60 million between 1991 and 2000.

A study conducted in Slovenia found that TBE vaccination is cost-effective from a healthcare payer's perspective when vaccination begins at 18 years of age and continues until the age of 80.[149]

In 1996, a cost-effectiveness estimation of TBE vaccination in the Stockholm area was performed and it was calculated that, based on the TBE incidence at that time and the cost of vaccination, mass vaccination would be an unrealistic alternative.[150] However, more than 20 years later, much higher incidences in the unvaccinated population were reported. A health economic analysis was conducted in Sörmland County, which is a highly TBE-endemic area adjacent to Stockholm County. The analysis calculated that the costs per QALY (quality adjusted life year) for a fully free of charge vaccination program would come much closer to the generally acceptable cost-effectiveness threshold in Sweden. The authors concluded that introducing a structured vaccination program would be cost-effective at all ages. However, it would be particularly cost-effective if implemented in childhood.[77]

Estimating the economic impact of a disease requires an assessment of its disease burden, in addition to cost-benefit analyses. The Burden of Communicable Diseases in Europe study computed disability-adjusted life years (DALYs) for 31 selected diseases, including tick-borne encephalitis, in the European Union and European Economic Area.[151] DALYs represent the equivalent of a year of full health lost and are the sum of the years of life lost due to premature mortality and the years lived with a disability. The calculation of DALYs relies on the incidence of acute, symptomatic disease as a crucial input variable. Furthermore, it requires several age-group and sex-specific variables, such as the risk of developing short- and long-term complications, their duration, and weights reflecting their severity. The study found that the median annual burden of TBE was 0.69 (0.65 -0.74) DALYs per 100,000 population[151]. It is worth noting that a Slovenian study found a much higher disease burden on the country level (11.0 (10.2-11.7) per 100,000).[152] Thus, differences in underlying assumptions and disease modelling approaches heavily influence the outcomes of such analyses. Although DALYs provide useful information for prioritization and planning in public health, they do not fully encompass all unknowns, uncertainties, variability and other “softer” criteria such as public perception.[153]

A TBE vaccination program must be evaluated against other healthcare resources. To determine if funding a TBE vaccination program yields better health outcomes at a reasonable cost, it is important to establish the long-term costs and health outcomes of a local TBE vaccination strategy.[154] Furthermore, TBE can result in high productivity loss beyond the healthcare sector. Increasing vaccination rates across all age groups is the most effective and efficient strategy to reduce the burden of TBE and protect the overall population's health.[155] Therefore, a vaccination program or at least a vaccination recommendation should be considered. It is important to note that out-of-pocket costs may have a positive impact on an individual's private consumption, which is not included in the health care analysis.

Health economic evaluations play a crucial role in informing decisions regarding the implementation of TBE vaccination programs. While the cost-effectiveness of such programs varies depending on factors such as incidence rates and population demographics, evidence suggests that TBE vaccination can yield significant economic benefits by reducing healthcare costs and productivity losses. Despite challenges in estimating disease burden and modelling economic impacts, prioritizing TBE vaccination efforts across age groups remains a cost-effective strategy for mitigating the overall burden of the disease and safeguarding public health.

The One Health approach

The One Health approach is a collaborative and holistic strategy that recognizes the interconnectedness of human, animal, and environmental health.[156] TBE involves a complex ecosystem in which the virus circulates between ticks, animals (such as small mammals and deer), and humans.[157–159] The One Health approach considers the interdependence of these systems with the environment and seeks to understand how changes in one component can affect the entire ecosystem.

As discussed earlier in this chapter, the tick species *Ixodes ricinus* is the predominant TBEV vector in Europe, while *Ixodes persulcatus* and *Haemaphysalis concinna* are found in Russia and Asia.[158,160] The main reservoir hosts for ticks are small mammals or insectivores such as rodents, hedgehogs, shrews and hares. While their small size makes them easy targets for ticks, especially nymphs, their biological characteristics allow TBEV to circulate in the bloodstream at levels that allow the virus to be transmitted to feeding ticks without killing them. As these hosts have a high reproductive rate and short lifespan, there are always enough animals naive to the virus for it to spread.[158] Larger animals, such as deer, serve as hosts for adult ticks.[161] With a lag time of one year, a study in Sweden showed that the number of roe deer and hares was positively correlated with the number of TBE cases in the region.[162]

Tick populations are also strongly influenced by environmental factors such as climate, vegetation, habitat and human activity.[157] As discussed earlier, climate change can influence the survival, abundance and activity of ticks and their hosts by affecting the vegetation and their habitat through prolonged higher temperatures and relative humidity.[35–38] Human activity has also changed over the years, which has contributed to the increase in TBE cases. In addition to heightened awareness of the diagnosis of the disease, the number of TBE cases could be affected by farming and global tourism (both recreational and business). This increases the possibility of human and tick contact when individuals travel from a non-TBE endemic region to a TBE endemic region.[1,35–38,137,138,157] Surveillance of tick, animal, and human activities can aid in tracking the prevalence of TBEV, identifying potential hotspots, assessing the risk of human exposure, and exploring the dynamics of cross-species transmission to reduce the risk of spillover events.

A model incorporating data on climate, forest cover, water, tick abundance, and sheep (as an indicator species) identified an increase in TBE incidence in the Örebro region of Sweden during the study period.[163] They found a variation in hotspots across the region. The risk of acquiring TBE increased by 12.5% for every 1% increase in relative humidity and by 72.3% for every 1% increase in the proportion of wetland forest. However, as the model had a low goodness of fit, other variables, such as human behavior could help create a stronger model for understanding the spatial distribution of ticks. Historical data on TBE cases, human population demographics and migration, climate teleconnection, beech fructification (used as a proxy for rodent density, which acts as a host for the TBE virus vector), and annual sunshine duration were used to forecast TBE incidences for Austria, Germany, and Switzerland from 2019 to 2021.[45] The first verified forecasting results for 2019 were highly reliable, but could be improved for better accuracy.[164]

The most common way to contract TBE is through a tick bite. However, it is also possible to acquire TBE through the consumption of unpasteurized TBEV-contaminated dairy products from goats, cows and sheep.[61] The largest outbreak of TBE occurred in 1951 in the former Czechoslovakia, where over 600 cases were reported due to the consumption of contaminated, unpasteurized cow and goat milk.[61,165] An analysis of TBE outbreaks in Slovakia from 2007 to2016 revealed that 17% of all TBE cases were due to consumption of dairy products.[166] This percentage showed an increasing linear trend throughout the study period. Notably, none of these cases reported a tick bite, nor were they vaccinated against TBE.[166] A systematic review and meta-analysis of 410 cases of foodborne-TBE (FB-TBE) between 1980 and 2021 confirmed that the majority of cases were located in Central and Eastern Europe (the so-called FB-TBE triangle) and Russia.[63] The clinical presentation is similar to non FB-TBE infections, and neuroinvasive disease is common in 39% of cases. However, the median incubation time is shorter at 3.5 days. None of the cases were vaccinated, except for one whose last booster was more than 15 years ago. The clinical attack rate was 14% in outbreaks with 10 or more cases, with significant heterogeneity.[63] These FB-TBE outbreaks have the potential to cause a significant public health issue, despite their infrequency. However, unlike non FB-TBE cases, patients with mild and nonspecific symptoms can be actively contacted during an epidemiological investigation to locate the source of the outbreak. FB-TBE cases can be prevented by vaccination and avoidance of unpasteurized dairy products in TBE-endemic areas.[100]

In April 2020, the first FB-TBE outbreak occurred in France where the virus had never been detected before.[167] The research team utilized the One Health approach to investigate the outbreak.[159] Forty-two out of 43 cases of FB-TBE were linked to the consumption of unpasteurized raw goat cheese from a local producer. The methodology of investigation included screening for TBEV in cheese and milk products to identify the source of infection, serological testing of all animals on the suspected farm and surrounding farms, landscape analysis and localization of the wooded area, ticks, and small animal surveys for virus detection and virus isolation and genome sequencing. Information gained from this thorough and integrative approach should help the farmers and health authorities assess the risk of infection and develop control strategies. This outbreak underscored the need to improve surveillance, detection and prevention of FB-TBE in France, particularly given the increasing global trend toward the consumption of local and traditional delicacies.[168]

In summary, the One Health approach provides a robust framework for understanding and addressing the complexity of TBE. By acknowledging the interdependence of human, animal, and environmental health and involving health authorities and local communities, this collaborative strategy enables comprehensive surveillance, targeted interventions and effective control measures. Interdisciplinary collaboration and integrated surveillance systems are essential steps in reducing the burden of TBE and protecting public health.

Recommendations

TBE is considered an emerging disease and a growing public health concern. A One Health approach should be considered to combat this complex problem, as it emphasizes the importance of interdisciplinary collaboration in addressing complex health challenges by highlighting the interconnectedness of tick, human, animal, and environmental health.

Although there is considerable variation in national

reporting of annual cases, the cumulative number of reported TBE cases across Europe increases, highlighting the need for improved TBE risk management.[1,9,10] Surveillance methods for TBE vary across Europe, with countries using different diagnostic criteria, access to diagnostic tests and knowledge on their appropriate, and approaches to national and regional surveillance.[1,9,13] Surveillance of TBE in Europe is currently incomplete, which means that reported cases are likely to only partially reflect the true risk, and that the true burden of TBE is significantly underestimated.[1,9,67,106] Experts on TBE have suggested the following measures to improve the surveillance of TBE throughout Europe:[1]

- Use of a single TBE case definition across Europe to ensure comparability of data;
- Testing all cases of aseptic meningitis/encephalitis of unknown etiology for TBEV infection;
- Rapidly extend testing to all patients with either a fever of unknown origin or CNS symptoms who live in or have visited an endemic, probable, or potential endemic area or who have received a tick bite;
- Improved funding for and access to diagnostic tests and testing facilities;
- Establishment of nationwide surveillance systems in countries that do not have them by implementing active surveillance systems with interactive maps of Ixodid tick activity across Europe; and
- Implementing active surveillance systems throughout Europe.

The national TBE disease burden and funding constraints will largely determine the extent to which these measures are implemented.[1]

Other recommendations to address the challenges as outlined in this chapter include:

- Climate change: The influence of climate change on the transmission of tick-borne diseases includes its impact on the survival, abundance, and activity of ticks, as well as their hosts. Changes in temperature, precipitation, and vegetation are expected to alter the geographic distribution and prevalence of diseases like TBE.[35–38] The spread of TBE to new regions in Europe presents a significant public health challenge. This challenge involves implementing measures to prevent TBE in regions not previously affected by the disease and where awareness of the disease is low. Such measures include establishing a surveillance system, recommending vaccination, and conducting awareness-raising campaigns.

- TBE vaccination recommendation: Vaccination remains the most effective method of protection against TBE. However, National Immunization Technical Advisory Groups in some European countries with TBE-endemic areas do not recommend TBE vaccines,[96] and only a few European countries have universal vaccination recommendations.[1,9] The unpredictability of TBEV microfoci and the difficulty in identifying TBE-endemic areas raise questions about the suitability of vaccine recommendations that focus solely on these areas. It may be advisable to expand TBE vaccine recommendations to cover the entire population, rather than just those residing in or travelling to currently identified endemic areas.[1] Alternatively, if TBE risk is limited to specific areas or if vaccination poses a significant burden on national or local healthcare services, vaccine recommendations should be simplified and standardized for healthcare practitioners and the public. TBE experts believe that this will aid the public in comprehending the recommendations and minimizing confusion.[1] In order for TBE vaccine recommendations to be effective, it is crucial that the public trusts the recommendations, understands the health risks associated with tick bites, has knowledge of TBE, and has easy access to vaccination services.[1,67,169]

- TBE vaccination rates: Uptake and compliance with TBE vaccination in Europe vary greatly, with overall low rates.[1,67,106] The uptake of the TBE vaccine is influenced by various factors, including specific recommendations, public awareness programs, vaccine awareness, perceptions of vaccine safety and reimbursement.[67] In many countries where TBE vaccines are recommended, vaccine uptake is low due to limited reimbursement of vaccine costs.[96] Although some countries have achieved good levels of vaccine uptake without a comprehensive national program,[67,106] vaccine reimbursement could lead to improved vaccine uptake, especially in low-income households.[169] However, in some countries, TBE vaccines are recommended and available at low cost, but vaccine uptake remains inadequate due to limited awareness of the disease burden and understanding of the risk.[96] Therefore, in countries where high awareness of the disease and vaccine does not directly translate into high vaccine uptake, motivators and barriers to vaccination must be analyzed to increase vaccine uptake. In countries where low vaccine awareness is associated with limited vaccine uptake, it is necessary to improve public awareness of TBE vaccines. In countries with low vaccine compliance, it is important to emphasize the need for booster shots.[67]

- TBE awareness and risk exposure: The incidence of TBE has increased over the past 25 years, posing a risk to individuals living in both TBE endemic and non-endemic countries, especially with the growth in international tourism.[137,138,157] Although TBE mortality rates are low, long-term morbidity underscores the importance of prevention. Therefore, safe and effective TBE

vaccination is strongly recommended for travellers from non-endemic areas with a high risk of tick exposure. Studies show that awareness of TBE varies among individuals.[1,67,138,170] Therefore, comprehensive risk assessments that include environmental and personal factors are necessary. Targeted awareness campaigns and the involvement of health professionals are essential to promote preventive measures. These campaigns should focus on risk areas, risk perception, and the benefits of vaccination to address barriers and misconceptions. These campaigns should improve access to vaccination while tailoring interventions to specific populations, such as the elderly, immunocompromised individuals, individuals with comorbidities and behavioral and occupational risks, and travellers.

- TBE vaccination for children: Evidence strongly supports the safety and efficacy of TBE vaccination in children, with seropositivity comparable to adults and high long-term protection rates.[24,107,108,110,111,113] However, despite its proven benefits, vaccination rates remain conservative, possibly due to lower disease incidence in children and underreporting of TBE cases. In recent years, there has also been an increase in cases of neurological sequelae and long-term cognitive impairment in children diagnosed with TBE.[24,72,118,133] To address this, there should be a concerted effort to increase vaccination uptake among children and adolescents, particularly in endemic areas. Given the potential underreporting or missed diagnoses of TBE, particularly in preschool children, pediatricians in TBE-endemic regions should remain vigilant for TBEV infection in children presenting with non-specific central nervous system symptoms. It is imperative for them to ensure comprehensive clinical follow-up for children diagnosed with TBE to address potential long-term morbidity.

- Economic impact: Health economic evaluations are essential to guide decisions about the implementation of TBE vaccination programs. Despite the limited number of cost-effectiveness analyses of the TBE vaccine, studies have demonstrated its economic benefits, particularly in reducing healthcare costs and productivity losses. Evaluating the long-term costs and health outcomes of local vaccination strategies is essential to determine their effectiveness and prioritize resource allocation. Increasing vaccination coverage across all age groups has been identified as the most effective strategy for reducing the burden of TBE and protecting public health. Despite challenges in estimating disease burden and economic impact, prioritizing TBE vaccination efforts is considered cost-effective and essential to reduce the overall burden of the disease.

Contact: phung.lang@uzh.ch,
rahel.ackermann@ne.ch

Citation: Lang P, Ackermann-Gäumann R. Public health aspects of TBE. Chapter 14. In: Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2024. 10.33442/26613980_14-7

References

1. Kunze M, Banović P, Bogovič P, et al. Recommendations to Improve Tick-Borne Encephalitis Surveillance and Vaccine Uptake in Europe. *Microorganisms*. 2022;10(7):1283. doi:10.3390/microorganisms10071283
2. Rosicky B. Notes on the classification of natural foci of tick-borne encephalitis in Central and South-East Europe. *J Hyg Epidemiol Microbiol Immunol*. 1959;3:431-443.
3. Blaskovic D, Nosek J. The ecological approach to the study of tick-borne encephalitis. *Prog Med Virol*. 1972;14:275-320.
4. Dobler G, Erber W, Bröker M, Chitimia-Dobler L, Schmitt HJ. Global distribution of the TBEV. Chapter 12c. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. Global Health Press; 2023.
5. Centers for Disease Control and Prevention. Tickborne Diseases Abroad. Published 2022. Accessed February 7, 2024. https://www.cdc.gov/ticks/tickbornediseases/abroad.html
6. Im JH, Baek JH, Durey A, Kwon HY, Chung MH, Lee JS. Geographic distribution of Tick-borne encephalitis virus complex. *J Vector Borne Dis*. 2020;57(1):14-22. doi:10.4103/0972-9062.308794
7. Jenkins VA, Silbernagl G, Baer LR, Hoet B. The epidemiology of infectious diseases in Europe in 2020 versus 2017–2019 and the rise of tick-borne encephalitis (1995–2020). *Ticks and Tick-borne Diseases*. 2022;13(5):101972. doi:10.1016/j.ttbdis.2022.101972
8. Steffen R, Lautenschlager S, Fehr J. Travel restrictions and lockdown during the COVID-19 pandemic-impact on notified infectious diseases in Switzerland. *J Travel Med.* 2020;27(8). doi:10.1093/jtm/taaa180
9. Erber W, Schmitt HJ, Jankovic TV. TBE-epidemiology by country—an overview. Chapter 12a. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. Global Health Press; 2023. doi:10.33442/26613980_12a-6
10. Daniel M, Danielová V, Kríz B, Jirsa A, Nozicka J. Shift of the tick *Ixodes ricinus* and tick-borne encephalitis to higher altitudes in central Europe. *Eur J Clin Microbiol Infect Dis*. 2003;22(5):327-328. doi:10.1007/s10096-003-0918-2
11. Van Heuverswyn J, Hallmaier-Wacker LK, Beauté J, et al. Spatiotemporal spread of tick-borne encephalitis in the EU/EEA, 2012 to 2020. *Euro Surveill.* 2023;28(11). doi:10.2807/1560-7917.Es.2023.28.11.2200543
12. European Centre for Disease Prevention and Control ECDC. Tick-borne encephalitis Annual Epidemiological Report for 2020. Published online 2022. Accessed December 29, 2023.

13. European Centre for Disease Prevention and Control ECDC. Surveillance Systems Overview for 2022. Available Online: Https://Www.Ecdc.Europa.Eu/En/Publications-Data/Surveillance-Systems-Overview-2022. Accessed December 29, 2023.
14. European Centre for Disease Prevention and Control (ECDC). EU Case Definitions.(2018). Accessed December 29, 2023. https://www.ecdc.europa.eu/en/all-topics/eu-case-definitions
15. Hellenbrand W, Kreusch T, Böhmer MM, et al. Epidemiology of Tick-Borne Encephalitis (TBE) in Germany, 2001–2018. *Pathogens.* 2019;8(2). doi:10.3390/pathogens8020042
16. 16. Zavadska D, Anca I, André F, et al. Recommendations for tick-borne encephalitis vaccination from the Central European Vaccination Awareness Group (CEVAG). *Hum Vaccin Immunother.* 2013;9(2):362-374. doi:10.4161/hv.22766
17. Kollaritsch H, Paulke-Korinek M, Holzmann H, Hombach J, Bjorvatn B, Barrett A. Vaccines and vaccination against tick-borne encephalitis. *Expert Rev Vaccines.* 2012;11(9):1103-1119. doi:10.1586/erv.12.86
18. Bogovic P, Lotric-Furlan S, Strle F. What tick-borne encephalitis may look like: clinical signs and symptoms. *Travel Med Infect Dis*. 2010;8(4):246-250. doi:10.1016/j.tmaid.2010.05.011
19. Ruzek D, Avsic Zupanc T, Borde J, et al. Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. *Antiviral Res*. 2019;164:23-51. doi:10.1016/j.antiviral.2019.01.014
20. Erber W, Khan F, Zavadska D, et al. Effectiveness of TBE vaccination in southern Germany and Latvia. *Vaccine.* 2022;40(5):819-825. doi:10.1016/j.vaccine.2021.12.028
21. Santonja I, Stiasny K, Essl A, Heinz FX, Kundi M, Holzmann H. Tick-Borne Encephalitis in Vaccinated Patients: A Retrospective Case-Control Study and Analysis of Vaccination Field Effectiveness in Austria From 2000 to 2018. *J Infect Dis*. 2023;227(4):512-521. doi:10.1093/infdis/jiac075
22. Zavadska D, Odzelevica Z, Karelis G, et al. Tick-borne encephalitis: A 43-year summary of epidemiological and clinical data from Latvia (1973 to 2016). *PLoS One*. 2018;13(11):e0204844. doi:10.1371/journal.pone.0204844
23. Bogovič P, Kastrin A, Lotrič-Furlan S, et al. Clinical and Laboratory Characteristics and Outcome of Illness Caused by Tick-Borne Encephalitis Virus without Central Nervous System Involvement. *Emerg Infect Dis*. 2022;28(2):291-301. doi:10.3201/eid2802.211661
24. Steffen R. Tick-borne encephalitis (TBE) in children in Europe: Epidemiology, clinical outcome and comparison of vaccination recommendations. *Ticks and Tick-borne Diseases*. 2019;10(1):100-110. doi:10.1016/j.ttbdis.2018.08.003
25. Hansson MEA, Örvell C, Engman ML, et al. Tick-Borne Encephalitis in Childhood: Rare or Missed? *The Pediatric Infectious Disease Journal*. 2011;30(4):355. doi:10.1097/INF.0b013e3181fe3b5a
26. Holzmann H. Diagnosis of tick-borne encephalitis. *Vaccine.* 2003;21 Suppl 1:S36-40. doi:10.1016/s0264-410x(02)00819-8
27. Lindquist L. Tick-borne encephalitis. In: Tselis AC, Booss J, eds. *Handbook of Clinical Neurology*. Vol 123. Elsevier B.V.; 2014.
28. Banović P, Obregón D, Mijatović D, et al. Tick-Borne Encephalitis Virus Seropositivity among Tick Infested Individuals in Serbia. *Pathogens*. 2021;10(3). doi:10.3390/pathogens10030301
29. Yoshii K, Ikawa A, Chiba Y, et al. Establishment of a neutralization test involving reporter gene-expressing virus-like particles of tick-borne encephalitis virus. *Journal of Virological Methods*. 2009;161(1):173-176. doi:10.1016/j.jviromet.2009.05.016
30. Haviernik J, Eyer L, Yoshii K, et al. Development and characterization of recombinant tick-borne encephalitis virus expressing mCherry reporter protein: A new tool for high-throughput screening of antiviral compounds, and neutralizing antibody assays. *Antiviral Res*. 2021;185:104968. doi:10.1016/j.antiviral.2020.104968
31. Domanovic D, Giesecke J. How to define an area where transmission of arthropod-borne disease is occurring? *Euro Surveill*. 2012;17(20).
32. Stefanoff P, Polkowska A, Giambi C, et al. Reliable surveillance of tick-borne encephalitis in European countries is necessary to improve the quality of vaccine recommendations. *Vaccine.* 2011;29(6):1283-1288. doi:10.1016/j.vaccine.2010.11.077
33. Braks M, van der Giessen J, Kretzschmar M, et al. Towards an integrated approach in surveillance of vector-borne diseases in Europe. *Parasit Vectors*. 2011;4:192. doi:10.1186/1756-3305-4-192
34. Stefanoff P, Zielicka-Hardy A, Hlebowicz M, et al. New endemic foci of tick-borne encephalitis (TBE) identified in districts where testing for TBE was not available before 2009 in Poland. *Parasit Vectors*. 2013;6:180. doi:10.1186/1756-3305-6-180
35. Gray JS, Dautel H, Estrada-Pena A, Kahl O, Lindgren E. Effects of climate change on ticks and tick-borne diseases in Europe. *Interdiscip Perspect Infect Dis*. 2009;2009:593232. doi:10.1155/2009/593232
36. Bouchard C, Dibernardo A, Koffi J, Wood H, Leighton PA, Lindsay LR. N Increased risk of tick-borne diseases with climate and environmental changes. *Can Commun Dis Rep*. 2019;45(4):83-89. doi:10.14745/ccdr.v45i04a02
37. Randolph SE. [Fauna, climate and politics: possible causes for the recent increases in tick-borne zoonoses]. *Arch Pediatr.* 2004;11(10):1282-1285. doi:10.1016/j.arcped.2003.12.019
38. Randolph SE. Is expert opinion enough? A critical assessment of the evidence for potential impacts of climate change on tick-borne diseases. *Anim Health Res Rev.* 2013;14(2):133-137. doi:10.1017/S1466252313000091
39. Bouchard C, Aenishaenslin C, Rees EE, et al. Integrated Social-Behavioral and Ecological Risk Maps to Prioritize Local Public Health Responses to Lyme Disease. *Environ Health Perspect*. 2018;126(4):047008. doi:10.1289/ehp1943

40. Aenishaenslin C, Bouchard C, Koffi JK, Ogden NH. Exposure and preventive behaviours toward ticks and Lyme disease in Canada: Results from a first national survey. *Ticks Tick Borne Dis*. 2017;8(1):112-118. doi:10.1016/j.ttbdis.2016.10.006

41. Aenishaenslin C, Bouchard C, Koffi JK, Pelcat Y, Ogden NH. Evidence of rapid changes in Lyme disease awareness in Canada. *Ticks Tick Borne Dis*. 2016;7(6):1067-1074. doi:10.1016/j.ttbdis.2016.09.007

42. Gray JS. Ixodes ricinus seasonal activity: Implications of global warming indicated by revisiting tick and weather data. *International Journal of Medical Microbiology*. 2008;298:19-24. doi:10.1016/j.ijmm.2007.09.005

43. Brugger K, Walter M, Chitimia-Dobler L, Dobler G, Rubel F. Seasonal cycles of the TBE and Lyme borreliosis vector Ixodes ricinus modelled with time-lagged and interval-averaged predictors. *Exp Appl Acarol.* 2017;73(3-4):439-450. doi:10.1007/s10493-017-0197-8

44. Tkadlec E, Václavík T, Široký P. Rodent Host Abundance and Climate Variability as Predictors of Tickborne Disease Risk 1 Year in Advance. *Emerg Infect Dis*. 2019;25(9):1738-1741. doi:10.3201/eid2509.190684

45. Rubel F, Walter M, Vogelgesang JR, Brugger K. Tick-borne encephalitis (TBE) cases are not random: explaining trend, low- and high-frequency oscillations based on the Austrian TBE time series. *BMC Infect Dis*. 2020;20(1):448. doi:10.1186/s12879-020-05156-7

46. Marini G, Tagliapietra V, Cristofolini F, et al. Correlation between airborne pollen data and the risk of tick-borne encephalitis in northern Italy. *Sci Rep*. 2023;13(1):8262. doi:10.1038/s41598-023-35478-w

47. Bogdziewicz M, Kelly D, Thomas PA, Lageard JGA, Hacket-Pain A. Climate warming disrupts mast seeding and its fitness benefits in European beech. *Nat Plants*. 2020;6(2):88-94. doi:10.1038/s41477-020-0592-8

48. Bouchard C, Leighton PA, Beauchamp G, et al. Harvested white-tailed deer as sentinel hosts for early establishing Ixodes scapularis populations and risk from vector-borne zoonoses in southeastern Canada. *J Med Entomol*. 2013;50(2):384-393. doi:10.1603/me12093

49. Levi T, Kilpatrick AM, Mangel M, Wilmers CC. Deer, predators, and the emergence of Lyme disease. *Proc Natl Acad Sci U S A*. 2012;109(27):10942-10947. doi:10.1073/pnas.1204536109

50. Roy-Dufresne E, Logan T, Simon JA, Chmura GL, Millien V. Poleward expansion of the white-footed mouse (Peromyscus leucopus) under climate change: implications for the spread of lyme disease. *PLoS One*. 2013;8(11):e80724. doi:10.1371/journal.pone.0080724

51. Simon JA, Marrotte RR, Desrosiers N, et al. Climate change and habitat fragmentation drive the occurrence of Borrelia burgdorferi, the agent of Lyme disease, at the northeastern limit of its distribution. *Evol Appl*. 2014;7(7):750-764. doi:10.1111/eva.12165

52. Kilpatrick AM, Dobson ADM, Levi T, et al. Lyme disease ecology in a changing world: consensus, uncertainty and critical gaps for improving control. *Philos Trans R Soc Lond B Biol Sci*. 2017;372(1722). doi:10.1098/rstb.2016.0117

53. Elväng A, Melik W, Bertrand Y, Lönn M, Johansson M. Sequencing of a tick-borne encephalitis virus from *Ixodes ricinus* reveals a thermosensitive RNA switch significant for virus propagation in ectothermic arthropods. *Vector Borne Zoonotic Dis*. 2011;11(6):649-658. doi:10.1089/vbz.2010.0105

54. Friedsam AM, Brady OJ, Pilic A, Dobler G, Hellenbrand W, Nygren TM. Geo-Spatial Characteristics of 567 Places of Tick-Borne Encephalitis Infection in Southern Germany, 2018-2020. *Microorganisms.* 2022;10(3). doi:10.3390/microorganisms10030643

55. Alkishe AA, Peterson AT, Samy AM. Climate change influences on the potential geographic distribution of the disease vector tick *Ixodes ricinus*. *PLoS One*. 2017;12(12):e0189092. doi:10.1371/journal.pone.0189092

56. Jaenson TG, Jaenson DG, Eisen L, Petersson E, Lindgren E. Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. *Parasit Vectors*. 2012;5:8. doi:10.1186/1756-3305-5-8

57. Heinz FX, Stiasny K, Holzmann H, et al. Emergence of tick-borne encephalitis in new endemic areas in Austria: 42 years of surveillance. *Eurosurveillance.* 2015;20(13):21077. doi:10.2807/1560-7917.ES2015.20.13.21077

58. Beermann S, Dobler G, Faber M, et al. Impact of climate change on vector- and rodent-borne infectious diseases. *J Health Monit*. 2023;8(Suppl 3):33-61. doi:10.25646/11401

59. Walter M, Brugger K, Rubel F. The ecological niche of *Dermacentor marginatus* in Germany. *Parasitol Res*. 2016;115(6):2165-2174. doi:10.1007/s00436-016-4958-9

60. Kunze M, Erber W, Haditsch M. TBE as a matter of public health. Chapter 13. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. The TBE Book. 6th ed. Global Health Press, Singapore, 2023. doi: 10.33442/26613980_13-6

61. Ruzek D, Kaucka K. A brief tale of two pioneering moments: Europe's first discovery of Tick-Borne Encephalitis (TBE) virus beyond the Soviet Union and the largest alimentary TBE outbreak in history. *Ticks Tick Borne Dis*. 2024;15(3):102314. doi:10.1016/j.ttbdis.2024.102314

62. Buczek AM, Buczek W, Buczek A, Wysokińska-Miszczuk J. Food-Borne Transmission of Tick-Borne Encephalitis Virus—Spread, Consequences, and Prophylaxis. *Int J Environ Res Public Health*. 2022;19(3):1812. doi:10.3390/ijerph19031812

63. Elbaz M, Gadoth A, Shepshelovich D, Shasha D, Rudoler N, Paran Y. Systematic Review and Meta-analysis of Foodborne Tick-Borne Encephalitis, Europe, 1980-2021. *Emerg Infect Dis.* 2022;28(10):1945-1954. doi:10.3201/eid2810.220498

64. Zimmermann H, Koch D. [Epidemiology of tick-borne encephalitis (TBE) in Switzerland 1984 to 2004]. Ther Umsch. 2005;62(11):719-725. doi:10.1024/0040-5930.62.11.719

65. Altpeter E, Zimmermann H, Oberreich J, Péter O, Dvořák C, Network SSS. Tick related diseases in Switzerland, 2008 to 2011. *Swiss Medical Weekly*. 2013;143(0102):w13725-w13725. doi:10.4414/smw.2013.13725

66. Schuler M, Zimmermann H, Altpeter E, Heininger U. Epidemiology of tick-borne encephalitis in Switzerland, 2005 to 2011. *Eurosurveillance*. 2014;19(13). doi:10.2807/1560-7917.ES2014.19.13.20756

67. Pilz A, Erber W, Schmitt HJ. Vaccine uptake in 20 countries in Europe 2020: Focus on tick-borne encephalitis (TBE). *Ticks and Tick-borne Diseases*. 2023;14(1):102059. doi:10.1016/j.ttbdis.2022.102059

68. Baroutsou V, Zens KD, Sinniger P, Fehr J, Lang P. Analysis of Tick-borne Encephalitis vaccination coverage and compliance in adults in Switzerland, 2018. *Vaccine*. 2020;38(49):7825-7833. doi:10.1016/j.vaccine.2020.10.022

69. Krawczuk K, Czupryna P, Pancewicz S, Ołdak E, Moniuszko-Malinowska A. Comparison of tick-borne encephalitis between children and adults—analysis of 669 patients. *J Neurovirol*. 2020;26(4):565-571. doi:10.1007/s13365-020-00856-x

70. Kohlmaier B, Schweintzger NA, Sagmeister MG, et al. Clinical Characteristics of Patients with Tick-Borne Encephalitis (TBE): A European Multicentre Study from 2010 to 2017. *Microorganisms*. 2021;9(7):1420. doi:10.3390/microorganisms9071420

71. Nygren TM, Pilic A, Böhmer MM, et al. Tick-borne encephalitis: Acute clinical manifestations and severity in 581 cases from Germany, 2018–2020. *Journal of Infection*. 2023;86(4):369-375. doi:10.1016/j.jinf.2023.02.018

72. Nygren TM, Pilic A, Böhmer MM, Wagner-Wiening C, Wichmann O, Hellenbrand W. Recovery and sequelae in 523 adults and children with tick-borne encephalitis in Germany. *Infection.* 2023;51(5):1503-1511. doi:10.1007/s15010-023-02023-w

73. Caracciolo I, Bassetti M, Paladini G, et al. Persistent viremia and urine shedding of tick-borne encephalitis virus in an infected immunosuppressed patient from a new epidemic cluster in North-Eastern Italy. *J Clin Virol*. 2015;69:48-51. doi:10.1016/j.jcv.2015.05.019

74. de Bruijn M, van der Lely N, Marcelis J, Roks G. ['Tick-borne' encephalitis in an immunocompromised patient]. *Ned Tijdschr Geneeskd*. 2015;159:A9067.

75. Chmelík V, Chrdle A, Růžek D. Fatal tick-borne encephalitis in an immunosuppressed 12-year-old patient. *Journal of Clinical Virology*. 2016;74:73-74. doi:10.1016/j.jcv.2015.11.029

76. Lipowski D, Popiel M, Perlejewski K, et al. A Cluster of Fatal Tick-borne Encephalitis Virus Infection in Organ Transplant Setting. *The Journal of Infectious Diseases*. 2017;215(6):896-901. doi:10.1093/infdis/jix040

77. Askling HH, Insulander M, Hergens MP, Leval A. Tick borne encephalitis (TBE)-vaccination coverage and analysis of variables associated with vaccination, Sweden. *Vaccine*. 2015;33(38):4962-4968. doi:10.1016/j.vaccine.2015.07.030

78. Riccò M, Corrado S, Marchesi F, Bottazzoli M. Tick-Borne Encephalitis Virus Vaccination among Tourists in a High-Prevalence Area (Italy, 2023): A Cross-Sectional Study. *Trop Med Infect Dis*. 2023;8(11):491. doi:10.3390/tropicalmed8110491

79. Nygren TM, Pilic A, Böhmer MM, et al. Tick-borne encephalitis vaccine effectiveness and barriers to vaccination in Germany. *Sci Rep*. 2022;12:11706. doi:10.1038/s41598-022-15447-5

80. Stefanoff P, Rosinska M, Samuels S, White DJ, Morse DL, Randolph SE. A National Case-Control Study Identifies Human Socio-Economic Status and Activities as Risk Factors for Tick-Borne Encephalitis in Poland. *PLoS One*. 2012;7(9):e45511. doi:10.1371/journal.pone.0045511

81. Caputo M, Stumpe V, Rübsamen N, Mikolajczyk RT, Karch A. Implementation of preventive measures against tick-borne infections in a non-endemic area for tick-borne encephalitis—Results from a population-based survey in Lower Saxony, Germany. *Ticks and Tick-borne Diseases*. 2019;10(3):614-620. doi:10.1016/j.ttbdis.2019.02.005

82. Kubinski M, Beicht J, Gerlach T, Volz A, Sutter G, Rimmelzwaan GF. Tick-Borne Encephalitis Virus: A Quest for Better Vaccines against a Virus on the Rise. *Vaccines (Basel).* 2020;8(3). doi:10.3390/vaccines8030451

83. Barrett PN, Schober-Bendixen S, Ehrlich HJ. History of TBE vaccines. *Vaccine*. 2003;21 Suppl 1:S41-9. doi:10.1016/s0264-410x(02)00814-9

84. Lu Z, Broker M, Liang G. Tick-borne encephalitis in mainland China. *Vector Borne Zoonotic Dis*. 2008;8(5):713-720. doi:10.1089/vbz.2008.0028

85. Xing Y, Schmitt HJ, Arguedas A, Yang J. Tick-borne encephalitis in China: A review of epidemiology and vaccines. *Vaccine*. 2017;35(9):1227-1237. doi:10.1016/j.vaccine.2017.01.015

86. Federal Office for Public Health Switzerland. *Federal Commission for Vaccination Issues, Swiss Vaccination Plan 2023*. Bundesamt für Gesundheit, Direktionsbereich Prävention und Gesundheitsversorgung, Abteilung Übertragbare Krankheiten; 2023:46. Accessed December 18, 2023. https://www.bag.admin.ch/bag/de/home/gesund-leben/gesundheitsfoerderung-und-praevention/impfungen-prophylaxe/schweizerischer-impfplan.html

87. *Finnisch Institute for Health and Welfare. Finnish National Vaccination Programme*.; 2023. Accessed December 29, 2023. https://thl.fi/en/web/infectious-diseases-and-vaccinations/information-about-vaccinations/finnish-national-vaccination-programme

88. *Public Service Health, Food Chain Safety and Environment, Belgium. Superior Health Council*. Vaccination against Tick-Borne Encephalitis (TBE). Brussels: SHC; 2019. Report 9435.; 2019. Accessed December 29, 2023. www.css-hgr.be

89. Sākumlapa | Slimību profilakses un kontroles centrs. Accessed March 10, 2024. https://www.spkc.gov.lv/lv?

90. Latvia extends encephalitis vaccine interval – Global Health Press. Accessed March 10, 2024. https://id-ea.org/latvia-extends-encephalitis-vaccine-interval/

91. Schelling J, Einmahl S, Torgler R, Larsen CS. Evidence for a 10-year TBE vaccine booster interval: an evaluation of current data. *Expert Review of Vaccines*. 2024;23(1):226-236. doi:10.1080/14760584.2024.2311359

92. Kind A, Ritzmann P, Marty F, Zimmermann H. Der Impfschutz gegen die Zeckenenzephalitis hält viel länger als bisher angenommen. Tick-borne encephalitis (TBE) - antibody titers and long-term immunity. Z Allg Med. 2008;84:153-156.

93. Steffen R, Erber W, Schmitt HJ. Can the booster interval for the tick-borne encephalitis (TBE) vaccine "FSME-IMMUN" be prolonged? - A systematic review. *Ticks Tick Borne Dis*. 2021;12(5):101779. doi:10.1016/j.ttbdis.2021.101779

94. Federal Office for Public Health Switzerland. Cantonal Vaccination Monitoring.; 2022. Accessed February 6, 2024. https://www.bag.admin.ch/bag/de/home/gesund-leben/gesundheitsfoerderung-und-praevention/impfungen-prophylaxe/informationen-fachleute-gesundheitspersonal/durchimpfung.html

95. Schmidt AJ, Altpeter E, Graf S, Steffen R. Tick-borne encephalitis (TBE) in Switzerland: does the prolongation of vaccine booster intervals result in an increased risk of breakthroughs? *J Travel Med*. 2022;29(2). doi:10.1093/jtm/taab158

96. Angulo FJ, Zhang P, Halsby K, et al. A systematic literature review of the effectiveness of tick-borne encephalitis vaccines in Europe. *Vaccine*. 2023;41(47):6914-6921. doi:10.1016/j.vaccine.2023.10.014

97. Kunz C. TBE vaccination and the Austrian experience. *Vaccine*. 2003;21 Suppl 1:S50-5. doi:10.1016/s0264-410x(02)00813-7

98. Heinz FX, Holzmann H, Essl A, Kundi M. Field effectiveness of vaccination against tick-borne encephalitis. *Vaccine*. 2007;25(43):7559-7567. doi:10.1016/j.vaccine.2007.08.024

99. Heinz FX, Stiasny K, Holzmann H, et al. Vaccination and tick-borne encephalitis, central Europe. *Emerg Infect Dis*. 2013;19(1):69-76. doi:10.3201/eid1901.120458

100. Chitimia-Dobler L, Lindau A, Oehme R, et al. Tick-Borne Encephalitis Vaccination Protects from Alimentary TBE Infection: Results from an Alimentary Outbreak. *Microorganisms*. 2021;9(5):889. doi:10.3390/microorganisms9050889

101. Pugh SJ, Moïsi JC, Kundi M, et al. Effectiveness of two doses of tick-borne encephalitis (TBE) vaccine. *J Travel Med*. 2022;29(2). doi:10.1093/jtm/taab193

102. Zens KD, Haile SR, Schmidt AJ, Altpeter ES, Fehr JS, Lang P. Retrospective, matched case-control analysis of tickborne encephalitis vaccine effectiveness by booster interval, Switzerland 2006-2020. *BMJ Open*. 2022;12(4):e061228. doi:10.1136/bmjopen-2022-061228

103. Zavadska D, Freimane Z, Karelis G, et al. Effectiveness of tick-borne encephalitis vaccination in Latvia, 2018-2020: an observational study. *Clin Microbiol Infect*. Published online July 6, 2023. doi:10.1016/j.cmi.2023.06.028

104. Zavadska D, Freimane Z, Karelis G, et al. Effectiveness of Tick-borne Encephalitis Vaccines in Children, Latvia, 2018-2020. *Pediatr Infect Dis J*. 2023;42(10):927-931. doi:10.1097/inf.0000000000004034

105. Kynčl J, Angulo FJ, Orlikova H, et al. Effectiveness of vaccination against tick-borne encephalitis in the Czech Republic, 2018-2022: an observational study. Submitted. Published online 2023.

106. Erber W, Schmitt HJ. Self-reported tick-borne encephalitis (TBE) vaccination coverage in Europe: Results from a cross-sectional study. *Ticks Tick Borne Dis*. 2018;9(4):768-777. doi:10.1016/j.ttbdis.2018.02.007

107. Zens Kyra D, Altpeter Ekkehardt, Wymann Monica N, Mack Annora, Baer Nora B, Haile Sarah R, Steffen Robert, Fehr Jan S, Lang Phung. A combined cross-sectional analysis and case-control study evaluating tick-borne encephalitis vaccination coverage, disease and vaccine effectiveness in children and adolescents, Switzerland, 2005 to 2022. Euro Surveill. 2024;29(18):pii=2300558. https://doi.org/10.2807/1560-7917.ES.2024.29.18.2300558

108. Rampa JE, Askling HH, Lang P, et al. Immunogenicity and safety of the tick-borne encephalitis vaccination (2009–2019): A systematic review. *Travel Medicine and Infectious Disease*. 2020;37:101876. doi:10.1016/j.tmaid.2020.101876

109. World Health Organization. Tick-borne encephalitis. Accessed March 11, 2024. https://www.who.int/health-topics/tick-borne-encephalitis

110. Ehrlich HJ, Pavlova BG, Fritsch S, et al. Randomized, phase II dose-finding studies of a modified tick-borne encephalitis vaccine: evaluation of safety and immunogenicity. *Vaccine*. 2003;22(2):217-223. doi:10.1016/S0264-410X(03)00563-2

111. Zent O, Banzhoff A, Hilbert AK, Meriste S, Służewski W, Wittermann C. Safety, immunogenicity and tolerability of a new pediatric tick-borne encephalitis (TBE) vaccine, free of protein-derived stabilizer. *Vaccine*. 2003;21(25):3584-3592. doi:10.1016/S0264-410X(03)00421-3

112. Pöllabauer EM, Fritsch S, Pavlova BG, et al. Clinical evaluation to determine the appropriate paediatric formulation of a tick-borne encephalitis vaccine. *Vaccine*. 2010;28(29):4558-4565. doi:10.1016/j.vaccine.2010.04.075

113. Wittermann C, Izu A, Petri E, Gniel D, Fragapane E. Five year follow-up after primary vaccination against tick-borne encephalitis in children. *Vaccine.* 2015;33(15):1824-1829. doi:10.1016/j.vaccine.2015.02.038

114. Pöllabauer EM, Pavlova BG, Löw-Baselli A, et al. Comparison of immunogenicity and safety between two paediatric TBE vaccines. *Vaccine.* 2010;28(29):4680-4685. doi:10.1016/j.vaccine.2010.04.047

115. Kaiser R. Frühsommermeningoenzephalitis im Kindes- und Jugendalter. *Monatsschr Kinderheilkd*. 2006;154(11):1111-1116. doi:10.1007/s00112-005-1184-4

116. Bogdanavičienė K, Gudavičiūtė G, Šeškutė M. A Retrospective Analysis of Tick-borne Encephalitis in Children Treated in Kaunas Hospital During 2012 to 2019. *The Pediatric Infectious Disease Journal*. 2022;41(9):702. doi:10.1097/INF.0000000000003595

117. Lesnicar G, Poljak M, Seme K, Lesnicar J. Pediatric tick-borne encephalitis in 371 cases from an endemic region in Slovenia, 1959 to 2000. *Pediatr Infect Dis J.* 2003;22(7):612-617. doi:10.1097/01.inf.0000073202.39700.a0

118. Krbková L, Štroblová H, Bednářová J. Clinical course and sequelae for tick-borne encephalitis among children in South Moravia (Czech Republic). *Eur J Pediatr*. 2015;174(4):449-458. doi:10.1007/s00431-014-2401-8

119. Bojkiewicz E, Toczylowski K, Grygorczuk S, et al. The Prevalence of Asymptomatic Infections with Tick-Borne Encephalitis Virus and Attitude towards Tick-Borne Encephalitis Vaccine in the Endemic Area of Northeastern Poland. *Vaccines*. 2022;10(8):1294. doi:10.3390/vaccines10081294

120. Sundin M. Chapter 6: TBE in children. TBE Book. Published December 28, 2016. Accessed February 13, 2024. https://tbenews.com/tbe/tbe6/

121. Fritsch P, Gruber-Sedlmayr U, Pansi H, et al. Tick-borne encephalitis in Styrian children from 1981 to 2005: a retrospective study and a review of the literature. *Acta Paediatrica*. 2008;97(5):535-538. doi:10.1111/j.1651-2227.2008.00763.x

122. Parfut A, Laugel E, Baer S, et al. Tick-borne encephalitis in pediatrics: An often overlooked diagnosis. *Infectious Diseases Now*. 2023;53(2):104645. doi:10.1016/j.idnow.2023.01.005

123. Stähelin-Massik J, Zimmermann H, Gnehm HE. Tick-Borne Encephalitis in Swiss Children 2000–2004: Five-Year Nationwide Surveillance of Epidemiologic Characteristics and Clinical Course. *The Pediatric Infectious Disease Journal*. 2008;27(6):555. doi:10.1097/INF.0b013e318165c195

124. Iff T, Meier R, Olah E, Schneider JFL, Tibussek D, Berger C. Tick-borne meningo-encephalitis in a 6-week-old infant. *Eur J Pediatr*. 2005;164(12):787-788. doi:10.1007/s00431-005-1753-5

125. Schmolck H, Maritz E, Kletzin I, Korinthenberg R. Neurologic, Neuropsychologic, and Electroencephalographic Findings After European Tick-borne Encephalitis in Children. *J Child Neurol*. 2005;20(6):500-508. doi:10.1177/088307380502000606

126. Zenz W, Pansi H, Zoehrer B, et al. Tick-Borne Encephalitis in Children in Styria and Slovenia Between 1980 and 2003. The Pediatric Infectious Disease Journal. 2005;24(10):892. doi:10.1097/01.inf.0000180506.76201.43

127. Lindquist L, Vapalahti O. Tick-borne encephalitis. *The Lancet*. 2008;371(9627):1861-1871. doi:10.1016/S0140-6736(08)60800-4

128. Haglund M, Günther G. Tick-borne encephalitis—pathogenesis, clinical course and long-term follow-up. *Vaccine*. 2003;21:S11-S18. doi:10.1016/S0264-410X(02)00811-3

129. Karelis G, Bormane A, Logina I, et al. Tick-borne encephalitis in Latvia 1973–2009: epidemiology, clinical features and sequelae. *European Journal of Neurology*. 2012;19(1):62-68. doi:10.1111/j.1468-1331.2011.03434.x

130. Mickienė A, Laiškonis A, Günther G, Vene S, Lundkvist Å, Lindquist L. Tickborne Encephalitis in an Area of High Endemicity in Lithuania: Disease Severity and Long-Term Prognosis. *Clinical Infectious Diseases*. 2002;35(6):650-658. doi:10.1086/342059

131. Veje M, Nolskog P, Petzold M, et al. Tick-Borne Encephalitis sequelae at long-term follow-up: a self-reported case–control study. *Acta Neurologica Scandinavica*. 2016;134(6):434-441. doi:10.1111/ane.12561

132. Bogovič P, Stupica D, Rojko T, et al. The long-term outcome of tick-borne encephalitis in Central Europe. *Ticks and Tick-borne Diseases*. 2018;9(2):369-378. doi:10.1016/j.ttbdis.2017.12.001

133. Fowler Å, Forsman L, Eriksson M, Wickström R. Tick-Borne Encephalitis Carries a High Risk of Incomplete Recovery in Children. *The Journal of Pediatrics*. 2013;163(2):555-560. doi:10.1016/j.jpeds.2013.01.037

134. Engman ML, Lindström K, Sallamba M, et al. One-year Follow-up of Tick-borne Central Nervous System Infections in Childhood. *The Pediatric Infectious Disease Journal*. 2012;31(6):570. doi:10.1097/INF.0b013e31824f23c0

135. Henrik U, Åsa F, Ronny W. Increased working memory related fMRI signal in children following Tick Borne Encephalitis. *European Journal of Paediatric Neurology*. 2016;20(1):125-130. doi:10.1016/j.ejpn.2015.09.004

136. UN Tourism Dashboard. Global and regional tourism performance. Available online: Accessed January 30, 2024. https://www.unwto.org/tourism-data/global-and-regional-tourism-performance

137. Banzhoff A, Bröker M, Zent O. Protection against tick-borne encephalitis (TBE) for people living in and travelling to TBE-endemic areas. *Travel Medicine and Infectious Disease*. 2008;6(6):331-341. doi:10.1016/j.tmaid.2008.06.011

138. Steffen R. Epidemiology of tick-borne encephalitis (TBE) in international travellers to Western/Central Europe and conclusions on vaccination recommendations. *Journal of Travel Medicine*. 2016;23(4):taw018. doi:10.1093/jtm/taw018

139. Mansfield KL, Johnson N, Phipps LP, Stephenson JR, Fooks AR, Solomon T. Tick-borne encephalitis virus – a review of an emerging zoonosis. *Journal of General Virology*. 2009;90(8):1781-1794. doi:10.1099/vir.0.011437-0

140. Chrdle A, Chmelík V, Růžek D. Tick-borne encephalitis: What travelers should know when visiting an endemic country. *Hum Vaccin Immunother.* 2016;12(10):2694-2699. doi:10.1080/21645515.2016.1218098

141. World Health Organization. Position paper on Tick-borne Encephalitis 2011. Accessed March 11, 2024. https://iris.who.int/bitstream/handle/10665/241769/WER8624_241-256.PDF?sequence=1

142. Federal Office for Public Health Switzerland. Zeckenübertragene Krankheiten – Lagebericht Schweiz. Accessed March 12, 2024. https://www.bag.admin.ch/bag/de/home/krankheiten/ausbrueche-epidemien-pandemien/aktuelle-ausbrueche-epidemien/zeckenuebertragene-krankheiten.html

143. Briggs BJ, Atkinson B, Czechowski DM, et al. Tick-Borne Encephalitis Virus, Kyrgyzstan. *Emerg Infect Dis*. 2011;17(5):876-879. doi:10.3201/eid1705.101183

144. Marano C, Moodley M, Melander E, De Moerlooze L, Nothdurft HD. Perceptions of tick-borne encephalitis risk: a survey of travellers and travel clinics from Canada, Germany, Sweden and the UK. *Journal of Travel Medicine*. 2019;26 (Supplement_1):S10-S16. doi:10.1093/jtm/tay063

145. Poulos C, Boeri M, Coulter J, Huang L, Schley K, Pugh SJ. Travelers' preferences for tick-borne encephalitis vaccination. *Expert Review of Vaccines*. 2022;21(10):1495-1504. doi:10.1080/14760584.2022.2108798

146. Hills SL, Broussard KR, Broyhill JC, et al. Tick-borne encephalitis among US travellers, 2010–20. *Journal of Travel Medicine*. 2022;29(2):taab167. doi:10.1093/jtm/taab167

147. Park M, Jit M, Wu JT. Cost-benefit analysis of vaccination: a comparative analysis of eight approaches for valuing changes to mortality and morbidity risks. *BMC Medicine*. 2018;16 (1):139. doi:10.1186/s12916-018-1130-7

148. Schwarz B. [Health economics of early summer meningoencephalitis in Austria. Effects of a vaccination campaign 1981 to 1990]. *Wien Med Wochenschr*. 1993;143 (21):551-555.

149. Smit R. Cost-effectiveness of tick-borne encephalitis vaccination in Slovenian adults. *Vaccine*. 2012;30(44):6301-6306. doi:10.1016/j.vaccine.2012.07.083

150. Haglund M, Forsgren M, Lindh G, Lindquist L. A 10-year follow-up study of tick-borne encephalitis in the Stockholm area and a review of the literature: need for a vaccination strategy. Scand J Infect Dis. 1996;28(3):217-224. doi:10.3109/00365549609027160

151. Cassini A, Colzani E, Pini A, et al. Impact of infectious diseases on population health using incidence-based disability-adjusted life years (DALYs): results from the Burden of Communicable Diseases in Europe study, European Union and European Economic Area countries, 2009 to 2013. *Euro Surveill*. 2018;23(16). doi:10.2807/1560-7917.Es.2018.23.16.17-00454

152. Fafangel M, Cassini A, Colzani E, et al. Estimating the annual burden of tick-borne encephalitis to inform vaccination policy, Slovenia, 2009 to 2013. *Euro Surveill.* 2017;22(16). doi:10.2807/1560-7917.Es.2017.22.16.30509

153. Cassini A, Colzani E, Kramarz P, Kretzschmar ME, Takkinen J. Impact of food and water-borne diseases on European population health. *Current Opinion in Food Science*. 2016;12:21-29. doi:10.1016/j.cofs.2016.06.002

154. Shedrawy J, Henriksson M, Hergens MP, Askling HH. Estimating costs and health outcomes of publicly funded tick-born encephalitis vaccination: A cost-effectiveness analysis. *Vaccine*. 2018;36(50):7659-7665. doi:10.1016/j.vaccine.2018.10.086

155. Cizman M, Rakar R, Zakotnik B, Pokorn M, Arnez M. Severe forms of tick-borne encephalitis in children. *Wien Klin Wochenschr*. 1999;111(12):484-487.

156. Prata JC, Ribeiro AI, Rocha-Santos T. Chapter 1 - An introduction to the concept of One Health. In: Prata JC, Ribeiro AI, Rocha-Santos T, eds. *One Health.* Academic Press; 2022:1-31. doi:10.1016/B978-0-12-822794-7.00004-6

157. Süss J. Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine*. 2003;21:S19-S35. doi:10.1016/S0264-410X(02)00812-5

158. Michelitsch A, Wernike K, Klaus C, Dobler G, Beer M. Exploring the Reservoir Hosts of Tick-Borne Encephalitis Virus. *Viruses*. 2019;11(7):669. doi:10.3390/v11070669

159. Gonzalez G, Bournez L, Moraes RA, et al. A One-Health Approach to Investigating an Outbreak of Alimentary Tick-Borne Encephalitis in a Non-endemic Area in France (Ain, Eastern France): A Longitudinal Serological Study in Livestock, Detection in Ticks, and the First Tick-Borne Encephalitis Virus Isolation and Molecular Characterisation. *Front Microbiol*. 2022;13:863725. doi:10.3389/fmicb.2022.863725

160. Süss J. Tick-borne encephalitis 2010: Epidemiology, risk areas, and virus strains in Europe and Asia—An overview. *Ticks and Tick-borne Diseases*. 2011;2(1):2-15. doi:10.1016/j.ttbdis.2010.10.007

161. Hofmeester TR, Sprong H, Jansen PA, Prins HHT, van Wieren SE. Deer presence rather than abundance determines the population density of the sheep tick, *Ixodes ricinus*, in Dutch forests. *Parasit Vectors*. 2017;10:433. doi:10.1186/s13071-017-2370-7

162. Jaenson TGT, Petersson EH, Jaenson DGE, et al. The importance of wildlife in the ecology and epidemiology of the TBE virus in Sweden: incidence of human TBE correlates with abundance of deer and hares. *Parasites & Vectors*. 2018;11 (1):477. doi:10.1186/s13071-018-3057-4

163. Kjær LJ, Johansson M, Lindgren PE, et al. Potential drivers of human tick-borne encephalitis in the Örebro region of Sweden, 2010–2021. *Sci Rep.* 2023;13(1):7685. doi:10.1038/s41598-023-34675-x

164. Rubel F, Brugger K. Operational TBE incidence forecasts for Austria, Germany, and Switzerland 2019-2021. *Ticks Tick Borne Dis*. 2021;12(1):101579. doi:10.1016/j.ttbdis.2020.101579

165. Kríz B, Benes C, Daniel M. Alimentary transmission of tick-borne encephalitis in the Czech Republic (1997-2008). *Epidemiol Mikrobiol Imunol*. 2009;58(2):98-103.

166. Kerlik J, Avdičová M, Štefkovičová M, et al. Slovakia reports highest occurrence of alimentary tick-borne encephalitis in Europe: Analysis of tick-borne encephalitis outbreaks in Slovakia during 2007–2016. *Travel Medicine and Infectious Disease*. 2018;26:37-42. doi:10.1016/j.tmaid.2018.07.001

167. French Agency for Food, Environmental and Occupational Health & Safety. Tick-borne encephalitis : tracing the origin of cases of transmission via cheese. Anses - Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail. Published October 4, 2022. Accessed March 11, 2024. https://www.anses.fr/en/content/tick-borne-encephalitis-transmission-cheese

168. 21bites. Embracing the Future of Food: Top Food Trends to Watch in 2024. 21bites. Accessed March 7, 2024. https://21bites.com/blogs/blog/embracing-the-future-of-food-top-food-trends-to-watch-in-2024

169. Slunge D. The Willingness to Pay for Vaccination against Tick-Borne Encephalitis and Implications for Public Health Policy: Evidence from Sweden. *PLoS One*. 2015;10(12):e0143875. doi:10.1371/journal.pone.0143875

170. Kollaritsch H, Chmelík V, Dontsenko I, et al. The current perspective on tick-borne encephalitis awareness and prevention in six Central and Eastern European countries: Report from a meeting of experts convened to discuss TBE in their region. *Vaccine*. 2011;29(28):4556-4564. doi:10.1016/j.vaccine.2011.04.061

Chapter 15

TBE vaccines licensed around the globe

Eva Maria Pöllabauer and Herwig Kollaritsch

Key Points

- Worldwide there are 6 different TBE vaccines – two from Western Europe, three from Russia and one from China. The two western European vaccines and one of the Russian vaccines have an adult and a pediatric formulation.
- The products names are FSME IMMUN and FSME-IMMUN Junior; Encepur adults and Encepur children, Klesch-E-Vac, EnceVir and EnceVir Neo, Dry lyophilized TBE Moscow and Sen Tai Bao.
- All TBE vaccines except the one from China have similar but not identical immunization schedules with primary immunization (3 to 4 doses according to vaccine) and regular booster vaccinations. For FSME-IMMUN, Encepur and EnceVir rapid immunization schedules are also licensed. The Chinese vaccine is given with 2 primary doses 2 weeks apart followed by annual boosters.
- Both - FSME-IMMUN and Encepur are well tolerated with a well-established safety profile. TBE-Moscow and EnceVir appear to be somewhat more reactogenic.
- All vaccines induce significant immune responses. In the absence of a formal correlate of protection, the presence of neutralizing antibodies is used as a surrogate marker for protection. More recent investigations indicate that in addition to the presence of neutralizing antibodies, immunologic memory and boostability seem to play a more important role than expected at time of first licensure.
- Clinical studies show long-term seropersistence of TBE antibodies after the first and subsequent booster vaccination with the two European vaccines.
- An effectiveness of approximately 99% (years 2000–2006) and 98.7% (years 2000-2011) was calculated for regularly vaccinated persons in Austria, a country with established high vaccination uptake. Recent studies show that vaccine effectiveness (VE) increases gradually with the number of vaccinations and seems to be optimal after 4 and more doses.
- Booster immunizations every 5 or 3 years, depending on age, are licensed beyond the 4th vaccination for the European vaccines. Recent data from Germany and Switzerland provide some evidence to support extension of booster intervals (up to ten years) for certain parts of the population.
- Whereas in Western Europe post-exposure prophylaxis with immunoglobulins was discontinued in the late 1990s, due to safety and efficacy concerns, in the highly endemic regions of Russia it continues to be common practice.

Active immunization

The first generation of TBE vaccines was produced in Russia. These vaccines were based on the TBEV-FE strain Sofjin, and were mouse-brain propagated. Over several decades, formulations and growth media were adapted step-by-step to result in the currently used TBE vaccines, details of which are summarized in Table 1. The two so called 'Western vaccines' are FSME-IMMUN, which is licensed through the mutual recognition procedure (MRP) of the European Medicines Agency (EMA), and Encepur, which has several national licenses. These two vaccines are distributed mainly in Europe and Israel, while the other TBE vaccines are predominantly produced for local markets. Since 2021 FSME-IMMUN is also licensed in the USA under the name TICOVAC.

Table 1: Basic characteristics of all licensed TBE vaccines

Vaccine name/ Manufacturer	FSME-IMMUN ® Pfizer	Encepur ® Bavarian Nordic	TBE-Moscow / Klesch-E-Vac Federal state scientific institution Chumakov	EnceVir® and EnceVir®Neo NPO Microgen	Dry -lyophilized TBE-Moscow scientific institution Chumakov	Sen Tai Bao Changchun Institute of Biological Products
Antigen						
Strain	TBEV-Eu Neudörfl	TBEV-Eu K23	TBEV-Fe Sofjin	TBEV-Fe Strain 205	TBEV-Fe Sofjin	TBEV-Fe, Mori-Jang
Passages	PCEC	PCEC	PCEC	PCEC	PCEC	NK
Production	PCEC	PCEC	PCEC	PCEC	PCEC	GKMC
Amount of antigen	2.4 µg adult / 1.2 µg children	1.5 µg adult / 0.75 µg children	0.5-0.75 µg (titer ≥ 1:128)	EnceVir® -0,6-3,0 µg/ EnceVir®Neo -0,3-1.5 µg	titer ≥1:128	Not specified
Excipients						
Adjuvant	$Al(OH)_3$	$Al(OH)_3$	$Al(OH)_3$	$Al(OH)_3$	$Al(OH)_3$	$Al(OH)_3$
Preservative	no	no	no	no	no	Thiomersal
Stabilizer	HSA	Sucrose	Sucrose, HSA	Sucrose, HSA	Sucrose, HSA	HSA
Presentation						
Formulation	0.5 mL/0.25 mL liquid	0.5 mL/0.25 mL liquid	0.5 mL/0.25 mL liquid	0.5 mL/0.25 mL liquid	0.5 mL Dry	5 mL multidose vials
Packaging	prefilled syringe	prefilled syringe	in ampoules	in ampoules	in ampoules	ampoules
Shelf-life	30 months (2°-8°C)	24 months (2°-8°C)	24 months (2°-8°C)	24 months (2°-8°C)	36 months (2°-8°C)	21 months in dark storage (2-8°C)

***Abbreviations**: HSA: Human Serum Albumin; PCEC: Primary Chicken Embryonic Cells; PHKC: primary hamster kidney cells; $Al(OH)_3$: Aluminum hydroxide; GKMC: Gopher Kidney Monolayer Cells; NK: Not known*

Manufacturer and products

TBE vaccines are produced commercially by five manufacturers. Two are produced in Europe, one by Pfizer (Vienna, Austria), one by GSK Vaccines (Marburg, Germany; bought by Bavarian Nordic, Kvistgaard, Denmark end 2019); 2 in Russia: IPVE (Moscow, Russia) and Microgen (Tomsk, Russia); and one in China: Sen Tai Bao (Changchun Institute of Biological Products Co., Ltd.; CIBP). The two manufacturers in Europe use very similar manufacturing processes but different virus strains and stabilizers. Both of them have licensed formulations for adults (Pfizer: FSME-IMMUN; Bavarian Nordic: Encepur) and for children older than one year (Pfizer: FSME-IMMUN Junior; Bavarian Nordic: Encepur-Children). FSME-IMMUN Junior is licensed for children up to and including 15 years of age, whereas Encepur-Children is licensed up to and including twelve years of age. In some countries, FSME-IMMUN is marketed as TicoVac. FSME-IMMUN, Encepur as well as EnceVir have (half dose) formulations for children and the TBE-Moscow vaccine is approved for use in children age 3 years or older. Human serum albumin (HSA) is used as a stabilizer by Pfizer, IPVE, CIBP, and Microgen, whereas Bavarian Nordic uses an increased amount of sucrose for this purpose. An overview of the excipients of the European and Russian vaccines is shown in Table 1.

FSME-IMMUN

This vaccine is based on the Austrian TBE strain Neudörfl (TBEV-Eu) and was licensed first in 1976. The virus was primarily passaged in the brains of specific pathogen-free (SPF) baby mice and then propagated in primary SPF chicken embryo cells. The vaccine formulation underwent several changes over subsequent decades until 2000. The actual licensed vaccine is a formaldehyde-inactivated, whole-virus vaccine (2.4 mcg antigen per dose), adjuvanted with aluminum hydroxide and containing HSA as an essential stabilizer. Details of the actual formulation are described in Table 1. A pediatric formulation containing half of the adult dose (FSME-IMMUN Junior) was licensed in 2002. The current manufacturer of FSME-IMMUN is Pfizer.

Encepur

This vaccine is based on the European subtype virus strain K23, isolated in Karlsruhe in southern Germany and originally licensed first in Germany in 1991 as Encepur by Chiron Behring, Marburg, Germany.[1] Similar to FSME-IMMUN, the seed virus for this vaccine is grown on primary chick embryo cells. The virus is inactivated by formaldehyde, adsorbed to aluminum hydroxide, and contains 1.5 mcg of antigen. A pediatric formulation containing half the adult dose (Table 1) has been available since 1994.[2] The genomic sequence of the K23 vaccine virus in the Encepur formulation has mutations compared to the originally published sequence.[90] However, the clinical impact of the modified primary amino acid sequence is unknown. In the year end of 2019 Bavarian Nordic acquired Encepur from GSK. According to communications by GSK and Bavarian Nordic, vaccine manufacturing will be transferred over the next 5 years, sales and marketing responsibility was assumed in 2020.

Russian vaccines

Three TBE vaccines have been developed and are marketed in Russia (see Chapter 12b: Russia). All of them are cultured on chick embryo cells and are formalin-inactivated. EnceVir, manufactured by Microgen, Tomsk, is based on the TBEV-FE subtype strain 205.[4]

There is a vaccine for adults (EnceVir (0.5) and as of 2014 also a pediatric formulation (EnceVir Neo (0.25) for children 3-17 years). Klesch-E-Vac is based on the TBEV-Fe prototype strain Sofjin, and manufactured by the Federal State Enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides (IPVE). It is provided as a suspension for injection.[3] Klesch-E-Vac has an adult (0.5mL) and also a pediatric formulation licensed for use as of 12 months to 16 years of age (half of the adult dose, i.e. 0.25 mL).

In addition, there is a dry-lyophilized TBE-Moscow vaccine (no specific trade name), based on the Sofjin strain.3 The producer is also the Federal State Enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides (IPVE). The product is approved for use in patients from 3 years of age as a unified formulation.

Sen Tai Bao

The Sen Tai Bao (Changchun Institute of Biological Products Co. Ltd: CIBP; in Changchun, Jilin Province, China) TBE vaccine is manufactured by the Changchun Institute of Biological Products (CIBP) and marketed in China only.[5] There a first vaccine against TBE was developed in 1953, by propagating the TBEV on mouse brain tissue followed by inactivation. It was an inactivated TBEV grown on infected mouse brain tissues. Between 1953 and now several vaccine formulations have been developed and used. Some of the earlier vaccines were grown on chicken embryo cells.[91] The current formalin-inactivated vaccine formulation is based on the TBEV-FE Mori-Jang strain, grown on monolayer gopher kidney cells. It uses HSA as the stabilizer and aluminum hydroxide as adjuvant and thiomersal as preservative. This vaccine has been approved for use in adults and children 8 years of age or older since 2004.[6] To reduce reactogenicity, it is recommended to add 0.2 mL of sodium bisulfite solution to each 5 mL dose, which will turn the color of the product from red to yellow. The vaccine should be administered subcutaneously into the lateral deltoid muscle region. First and second injections are

Table 2: Immunization schedules for TBE vaccines according to WHO recommendations

Dose 1 considered to be given on day „0“, intervals in table below given in months unless stated otherwise.

Vaccine schedule	Primary series*				Boosters
	Dose 1	Dose 2	Dose 3	Dose 4	Following doses
FSME-IMMUN *Regular*	Day 0	1-3 months	5-12 months	3 years	5 years (<60 years old)** (3 years if ≥60 years old)
FSME-IMMUN *Rapid*		14 days	5-12 months	3 years	5 years (<60 years old)** (3 years if ≥60 years old)
ENCEPUR *Regular*		2 weeks – 3 months	9-12 months	3 years	5 years (<60 years old)** (3 years if ≥60 years old)
ENCEPUR *Rapid*		Day 7	Day 21	12 – 18 months	5 years (<60 years old)** (3 years if ≥60 years old)
TBE-Moscow *Regular*		1-7 month	12 month	3 years	3 years
TBE-Moscow (only Klesch-E-vac) *Rapid*		14 days	12 month	3 years	3 years
EnceVir *Regular*		1-7 month	12 month	3 years	3 years
EnceVir *Rapid*		14 days	12 month	3 years	3 years
SenTai Bao		7-10 days	Boosters every year***		

* *Dose 3 resp. dose 4 have to be regarded immunologically as “first booster” doses if interval to second/third vaccine dose exceeds 4 months.*

** *50 years (instead of 60 years) in Germany*

*** *annual dose before the start of the season*

administered 7-10 days apart, the third and following doses are given annually. Dosing by age is done by volume adjustment, i.e. children 2-6 years receive 0.5 mL/dose; 7-10 years 1.0 mL/dose; and 11-15 years 1.5mL/dose. Subjects 16 years and older receive 2.0 mL, 3.0 mL and 3.0 mL as dose 1, 2, and 3, respectively.

Details on the schedules for the different licensed vaccines are summarized in Table 2. In brief, the basic immunization protocol for all vaccines consists of 3 doses (except the Sen Tai Bao, which has only 2 doses), similar to conventional immunization schedules with other aluminum-adjuvanted, inactivated vaccines: the first vaccination is followed by a second dose 4-12 weeks later, and a third shot is administered 5-12 months later. However, considerable differences still exist between vaccine brands, primarily based on the schedules used in licensing studies. Extension of intervals between doses, particularly after the second dose, will not hamper successful continuation of vaccination. For Encepur and FSME-IMMUN, a rapid or accelerated immunization schedule is licensed for children and adults (Table 2). In the context of the conventional immunization schedule for any of the 4 non-Chinese vaccine brands, the first TBE booster immunization is recommended 3 years following the third vaccination of the primary series. Subsequent boosters for the European vaccines are following the licensed schedules and recommend boosters

at intervals of 5 years in persons below 50 and 60 years of age for Encepur and FSME-IMMUN, respectively, and every 3 years for persons older than 50 or 60 years of age, respectively. Booster doses for the Russian vaccines are recommended every 3 years for all age groups. Switzerland and Finland changed their national immunization schedule to subsequent boosters every 10 years, supported by newer data (see below). In February 2024, Latvian health authorities also extended the recommended booster interval after the 4th dose to 10 years. The FDA licensed FSME-IMMUN, under the name TICOVAC, for the first time in 2021 in the USA for travelers and laboratory workers.[116] A respective ACIP (Advisory Committee on Immunization Practices) recommendation was published in the MMWR in 2023. Along with a detailed exposée on the TBE virus, the disease and diagnostics, disease incidence, vaccine immunogenicity and effectiveness, vaccine safety, etc., a recommendation for a primary immunization is provided. Recommended is a 3-dose schedule for both - the adult and pediatric formulations, similar to that licensed in Europe. A booster vaccination can be administered 3 years later, in case of ongoing exposure. No ACIP recommendations are made on the need for subsequent booster doses.

Contraindications and precautions

In general, for all TBE vaccines, hypersensitivity to the active substances, any of the excipients, or production residues constitutes a contraindication to immunization (Table 1). For the four non-Chinese TBE vaccines, severe hypersensitivity to egg, chicken proteins, or latex may cause severe allergic reactions in sensitized individuals. A moderate allergy to egg proteins (defined as hives after consumption/injection) does not constitute a contraindication for TBE vaccination with either vaccine. However, patients with moderate egg allergy should be monitored for one hour after application. Therefore, persons with proven “non-severe egg allergy” can receive a TBE vaccination. In case of a moderate or severe acute illness with or without fever, TBE vaccination should be postponed.

Previous exposure to other flaviviruses or flavivirus vaccines (for example, against Yellow fever [YF], Japanese encephalitis virus [JEV], or dengue virus) has been suggested to affect the immune response to TBE vaccination. While for a long time this was not adequately studied in humans, a new study became available in 2019[101], which investigated the influence of pre-existing YF vaccine-derived immunity on the antibody response to TBE vaccination. By comparing samples from YF pre-vaccinated and flavivirus-naive individuals, it could be shown that YF immunity not only caused a significant impairment of the neutralizing antibody response to TBE vaccination but also a reduction of the specific TBE virus neutralizing activities (NT and ELISA-titer ratios). Although the clinical relevance of these findings remains unclear, in practice, an increased awareness of the possible impact of pre-existing flavivirus immunity in the assessment of flavivirus vaccines appears to be warranted. In contrast, TBE vaccination has been shown to enhance the immune response to an inactivated JEV vaccine,[7] but even though cross-reactive antibodies have been described, there is no evidence of actual cross-protection between JEV and TBE vaccines.

For both European TBE vaccines, there is no data on their use during pregnancy and lactation. As with all other inactivated vaccines, vaccine administration during pregnancy may be considered after carefully weighing risk and benefit.

Vaccine stability and storage

FSME-IMMUN is available as a pre-filled syringe without needle. The vaccine must be refrigerated at 2°C to 8°C. The shelf life is 30 months. Encepur is available as a pre-filled syringe with and without needle and must be stored at the same temperature (between 2°C and 8°C). The shelf life is 24 months. TBE-Moscow vaccine has a shelf life of 24 months and EnceVir of 36 months, both with the same temperature requirements as the European vaccines. The currently licensed Chinese vaccine has a shelf life of 21 months.

Induction of immunity

No clinical studies with efficacy endpoints have been conducted on any of the licensed TBE vaccines. These vaccines have been registered on the basis of immunogenicity and safety studies, which consistently show a significant rise in neutralizing antibodies after primary vaccination with the vaccine. A Cochrane Collaboration review published in 2009 summarized 11 randomized clinical trials (10 publications), conducted with 3 different TBE vaccines (IPVE, FSME-IMMUN, and Encepur) and involving 8,184 subjects (6,586 adults and 1,598 children).[8] Overall seroconversion rates exceeding 87% were observed. Studies conducted by the respective manufacturers report seroconversion rates in the range of 92%–100% for Encepur and FSME-IMMUN, as measured by a commercial enzyme-linked immunosorbent assay (ELISA) or neutralization test (NT), with seroconversion being defined as NT =1:10, or according to the recommendations of the ELISA manufacturer.[9–12] “Low responders” after TBE vaccination are seen very rarely, there is no obvious “personal constellation” (except immunosuppression) that predisposes for insufficient immune response.[112]

Correlates of protection

Neutralizing antibodies directed against the protein E represent the most important mechanism of protection

against TBEV, not only after natural infection but also after vaccination, even if antibody responses in both cases differ.[39] According to the World Health Organization (WHO), in the absence of a formal correlate of protection for TBE vaccines, these neutralizing antibodies can be used as a surrogate marker for immunity.[33] Unfortunately, there is no generally accepted, standardized neutralization test nor are there any international reference reagents. In general, a titer ≥1:10 is considered seroprotective;[40] however, in the context of some vaccine licensure studies, titers of ≥1:2 were accepted as a correlate for a significant immune response.[41] Neutralization assays as used in various studies to determine seroprotection after vaccination differed to a large extent: their sensitivity differed and different test protocols were used, which makes a comparison of results difficult. There is only one occasion of directly comparable TBE antibody test results with standardized serum samples available and even in this study different NT test results were shown. Moreover, detection of virus-neutralizing antibodies in vitro was never correlated with serum antibody concentration in vivo necessary to achieve solid protection in a subject.

ELISA results are not suitable as reliable surrogate markers for neutralizing antibodies due to cross-reactivity with other flaviviruses (specifically antibodies resulting from infection or vaccination). Moreover, the ELISA assay does not distinguish between antibodies with low and high avidity, hence determining also antibodies without neutralizing capacity. Therefore, ELISA measurements are primarily useful for screening purposes. The HI test, which has been broadly used in the past, is no longer considered state of the art.

Clinical study program with the different brands

FSME-IMMUN

The clinical development program for FSME-IMMUN included 13 studies that investigated the immunogenicity and safety of the vaccine in approximately 5,180 adults and 6,430 children. An additional 4 studies on FSME-IMMUN were identified after review and analysis of published literature.[9] The seroconversion rate in adults 16 to 65 years of age, vaccinated according to the conventional schedule, was 97% after the second dose and ranged between 99.5% and 100% after the third dose, as measured by ELISA and/or NT.[9] When the rapid immunization schedule (Table 2) was used, seroconversion rates in NT after the second vaccination were 98.0% and 89.9% in adults younger or older than age 50, respectively, and 100% and 99.3% in those 2 age groups after the third vaccination, respectively. Two pediatric studies (a dose-finding study with more than 400 children who received the later licensed pediatric dose and a large safety study with an immunogenicity subset that included approximately 370 children, all between the ages of 1 and 15 years) found seroconversion rates (ELISA) of 96% to 100% (depending on the age sub-group) after the second vaccination and almost 100% in all age subgroups after the third vaccination.[13]

Another pediatric study investigated immune response in 149 and 152 children 1–11 years of age, who were vaccinated with FSME-IMMUN Junior and Encepur Children, respectively, in the context of a primary immunization schedule. According to the NT based on the Neudörfl strain, seropositivity rates after the second vaccination in the combined age groups was 100.0% in children who received FSME-IMMUN Junior and 97.8% in those who received 2 vaccinations with Encepur Children.[14] A third vaccination with FSME-IMMUN Junior induced 100% seropositivity in both study groups.[15]

An earlier pediatric study, which investigated the immune response in 334 children to both FSME-IMMUN Junior and Encepur Children for the first 2 vaccinations, using the conventional as well as the rapid immunization schedule, found higher seropositivity rates (NT ≥10) in the Encepur-immunized group versus the group that received FSME-IMMUN Junior, using either vaccination schedule. Upon completion of the primary vaccination course, and after the third dose (given with Encepur Children), >95% of all children achieved an NT ≥10.16 Both studies confirmed the interchangeability of the 2 TBE vaccines when given as a third dose in the context of a conventional or rapid primary immunization schedule.

Encepur

Data on the immunogenicity of Encepur from 8 clinical and post-marketing studies, which included 7,500 subjects, showed 100% seroconversion or a 4-fold rise in anti-TBEV antibodies after primary immunization.[17] Similar immunogenicity was achieved with either conventional or rapid immunization schedules (see Table 2).[12]

In 3 studies, comprising a total of 3,118 subjects between the ages of 12 and 76 years, the non-inferiority of the new polygeline-free formulation to the former vaccine containing polygeline was demonstrated.[18] In addition, the rapid immunization schedule using the new formulation was investigated.[17,19,20] The new formulation was also shown to be safe and immunogenic in a review of data from clinical trials and post-marketing experience in approximately 7,500 subjects aged 1 to 77 years.[20] The immunogenicity of the vaccine and the advantages of the rapid immunization schedule were further confirmed in a number of pediatric trials that enrolled more than 3,500 children 1–11 years of age.[21,22] The immunogenicity of the rapid schedule in children, as well as the interchangeability with FSME-IMMUN when given as a third dose, was shown by Wittermann et al.[23] Seropositivity rates of 99% and 100%

were determined at 3 and 5 years, respectively, after booster doses in children 1–11 years of age.[16]

Russian vaccines

The Russian vaccines, TBE-Moscow (Klesch-E-Vac) and EnceVir, have been evaluated in 2 clinical studies, each involving 200 adults. Antibody titers ≥1:80 (hemagglutination inhibition [HI] test) were detected following 2 doses, 2 or 5 months apart, in 84% and 93% of subjects receiving TBE-Moscow vaccine and in 82% and 89% of the vaccinees who received EnceVir, respectively.[24,25]

Another study with an age-stratified analysis of 325 subjects found at least a 4-fold increase of HI-antibody titers in 96%, 93%, and 89%, respectively, for each of 3 age groups: 3–6 years, 7–14 years, and 15–18 years, after vaccination with TBE-Moscow vaccine, versus 84%, 97%, and 92%, respectively, for the same age groups after receiving the EnceVir vaccine.[23]

No significant differences regarding immunogenicity against different TBEV strains could be found between TBE-Moscow vaccine and FSME Immun Inject (FSMEV propagated in mouse brain cells).[4] After 2 doses of the TBE-Moscow vaccine given 4 months apart, 92% of children and adolescents aged 7–17 years achieved a 4-fold rise in antibody levels compared with baseline.[4] Based on these results, the vaccine was recommended first for use in children and later for use in adults.[4]

A study comparing EnceVir and TBE-Moscow vaccine (N=400) found seropositivity (HI test) in 82% and 89% of patients, respectively, after 2 doses of EnceVir given 2 or 5 months apart, whereas the seropositivity rates with the TBE-Moscow vaccine were 84% and 93%, respectively.26–28 Furthermore, the 2 vaccines were also compared in 325 children who received 2 doses of either vaccine. A 4-fold rise in HI titer was achieved in 84% to 97% of the children with EnceVir and in 96% to 98% with TBE-Moscow vaccine, respectively.[29] Twelve months after the last dose of EnceVir or TBE-Moscow vaccine, 72% and 87%, respectively, of the vaccinated individuals were still seropositive. A booster response was efficacious in all of the 131 children who received a third dose 1 year after the first 2 vaccinations.[30]

In studies comparing the available Russian TBE vaccines, seroconversion rates of 59% and 83%, after 1 and 2 doses, respectively, were achieved with TBE-Moscow vaccine, versus 75% and 85%, respectively, with EnceVir.[31] Even without randomized controlled efficacy trials, the field effectiveness of the 2 Russian vaccines has been proven in highly endemic regions, e.g., in Krasnoyarsk and Sverdlovsk.[31–33, 102]

Sen Tai Bao

According to an English-language article summarizing five clinical studies investigating the current Chinese TBE vaccine in children 8–17 years of age (N=616), in adults <60 years of age (N≈5600), and in elderly individuals >60 years of age (N=166), seropositivity rates (as measured by plaque reduction neutralization test and/or ELISA) ranged between 86.4% and 98.8% after 2 doses.[6] In the group of subjects ≥60 years old, the seropositivity rate 28 days after the second vaccination was 97.3%. In one of the studies, seropersistence rates of 86.5% and 76.9% were observed 6 and 12 months after the second vaccination, respectively.

Comparative studies

There is only one study in which the immunogenicity of TBE-Moscow, EnceVir, FSME-IMMUN, and Encepur Adults was directly compared by using the Far-Eastern virus strain P-73 in adults.[34] All vaccines induced neutralizing antibodies against the tested strain with TBE-Moscow; neutralizing antibodies were detected in 100% and 94% of the vaccinees after 2–5 months and 2 years, respectively. With EnceVir, neutralizing antibody detection rates were 88% and 84%; with FSME-IMMUN, 88.2% and 78.1%; and with Encepur, 100% and 100%, respectively.

Irregular vaccination

Even irregular vaccination schedules confer good protection for the vaccinee. An investigation of the field effectiveness of TBE vaccination in Austria – a country in which 88% of the total population is vaccinated against TBE at least once and 58% is regularly vaccinated according to the recommended schedule – found an overall effectiveness in regularly vaccinated persons of about 99%, and 95% in subjects with a record of irregular vaccination.[35,36] A later investigation of the effectiveness of two or > 3 doses of a TBE vaccine found consistently high VE across both groups (94.5% and 97.4%, respectively).[103] These findings are especially important for travelers with insufficient time to complete the primary immunization schedule. Nevertheless, according to the ACIP recommendation for US travelers, the 3rd dose of the primary series should be completed at least one week before potential exposure. For persons who cannot complete the 3-dose primary series, a reference is made to immunogenicity and effectiveness after incomplete primary series (1 or 2 Doses) in Adults and Children.[116]

Furthermore, in a cohort study of more than 1,100 persons whose vaccination deviated from the recommended schedule, a single booster immunization with FSME-IMMUN was administered up to 20 years after 1, 2, or 3 primary vaccinations.[37] The results of this study demonstrated that, independent of the interval since last vaccination and the age of the vaccinee, a sufficient booster response was

induced if at least 2 or 3 primary vaccinations were previously administered.[37,38] In addition, similar results have been seen with Encepur, given as a catch-up vaccination after primary or primary + booster vaccination.[51] Altogether study results suggest that even initial irregular vaccination schedules do not implicate a complete "restart" of vaccination series, regular completion of vaccination course is sufficient to induce an adequate immune response.

Cell mediated immunity

Until recently little was known about the cellular immune response after TBE vaccination. Immunization with inactivated TBE vaccine has been reported to induce primarily a CD4+ T-cell response with a very low induction of CD8+ cells.[60,61] More recent investigations of TBE 'low-responders' after vaccination showed a positive correlation with humoral and cellular immune responses upon booster vaccination: high or low TBE titers were associated with sufficient or lack of Ag-specific T-cell proliferation, respectively.[62]

Research published in 2016 reported on the cellular immune response after a booster vaccination of FSME-IMMUN, administered by subcutaneous and intramuscular routes, revealing that interleukin-2 (IL-2), interferon (IFN) gamma, and interleukin-10 (IL-10) levels, produced upon antigen re-stimulation of peripheral blood mononuclear cells (PBMCs), were already elevated prior to vaccination.[63] This observation is in line with the fact that all study subjects had received multiple TBE vaccinations in the past and therefore had high numbers of TBE-specific effector memory T cells. Quantification of different T-cell subpopulations (naïve, memory, and suppressor T cells) before and 1 week after booster vaccination showed a relative decrease in regulatory T cells after vaccination. This is most likely due to an effector T-cell expansion induced by the booster vaccination and not the result of a decrease in the total number of regulatory T cells.[63] Moreover, the investigators observed an increase in the percentage of CD4+ T cells combined with a slight relative decrease of CD8+ T cells after intramuscular vaccination and a relative decrease of effector memory CD4+ T cells after subcutaneous vaccination. However, the observed changes in the CD4+ and CD8+ T-cell sub-populations were very small and had no influence on neutralizing antibody titers.[63] Whereas all these data were obtained after TBE booster immunization in previously vaccinated individuals, data are lacking on the cellular immune response in the context of TBE primary vaccination.

In order to provide an answer to this question the age-related differences in the humoral and cellular immune response after primary immunization was investigated using another flavivirus vaccine – an inactivated, adjuvanted Japanese Encephalitis vaccine.[109] Both, humoral and cellular immune responses were analyzed in elderly (mean age 69y) and younger (mean age 24y) subjects according to age and cytomegalovirus (CMV) seropositivity. A reduced humoral immune response was found in the elderly group. This was paralleled by a reduced cytokine production, such as Interferon gamma in vitro, as well as higher frequencies of late differentiated effector and effector memory cells and T regulatory cells. The described cellular changes combined with lower humoral responses were in particular prominent in CMV seropositive elderly people. The finding of this study, although based on results after JE-vaccination, once more confirms the importance of maintaining the existing booster intervals for individuals who were primed after the age of 60 years in order to ensure sufficient long-lasting protection.

Vaccine interchangeability and cross-protection

In general, it is preferred that the same vaccine brand is used for the complete primary immunization series. However, in order not to interrupt a vaccination series in case of unavailability of a certain vaccine, the immunization series can be completed with a different brand of TBE vaccine. Several studies confirmed that FSME-IMMUN and Encepur can be safely interchanged for the third vaccination in the context of the conventional primary immunization of adults and children, as well as for subsequent booster vaccinations.[11,15,23] In two studies – one in adults and one in children aged 12 years and younger - FSME-IMMUN was administered as the 3rd dose of the primary schedule after two doses of Encepur;[11,15] in a third pediatric study Encepur was given for the 3rd dose after two doses of FSME-IMMUN.[23]

A review describing 3 studies in which Encepur was given as a booster after a complete primary immunization with FSME-IMMUN (with or without booster) and further 3 studies in which Encepur or FSME-IMMUN was given for the third vaccination after two doses of the respective other brand in the context of the conventional schedule come to the same conclusion, irrespective of the somewhat differing immunogenicity results.[92] These differences, as mentioned several times throughout this chapter, are primarily due to the different test systems used – utilizing a homologous or heterologous TBE virus strain.

A switch from Encepur to FSME-IMMUN for the 3rd vaccination of the rapid immunization schedule (1-7-21), as well as a switch between first and second vaccination in the conventional schedule for FSME- IMMUN as well as for Encepur should be considered only under exceptional

circumstances, as these schedules are not licensed.

Evidence exists that TBE vaccines protect not only against the homologous subtype, but also against heterologous subtypes (European, Siberian, and Far-Eastern TBEV subtypes). In vitro and in vivo studies have shown broad cross-neutralizing capacity of vaccine-induced antibodies by either vaccine.[24,25,34,42,43] Moreover, a systematic review[44] published a few years ago supports robust cross-neutralization with the exception of 1 strain (TBEV-Fe P-69), for which a significantly lower level of neutralization was determined. In contrast, there is no evidence from human studies (except against Omsk HF)[43] that vaccine-induced TBEV antibodies provide cross-protection against other flaviviruses.

To overcome the problem of missing comparability data between immune responses to different TBEV strains, due to a poorly standardized methodology, a novel test system that uses hybrid viruses was developed; this system allows an unbiased head-to-head comparison of the humoral responses against different TBEVs from all 3 subtypes. Studies using this new technique have found comparable vaccine-induced neutralizing titers against TBEVs of all subtypes, in sera of subjects who received 2 doses of FSME IMMUN Junior, and somewhat reduced, but still protective, neutralization capacity against Omsk hemorrhagic fever virus (OHFV).[43] Another study found differences in the ability of 2 European pediatric TBE vaccines to induce antibodies capable of neutralizing heterologous TBEV strains.[45]

While it has been shown that an immunization with Encepur in subjects living in regions with Far Eastern TBEV circulation induced higher immune responses in originally seropositive as compared to seronegative individuals, similar data with vaccines based on the Far Eastern TBEV strains are limited.[94]

A recently published study found statistically significant differences in the immune response in subjects with pre-existing immunity to the TBEV FE strain Sofjin or Siberian strain Ekaterinburg-27-11-06 as compared to seronegative individuals, only after the first vaccination with one of the two Russian TBE vaccines (Tick-E-Vac based on FE strain Sofjin and EnceVir based on FE strain 205). After the second dose, the difference was insignificant.[95]

Antibody persistence and boosting properties

Up to the year 2004, 3-year booster intervals were recommended for the 2 European TBE vaccines. However, in 2004 and 2006 data suggesting a longer seropersistence became available.[38,46] Since then, studies investigating the seropersistence after primary and booster vaccinations with both European vaccines have been conducted.[16,19,47–49]

The seropersistence of TBEV antibodies in 347 adults between the ages of 18 and 67 years was evaluated 2 and 3 years after completion of the primary vaccination, with the first 2 doses being either FSME-IMMUN or Encepur. The third dose consisted of FSME-IMMUN for all study subjects.[50] Seropositivity rates of 96.8% and 95.4% were determined using NT 2 and 3 years after the third dose of the primary series, respectively. All subjects (100%) achieved seropositivity after the subsequently administered first booster vaccination.

A subsequent long-term investigation of seropersistence after an Encepur booster vaccine was initiated,47,48,52 and seropositive rates (SPR) were evaluated from 2 to 10 years after the booster was given. After 2, 3, and 4 years, SPR of 95.9%, 96.7%, and 93.8% were found. In subjects 50–60 and >60 years of age, SPR dropped after 4 years to 93.0% and 91.7% for the 2 age groups, respectively. After 5 and 6 years, SPR in subjects below age 60 dropped to 96% and 94%, while for subjects age 60 years and older, rates of 89% and 86% were detected, respectively. Geometric mean titers (GMTs) were also lower not only in subjects age 60 years and older, but also in subjects older than 50 years. At the end of the study, 8 and 10 years after the booster, SPR were 86.8% and 77.3%, with a pronounced age correlation, while in subjects younger than 50 years of age, seropositivity rates of 83.9% could be detected after 10 years. In the age group older than 50 years, only 66% of these subjects remained seropositive.[47] Similar to observations in young adults, seropersistence over a 5-year period was shown for adolescents who received their primary immunization according to different immunization schedules.[16,53]

A prospective investigation of seropersistence of TBE antibodies was published by Konior et al.[88] The study – a follow-up study of the one described above in 347 adults, investigated the seropersistence of TBE antibodies up to 10

years after a primary immunization and first booster with FSME-IMMUN. The necessity for a booster vaccination was evaluated on the basis of yearly NT determinations. As expected, the decrease in seropositivity was more pronounced in elderly as compared to younger individuals - the proportion of subjects left potentially unprotected by prolonging the booster interval beyond 5 years was 7% in the 18–49 years age group and 18% in the 50–60 years age group. By 10 years, these proportions increased to 11% and 26% in the 18–49 years and 50–60 years age groups, respectively. Nevertheless, overall, a total of only 47 subjects (14.9%) received the second booster dose over the follow-up period, and 84.9% of the study subjects were still seropositive after 10 years. Seropositivity rates were even higher (88.6%) in subjects below 50 years of age.

In a phase IV follow-up study published by Beran et al.[89] adults and adolescents who had received 3 different primary vaccination schedules (rapid, conventional and accelerated conventional) in a predecessor study and a booster dose 12-18 months or 3 years after the primary series were followed for the persistence of their TBE antibodies by yearly NT determinations. Overall, ≥97% of the study subjects in the per protocol set were seropositive (NT titers ≥10) across all timepoints, regardless of the primary vaccination schedule; however, older age groups showed overall lower GMTs.

Long-lasting seropersistence of TBEV antibodies after the first booster was confirmed also by a another published study98 investigating the antibody persistence in children, adolescents and young adults who received their primary immunization with FSME-IMMUN Junior when they were aged 1-15 years and an age-appropriate booster with either FSME-IMMUN or FSME-IMMUN Junior 4-5 years after the primary schedule. Seropositivity rates as determined by NT were 99.4% after 5 years and 90.3% after 10 years.

Furthermore, seropersistence of TBE antibodies after the 3rd dose of the primary immunization has been investigated 2 and 3 years thereafter: 50 subjects aged 18-50 years showed higher seropositivity rates (88.7% and 92.3%, after 2 and 3 years, respectively) than those aged 51-67 years (65.5% and 70.9% after 2 and 3 years, respectively), thus confirming the still existing manufacturer recommendation for the administration of the first booster dose 3 years after completion of the primary series.

The seropersistence studies with both European vaccines show long-term anti-TBEV antibody persistence after the first booster vaccination, especially in the population below 50-60 years of age, as well as excellent boostability in all age groups, indicating the establishment of a strong immune memory. It is not clear if permanent presence of neutralizing antibodies is a prerequisite for protection against clinical disease, as rapid recall of immune memory after vaccination may contribute as well to protection. However, there is no substantial evidence that immune memory alone will protect the patient from TBE in case of infection, particularly in the elderly and in immunocompromised persons.

More recent investigations in Germany and Latvia found high vaccine effectiveness after 2, 3 and > 4 doses not only for subjects vaccinated according to the licensed schedules, but also for those immunized outside the regular schedule, whereby delayed boostering did not cause significant differences in VE.

There is no data on long-term seropersistence for the 2 Russian and the Chinese vaccines. Twelve months after primary immunization, seropositivity rates of 72%, 87%, and 77% were determined for EnceVir, TBE-Moscow, and the Chinese Vaccine, respectively.[6]

Even before results on long term seropersistence became available, a recommendation for a 10-year booster interval starting directly after the 3rd vaccination of the primary series was introduced in 2006 in Switzerland. Meanwhile Finland, and very recently, in 2024, also Latvia adopted a 10-year booster interval recommendation, however, after the 4th dose. The primary goal of the change in Switzerland was to increase the vaccine coverage, which was achieved only to a moderate extent in some Swiss cantons in the years thereafter.[89] However, according to a more recent survey, a public health benefit resulting from an increased acceptability of TBE vaccination, was noted. [105]Nationwide, a coverage of 41.7% was found for 1 dose and 32.9% for a complete primary series. According to the authors 135 TBE cases were prevented in 2018 due to vaccination. A TBE incidence rate of 6.83/100,000 among the unvaccinated population was calculated and a VE of 91.5% was estimated. Furthermore, a retrospective analysis of surveillance data, collected between the years 2000 and 2019, which compared breakthrough infections and the breakthrough rate 0-3 years and >3-10 years after the 3rd dose across time periods and age groups, found no indication that

Table 3: Safety and Reactogenicity of FSME-IMMUN and Encepur (source: SMPCs)

Probability	≥1/10	≥1/100 <1/10	≥1/1000 <1/100	≥1/10.000 <1/1000	Not known
FSME-Immun 1st vaccination: n=3512 2nd vaccination: n=3477 3rd vaccination: n=3277	Local reaction at injection site: e.g., Injection-site pain	Headache, nausea, myalgia arthralgia, malaise, fatigue.	Lymphadeno-pathy, vomiting, fever (only exceptionally >39°C), injection-site hemorrhage.	Acute allergic reactions, somnolence, diarrhea, abdominal pain, vertigo, local reaction at injection site: redness, swelling, induration, pruritus, paraesthesia, inflammation	Herpes Zoster (in pre-exposed individuals), aggravation of autoimmune disease, anaphylactic reaction, visual impairment, photophobia, eye pain, demyelinating disorders, meningismus, encephalitis, neuritis, neuralgia, tachycardia, tinnitus, dyspnea, urticaria, rash, pruritus, dermatitis, erythema, hyperhidrosis, back pain, joint swelling, neck pain, musculoskeletal stiffness, pain in extremity, gait disturbance, chills, flu-like symptoms, weakness, edema
Encepur *(Pooled data from clinical studies and post-marketing surveillance)*	Transient pain at injection site, general malaise, myalgia, headache	Redness, swelling at injection site, flu-like symptoms, fever ≥38°, nausea, arthralgia	Arthralgia and myalgia (neck), vomiting	Granuloma at injection site, diarrhea, arthralgia and myalgia in the neck region, lymphadenopathy, neuritis-like symptoms, systemic allergic reactions - like urticaria, dyspnea, bronchospasm, hypotension, transient thrombocytopenia	Extremely rare: Guillain-Barre Syndrome

extended booster intervals resulted in higher rate of breakthrough infections. Moreover, a marked public health benefit was observed with respect to increased acceptability of TBE vaccination.[107] Nevertheless, due to the increased endemicity of TBE in Switzerland and the overall still low vaccine coverage, in 2019 vaccination recommendations were geographically extended to the entire country except the cantons of Geneva and Ticino.[97]

A systematic literature review[106] suggests that seropersistence alone does not explain the high effectiveness of TBE vaccination irrespective of the time since the last vaccine dose. While in >90% of younger subjects seropositivity persisted for more than 10 years, only 37.5% of subjects over 60 years of age were still seropositive, which is in contrast to the high VE, even in irregularly vaccinated individuals. The authors believe that Immunological memory is an alternative mechanism of action for protection against TBE, however, there is no proof for this assumption so far.

Vaccine effectiveness

Austria is a highly endemic country for TBE with a very long history of TBE immunization. Vaccination coverage has increased steadily since the 1970s, when the first TBE vaccine – FSME-Immun – was initially licensed. According to an investigation of the field effectiveness of TBE vaccines in Austria during the years 2000–2006, 88% of the Austrian population has a history of TBE vaccination, and 58% were

vaccinated according to the licensed schedule.[35] For the above-mentioned period, when FSME-IMMUN comprised 90% to 95% of the TBE vaccines administered in Austria, an effectiveness of approximately 99% was calculated for regularly vaccinated persons, with no statistically significant difference between age groups.[35] Not a single case of TBE was recorded within the first year after a documented history of 2 vaccinations, thus achieving a vaccine effectiveness of 100% after 2 vaccinations. A later investigation of vaccine effectiveness for the years 2000-2011[36] showed a slight decrease of vaccination coverage to 85% in 2011. Nevertheless, similarly high rates of effectiveness were seen: 98.7% and 96.3% for regularly vaccinated subjects under best- and worst-case assumptions, respectively, and 92.5% and 91.3% for irregularly vaccinated subjects under best- and worst-case scenarios, respectively. These findings highlight the importance of adhering to the recommended vaccination schedule in high-risk regions, as there is a considerably higher risk of acquiring TBE in irregularly vaccinated subjects. As a result of the high vaccination uptake in Austria, an estimated 4,000 TBE cases and 20 deaths were prevented between 2000 and 2011.35,36 During the same time, neighboring countries including the Czech Republic and Slovenia, which are also highly endemic for TBE but with very low vaccination coverage (16% in 2009 and 12% in 2008, respectively),[36,64] experienced an increase in disease incidence.

A recent survey conducted in Southern Germany and Latvia revealed a VE of 97.2%, 95.0% and 95.4% after 2, 3 and > 4 doses, respectively for Germany and 98.1%, 99.4% and 98.8%, respectively, for Latvia, among subjects vaccinated on schedule.[104] Only marginal differences in VE was observed for subjects vaccinated outside the regular schedule. According to the authors of this survey delayed timing after the 4th vaccination did not result in significant differences in VE for any age group.

As presented above, more recent investigations in Germany and Latvia found high vaccine effectiveness after 2, 3 and > 4 doses not only for subjects vaccinated according to the licensed schedules, but also for those immunized outside the regular schedule, whereby delayed boostering did not

Table 4: Post-exposure prophylaxis according to vaccination status

Vaccination history (written documentation)	Interval between last immunization and tick sting	Interval between tick sting and physicians visit[b]	Recommendation
Unvaccinated or unknown	Not applicable	<4 weeks	Wait until ≥4 weeks after sting, then initiate immunization series
1 dose	≤ 14 days	Not relevant	Wait until ≥4 weeks after sting, then administer 2nd dose
	15 days - 1 year	<48 hours	Administer 2nd dose immediately
		≥48 h	Wait until ≥4 weeks after sting, then administer 2nd dose[a]
	≥1 year	<48 h	Administer 2nd dose immediately[a]
		≥ 48 h	Wait until ≥4 weeks after sting, then administer 2nd dose[a]
≥2			Additional vaccination according to regular schedule

Austrian Immunization Plan 2017[79] (http://www.bmgf.gv.at/cms/home/attachments/2/8/1/CH1100/CMS1452867487477/impfplan.pdf)

[a] *Testing of antibody response recommended. If not possible, count this vaccination as the first one in basic immunization schedule*

[b] *If time elapsed is not to be determined, use schedule: >48 h after tick bite*

cause significant differences in VE.

A systematic literature review of the effectiveness of tick-borne encephalitis vaccines in Europe identified a total of 13 studies, conducted in Austria, the Czech Republic, Latvia, Germany and Switzerland, published between 2003 and 2023. TBE vaccine effectiveness was estimated >92% against TBEV infection in all age groups. Studies in Austria, the Czech Republic, Latvia, and Switzerland estimated that TBE vaccines prevented >1,000 TBE cases a year.[114] An Abstract Disposition Report from Boston, Massachusetts, October, 2023, on effectiveness of vaccination in the Czech Republic between 2018 and 2022 concluded that TBE vaccination averted an estimated 1,020 TBE cases in the Czech Republic in the investigated time period.[115]

Based on the meanwhile accumulating amount of vaccine effectiveness data, a prolongation of the booster intervals appears feasible, especially for younger and fully immunocompetent persons. Primarily in countries with very low vaccination coverage this could have a positive effect. A potential negative effect for countries with very well-established vaccination programs and high vaccination uptake should be avoided through appropriate national recommendations. Such recommendations have however, to take carefully into account individual risk factors as well as the local epidemiological situation. Important points to consider in this regard are immunocompetence and age. Therefore, a general prolongation of booster intervals seems well reasonable only after the 4th dose, especially for subjects who received their primary vaccination after the age of 60 years, as the formation of immune memory is impaired with increasing age.[111] This is supported by a Swedish study on vaccination failures (see below), which indicates that additional vaccinations in the elderly might overcome the problem of an age-related impaired immune response[99].

Impaired immune response

Most of the studies conducted in elderly individuals have shown consistently lower antibody concentrations compared with younger age groups.[54-57] A cross-sectional study from the highly endemic Åland Islands found that age of the individual and number of vaccine doses were the 2 most important factors for determining the immune response to vaccination.[50,55] The majority of these studies included subjects who received their primary vaccination series below the age of 50 years, which might have influenced the duration of seropositivity and B-cell memory.[47,53] This is well in accordance with data on vaccine failures, which are significantly more often seen in older persons. Unfortunately, few data exist on primary vaccination in individuals of more advanced age and eventual immunological consequences.

An observational study with FSME-IMMUN and Encepur administered to previously unvaccinated elderly subjects reported seropositivity rates of 95% and 80%, respectively, for subjects vaccinated with FSME-IMMUN (as measured by the Immunozym and Enzygnost ELISA Kits) and 65% and 80%, respectively, for subjects vaccinated with Encepur (as measured by the Immunozym and Enzygnost ELISA Kits).[56] This study illustrates not only the reduced immune response after TBE vaccination seen in the elderly population, but it also gives evidence for the dependence of serologic results on the commercial ELISA test systems. Unfortunately, NT was not evaluated in the context of this study. Another study, which compared the primary immune response in older and younger subjects, showed that those primed after the age of 50 years achieve not only lower titers but also experience a more rapid decline of neutralizing antibodies as compared to subjects primed at a younger age. Of note, almost no difference in the booster response was found between the 3 older age groups: 50–59 years, 60–69 years, and >69 years of age, indicating that responsiveness to vaccination is impaired already by the age of 50.[54]

The immune response to a conventional primary immunization schedule with FSME-IMMUN in previously unvaccinated subjects >70 years of age was investigated in another study.[58] Four weeks after the second and third vaccinations, 98.5% and 99.3% of subjects were seropositive (≥10) by NT, even if GMTs were generally lower. Although antibody concentrations are lower in the elderly, booster doses have been shown to increase sufficiently the antibody levels, indicating an adequate immune memory response in the elderly population as well. Moreover, the quality of antibodies as measured by antibody avidity were shown to be intact despite the lower antibody titers.[59]

Due to the concern of waning immune response with age, a Swedish study investigated the immunogenicity in subjects > 50 years of age using the standard 3-dose primary

schedule and alternatively two different 4-dose schedules (0-7-21-360 or 0-30-90-360).[108] Immune response was measured by NT at days 0-60, 120, 360 and 400. The 0-7-21-360 schedule showed higher titers in the older age group than the standard 3-dose schedule for all investigated timepoints. The second 4-dose schedule did not show such differences on day 400.

All findings described above underscore the importance of establishing well differentiated and personalized vaccination recommendations, which allow safe extension of booster intervals in order to simplify immunization schedules and improve vaccine coverage in affected geographies on the one hand, but, on the other hand, not increasing the risk of being insufficiently protected for immunocompromised groups of the population or subjects who received their primary immunization after the age of 60 years. Furthermore, if prolonged booster intervals should be applied, additional data are also needed for children, particularly when the primary vaccination course is applied at a very young age.[113] In these children an additional dose, for instance at school entry, could be considered, assuming that an interval of at least 3 years since the primary vaccination has passed.

In the context of a mass immunization program that started in 1996 in the highly endemic region of Sverdlovsk in Russia, an impressive decrease in TBE incidence could be achieved – from 42.1/100,000 in 1996 to 9.7/100,000 in 2000 to 5.1/100,000 in 2006. The vaccines used were TBE-Moscow (market share 80%); EnceVir (market share 6%); FSME-IMMUN (market share 12%); and Encepur (market share 2%). Based on these data, an overall vaccine effectiveness of 62% and 89% was estimated for the years 2000 and 2006, respectively.[31] Nevertheless, rare cases of TBE breakthrough disease, primarily in subjects older than 50 years of age, have been reported after primary TBE vaccination but not after booster immunization.[65-68]

No effectiveness data are available for the Chinese vaccine. There is only a single report, from the Center for Disease Control and Prevention, of the Hailar Railway, which showed that since the use of the current generation TBE vaccine, no TBE cases had been reported in 2009 and 2010.[6] However, details of the vaccination program (vaccination schedule, type of surveillance, etc.) are largely unknown.

Vaccine failures

Vaccine failures have been reported only occasionally. A retrospective investigation of breakthrough cases over a period of 8 years was conducted in Sweden.[65] During this period, 19 verified and 8 probable cases of TBE vaccine failures were reported. No accepted and plausible rationale exists to explain the immunological mechanisms leading to a vaccination failure. Therefore, it is not clear whether primary low-level responsiveness after regular TBE vaccination may be a risk factor for vaccine breakthrough. In contrast to unvaccinated subjects, most patients with breakthrough disease already had high antibody avidity and strong neutralizing antibodies in the first sample taken after hospitalization. When combined with an observed delayed immunoglobulin M (IgM) antibody response, and therefore presenting the features of an anamnestic response, this immune profile was obviously not sufficient to prevent the disease.[68] In 2019 a second retrospective study[99] on vaccine breakthroughs in Sweden was published and identified particularly i) older age (over 50 years of age), ii) immunocompromising comorbidities and iii) number of preceding vaccinations as key parameters for a higher risk of vaccine failures. The authors recommend for those persons, who start with their primary immunization series after the age of 50 an “extra” priming dose to reduce this risk. In addition, this study could for the first time define the probability of vaccine failures with 5% in a vaccinated population. While the Swedish study found there is an indication for more severe disease courses in older age, a retrospective study on clinical severity of vaccine breakthroughs from Germany,[100] however, could not identify a higher risk of more severe clinical disease in these patients.

A more recent retrospective case-control study investigated the occurrence of severe and mild TBE in hospitalized vaccinated and unvaccinated patients in Austria from 2000 to 2018. Of 1,545 hospitalized patients, 206 were vaccinated; in those, a higher proportion of severe disease course was observed, especially in children.[110] According to the authors the higher proportion of severe courses is not the result of an increased risk associated with vaccination, but rather can be explained by the lower field effectiveness against severe than against mild disease. This difference is especially pronounced in children (Field Effectiveness of 82.7% for severe vs 94.7% for mild disease). Impressively, this study found that in Austria vaccinated patients with

TBE were significantly younger than non-vaccinated; the proportion of patients below the age of 16 years was 2-fold higher in the group of vaccinated than in unvaccinated patients. A potential explanation of this striking finding could be the pediatric dose (half of the adult dose). In this regard the authors examined records of TBE in vaccinated children before the introduction of the pediatric dose and found only 2 cases among vaccinated children between 1979 and 2003. Taking into account increased awareness and improved diagnostics, which could have influenced this difference over time, this finding should result in a special vigilance when considering prolongations of booster intervals for children. On the contrary, the authors of this study suggest adding an additional priming dose for children in order to confer protection against severe disease.

Safety and tolerability

The currently available European TBE vaccines have a well-established safety record.[8,33] Safety and tolerability have been investigated in a number of clinical studies conducted in children and adults. Broad experience also comes from the field, with extensive pharmacovigilance over many years. Over the past decades, TBE vaccine formulations have been refined, thereby significantly reducing reactogenicity. In contrast, little published data are available on the safety of the 2 Russian vaccines and almost no data are available on the Chinese vaccine.[69] Frequently reported reactions after TBE vaccination basically do not differ from those occurring after vaccination with other aluminum-adjuvanted vaccines, e.g., local pain, redness, and swelling at the injection site, as well as headache, fatigue, malaise, muscle pain, joint pain, and fever.

Safety has been investigated in the context of many clinical studies with FSME-IMMUN, involving more than 13,800 children and adults.[9-11,13,14,50] All adverse reactions observed during clinical studies and relevant reports to the pharmacovigilance departments of the manufacturers are summarized in the Summary of Product Characteristics, Table 3. The most frequently reported reactions to the vaccination are local pain (≥1/10), headache, fatigue, malaise, myalgia, and arthralgia (1/100 and <1/10), whereas the frequency of fever was uncommon (≥1/1,000 and <1/100). Adverse reactions to vaccination seen in children are similar to those observed in adults. However, children more frequently experience fever, especially young children after the first vaccination. In addition, young children commonly react to vaccination with irritability, appetite loss, and disturbed sleep.

Similarly, at least 4 clinical trials have established the safety profile of Encepur in children and adults[12,18,20,22] (Table 3). Similar to FSME-IMMUN, the most frequently reported reactions to vaccination with Encepur are local pain, malaise, myalgia, and headache (>10% of vaccinees), whereas local redness, swelling, flu-like symptoms, nausea, arthralgia, and fever (primarily after the first vaccination) were observed in 1–10% of the vaccinees.

As of 2002, 2 TBE pediatric vaccines, FSME-IMMUN Junior (Baxter) and Encepur Children (Novartis/GSK), were marketed and at that time a post-marketing sentinel study was carried out in Austria. The study was conducted by the Institute for Vaccine Safety of the Austrian Green Cross and included 500 selected pediatricians and general practitioners who generated data on more than 25,000 vaccinations (85% with FSME-IMMUN). A total of 107 adverse events (AEs) were reported, with 69 (64.5%) of these occurring in children below the age of 2 years; also, 75.8% of the AEs were reported in association with the first vaccination. Fever was reported in 63 cases; 45 of these cases were mild, 15 moderate, and 3 severe (fever >39.5° C).[70]

Data derived from spontaneous reporting to the pharmacovigilance departments of manufacturers of both vaccines (FSME-IMMUN, for the period between 2001 and 2009, and Encepur, for the period between 2002 and 2009) indicate comparable rates of serious AEs (1.57 per 100,000 doses administered).[41] According to safety grading, as published in a WHO position paper in 2011, currently available TBE vaccines are not causally associated with serious adverse vaccine reactions.[71] Finally, although the safety sections of the SMPCs for FSME IMMUN and Encepur show some differences, it can be concluded that both vaccines have a similar safety and reactogenicity profile.

According to the Russian National Regulatory Authority, both Russian vaccines – TBE-Moscow and EnceVir – are safe and well tolerated,[33,41] and their manufacturing process fulfills WHO standards. However, no official documentation of quality control exists and no published data from large, controlled safety trials are available. Small-scale observational studies with TBE-Moscow and EnceVir have suggested a moderate reactogenicity profile with no

significant differences between the 2 vaccines. Post-marketing surveillance data did not identify any serious AEs.[26,32,72]

A study in children between 7 and 17 years of age comparing TBE-Moscow vaccine and FSME-Immun (old formulation; adult dose used also for children) found that fever was reported more frequently with TBE-Moscow vaccine; however, the differences were not significant.[4]

A passive, post-marketing surveillance review of EnceVir did not reveal any serious AEs up to 2010.[72] In 2010 and 2011, some lots of EnceVir were associated with a high incidence of fever and allergic reactions, particularly in children and adolescents. As a result, these lots were withdrawn from the market and the vaccine indication was restricted to adults above the age of 17 years.[73]

No published safety data are available for the Chinese TBE vaccine.

Passive Immunization and post-exposure prophylaxis

For many years, passive immunization as well as post-exposure prophylaxis with TBEV IgG preparations (immune globulin concentrate) was a state of the art treatment following a tick bite in unvaccinated subjects in Europe and Russia. Administration of an immunoglobulin concentrate for passive immunization was expected to protect against disease. However, passive immunization was blamed for antibody-mediated enhancement (ADE) of TBE infection in children,[74] like ADE phenomena in Dengue infections. In the late 1990s, the use of these immunoglobulins after tick exposure in a TBE-endemic area was discontinued even if the enhancement of TBEV infection could not be proven, either in humans or in a mouse model.[75,76] In Russia, especially in the highly endemic regions, post-exposure prophylaxis with immunoglobulins continues to be common practice. Russian studies report that timely administration of specific immunoglobulin after a tick bite can prevent clinical disease in about 80% of cases. The recommended dose is 0.05 mL/kg body weight of TBE immunoglobulin, whereby the antibody titer should not be less than 1:80.[77,78] However, investigations of the TBE-specific neutralizing antibody titers in IVIG (intravenous immunoglobulin) preparations from different geographic regions showed significantly lower TBEV neutralization titers in Russian-IVIG preparations compared with European IVIG preparations.[78]

Post-exposure prophylaxis with TBE vaccines in persons with a tick bite has to take into account the vaccination status and the incubation period of the disease. An accepted approach is summarized in Table 4.[79]

TBE vaccination in special patient groups

Underlying medical conditions can influence the outcome of vaccination by reducing the immune response. Alternatively, vaccination can theoretically cause a deterioration or exacerbation of the underlying condition. Therefore, the decision to vaccinate or not in subjects with serious medical conditions must be based on a careful risk/benefit analysis. Several studies have investigated immune response effects or influence on the course of the disease in the context of TBE immunization.

A controlled trial on TBE vaccination in patients with multiple sclerosis found no association between the vaccination and disease activity (as detected by magnetic resonance imaging [MRI]), clinical relapse, or disease progression.[80]

Another study investigated the effect of TBE vaccination in medically immunosuppressed patients with rheumatoid arthritis.[81] The patients (N=66) received a TBE primary immunization series while they were on regular treatment with a tumor necrosis factor inhibitor (TNFi) and/or methotrexate (MTX) for at least 1 year. One month after the third dose, 39% (26/66) of the patients and 79% (44/56) of the healthy controls had seroprotective NT levels. The relatively low SPR observed in the control group may be attributed to the fact that 37 and 35 of the patients and controls, respectively, were 60 years of age and older. Interestingly, the group of patients receiving a combined treatment (TNFi + MTX) had a significantly lower protection rate compared with healthy controls (36% vs 87%), while rates in patients treated with only a single medication did not differ from those seen in healthy controls. The significant difference in SPR remained even when an additional priming dose was given to all patients and healthy controls who were ≥60 years old: 31% (9/29) in the patient group compared with 81% (17/21) in the control group. In addition, this study demonstrated that in older patients (>60 years of age) immunosenescence apparently added to the treatment effects, leading to seroconversion rates of only around 30% after 4 doses of TBE vaccine in patients with combined immunosuppressive treatments.

The effect of TBE vaccination using an abbreviated

immunization schedule was also compared in 31 heart transplant recipients, under cyclosporine-based immunosuppression, and 29 controls.[82] Immune response (seroconversion rates [SCRs] and GMTs) were markedly reduced in the transplant recipients as compared with the control group. Even though the vaccine used in this study is no longer on the market (previous generation of Encepur, stabilized with polygeline), the findings are consistent with more recent investigations.

Public health considerations

While no formal vaccine efficacy study has been conducted with any TBE vaccine, effectiveness and pharmacoeconomic studies have been conducted, and the evidence for the public health impact of TBE immunization is indisputable. The most impressive example can be obtained from Austria, a country with a longstanding tradition of TBE immunization and reliable epidemiological data since the early 1970s. Since that time, vaccination coverage has increased steadily with currently 85% to 88% of the population having received at least 1 dose of TBE vaccine.[36] As a result, disease incidence dropped from approximately 700 to fewer than 100 cases per year, while in neighboring countries, with low vaccine coverage, the disease incidence has increased (see chapter on epidemiology).

As TBE disease was believed to be less severe in children, some countries had recommendations for adults only. More recent publications on severe disease courses and underestimation of long-term sequelae in children have led to adaptations of the vaccination recommendations for children in some countries. For instance, in Sweden, the age cut-off was reduced in 2012 from 7 years to 3 years of age and in 2013 from 3 years to 1 year of age.

In 2011, the WHO published a position paper on TBE vaccination33 recommending vaccination of all age groups in areas of high pre-vaccination disease incidence, defined as an incidence of ≥5/100,000 population per year, while in regions with lower incidence, vaccination recommendations should be confined to groups of the population exposed to a particular risk. Furthermore, the WHO also recommends vaccination of travelers planning outdoor activities in endemic areas during the active tick season.[84] In 2012, TBE became notifiable on the European level at the European Centre for Disease Prevention and Control (ECDC), which is a further, important step towards comprehensive and continuous assessment of the disease epidemiology across Europe.

Based on the newly arising vaccine effectiveness data it appears strategically correct to prolong the booster intervals up to 10 years after the 4th dose for certain parts of the population. This would partly align the booster intervals with those of other routinely administered vaccines, leading to a simplification of immunization schedules for the TBE vaccine, but also in general, with the goal of improving vaccine uptake and coverage. For immunocompromised individuals and those who received their primary immunization after the age of 60 years the currently licensed intervals should remain valid. Due to the fact that respective data for children are still missing, the current intervals should remain valid for the pediatric population as well. Moreover, as recent retrospective investigations provide some indications that the pediatric dose might be insufficient to confer long-lasting protection against severe disease.

Little information is published on the economic burden of TBE disease. Based on the finding that the Austrian TBE vaccination campaigns for the period 1981–1990 led to a reduction of more than 50% of clinical TBE cases, a benefit of €24 million was calculated versus the pre-vaccination era. Using a linear trend prognostic model for the further decline of TBE cases while vaccination coverage reached 85% by 2000, the author concluded that for the period 1991 to 2000, a total cost saving of €60 million can be estimated.[83] Epidemiological trends and progress in vaccination coverage have confirmed these assumptions.36 The majority of endemic countries in Europe, as well as Russia, have TBE vaccination recommendations in place, targeting primarily at-risk groups. More recently, recommendations for travelers to endemic regions were issued in many countries (see Chapter 12b).

More recently, in 2018, a cost/benefit analysis became available for Sweden. In the highly endemic area of Stockholm, where the number of cases is increasing despite the increased uptake of TBE vaccines, earlier studies showed that low-income households have lower vaccination coverage even when they are at high risk. The newly performed analysis showed again in cost per QALY (Quality-adjusted Life Years) of a free vaccinations program for the Stockholm County, especially for children of 3 years old, below generally acceptable cost-effectiveness thresholds in Sweden.[96]

Contact: eva.poellabauer@meduniwien.ac.at

Citation:

Pöllabauer EM, Kollaritsch H. TBE vaccines licensed around the globe. Chapter 15. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 7th ed. Singapore: Global Health Press; 2023. doi: 10.33442/26613980_15-7

References

1. Klockmann U, Bock HL, Franke V, Hein B, Reiner G, Hilfenhaus J. Preclinical investigations of the safety, immunogenicity and efficacy of a purified, inactivated tick-borne encephalitis vaccine. *J Biol Stand*. 1989;17:331-42.
2. Girgsdies OE, Rosenkranz G. Tick-borne encephalitis: development of a paediatric vaccine. A controlled, randomized, double-blind and multicentre study. *Vaccine.* 1996;14:1421-8.
3. Vorob'eva MS, Rasshchepkina MN, Ladyzhenskaia IP. [Vaccines, immunoglobulins, and test systems for the prevention and diagnosis of tick-borne encephalitis]. *Vopr Virusol*. 2007;52:30-6.
4. Pavlova LI, Gorbunov MA, Vorob'eva MS, et al. [A cultured concentrated inactivated vaccine against tick-borne encephalitis studied during the immunization of children and adolescents]. *Zh Mikrobiol Epidemiol* Immunobiol. 1999:50-3
5. Lu Z, Bröker M, Liang G. Tick-borne encephalitis in mainland China. *Vector Borne Zoonotic Dis*. 2008;8:713-20.
6. Xing Y, Schmitt HJ, Arguedas A, Yang J. Tick-borne encephalitis in China: A review of epidemiology and vaccines. *Vaccine*. 2017;35:1227-37.
7. Schuller E, Klade CS, Heinz FX, et al. Effect of pre-existing anti-tick-borne encephalitis virus immunity on neutralising antibody response to the Vero cell-derived, inactivated Japanese encephalitis virus vaccine candidate IC51. *Vaccine*. 2008;26:6151-6.
8. Demicheli V, Debalini MG, Rivetti A. Vaccines for preventing tick-borne encephalitis. Cochrane Database *Syst Rev*. 2009:CD000977.
9. Loew-Baselli A, Poellabauer EM, Pavlova BG, et al. Prevention of tick-borne encephalitis by FSME-IMMUN® vaccines: review of a clinical development programme. *Vaccine*. 2011;29:7307- 19.
10. Ehrlich HJ, Pavlova BG, Fritsch S, et al. Randomized, phase II dose-finding studies of a modified tick-borne encephalitis vaccine: evaluation of safety and immunogenicity. *Vaccine*. 2003;22:217-23.
11. Loew-Baselli A, Konior R, Pavlova BG, et al. Safety and immunogenicity of the modified adult tick-borne encephalitis vaccine FSME-IMMUN: results of two large phase 3 clinical studies. *Vaccine*. 2006;24:5256-63.
12. Schöndorf I, Beran J, Cizkova D, Lesna V, Banzhoff A, Zent O. Tick-borne encephalitis (TBE) vaccination: applying the most suitable vaccination schedule. *Vaccine.* 2007;25:1470-5.
13. Pöllabauer EM, Fritsch S, Pavlova BG, et al. Clinical evaluation to determine the appropriate paediatric formulation of a tick- borne encephalitis vaccine. *Vaccine*. 2010;28:4558-65.
14. Pöllabauer EM, Pavlova BG, Löw-Baselli A, et al. Comparison of immunogenicity and safety between two paediatric TBE vaccines. *Vaccine*. 2010;28:4680-5.
15. Prymula R, Pöllabauer EM, Pavlova BG, et al. Antibody persistence after two vaccinations with either FSME-IMMUN® Junior or ENCEPUR® Children followed by third vaccination with FSME-IMMUN® Junior. *Hum Vaccin Immunother*. 2012;8:736-42
16. Wittermann C, Petri E, Zent O. Long-term persistence of tick-borne encephalitis antibodies in children 5 years after first booster vaccination with Encepur Children. *Vaccine*. 2009;27:1585-8.
17. Zent O, Bröker M. Tick-borne encephalitis vaccines: past and present. *Expert Rev Vaccines.* 2005;4:747-55.
18. Zent O, Beran J, Jilg W, Mach T, Banzhoff A. Clinical evaluation of a polygeline-free tick-borne encephalitis vaccine for adolescents and adults. *Vaccine.* 2003;21:738-41.
19. Zent O, Jilg W, Plentz A, et al. Kinetics of the immune response after primary and booster immunization against tick-borne encephalitis (TBE) in adults using the rapid immunization schedule. *Vaccine*. 2003;21:4655-60.
20. Zent O, Hennig R, Banzhoff A, Bröker M. Protection against tick-borne encephalitis with a new vaccine formulation free of protein-derived stabilizers. *J Travel Med*. 2005;12:85-93.
21. Schoendorf I, Ternak G, Oroszlàn G, Nicolay U, Banzhoff A, Zent O. Tick-borne encephalitis (TBE) vaccination in children: advantage of the rapid immunization schedule (i.e., days 0, 7, 21). *Hum Vaccin*. 2007;3:42-7.
22. Zent O, Banzhoff A, Hilbert AK, Meriste S, Sluzewski W, Wittermann C. Safety, immunogenicity and tolerability of a new pediatric tick-borne encephalitis (TBE) vaccine, free of protein-derived stabilizer. Vaccine. 2003;21:3584-92.
23. Wittermann C, Schöndorf I, Gniel D. Antibody response following administration of two paediatric tick-borne encephalitis vaccines using two different vaccination schedules. *Vaccine.* 2009;27:1661-6.
24. Leonova GN, Ternovoi VA, Pavlenko EV, Maistrovskaya OS, Protopopova EV, Loktev VB. Evaluation of vaccine Encepur Adult for induction of human neutralizing antibodies against recent Far Eastern subtype strains of tick-borne encephalitis virus. *Vaccine.* 2007;25:895-901.
25. Chiba N, Osada M, Komoro K, Mizutani T, Kariwa H, Takashima I. Protection against tick-borne encephalitis virus isolated in Japan by active and passive immunization. *Vaccine*. 1999;17:1532-9

26. Krasilnikov I, Mischenko IA, Sharova OI, Vorob'eva M. Development of technology of tick-borne encephalitis vaccine (strain 205). *Int J Med Microbiol.* 2002;291:173.

27. Gorbunov MA, Pavlova LI, Vorob'eva MS, Raschepkina MN, Stronin OB. Results of clinical evaluation of EncoVir vaccine against tick-borne encephalitis. *Epidem Vaccinoprophil*. 2002;5:49.

28. 28. Krasilnikov IV, Mischenko IA, Sharova OI, et al. Vaccine "EnceVir": development and implementation in practical use. *Biopreparations*. 2004;2:21-4.

29. Pavlova LI, Stavitskaya IV, Gorbunov MA, al e. Immunizations of children and adolescents with concentrated purified vaccines against TBE. *Biopreparations*. 2003;1:24-8.

30. Stavitskaya IV, Shkuratova OV, Pavlova LI, Shutova NA, Sharova OI. Immunological effectiveness of EnceVir in children. *Biopreparations*. 2004;2:34–6.

31. Romanenko VV, Esiunina MS, Kiliachina AS. [Experience in implementing the mass immunization program against tick-borne encephalitis in the Sverdlovsk Region]. *Vopr Virusol*. 2007;52:22-5.

32. Borodina TN, Evtoushok GA, Tevelenok OG, Opeikina NN. Epidemiological effectiveness of vaccination against TBE in Krasnoyarsk region. *Biopreparations*. 2004;2:30-1.

33. WHO. Vaccines against tick-borne encephalitis (TBE): WHO position paper, 10 June, 2011. *Wkly Epidemiol Rec*. 2011;86:241-56.

34. Leonova GN, Pavlenko EV. Characterization of neutralizing antibodies to Far Eastern tick-borne encephalitis virus subtype and the antibody avidity for four tick-borne encephalitis vaccines in humans. *Vaccine*. 2009;27:2899-904.

35. Heinz FX, Holzmann H, Essl A, Kundi M. Field effectiveness of vaccination against tick-borne encephalitis. *Vaccine*. 2007;25:7559-67.

36. Heinz FX, Stiasny K, Holzmann H, et al. Vaccination and tick-borne encephalitis, central Europe. *Emerg Infect Dis*. 2013;19:69-76.

37. Schosser R, Reichert A, Mansmann U, Unger B, Heininger U, Kaiser R. Irregular tick-borne encephalitis vaccination schedules: the effect of a single catch-up vaccination with FSME-IMMUN. A prospective non-interventional study. *Vaccine*. 2014;32:2375-81.

38. Rendi-Wagner P, Kundi M, Zent O, et al. Immunogenicity and safety of a booster vaccination against tick-borne encephalitis more than 3 years following the last immunisation. *Vaccine.* 2004;23:427-34.

39. Jarmer J, Zlatkovic J, Tsouchnikas G, et al. Variation of the specificity of the human antibody responses after tick-borne encephalitis virus infection and vaccination. *J Virol*. 2014;88:13845-57.

40. Holzmann H, Kundi M, Stiasny K, et al. Correlation between ELISA, hemagglutination inhibition, and neutralization tests after vaccination against tick-borne encephalitis. *J Med Virol*. 1996;48:102-7.

41. Kollaritsch H, Krasilnikov V, Holzmann H, et al. Background Paper on Vaccines and Vaccination Against Tick-borne Encephalitis (TBE). Geneva, WHO Strategic Advisory Group of Experts on Immunization 2011.

42. Holzmann H, Vorobyova MS, Ladyzhenskaya IP, et al. Molecular epidemiology of tick-borne encephalitis virus: cross-protection between European and Far Eastern subtypes. *Vaccine*. 1992;10:345-9.

43. Orlinger KK, Hofmeister Y, Fritz R, et al. A tick-borne encephalitis virus vaccine based on the European prototype strain induces broadly reactive cross-neutralizing antibodies in humans. *J Infect Dis*. 2011;203:1556-64.

44. Domnich A, Panatto D, Arbuzova EK, et al. Immunogenicity against Far Eastern and Siberian subtypes of tick-borne encephalitis (TBE) virus elicited by the currently available vaccines based on the European subtype: systematic review and meta-analysis. *Hum Vaccin Immunother*. 2014;10:2819- 33.

45. Beck Y, Fritz R, Orlinger K, et al. Molecular Basis of the Divergent Immunogenicity of Two Pediatric Tick-Borne Encephalitis Virus Vaccines. *J Virol*. 2015;90:1964-72.

46. Rendi-Wagner P, Zent O, Jilg W, Plentz A, Beran J, Kollaritsch H. Persistence of antibodies after vaccination against tick-borne encephalitis. *Int J Med Microbiol*. 2006;296 Suppl 40:202-7.

47. Paulke-Korinek M, Kundi M, Laaber B, et al. Factors associated with seroimmunity against tick-borne encephalitis virus 10 years after booster vaccination. *Vaccine.* 2013;31:1293-7.

48. Paulke-Korinek M, Rendi-Wagner P, Kundi M, Laaber B, Wiedermann U, Kollaritsch H. Booster vaccinations against tick-borne encephalitis: 6 years follow-up indicates long-term protection. *Vaccine.* 2009;27:7027-30.

49. Zent O, Plentz A, Schwarz TF, et al. TBE booster immunization according to the rapid immunization schedule: are 3-year booster intervals really necessary? *Vaccine.* 2004;23:312-5.

50. Loew-Baselli A, Poellabauer EM, Pavlova BG, et al. Seropersistence of tick-borne encephalitis antibodies, safety and booster response to FSME-IMMUN 0.5 ml in adults aged 18-67 years. *Hum Vaccin.* 2009;5:551-6.

51. Rendi-Wagner P, Kundi M, Zent O, et al. Persistence of protective immunity following vaccination against tick-borne encephalitis—longer than expected? *Vaccine.* 2004;22:2743- 9.

52. Rendi-Wagner P, Paulke-Korinek M, Kundi M, Wiedermann U, Laaber B, Kollaritsch H. Antibody persistence following booster vaccination against tick-borne encephalitis: 3-year post-booster follow-up. *Vaccine*. 2007;25:5097-101.

53. Beran J, Xie F, Zent O. Five year follow-up after a first

booster vaccination against tick-borne encephalitis following different primary vaccination schedules demonstrates long-term antibody persistence and safety. *Vaccine*. 2014;32:4275-80.

54. Weinberger B, Keller M, Fischer KH, et al. Decreased antibody titers and booster responses in tick-borne encephalitis vaccinees aged 50-90 years. *Vaccine*. 2010;28:3511-5.

55. Lindblom P, Wilhelmsson P, Fryland L, et al. Factors determining immunological response to vaccination against tick-borne encephalitis virus in older individuals. *PLoS One*. 2014;9:e100860.

56. Jilkova E, Vejvalková P, Stiborová I, Skorkovský J, Král V. Serological response to tick-borne encephalitis (TBE) vaccination in the elderly—results from an observational study. *Exp Opin Biol Ther*. 2009;9:797-803.

57. Hainz U, Jenewein B, Asch E, Pfeiffer KP, Berger P, Grubeck - Loebenstein B. Insufficient protection for healthy elderly adults by tetanus and TBE vaccines. *Vaccine*. 2005;23:3232 -5.

58. Wanke K, von Braun A, Häberli L, et al. Immunogenicity and safety of tick-borne encephalitis vaccination in healthy elderly individuals. Paper presented at: ECCMID, 2012; London, UK.

59. Stiasny K, Aberle JH, Keller M, Grubeck-Loebenstein B, Heinz FX. Age affects quantity but not quality of antibody responses after vaccination with an inactivated flavivirus vaccine against tick-borne encephalitis. *PLoS One*. 2012;7:e34145.

60. Aberle JH, Aberle SW, Kofler RM, Mandl CW. Humoral and cellular immune response to RNA immunization with flavivirus replicons derived from tick-borne encephalitis virus. *J Virol.* 2005;79:15107-13.

61. Gomez I, Marx F, Saurwein-Teissl M, Gould EA, Grubeck-Loebenstein B. Characterization of tick-borne encephalitis virus-specific human T lymphocyte responses by stimulation with structural TBEV proteins expressed in a recombinant baculovirus. *Viral Immunol*. 2003;16:407-14.

62. Garner-Spitzer E, Wagner A, Paulke-Korinek M, et al. Tick-borne encephalitis (TBE) and hepatitis B nonresponders feature different immunologic mechanisms in response to TBE and influenza vaccination with involvement of regulatory T and B cells and IL-10. *J Immunol*. 2013;191:2426-36.

63. Hopf S, Garner-Spitzer E, Hofer M, Kundi M, Wiedermann U. Comparable immune responsiveness but increased reactogenicity after subcutaneous versus intramuscular administration of tick-borne encephalitis (TBE) vaccine. *Vaccine.* 2016;34:2027-34.

64. Stefanoff P, Polkowska A, Giambi C, et al. Reliable surveillance of tick-borne encephalitis in European countries is necessary to improve the quality of vaccine recommendations. *Vaccine*. 2011;29:1283-8.

65. Andersson CR, Vene S, Insulander M, Lindquist L, Lundkvist A, Gunther G. Vaccine failures after active immunization against tick-borne encephalitis. *Vaccine*. 2010;28:2827-31.

66. Koppi S, Faé P, Hartmann G, Höftberger R, Holzmann H. [Fatal outcome of tick-borne encephalitis despite complete active vaccination]. *Nervenarzt*. 2011;82:506, 8.

67. Plisek S, Honegr K, Beran J. TBE infection in an incomplete immunized person at-risk who lives in a high-endemic area—impact on current recommendations for immunization of high-risk groups. *Vaccine*. 2008;26:301-4.

68. Stiasny K, Holzmann H, Heinz FX. Characteristics of antibody responses in tick-borne encephalitis vaccination breakthroughs. *Vaccine*. 2009;27:7021-6.

69. Kollaritsch H, Paulke-Korinek M, Holzmann H, Hombach J, Bjorvatn B, Barrett A. Vaccines and vaccination against tick -borne encephalitis. *Exp Rev Vaccines.* 2012;11:1103-19.

70. Weinzettel R, Ertl S, Zwiauer K. [FSME monitoring: monitoring of adverse events of tick-borne-encephalitis vaccines by selected paediatricians and general practitioners]. *Wien Med Wochenschr*. 2007;157:107-10.

71. WHO. Vaccines against tick-borne encephalitis (TBE): WHO position paper, 10 June, 2011 [Grading safety]. 2011; available at: http://www.who.int/immunization/ TBE_grad_safety.pdf? ua=1.

72. Il'chenko TE, Bilalova GP, Stavitskaya NX, Solanik RG, Bistritskaya LD, Krasnilikov IV. Organziation of Public Health. *Siberian J Med*. 2009;2:50-5.

73. WHO. Vaccines against tick-borne encephalitis (TBE): WHO position paper, 10 June, 2011 [Grading crossprotection]. 2011; available at: http://www.who.int/immunization/ TBE_grad_crossprotection.pdf?ua=1.

74. Kluger G, Schöttler A, Waldvogel K, et al. Tick-borne encephalitis despite specific immunoglobulin prophylaxis. *Lancet.* 1995;346:1502.

75. Kreil TR, Maier E, Fraiss S, Eibl MM. Neutralizing antibodies protect against lethal flavivirus challenge but allow for the development of active humoral immunity to a nonstructural virus protein. *J Virol.* 1998;72:3076-81.

76. Bröker M, Kollaritsch H. After a tick bite in a tick-borne encephalitis virus endemic area: current positions about post-exposure treatment. *Vaccine*. 2008;26:863-8.

77. Pen'evskaia N, Rudakov N. [Efficiency of use of immunoglobulin preparations for the postexposure prevention of tick-borne encephalitis in Russia (a review of semi-centennial experience)]. *Med Parazitol (Mosk).* 2010;1:53-9.

78. Rabel PO, Planitzer CB, Farcet MR, Kreil TR. Tick-borne encephalitis virus-neutralizing antibodies in different immunoglobulin preparations. *Clin Vaccine Immunol*. 2012;19:623-5.

79. BMGF. Impfplan Österreich 2017. Vienna, Austria: Ministerium Frauen Gesundheit;2017.

80. Baumhackl U, Franta C, Retzl J, Salomonowitz E, Eder G. A controlled trial of tick-borne encephalitis vaccination in patients with multiple sclerosis. *Vaccine*. 2003;21 Suppl 1:S56- 61.

81. Hertzell KB, Pauksens K, Rombo L, Knight A, Vene S,

Askling HH. Tick-borne encephalitis (TBE) vaccine to medically immunosuppressed patients with rheumatoid arthritis: A prospective, open-label, multi-centre study. *Vaccine*. 2016;34:650-5.

82. Dengler TJ, Zimmermann R, Meyer J, Sack FU, Girgsdies O, Kübler WE. Vaccination against tick-borne encephalitis under therapeutic immunosuppression. Reduced efficacy in heart transplant recipients. *Vaccine*. 1999;17:867-74.

83. Schwarz B. [Health economics of early summer meningoencephalitis in Austria. Effects of a vaccination campaign 1981 to 1990]. *Wien Med Wochenschr*. 1993;143:551-5.

84. WHO. International Travel and Health, chapter 6. 2010; available at: http://www.who.int/ith/ITH2010chapter6.pdf.

85. Zavadska D, Anca I, André F, et al. Recommendations for tick-borne encephalitis vaccination from the Central European Vaccination Awareness Group (CEVAG). *Hum Vaccin Immunother.* 2013;9:362-74.

86. ECDC, European Center for Disease Prevention and Control. Epidemiological situation of tick-borne encephalitis in the European Union and European Free Trade Association countries. 2012; available at: http://ecdc.europa.eu/en/ publications/Publications/TBE-in-EU-EFTA.pdf.

87. Konior R, Brzostek J, Poellabauer EM, Jiang Q, Harper L, Erber W. Seropersistence of TBE virus antibodies 10 years after first booster vaccination and response to a second booster vaccination with FSME-IMMUN 0.5mL in adults. *Vaccine.* 2017 Jun 16;35(28):3607-3613

88. Beran J, Lattanzi M, Xie F, Moraschini L, Galgani I. Second five-year follow-up after a booster vaccination against tick-borne encephalitis following different primary vaccination schedules demonstrates at least 10 years antibody persistence. *Vaccine*. 2018 Feb 1

89. Steffen, NECTM, 2018

90. Bröker M, Eickmann M, Stadler K. Genetic Stability of a Tick-Borne Encephalitis (TBE) Virus Strain used for the Production of a TBE Vaccine. *J Vaccin Vaccination*. 2011;02 (01).

91. Xing Yi, Schmitt Heinz-Josef, Arguedas Adriano, Yang Junfeng. Tick-borne encephalitis in China: A review of epidemiology and vaccines. *Vaccine*. 2017;35:1227–1237

92. Bröker M, Schöndorf I. Are tick-borne encephalitis vaccines interchangeable? *Exp Rev Vaccines*. 2006;5 (4):461-466

93. Litzba N, Zelená H, Kreil TR, Niklasson B, Kühlmann-Rabens I, Remoli ME, Niedrig M. Evaluation of different serological diagnostic methods for tick-borne encephalitis virus: enzyme-linked immunosorbent, immunofluorescence, and neutralization assay. *Vector Borne Zoonotic Dis*. 2014;14 (2):149-59. doi: 10.1089/vbz.2012.1287. Epub 2013 Dec 20.

94. Leonova GN, Pavlenko EV. Characterization of neutralizing antibodies to far Eastern tick-borne encephalitis virus subtype and the antibody avidity for four tick-borne encephalitis vaccines in humans. *Vaccine*. 2009;27 (21):2899-2904.

95. Maikova GB, Chernokhaeva LL, Rogova YV, et al. Ability of inactivated vaccines based on far-eastern tick-borne encephalitis virus strains to induce humoral immune response in originally seropositive and seronegative recipients. *J Med Virol*. 2019;91(2):190-200.

96. Shedrawy J, Henriksson M, Hergens MP, Askling HH. Estimating costs and health outcomes of publicly funded tick-borne encephalitis vaccination: A cost-effectiveness analysis. *Vaccine*. 2018;36(50):7659-7665.

97. Bundesamt für Gesundheit (BAG) Bulletin 6/2019, 4 Feb., 2019

98. Poellabauer E, Angermayr R, Behre U, Zhang P, Harper L, Schmitt HJ, Erber W. Seropersistence and booster response following vaccination with FSME-IMMUN in children, adolescents, and young adults. *Vaccine*. 2019 May 27;37(24):3241-3250.

99. Hansson KE, Rosdahl A, Insulander M, Vene S, Lindquist L, Gredmark-Russ S, Askling HH. Tick-borne Encephalitis Vaccine Failures: A 10-year Retrospective Study Supporting the Rationale for Adding an Extra Priming Dose in Individuals Starting at Age 50 Years. *Clin Infect Dis*. 2020 Jan 2;70(2):245-251

100. Dobler G, Kaier K, Hehn P, Böhmer MM, Kreusch TM, Borde JP..Tick-borne encephalitis virus vaccination breakthrough infections in Germany - A retrospective analysis from 2001-2018. *Clin Microbiol Infect*. 2019 Dec 13. pii: S1198-743X(19)30653-6

101. Bradt V, Malafa S, von Braun A, Jarmer J, Tsouchnikas G, Medits I, Wanke K, Karrer U, Stiasny K, Heinz FX. Pre-existing yellow fever immunity impairs and modulates the antibody response to tick-borne encephalitis vaccination. *NPJ Vaccines*. 2019 Sep 6;4:38.

102. Vorovitch MF,Maikova GB, Chernokhaeva LL, Romanenko VV, Karganova GG, Ishmukhametov AA. Comparison of the Immunogenicity and Safety of Two Pediatric TBE Vaccines Based on the Far Eastern and European Virus Subtypes. *Adv Virol*. 2019 Dec 24;2019:5323428

103. Pugh S, Moisi J, Kundi M, et al. Effectiveness of Two Doses of Tick-borne Encephalitis (TBE) Vaccine. *J Travel Med.* 2022 Jan 6;

104. Erber W, Khan F, Zawadska D, et al. Effectiveness of TBE vaccination in southern Germany and Latvia. *Vaccine*. 2022 Jan 31; 40(5): 819-25

105. Zens KD, Baroutsou V, Siniger P, Lang P. A cross-sectional study evaluating tick-borne encephalitis vaccine uptake and timeliness among adults in Switzerland. *PLoS One*. 2021 Dec 14; 16(12):e0247216.

106. Steffen R, Erber W, Schmitt HJ, Can the booster interval for the tick-borne encephalitis (TBE) vaccine 'FSME-IMMUN' be prolonged? – A systematic review. Ticks and Tick-borne Diseases 12, 2021 101779

107. Schmidt AJ, Altpeter E, Graf S, Steffen R. Tick-borne

encephalitis (TBE) in Switzerland: does the prolongation of vaccine booster intervals result in an increased risk of breakthroughs? Paper presented at the XIVth international symposium on Ticks and Tick-borne Diseases, Weimar 24-26 March 2021.

108. Kantele A, Rombo L, Vene S, Kundi M, Lindquist L, Erra EO. Three-dose versus four-dose primary schedules for tick-borne encephalitis (TBE) vaccine FSME-immun for those aged 50 years or older: A single-centre, open-label, randomized controlled trial. *Vaccine*. 40(2022) 1299-1305

109. Wagner A, Garner-Spitzer E, Jasinska J, Kollaritsch H, Stiasny K, Kundi M, Wiedermann U. Age-related differences in humoral and cellular immune responses after primary immunization: indication for stratified vaccination schedules. Scientific Reports, 2018

110. Santonja I, Stiasny K, Essl A, Heinz FX, Kundi M, Holzmann H. Tick-borne encephalitis in vaccinated patients: a retrospective case-control study and analysis of vaccination field effectiveness in Austria from 2000 to 2018. JID, 2022:XX

111. Cisneros B, García-Aguirre I, Unzueta J, et al. Immune system modulation in aging: Molecular mechanisms and therapeutic targets. *Front Immunol*. 2022 Dec 15

112. Garner-Spitzer E, Wagner A, Paulke-Korinek M, Kollaritsch H, Heinz FX, Redlberger-Fritz M, Stiasny K, Fischer GF, Kundi M, Wiedermann U. Tick-borne encephalitis (TBE) and hepatitis B nonresponders feature different immunologic mechanisms in response to TBE and influenza vaccination with involvement of regulatory T and B cells and IL-10, *J Immunol.* 2013 Sep 1

113. Eder G, Kollaritsch H. Antigen dependent adverse reactions and seroconversion of a tick-borne encephalitis vaccine in children. *Vaccine*, 2003 Sep 8

114. Frederick J. Angulo, Pingping Zhang, Kate Halsby, Patrick Kelly, Andreas Pilz, Harish Madhava, Jennifer C. Moïsi, Luis Jodar. A systematic literature review of the effectiveness of tick-borne encephalitis vaccines in Europe. *Vaccine.* 41 (2023) 6914–6921

115. Kyncl J, Angulo FJ, Orlikova H, Zhang P, Vlckova I, Maly M, Krivohlavkova D, Harper LR, Edwards J, Bender C, Pilz A, Erber W, Madhava H, Moïsi JC. Effectiveness of vaccination against tick-borne encephalitis in the Czech Republic, 2018-2022. Abstract Disposition Report, Boston, Massachusetts, October 11-15, 2023

116. Susan L. Hills, MBBS1; Katherine A. Poehling, MD2; Wilbur H. Chen, MD3; J. Erin Staples, MD, PhD. Tick-Borne Encephalitis Vaccine: Recommendations of the Advisory Committee on Immunization Practices, United States, 2023, Recommendations and Reports / November 10, 2023 / 72(5);1–29