THE TBE BOOK

SIXTH EDITION

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In memory of

Christian Kunz

1927 – 2020

Father of the first TBE vaccine

Considered a pioneer of virology in Austria, Christian Kunz's interest in scientific research awoke in the 1950s and was supported by study visits to the then German strongholds for virology - Freiburg, Tübingen and Marburg.

His early publications received international attention and earned him a grant from the Rockefeller Foundation to continue his research at Rockefeller Laboratories in New York.

The experiences at research institutes and contacts with outstanding international scientists significantly shaped his further career.

Back in Vienna, he established the Institute of Virology with a research focus on arthropod-borne diseases and especially TBE, the by far most common virus-related disease of the central nervous system in endemic areas.

He was intensively engaged in virus diagnostics, basic medical virology, and the life cycle of the TBE virus in nature. Also, TBE-endemic areas throughout Austria were identified. He finally used all his knowledge to develop a highly effective vaccine against TBE, initially in cooperation with an English research institute and later with the Austrian pharmaceutical company IMMUNO.

The vaccine was first licensed in 1976 and ever since, the broad use of the vaccine in Austria has led to an impressive reduction of the TBE burden of disease.

Prof. Kunz was a founding member and for many years Chairman of the "European Group for Rapid Virus Diagnosis," which became the "European Society for Clinical Virology" in 1997, an association of leading medical virologists from across Europe, who focused primarily on the development of new methods for early detection of viral infections.

He was awarded the Loeffler-Frosch-Medal of the International Society of Virology for his outstanding achievements for the development of Virology in German-speaking countries.

We deeply appreciate Christian Kunz's scientific achievements, and the editors and publisher dedicate this 3rd Edition of "The TBE Book" (2020) to him in commemoration.

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Introduction to the 1st edition

While the number of vector-borne diseases and their incidence in Europe is much less than in tropical and/or developing countries, there are, nevertheless, a substantial number of such infections in Europe. The most important one is the zoonotic arbovirus infection Tick-Borne Encephalitis (TBE), a virus transmitted to humans by ticks or by consumption of unpasteurized dairy products from infected cows, goats, or sheep.

TBE is endemic in the non-tropical Eurasian forest belt with most cases occurring in Russia and in central and eastern parts of Europe. In endemic areas, TBE is one of the most important causes of viral meningitis/encephalitis and a major public health concern. Moreover, TBE is becoming more and more frequent in Europe due to the appearance of new endemic areas and increasing awareness.

However, it might be difficult to diagnose TBE, because clinical manifestations tend to be relatively nonspecific. Although a standardized case definition across the European Union has existed now for a few years, national implementation of TBE programs, including regular screening and diagnosis, are done in only very few countries. Therefore, wide differences in the intensity and quality of national surveillance of TBE cases still exist, and the true burden of disease and the areas with circulation of the TBE viral subtypes in Europe and Asia are not fully known. Moreover, although safe and effective vaccines are available, vaccination uptake in most endemic countries is too low to reduce the TBE burden significantly.

We therefore have tried to compile in this "working book" the most recent and relevant aspects of TBE. Digital technology allows us to continuously review and update the information in the e-book version almost in real time. Therefore, with the publication of this book, all authors and editors, as well as the publisher will continue to provide the best and latest data and the most current insights pertinent to the field of TBE. With this in mind, we urge all readers to communicate to us any news, comments, or scientific input that may be relevant to patients, their physicians, or decision makers.

The editors thank all authors, Global Health Press (GHP) and Thomas Gegeny (Engage Scientific) for their excellent work, contributions, and input.

Priv.-Doz. Dr. G. Dobler, Dr. W. Erber, Prof. Dr. H.J. Schmitt - Editors

Introduction to the 2nd edition

We thank the publisher, all authors, contributors and readers of the TBE-Book who made this such a big success resulting in the need for a second edition within less than one year! Here we present the new edition with some corrections, modifications, one full new chapter, and relevant updates particularly in the epidemiology chapters as well as an improved printing size which allows using larger fonts for easier readability. As before, the TBE Book is also available online and we encourage our readers to join the "TBE-family" under the link below which gives access to all chapters and all updates, access to the monthly newsletter and the weekly snapshots and which helps to exchange relevant information: https://id-ea.org/tbe

We also welcome Dr. Michael Bröker as the fourth Editor – already known to our online readers as the Editor of our monthly newsletter and weekly snapshot.

The editors thank all authors and Global Health Press (GHP) for their excellent work, contributions and input.

Munich (Germany), Vienna (Austria), Marburg (Germany), Collegeville, PA (USA), April 2019

Priv.-Doz. Dr. Gerhard Dobler, Dr. Wilhelm Erber, Dr. Michael Bröker, Prof. Dr. Heinz-Josef Schmitt

Introduction to the 3rd edition

We are grateful to the publisher and to all authors who support us with this 3rd "online only" edition of 'The TBE-Book'.

We requested from authors any relevant scientific updates, particularly for chapter 12b (TBE in countries), in order to make the latest number of TBE cases diagnosed publicly available.

Munich (Germany), Vienna (Austria), Marburg (Germany), Collegeville, PA (USA), April 2020

Prof. Dr. Gerhard Dobler, Dr. Wilhelm Erber, Dr. Michael Bröker, Prof. Dr. Heinz-Josef Schmitt

Introduction to the 4th edition

Working in the field of infectious diseases in 2020 meant to devote all available resources to focus on protecting, diagnosing, and treating almost 8 billion humans around the globe against COVID19. In contrast to many airborne diseases like influenza and RSV-infections and different from gastrointestinal- and travel-related infections, which all decreased or in some cases even virtually disappeared as a result from behavioral restrictions and hygiene measures, TBE cases increased, at least in central Europe. These developments are now reflected in this updated 4th, online-only edition of THE TBE BOOK.

Bearing all this in mind, we thank all country-authors who worked hard to provide the local 2020 TBE case numbers – often against the odds and often by working even more extra-hours. Their efforts are much appreciated by all the readers! We are also indebted to the wonderful team at Global Health Press who again collected, formatted, and put all new data as well as many "small" changes together in a publicly available online-format.

Munich (Germany), Vienna (Austria), Marburg (Germany), Collegeville, PA (USA), May 2021

Prof. Dr. Gerhard Dobler, Dr. Wilhelm Erber, Dr. Michael Bröker, Prof. Dr. Heinz-Josef Schmitt

Introduction to the 5th edition

During this spring of 2022, the reported number of COVID-19 cases has decreased to very low levels. Nevertheless, as pointed out in 2021, many infectious disease specialists, microbiologists and public health experts, are still heavily involved in work related to this pandemic. But even against these odds of limited available capacity by researchers, we again were able to capture the 2021 TBE cases reported from most of the affected countries in Eurasia. New publications on vaccine effectiveness, optimal vaccination schedules and epidemiology have come up, but we will only cover this in the next (6th) edition planned for May 2023, once the full picture has become available.

In this 5th edition, we want to highlight to the readers, particularly travelers and travel medicine advisors:

- Each country has different methods of detecting and reporting TBE cases, so the data in Chapter 12 must be interpreted cautiously, as they are minimal numbers only.
- No single country reports "true TBE incidence data" as nowhere the completeness of testing has been documented and as the impact of vaccine uptake has never been systematically considered anywhere.
- As a result of (first) testing, Tunisia (!) is now on the list of TBE-endemic countries, whereas the situation remains unclear for other Mediterranean countries due to a lack of appropriate surveillance.
- The impact of climate change and weather factors remain totally unclear as consistent testing for cases has never been established and as many factors may theoretically influence case numbers, most of which were never captured in a scientifically reproducible manner.

With all this in mind, the Editors are extremely pleased that the labelling of countries as "predisposed" (climate, landscape, ticks, etc., are all there, but evidence for autochthonous TBE does not exist); "imperiled" (TBEV detected, but no case yet reported); "affected" (sporadically autochthonous TBE cases reported) and "endemic" (regular report of cases of several continuous seasons) have been generally accepted – and as long as incidence data are missing, this may best reflect local situation.

Furthermore, we have now aimed at documenting TBE-risk areas (i.e., the risk for TBE is >0) in our Map in Chapter 12c down to the county level for each country. This is a technical masterpiece accomplished by our publisher and the IT team in Singapore, Brian Ong, Muhammad Shaqeez and Augustine Hong, as they managed to technically simplify the highly accurate representation of the maps, which before would have even made the best commercially available computers collapse upon login into the immense map database. Congratulations to our IT-colleagues and – last but not least – a big "thank you" to our publisher, Daniela La Marca who made this all happen again in no time.

May 2022, Munich – Wien – Marburg – Nierstein

Prof. Dr. Gerhard Dobler, Dr. Wilhelm Erber, Dr. Michael Bröker, Prof. Dr. Heinz-Josef Schmitt

Introduction to the 6th edition

The World Health Organization (WHO) just declared the end of the COVID-19 pandemic, which kept and keeps infectious diseases specialists extremely busy with taking care of infected patients. COVID case numbers largely declined, but now long/post COVID and organ, mental, and social complications have become a new clinical and research focus. With this in mind, we are extremely grateful to all country authors for providing us with the 2022 TBE case numbers – despite the little time they have due to the additional work created by the pandemic.

While many infectious diseases – specifically acute respiratory tract infections and travel-related diseases – dramatically decreased during the height of the COVID-19 pandemic, in contrast, TBE cases tended to increase in Central Europe and elsewhere during the same time. Here we provide this important new data to our global readers.

We are pleased with the fact that colleagues from the USA have meanwhile become our major readers, and that the graphs and tables provided in this book have been frequently used in a number of prominent other publications. We specifically are encouraged by the feedback to the changes introduced in the 5th edition in 2022, and we will therefore continue to classify regions and countries as “predisposed”, “imperiled”, “affected” and “endemic” as true incidence data is still largely missing.

Our gratitude goes to our publisher, Daniela La Marca, our language Editor, John Hatley, and our Global Health Press IT team, the latter specifically for creating the TBE world map allowing us to enter data down to the level of single counties (Chapter 12c). Without all the excellent hard work of our colleagues this update would not have been possible.

Singapore, Munich, Vienna, Marburg, Nierstein in May 2023

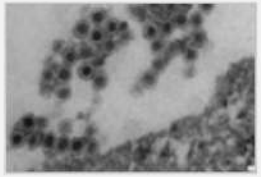
Prof. Dr. Gerhard Dobler, Dr. Wilhelm Erber, Dr. Michael Bröker, Dr. Lidia Chitimia-Dobler, Prof. Dr. Heinz-Josef Schmitt

TBE Milestones

20th Century

100 million years old: tick[1]

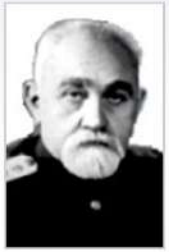
10,000 years old: TBE virus[2]



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 Kunz
Pioneer of the first 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 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 Maudrich Verlag, Vienna, 1932
4. Lev Zilber. Photo from the book *Lev Alexandrovich Zilber* written by his son 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: Priv.-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

A short history of TBE

Olaf Kahl, Vanda Vatslavovna Pogodina†, Tatyana Poponnikova, Jochen Süss and Vladimir Igorevich Zlobin

Key Points

- TBE virus is a flavivirus and a prominent tick-borne human pathogen occurring in parts of Asia and Europe.
- The virus was discovered by Lev A. Zilber and co-workers in the former USSR during an expedition in the Far Eastern taiga under the most difficult conditions in 1937.
- They and members of a second expedition under the leadership of the Academician Evgeny N. Pavlovsky 1938 elucidated the basic eco-epidemiology of the virus.
- In their natural foci, TBE virus circulates between vectors, certain ixodid ticks, and some of their hosts, so-called reservoir hosts, mostly small mammals.
- Five different subtypes of TBE virus have been described to date.

Introduction

Tick-borne encephalitis virus (TBEV) is an arthropod-borne human pathogen, ecologically known as an arbovirus.[1] Taxonomically, it is a member of the genus *Flavivirus* together with other medically relevant arboviruses (e.g., Yellow fever virus, Dengue virus). The virus is endemic in Asia and Europe where it circulates between its principal vectors, usually hard ticks of the genus *Ixodes*, and certain small mammals, referred to as 'reservoir hosts', fed on by virus-infected vector ticks. The bite of infected vector ticks is also the common route of infection for humans. Each year several thousand people fall ill with TBE. Long before Smith and Kilbourne[2] discovered that ticks can transmit pathogens to their hosts and before TBEV was discovered, the disease had been mentioned in the literature. Parish records from the Åland islands (Finland) contain case descriptions of a disease at least similar to TBE in the 18th century.[3] 'Taiga encephalitis' or 'biphasic meningoencephalitis' had been observed in eastern parts of the former USSR mostly in soldiers, railway workers and loggers, as the region began to develop in the 19th century.

Schneider[4] was the first to give a medical description of the 'Epidemische akute Meningitis serosa' (also known as 'Schneidersche Krankheit' in Austria), which was in fact TBE. Panov[5] gave the first detailed description of the clinical picture of the so-called 'summer encephalitis' in the Far East.

This chapter presents a brief synopsis of the major milestones in TBE and TBEV research, beginning with the discovery of the virus in the former USSR.

Discovery of TBEV in different regions of Eurasia

Molecular biological data indicate that TBEV has its origins in Western Siberia approximately 3100 [1800–4900] years ago.[6] From there the eastern TBEV groups spread to the east through Asia and the western TBEV groups to the west, and they might have reached central Europe approximately 2000 years ago. However, the first isolation of TBEV succeeded only in 1937.

In the 1930s, a large number of people living in the taiga in the Far East and Soviet troops located in that region fell ill with a serious neurological disease, with a frequently fatal outcome. The etiology of the disease was unknown, and first attempts to identify the pathogen failed. In 1937, the USSR Ministry of Health sent out an expedition, which was led by Lev A. Zilber, the head of the first medical virological laboratory in the country. Zilber put together a group of very capable and highly motivated young researchers and technical assistants. They worked in two teams at two different remote places in the taiga under extremely difficult conditions. The northern team was working in the Khabarovsk Territory (leader: Elizabeth N. Levkovich) and the southern team in Primorsky Territory (leader: Alexandra D. Sheboldaeva). No infrastructure for scientific research existed in the area of the taiga where the disease occurred, so the teams had to find simple, practical solutions for establishing what they called a scientific campus.

The teams started their practical work in mid-May 1937, and very soon the first relevant results were available. When investigating local people, they found numerous cases with neurological symptoms. Twelve out of 64 hospitalized patients died. The virus was isolated from 29

febrile patients, from diseased mice (after they had been infected with a tick suspension), and from ticks feeding on them. When team members warned local people to avoid tick bites, the number of new cases distinctly decreased. So there was compelling evidence at the end of that mission in mid-August that the team had found the causative viral agent, with *Ixodes persulcatus* ticks as the vectors.

Unfortunately, some team members became infected with TBEV and developed disease symptoms. Fortunately, nobody died – which appears almost unavoidable when taking into consideration the highly unsafe conditions and the highly contagious nature of the virus. As an example, Dr. Chumakov, who became a famous virologist later on, fell ill with a severe form of TBE after cutting his finger during an autopsy. Residual effects were right-arm paralysis and hearing loss. However, this did not prevent him from finding new TBEV foci in the Ural and Transural regions, far away from the Far East.[7]

Scientifically, the expedition was a great success. Zilber and the other team members had isolated the causative virus, elucidated the basic eco-epidemiology of the disease, and provided some effective prophylactic information on how to avoid an infection. Further expeditions were sent out to the Far East to learn more about the virus and the disease and its prophylaxis.[8]

The first cases of TBE in China were reported in 1943, and the causative virus was isolated in 1944 from brain samples of patients who had died (reviewed in Yoshii et al., 2017).[9] The discovery of TBE in Europe started with the clinical-epidemiological description of 24 cases of aseptic meningitis in the district of Neunkirchen (Lower Austria) by Schneider in 1931.[4] Although the outcome was described as benign, the convalescence of many patients was prolonged. In the early 1940s scientists of the Rockefeller Institute for Medical Research in New York showed serological cross-reactions between hyperimmune sera of Louping ill virus and Russian Spring Summer encephalitis virus.

The first documented TBEV isolation in Europe was made from *Ixodes ricinus* ticks (*strain 256*) in Belarus in 1939[10], and the second isolation was reported in former Czechoslovakia in 1948 (strain *Hanzalova*, isolated near Prague).[11] In 1952, a virus strain (*KEM I*) was isolated during an alimentary outbreak in Hungary. Other eastern European countries followed shortly after, and TBEV strains were isolated in Slovenia in 1953, in Poland in 1954, in Austria in 1954 (strain *Scharl*), and in Slovakia in 1958. The first TBEV strain in Finland was detected in 1959 (*Kumlinge* strain).[12] Sweden reported the first detection of TBEV in 1954, and Denmark reported the first clinical cases of TBE from Bornholm Island, also in the 1950s. In Norway, however, the first described human case of TBE occurred only in 1997.[14] In Germany, the first descriptions of TBE and the first virus isolations resulted from the late 1950s in the former German Democratic Republic.[15]

Photo of Lev Zilber from the book "Lev Alexandrovich Zilber" written by his son L.L. Kisselev and E.S. Levina, published in 2005 by the publishing house "Science" in the series "Scientific biographies"

Rehse-Küpper et al. (1978)[16] were probably the first who isolated TBEV strains in the former Federal Republic of Germany as the two virus strains isolated by Müller et al. (1970) were to the best of our knowledge never confirmed as being TBEV.[17]

France followed with the first isolation of TBEV from the Alsace region in 1970.[18] It was only in 2016 that the first autochthonous human cases of TBE were described in The Netherlands and a TBEV strain (strain *Sallandse*) was detected in ticks.[19]

The detection of the TBEV natural transmission cycle

Due to the pioneering research work by the Zilber expedition in the Far East, the basic outlines of TBEV eco-epidemiology were elucidated within a few months in 1937. They found that the pathogen is a virus that can be transmitted through the bite of *Ixodes persulcatus*, a hard tick (family Ixodidae).

Another expedition was sent out by the USSR Ministry of Health to the Far East under the leadership of E.N. Pavlovsky in 1938 to learn about the circulation of TBEV in the field and the involved reservoir hosts. Largely based on the findings during that expedition, Pavlovsky[20,21] developed the famous concept of 'The Natural Nidality of Transmissible Diseases', where he described the ecology of zoonoses. Arthropod vectors (ixodid ticks) that become infected with TBEV through a blood meal on an infective host carry the virus to the following life stage(s) and transmit it during the following blood meal(s) to a host. So-called reservoir hosts become infected through the bite of an infected tick, and in turn transmit the virus to other feeding ticks. Long-term virus circulation exists only in definite types of landscape with suitable abiotic conditions where all the necessary biotic partners (vectors, reservoir hosts) are present in sufficient densities.

Chumakov & Naidenova[22] (cited after[23]) found the hard tick *Ixodes ricinus*, a close relative of *I. persulcatus*, to be a vector of a milder form of TBE in some European areas of the former USSR. This was later confirmed by various European researchers. Rampas & Gallia[24] from Czechoslovakia were the first outside the former USSR to isolate TBEV from field-collected ticks.

An alternative alimentary route of human TBE infection became apparent in the European part of the former USSR from 1947 to 1951.[25] Groups of people contracted TBE after consuming unpasteurized goat milk or goat milk products (e.g., cheese) from viremic goats. Similar alimentary TBE epidemics occurred also in other TBE endemic countries, e.g., in Rožňava (south-eastern Slovakia) with more than 600 cases in 1951[23] and in Niesky (former German Democratic Republic) in 1961.[26]

Field work on TBEV decreased in several European countries in the 1970s and 1980s. TBEV ecology seemed to be well understood. The main interest of researchers focused more on the molecular biology of TBEV and also on the newly discovered *Borrelia burgdorferi*, the causative agent of human Lyme borreliosis. Interestingly, this coincided with the first European TBE vaccine becoming available in 1976,[27] and the TBE problem seemed to be solved.

Jones et al.[28] made the significant finding in the laboratory that guinea pigs can infect feeding *Rhipicephalus appendiculatus* with *Thogoto virus*, another tick-borne virus, without showing an apparent viremia. Encouraged by this finding, Alekseev & Chunikhin[29] and Labuda et al.[30] demonstrated non-viremic transmission of TBEV from small mammals (infected through tick bite) to uninfected feeding ticks. This was a major step forward in our understanding of the field ecology of the virus, and reactivated interest in TBEV ecology. Milan Labuda and various co-workers made a number of further relevant contributions to this topic.[31-33] They found (i) that TBEV is transported in Langerhans' cells in infected hosts, (ii) that non-viremic transmission also occurs in immune hosts, and (iii) that this kind of transmission happens in small, but not in larger mammals. The most commonly used term now is 'co-feeding transmission', although non-viremic transmission might technically be the better term.

The detection of different TBEV subtypes

Based on general viral properties such as viral morphology, physical and chemical properties, virion structure, arthropod carriers, and serological cross-reactions, the genus *Flavivirus* including TBEV was considered to be part of the family *Togaviridae*. This term was first offered by Lwoff and Tournier (1966).[34]

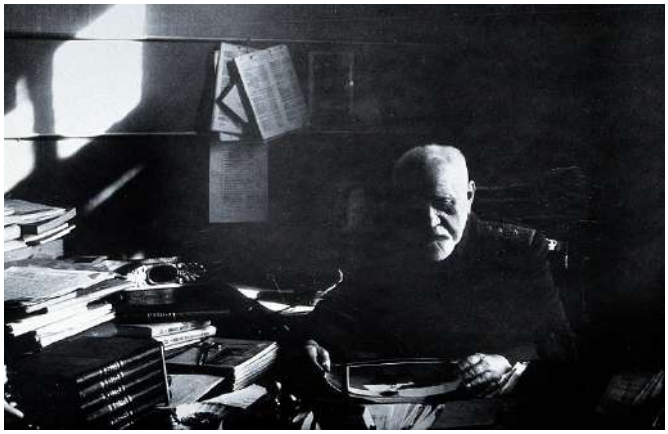


Photo of Evgeny N. Pavlovsky
Credit: LJ Bruce-Chwatt from the Wellcome Collection

The family *Togaviridae* consisted of the genera *Alphavirus* (former arbovirus group A), *Flavivirus* (former arbovirus group B) with *Dengue virus*, *type 1*, and some other viruses.[35,36]

De Madrid and Porterfield[37] divided the genus *Flavivirus* into 7 subgroups according to plaque reduction neutralization test (PRNT). The first subgroup includes tick-borne viruses such as TBEV, *Omsk hemorrhagic fever virus, Louping ill virus, Langat virus, Negishi virus,* and *Kyasanur forest disease virus.* Along with the above-mentioned viruses, the TBEV complex included *Alma Arasan, Apoi, Royal-Farm, Kadam, Powassan* viruses, and according to Gaidamovich and Loginova[38] also *Gadgets Gully, Saumarez Reef, Karshi,* and *Tyuleniy* viruses.

These viruses share some antigenic similarity but have different geographic distributions, associations with different ticks and vertebrate hosts, and a different pathogenic potential for humans. Due to a difference in the replication strategies of alpha- and flaviviruses, the family *Flaviviridae* was established as an independent family that comprises the genus *Flavivirus* with more than 70 species dividing into 10 serocomplexes.[39] According to modern classification, the family *Flaviviridae* comprises the genera *Flavivirus*, *Pestivirus*, and *Hepacivirus*. TBEV belongs to the mammalian tick-borne flavivirus group and comprises 3 subtypes: European, Far Eastern, and Siberian.[40]

Two geographic and antigenic variants of TBEV (Eastern and Western) had been known for 40 years.[1,41-44] Clarke[43] divided 28 strains in 2 antigen variants by the gel precipitation test with cross-absorbed sera. She concluded that there are 2 antigen subtypes: Eastern and Western (Central European). Chumakov et al.[45] considered that Eastern and Western subtypes differ within the species TBEV; they proposed a classification into 'Persulcatus' and 'Ricinus' antigen variants according to viral ecology. Votyakov et al.[44,46] argued that the infectious agents of Eastern and Western TBE are different species according to differences in antigen profiles, geography, clinical and pathological features in animals and humans.

Pletnev et al.[47,48] and Mandl et al.[49,50] decoded the complete genomes of Eastern (*Sofjin*) and European (*Neudoerfl*) strains and thereby started a new phase of intraspecific TBEV classification. The obtained data proved that the genetic differences between the Western and Eastern variants are significant with 16.8–16.9% of nucleotide substitutions and 6.9–7.2% of amino acid substitutions. Two Eastern strains in contrast have 3- and 4-times lower differences in nucleotide (4.6%) and amino acid (1.8%) substitutions, respectively.

Rubin and Chumakov[51] published the first results of the Siberian subtype. They demonstrated some peculiarities of the strain *Aina* isolated in the Irkutsk region, USSR, from a child with TBE. Pogodina et al.[52,53] described a group of strains isolated in Eastern Siberia from *I. persulcatus*, from rodents and patients serologically closely related to the strain *Aina*. Gritsun et al.[25,54] were the first to genotype strains of the Siberian subtype by gene E and complete genome sequencing. Two strains – *Vasilchenko* (L40361) and *Zausaev* (AF527415) – became prototype strains of 2 Siberian subtype clusters (reflecting their geographic localization).

Sequencing a gene E fragment (160 bp length) of 8 and thereafter 29 strains isolated in different geographic regions, carried out by Zlobin et al.[55-57] enabled the identification of 3 major genotypes (subtypes): (1) Far Eastern, (2) Western, and (3) Ural-Siberian (Siberian). According to Ecker,[58] TBEV consists of 3 subtypes corresponding to 3 major genotypes: European, Far Eastern, and Siberian. However, Grard[59] reinterpreted the data of the genetic relationships among arthropod-borne viruses. She suggested that TBEV should include 4 subtypes: (1) Louping ill virus (Spanish, British, and Irish subtypes), (2) TBEV (European subtype), (3) TBEV (Far Eastern and Siberian subtype), and (4) Turkish sheep encephalitis virus and its subtype, Greek goat encephalitis virus.

Beside the 3 described and accepted subtypes, 2 different strains – *178/79* and *886/84* – have been described by Russian researchers. These 2 strains have been shown not to be closely related to any of the 3 known subtypes.[60] Additional studies are needed to demonstrate whether these strains can be classified as new TBEV subtypes. These results mean also that further TBEV subtypes may be detected in future.

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Chapter 2a

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 *Flavivirus*, 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 Himalaya 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 *Flavivirus*, family *Flaviviridae* (from the Latin *flavus* – 'yellow', referring to the prototype virus, yellow fever virus).

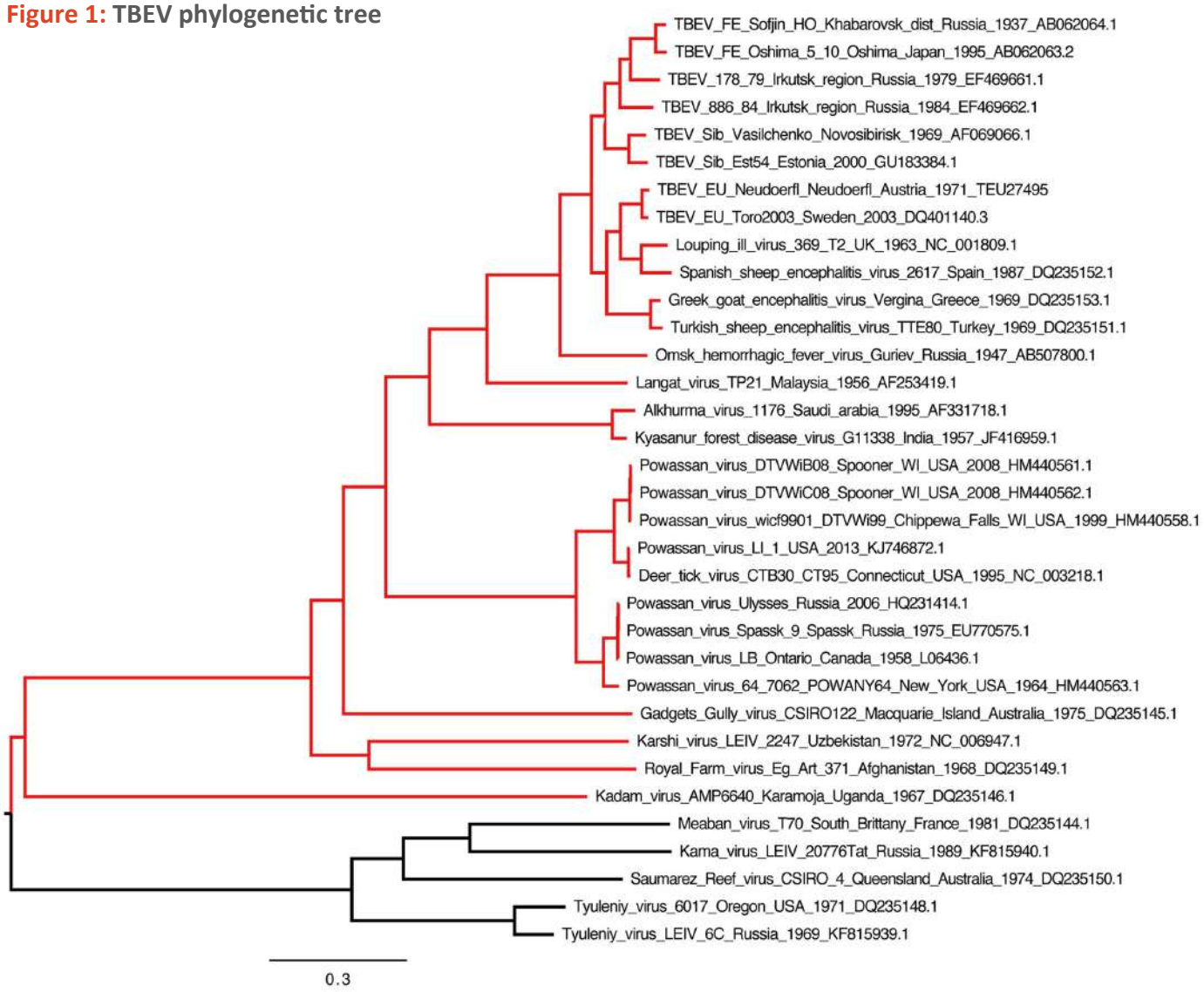
The genus *Flavivirus* comprises over 70 virus species, many of which are important human pathogens.[1] Besides TBEV, these include mosquito-borne viruses such as dengue viruses, Japanese encephalitis virus, yellow fever virus, Zika virus, and many others. Virtually the entire human population lives where at least one flavivirus species is endemic.[1] Moreover, many flaviviruses 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).[2–3] 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 flaviviruses are arboviruses – some are vertebrate-specific (also called 'No known vector' and further divided into rodent-specific and bat-specific flaviviruses)[4] while some are insect-specific.[5] These classifications reflect the adaptation of the viruses to particular invertebrate or vertebrate hosts, and modes of virus transmission in nature.

Tick-borne flaviviruses (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 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' (Fig. 1). All TBFVs are closely related

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 1000 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 (Norwegian Institute of Public Health, Oslo) who prepared and supplied the tree.

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.[6]

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.[7,8]

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).[10,11] 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.[115, 116]

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.[12] Another new potential TBEV subtype (Himalayan – TBEV-Him) was identified recently in wild rodents in Qinghai-Tibet Plateau in China.[117]

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 5000 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.[23]

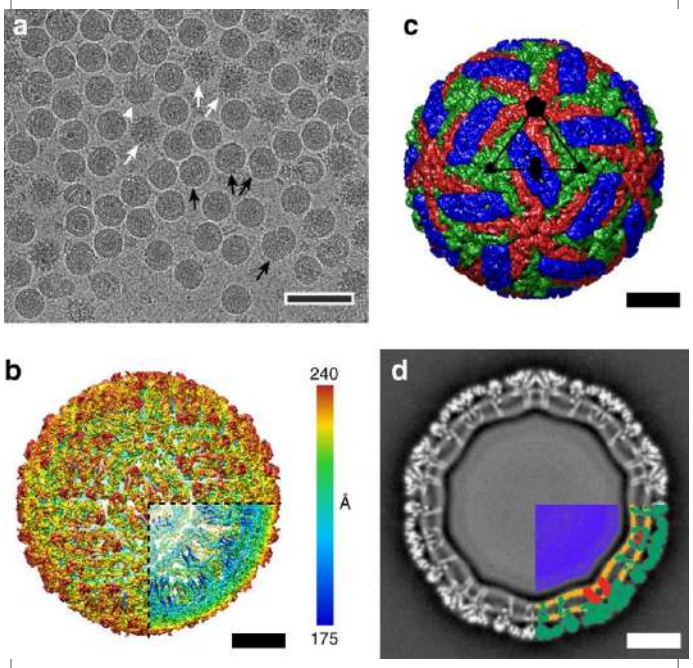
Virion structure and morphology

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 (Fig. 2).[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) (Fig. 2).

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 heterodimers and exist

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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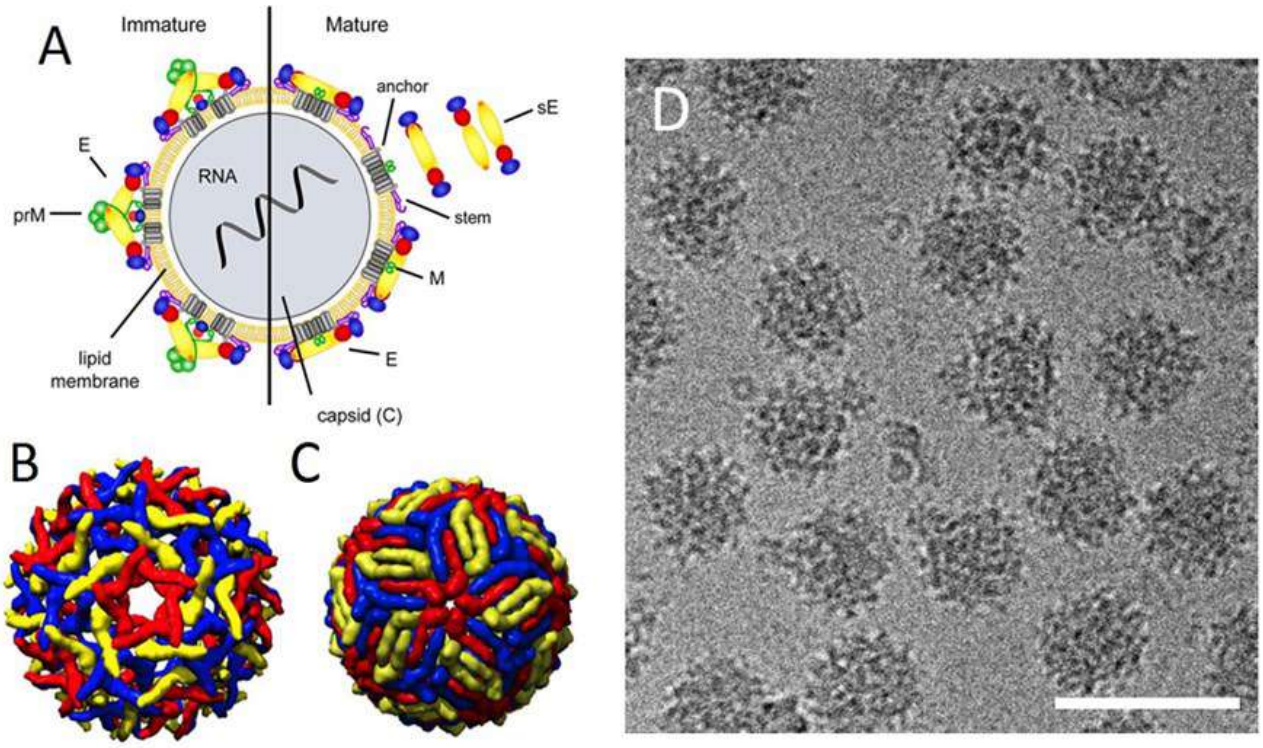
Figure 3



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 Vratskikh O, Stiasny K, Zlatkovic J, et al. Dissection of antibody specificities induced by yellow fever vaccination. PLoS Pathog 2013;9:e1003458. figshare: https://dx.doi.org/10.1371/journal.ppat.1003458.g001 (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.*

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 (Fig. 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.[25]

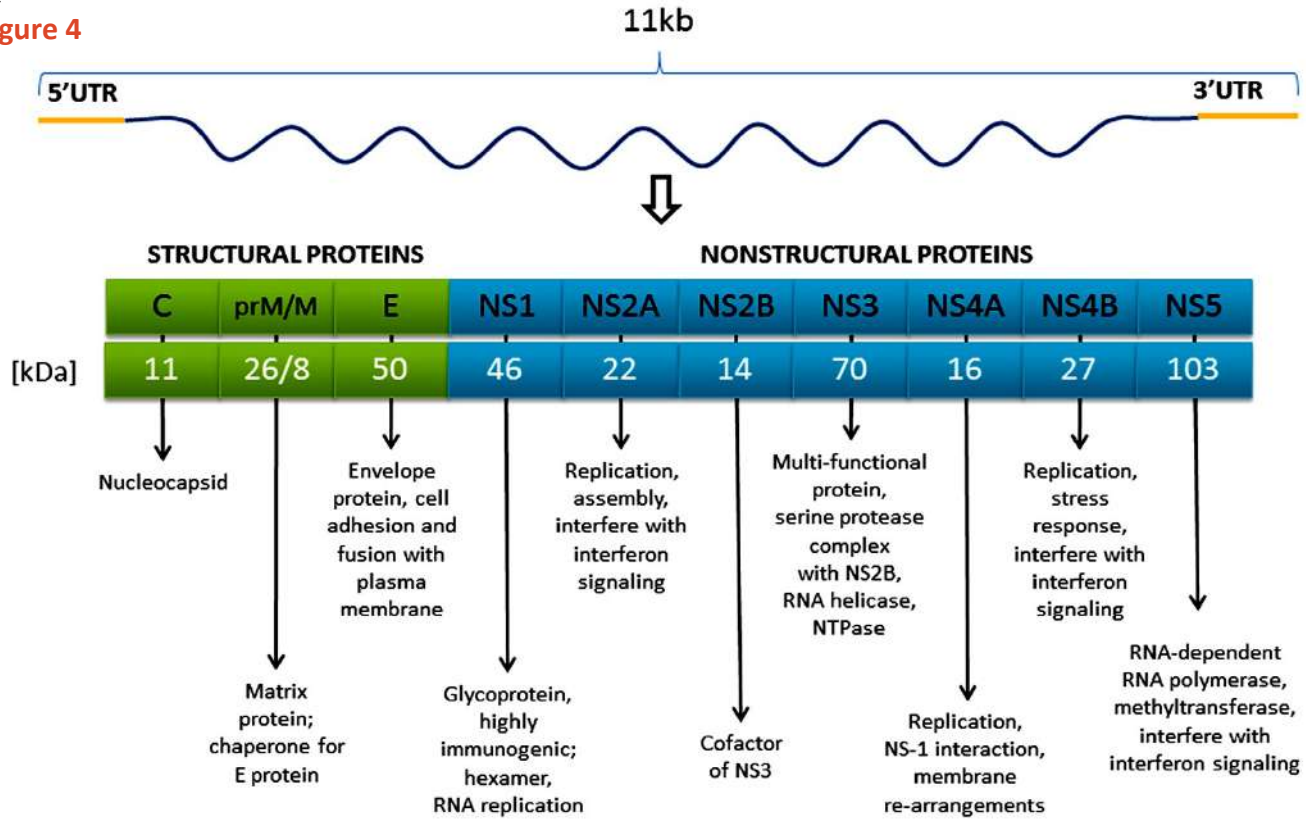
The structure of purified TBEV particles has recently been determined at near atomic resolution of 3.9 Å by reconstruction of cryo-electronmicroscopic images (Figure 2).[118] The study revealed a relatively smooth outer surface of the particle, and E and M proteins organized in a similar manner to that in other flaviviruses. The surface of the TBEV virion is covered with small protrusions formed by glycans attached to the E-protein molecules.[118] 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.[118]

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 established model system for flavivirus membrane fusion because they have fusion characteristics similar to those of infectious virions.[28]

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-

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 (kindly provided by Martin Palus, with permission).

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 cryo-electron microscopic reconstructions.[26] 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 nucleo-capsid 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.[27]

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 3400 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] (Fig. 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 not expressed or is present at undetectable concentrations, suggesting that this additional ORF has either 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.[38]

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.[39]

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.[37] 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.[44,45] A short poly(A) tract is genetically more stable compared with the virus having a long poly(A) tract.[46]

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.[37,38] 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.[37,48] 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.[38,48]

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

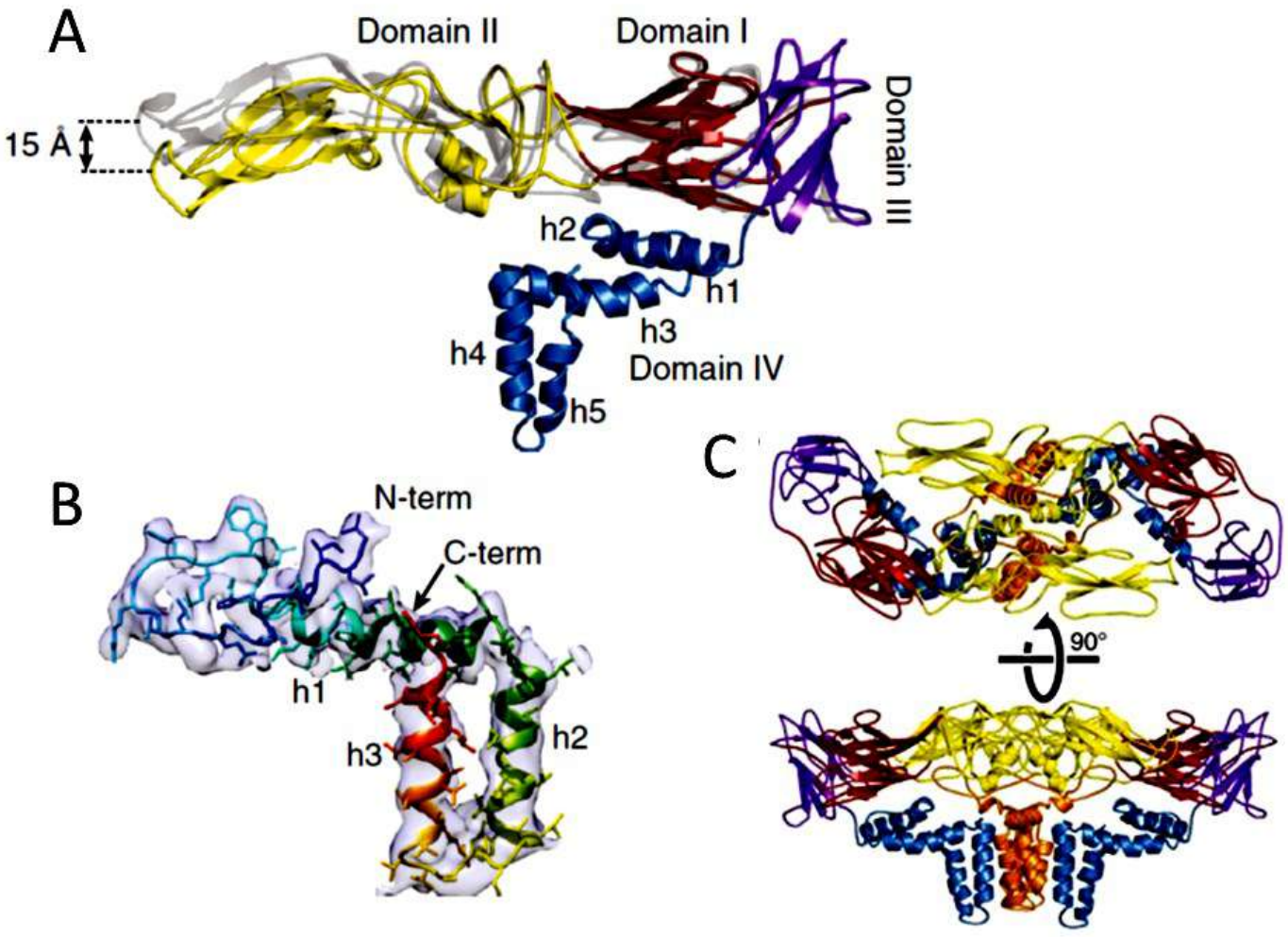
degradation of viral RNA by the cellular 5'-3' exoribonuclease XRN1.[49] The exoribonuclease activity stops at the highly ordered RNA secondary structures at the beginning of the 3'-UTR. SfRNA is involved in modulating multiple cellular pathways; e.g., inhibiting antiviral activity of type I interferons (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.[39] Within the ORF 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

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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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.[39,51] 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.[31,39,56] Six cysteine residues, all disulfide-bridged, are highly conserved. The C-terminal region contains an ectodomain and 2 potential membrane-spanning domains.[31] The cleavage of prM into pr and M occurs in the Golgi complex and is mediated by furin or a furin-like enzyme[57,58] leading to a conversion from immature to mature fusogenic and fully infectious viral particles (Fig. 3).[57] The pr fragment is then secreted.[39] A conserved region in the prM protein is a critical molecular determinant for the assembly and secretion of the virus.[59] The M-protein consists of an N-terminal loop and three helices (Fig. 5B). The first helix is situated as a perimembrane and the last two as transmembranes; 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.[118] 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 (Fig. 5C).[118]

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.[60] The three-dimensional structure of the E protein was studied at the resolution of 2.0 Å by X-ray crystallography[61] (Fig. 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.[118] 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 (Fig. 5A).[118] Such a bending of the ectodomain in the virion prevents induction of premature membrane fusion mediated by the E protein.[118] 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.[39]

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.[62] Conserved histidines in the E protein function as molecular switches and, by their protonation at acidic pH, control the fusion process.[63]

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 disulfide bridges, and an N-glycosylation site. 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.[64]

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 disulfide bridges.[61,65] 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.[66] 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.[63]

Domain III has the typical fold of an immunoglobulin constant (IgC) molecule.[65] 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.[61]

Amongst the most conserved parts of the E protein, there are 12 cysteine residues forming 6 disulfide bridges with conserved localization in common with all known flaviviruses.[67]

The E protein is also considered to be a major determinant of TBEV virulence. Amino acid substitutions in E protein often cause decrease in neuroinvasiveness, although neurovirulence is usually not reduced.[68] 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.[62] A number of identified substitutions causing escape of the virus from the neutralizing effect of monoclonal antibodies,[69] deficiency in the ability to agglutinate erythrocytes,[70] and a change in virus growth properties in cell cultures, mice, or ticks,[60,71-74] have been described.

Non-structural proteins

NS1 is a glycoprotein containing 2 or 3 potential glycosylation sites and 12 conserved cysteines forming disulfide bridges.[75] 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.[39,76] Alternatively, some species of the protein could be anchored into the membrane by glycosyl-phosphatidylinositol.[39,77] 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.[78] Secretion of NS1 protein into the extracellular space appears particularly in the form of pentamers or hexamers and occasionally as decamers or dodecamers.[79] This so-called 'soluble antigen', together with membrane-bound NS1 induces a protective immune response in the host.[80] NS1 protein is also known to activate the Toll-like receptors (TLRs),[81] and inhibit the complement system.[82–83]

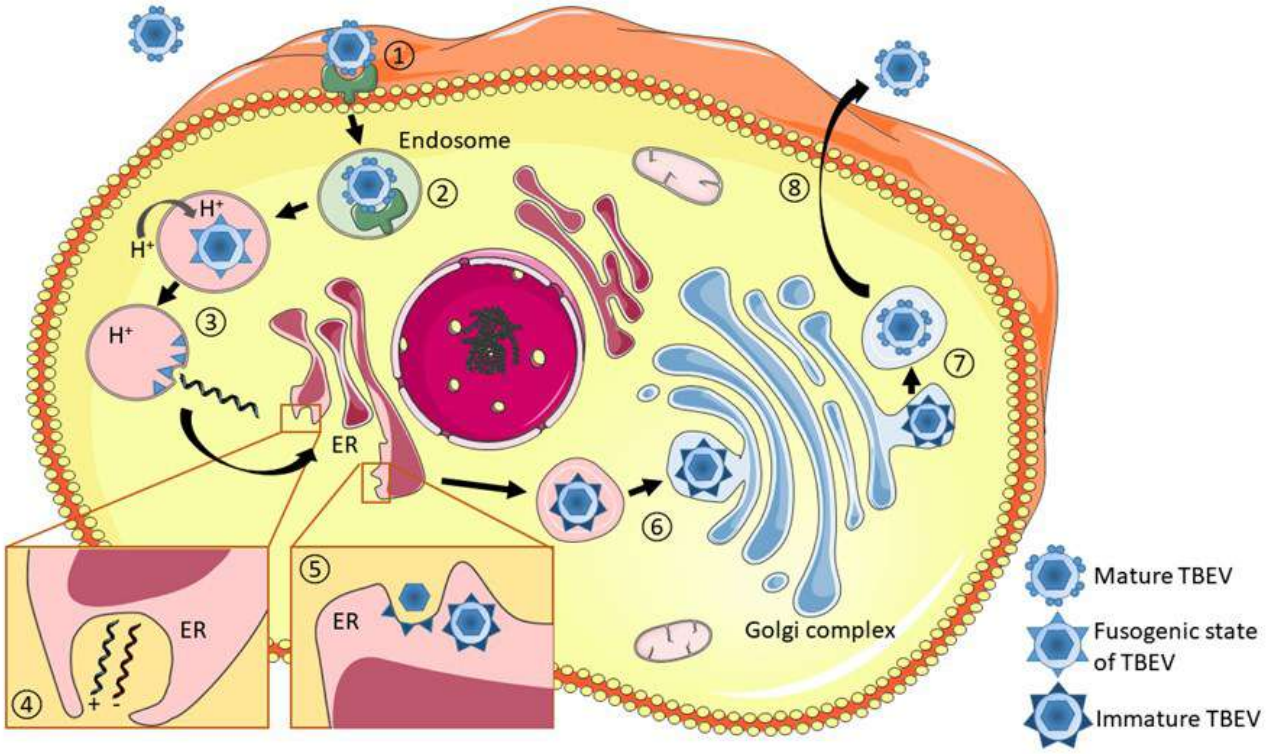
NS2A is a small, hydrophobic protein, currently with no defined function. It is believed to play a role in forming the RC.[39] A small membrane-associated protein, NS2B, serves as a crucial co-factor for protease activity of the NS3 protein. The central hydrophilic domain of the NS2B protein possibly interacts with the NS3 protein and it is flanked by hydrophobic regions probably anchored in the membrane.[85] 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.[39]

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.[39] 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 hetero-dimeric complex.[39,86] 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.[87] 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.[39] The C-terminal region also has RNA triphosphatase and 5'RNA phosphatase activities.[88] 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.[86,89]

NS4A and **NS4B** are small, hydrophobic proteins. NS4A is probably part of the replication complex.[90] NS4B, a transmembrane 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.[91] 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.[92]

NS5 is the largest (100 kDa) and most highly conserved viral protein serving as a viral RNA-dependent RNA polymerase.[93] Its C-terminus shares sequence homology with RNA-dependent RNA polymerases of other positive-stranded RNA viruses.[39,94] The N-terminal domain has a function as AdoMet-dependent methyltransferase involved in the mRNA capping process, transferring a methyl group from

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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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.[86] The NS5 proteins form complexes with NS3 proteins, which results in stimulation of the NS3 RNA nucleoside triphosphatase activity.[39,95]

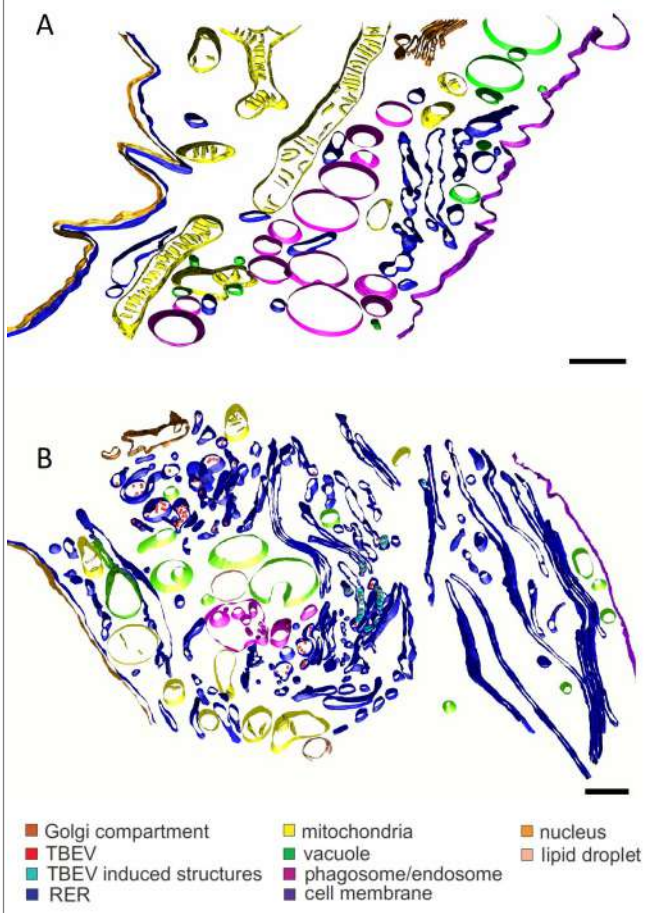
The NS5 protein is a promising target for specific antiviral inhibitors. Indeed, several nucleoside analogs targeting NS5 and causing premature termination of viral RNA synthesis were found to exhibit high inhibitory activity against TBEV.[96,97]

Apart from the main function as RNA-dependent RNA polymerase, the TBEV NS5 protein interferes with type I IFN JAK-STAT signaling.[98,99]

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.[100] 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.[100] It remains unclear whether TBEV uses single or multiple receptors on susceptible cells. Involvement of highly conserved glycosaminoglycans, such

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 Palus M, Bílý T, Elsterová J, et al. Infection and injury of human astrocytes by tick-borne encephalitis virus. J Gen Virol 2014;95(Pt 11):2411-26, with permission).

as heparan sulfate, 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.[101] 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.

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, the virus is internalized into clathrin-coated vesicles by the process of endocytosis (see Chapter 2b for details). 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). 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.[31] 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.[102]

TBEV replicates in the cytoplasm in close association with virus-induced intracellular membrane structures, also called replication compartments (Fig. 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.[103] 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 interconnected membranes (Fig. 6).[103]

The immature non-infectious virions containing proteins prM and E in heterodimeric association are transported to the Golgi complex, where the pr part of the prM molecule is cleaved, and the E protein is reorganized from trimers to form fusion-competent homodimers. These 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 (Fig. 6).[102]

TBEV infection is associated with dramatic morphological changes occurring in the infected cells (Fig. 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.[104–107] The infection is

commonly cytocidal; the infected cells often die by apoptosis or necrosis,[104] but some vertebrate cell types survive the lytic crisis and become chronically infected.[108]

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.[107,110–113] However, both vertebrate and tick cells activate innate defense mechanisms against the infection.[113]

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.[114]

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 a 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.

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Chapter 2b

The molecular and antigenic structure of TBEV

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Key Points

- TBEV-particles are assembled in an immature, noninfectious form in the endoplasmic reticulum by the envelopment of the viral core (containing the viral RNA) by a lipid membrane associated with two viral proteins, prM and E.
- Immature particles are transported through the cellular exocytic pathway and conformational changes induced by acidic pH in the trans-Golgi network allow the proteolytic cleavage of prM by furin, a cellular protease, resulting in the release of mature and infectious TBE-virions.
- The E protein controls cell entry by mediating attachment to as yet ill-defined receptors as well as by low-pH-triggered fusion of the viral and endosomal membrane after uptake by receptor-mediated endocytosis.
- Because of its key functions in cell entry, the E protein is the primary target of virus neutralizing antibodies, which inhibit these functions by different mechanisms.
- Although all flavivirus E proteins have a similar overall structure, divergence at the amino acid sequence level is up to 60 percent (e.g. between TBE and dengue viruses), and therefore cross-neutralization as well as (some degree of) cross-protection are limited to relatively closely related flaviviruses, such as those constituting the tick-borne encephalitis sero-complex.

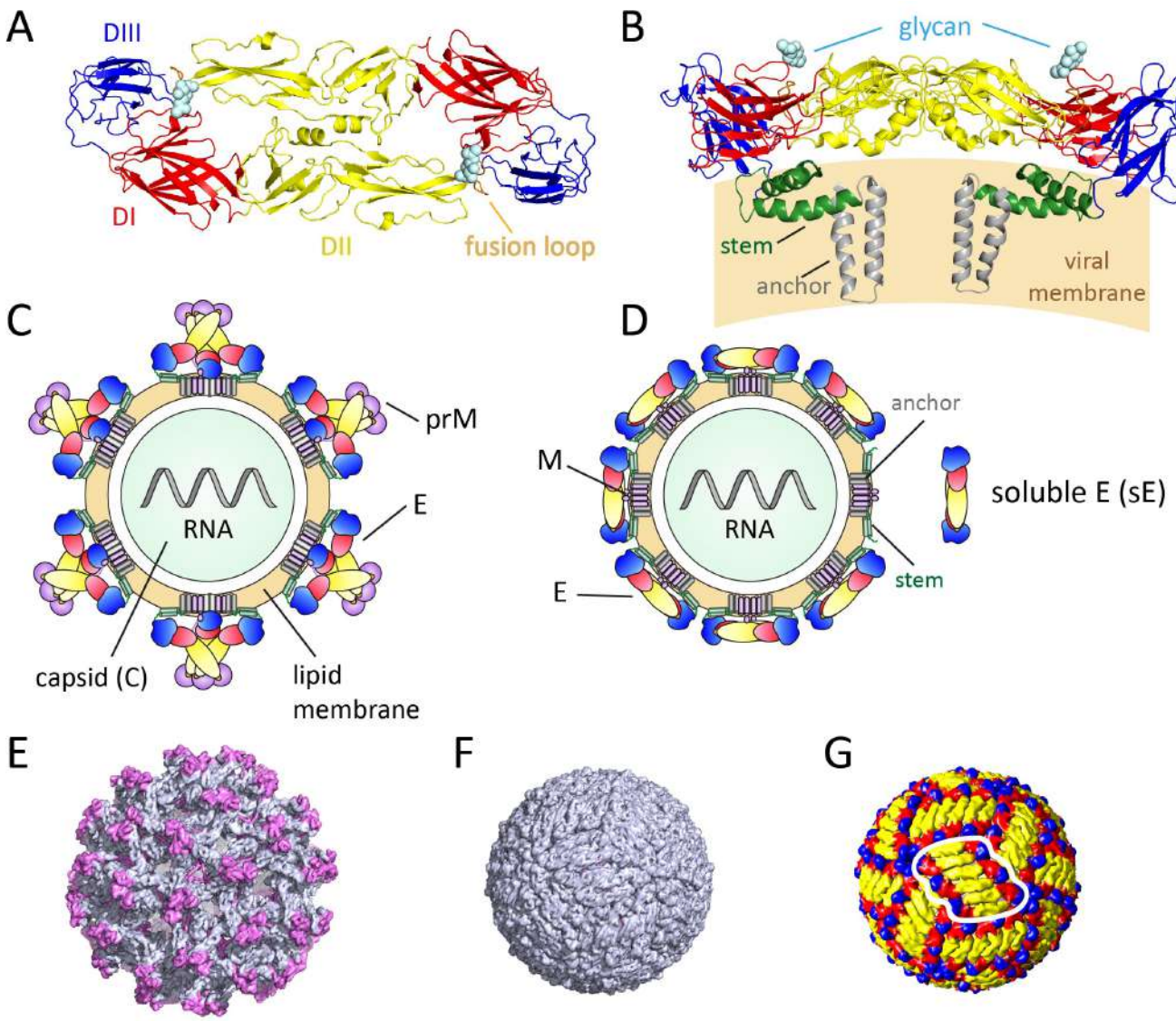
Introduction

Tick-borne encephalitis virus (TBEV) has played a pioneering role in the history of flavivirus structural biology, being the first of these viruses for which a high atomic resolution structure of the envelope glycoprotein E was determined[1] (Fig. 1A). This undertaking started 1987 in a collaboration between researchers at the Institute of Virology, University of Vienna, (now Center for Virology, Medical University of Vienna), and the Department of Biochemistry, Harvard University. The work took several years and required the purification of a total of 49 mg of a soluble form of the TBEV E protein (sE) that was isolated by trypsin cleavage from 401 mg of purified infectious TBEV. To obtain these amounts, 34,300 embryonated eggs were used for the preparation of primary chick embryo cells that were required for growing the virus. First structural details became visible in 1993, and the complete study was finally published in 1995.[1]

The structure of sE was a great surprise because of its unexpected features. In stark contrast to the prototypic influenza envelope glycoprotein (hemagglutinin, HA), which forms spiky projections of HA trimers at the viral surface, the TBEV E protein is an antiparallel dimer that is oriented horizontally to the viral membrane (Fig. 1A,B). Each of the monomeric sE subunits contains three domains (DI, DII, and DIII) that are connected to each other and the membrane-associated part of the protein by flexible linker regions. It took eight additional years until another flavivirus E protein structure (the dengue virus E protein) was published.[2] Meanwhile, atomic resolution structures of E proteins are available for several of the most important human pathogenic flaviviruses, including dengue viruses, West Nile virus (WNV), Japanese encephalitis virus (JEV), Zika virus and Yellow fever virus (YFV),[2-11] which gives the name to the genus *Flavivirus* in the family *Flaviviridae*.[12] All of these structures have the same overall protein architecture as the TBEV E protein.

In terms of their structure, flaviviruses are today among the best-studied enveloped viruses. Importantly, new technologies and instrumentation have led to the elucidation of structural details not only of the isolated E protein but also of whole virus particles using electron cryomicroscopy (cryo EM). Structures of both immature and mature virions are available for closely related mosquito-borne flaviviruses (such as dengue, West Nile, Japanese encephalitis, and Zika viruses)[13-25] and form the basis for understanding the viral life cycles and interactions with antibodies at a molecular level. Recently, a high-resolution cryo-EM structure of mature TBEV was published by Fuzik et al.,[26] providing for the first time details of the particle organization and interactions of proteins in a flavivirus transmitted by ticks (Fig. 1B,G).

Figure 1: Structural organization of flaviviruses



(A,B) Ribbon diagrams of the TBEV sE dimer [PDB code: 1SVB, (1)] and full-length E dimer [PDB code: 5O6A,[26]]. (A) Top view. (B) Side view. Color code E: domain I (DI), red; domain II (DII), yellow; domain III (DIII), blue; fusion loop (FL), orange; stem, green; membrane anchor, grey.

(C,D) Schematic representations of immature (C) and mature (D) virus particles.

(E,F) Electron cryo-microscopy structures of dengue virus serotype 1 particles. (E) Immature virion [PDB code: 4B03, [21]]. (F) Mature virion [PDB code: 4CCT, (21)]. The prM proteins are shown in purple, and the E proteins in gray. (G) Electron cryo-microscopy structure of TBEV [PDB code: 5O6A,[26]], with individual domains of E colored as in A to D. One raft consisting of 3 parallel E dimers is encircled in white.

Panels A, B, E and F were prepared with PyMOL (Schrödinger LLC), panel G with UCSF Chimera [[119], http://www.rbvi.ucsf.edu/chimera/].

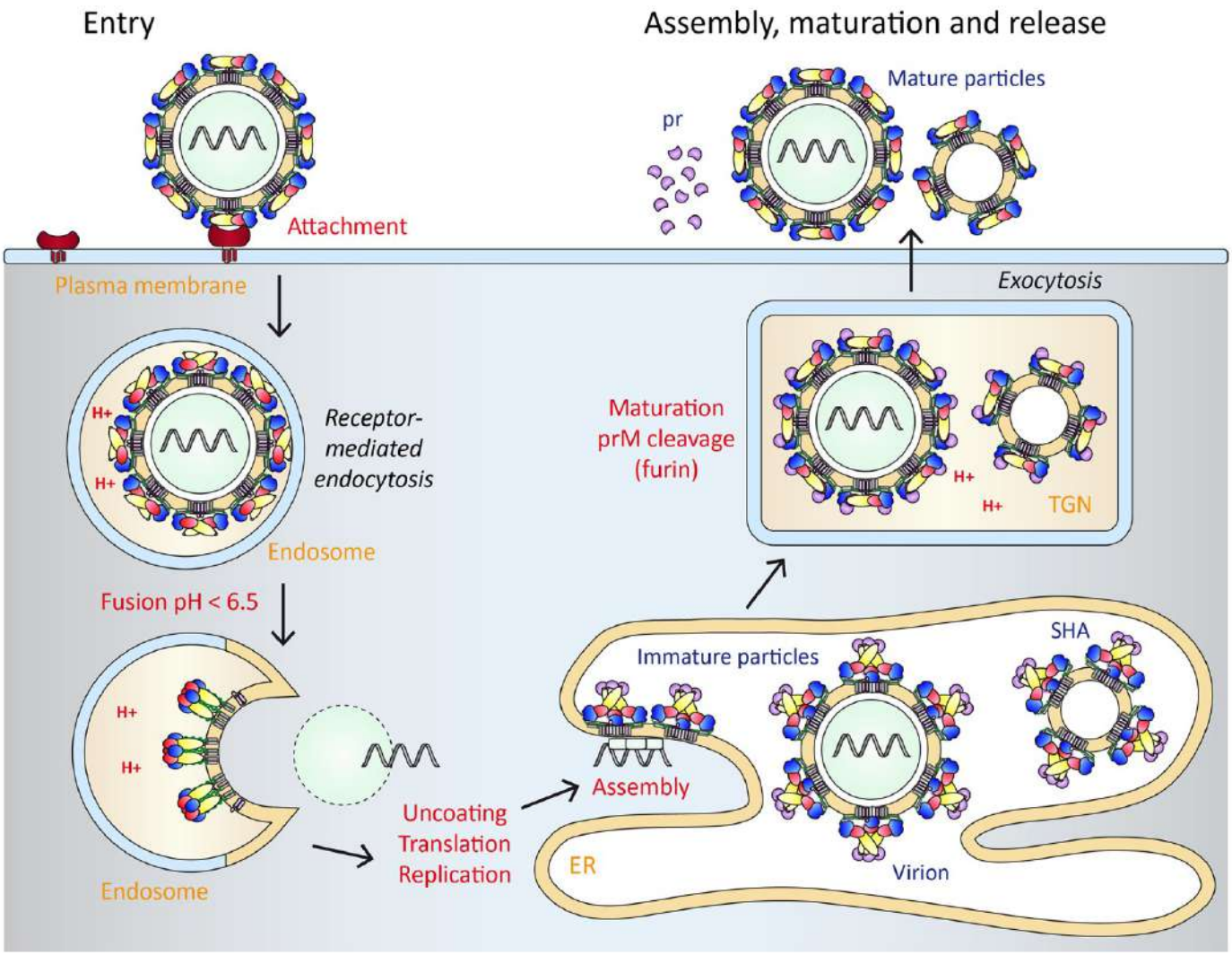
Here, we review the structure of TBEV with a focus on the role of E in the viral life cycle and as a major determinant for the induction of virus-neutralizing antibodies. These properties are discussed in the context of what is known for other flaviviruses, in order to provide a more rounded picture of TBEV structure-function relationships and to emphasize the gaps that still exist in our understanding of the structural foundations of TBEV biology.

Virus particle structures and life cycle

Virus assembly, maturation and release

Virus assembly takes place in the endoplasmic reticulum (ER) and leads to the formation of immature particles (Fig. 1C,E and Fig. 2).[27] This first assembly product contains three

Figure 2: Life-cycle of flaviviruses



Left: Virus entry. Viruses are taken up by receptor-mediated endocytosis and low pH in endosomes triggers viral membrane fusion, resulting in the release of the viral genome into the cytoplasm. Protein translation and RNA replication occur at virus-induced ER membranes.

Right: Virus assembly, maturation and release. Formation of immature virions takes place by a budding process into the ER. As a byproduct, subviral particles are formed that are devoid of a nucleocapsid. Particles are transported through the exocytic pathway. The acidic pH in the TGN causes a major structural rearrangement that leads to the formation of an E herringbone-like arrangement that is characteristic of mature virions (see Fig. 1) and exposes the furin cleavage site in prM. The cleaved-off pr segment of prM remains associated with E at acidic pH but falls off at the neutral pH of the extracellular fluid upon secretion of the particles.

Color code of prM and E as in Fig. 1.

structural proteins: C (capsid), forming an ill-defined spherical core together with the viral genomic RNA, and two membrane associated proteins, prM (precursor of M) and E in a heterodimeric complex. Trimers of these heterodimers form spikes at the surface of immature particles that are non-infectious (Fig. 1C,E).

Studies with TBEV have provided evidence that prM functions as a chaperone for the correct folding of E during its biosynthesis, at least in certain cellular environments.[28] Experiments with recombinantly expressed prM and E proteins in mammalian cells (COS-1) revealed that heterodimerization of the two proteins occurs rapidly and is important for the final folding steps. On the one hand, E apparently requires prM to reach its native conformation efficiently and on the other hand, prM needs E for rapid signal sequence cleavage at its N-terminus during viral polyprotein processing. After their formation in the ER, immature virus particles are transported through the exocytic pathway of the cell. As a crucial step of virus maturation, the prM protein is cleaved in the trans-Golgi network (TGN) by the cellular protease furin, generating membrane-anchored protein M and the proteolytic fragment pr.

Table 1. Furin cleavage sites of different flaviviruses

Virus	Strain	Amino acid (AA) sequence pr P14[b] P1 ↓[a]	AA sequence M P1' P6' [c]	GenBank Accession no.
TBEV	Neudoerfl	YGRCGKQEGS--RT**RR**	SVLIPSH	U27495
POW virus	LB	YGRCGRQAGS--RG**KR**	SVVIPTH	L06436
DEN-1 virus	SG/07K3640DK1/2008	YGTC-SQTGEHRRD**KR**	SVALAPH	GQ398255
Zika virus	H/PF/2013	YGTCHHKKGEARRS**RR**	AVTLPSH	KJ776791
WNV	NY_99	YGRC-TKTRHSRRS**RR**	SLTVQTH	DQ211652
YFV	Asibi	YGKC-DSAGRSRRS**RR**	AIDLPTH	AY640589

[a] *The arrow indicates the proteolytic cleavage site.*
[b] *Sequence positions P1 to P14 (TBEV numbers) upstream of cleavage site (pr part), dibasic motif in bold letters (P1, P2)*
[c] *Sequence positions P1' to P6' downstream of cleavage site (M protein)*

Increasing the pH in the TGN of TBEV-infected cells by acidotropic agents (such as ammonium-chloride) or by bafilomycin A1 (a specific inhibitor of the vacuolar type H+ ATPase) led to the release of immature particles with a 20 – 50 fold lower specific infectivity and hemagglutination (HA) activity than mature viruses.[29] This suggested that a conformational change in the prM-E complex is induced by the slightly acidic pH in the TGN, which is required for furin cleavage. Evidence that the maturation cleavage is conferred by the TGN-resident protease furin was obtained in experiments with a furin-deficient human cell line (LoVo), which produced only immature viruses, as well as a specific furin inhibitor that blocked furin cleavage, and by in vitro cleavage experiments with recombinant furin.[30] Treatment of immature TBEV particles with furin resulted in a 100-fold increase in specific infectivity and the acquisition of hemagglutination as well as membrane fusion activities.

Importantly, furin cleavage itself did not require an acidic pH, but the conformational change exposing the cleavage site in the prM-E complex was acid pH-dependent. The low-pH-induced reorganization of the protein complex was shown to be irreversible in the case of TBEV[30], but appears to be reversible in the case of dengue viruses.[31]

The furin cleavage site of TBEV corresponds to a consensus sequence also found in other flavivirus prM proteins (Table 1). The dependence of virus maturation on this conserved sequence element in prM was demonstrated directly by a genetic approach. A specific mutation in the furin recognition sequence engineered into an infectious TBEV clone (resulting in the deletion of one of the arginines at P1,P2; Table 1) did not impair the assembly of immature particles but completely abolished infectivity.[32] Infectivity could be restored by in vitro trypsin cleavage, which is likely to cleave at one of the R residues that was retained at the furin cleavage site (Table 1).

So far, the structure of immature virions has only been determined for mosquito-borne flaviviruses, which were shown to carry 60 spikes of trimers of prM-E heterodimers.[16,19,21,31] Considering the high degree of structural conservation of viral proteins and mature particles, it is justified to assume that immature TBE virions are similar to those of mosquito-borne flaviviruses. In the course of exocytosis of immature viral particles, the acidic pH in the TGN causes a major re-arrangement of the viral glycoprotein interactions, resulting in the conversion of the trimeric prM-E spikes into a herringbone-like shell of 90 E protein dimers (Fig. 1G). Data obtained with dengue virus show that the pr fragment remains associated with the particles at acidic pH after furin cleavage but dissociates at neutral pH when the particles are released from the cells (Fig. 2).[31]

Mature virions display the herringbone-like arrangement of E that was induced in immature particles when encountering the low pH in the TGN. The release of the pr fragment leaves E in a metastable conformation, poised to undergo dramatic low pH-induced structural changes that mediate viral fusion in endosomes upon virus entry (see below). The function of prM and the pr fragment is thus to protect E in the acidic TGN and to avoid membrane fusion already at this stage of the viral life cycle.[33]

The static pictures of fully immature and fully mature particle structures determined by cryo EM cannot be reconciled with all experimental data obtained in studies of flavivirus entry and virus interactions with antibodies.[34, 35] First, some antibodies binding to seemingly inaccessible (cryptic) epitopes in E neutralized viral infectivity in various flavivirus systems. These observations led to the concept of 'virus breathing' as a consequence of envelope glycoprotein dynamics,[36] reflecting the metastable nature of E which transiently exposes otherwise buried protein surfaces

within the E dimer or at the inter-dimer contact regions in the virion. Retrospectively, antibody-induced conformational changes, described for TBEV already in 1984, are also likely due to E protein dynamics and virus breathing.[37] Secondly, many data indicate that virus particles released from infected cells are a heterogeneous mixture of immature, partially mature and fully mature particles.[38,39] As a specific structural feature, partially mature and breathing particles expose the viral membrane, which has been shown to be a target for interactions with cellular lipid receptors that can mediate cell entry.[40,41]

It can be hypothesized that an ensemble of heterogeneous particles in combination with virus breathing may be important for flaviviruses to infect different tissues in their invertebrate and vertebrate hosts.[34] Heterogeneity may also be required to maintain these viruses in their natural cycles and constitute a powerful means to adapt to new environments or to acquire new pathogenic properties, such as those observed in the recent Zika virus epidemic.[42,43]

Subviral particles

Flavivirus-infected cells do not only secrete complete virus particles but also subviral particles that are non-infectious and smaller than whole viruses but have similar HA activity. Because of these properties they were described as 'slowly sedimenting hemagglutinin' (SHA) in the flavivirus literature.[44, 45]

Noninfectious subviral particles of TBEV were produced in recombinant form by the co-expression of the two viral glycoproteins prM and E in COS-1 cells.[46,47] These particles [designated 'recombinant subviral particles' (RSPs)] were secreted from transfected cells and had a density of approximately 1.14 g/cm³.[48,49] They were sensitive to disintegration by the detergent Triton X 100, consistent with the presence of a lipid membrane carrying the two viral envelope proteins.[48] The formation of RSPs could also be achieved by the expression of prM and E from separate plasmids, but was not possible with a soluble form of E that lacked its membrane anchor.[47] More detailed mapping studies allowed the identification of the so-called stem together with the first trans-membrane regions of E to be essential for particle formation.[50]

The ER was shown to be the site of assembly of RSPs by biochemical and electron microscopical analyses.[51] In addition to the rough ER, RSPs were observed in the smooth ER and downstream compartments of the secretory pathway. Approximately 75% of the particles had a diameter of 30 nm, but a number of larger particles and tubular structures were also seen in vesicular compartments of transfected cells.[51]

It is an important conclusion of these studies that the formation of prM-E heterodimers and their lateral interactions are sufficient to drive the budding of membrane-containing virus-like particles at the ER membrane, in the absence of any interactions with viral RNA or a capsid.

The 30 nm RSPs were the first flaviviral particles for which a cryo-EM structure was determined.[52] The 19Å resolution map revealed an arrangement of E protein dimers in a T=1 icosahedral surface lattice (different from that of the virion, Fig. 1G) and allowed the definition of interaction sites between E dimers, positions of M relative to E, and the assignment of transmembrane regions of E and M. When the prM furin cleavage site was deleted in the plasmid construct for RSP production by mutagenesis, a substantial number of particles were observed that had the same size as whole immature virions (diameter 60 nm), in addition to the 30 nm particles described before.[53] It was therefore concluded that the primary assembly products in prM-E expressing cells are immature particles of both size classes, but in their mature forms (i.e. after prM cleavage) the larger particles are less stable and therefore seen as a minority compared to the 30 nm particles secreted from transfected cells. Apparently, alternative assembly products can be formed by prM-E interactions. The role of subviral particles in natural TBEV infections of ticks and/or mammalian hosts remains to be elucidated.

The E protein in RSPs appears to be structurally and functionally identical to that at the surface of whole TBE virions.[48] As a consequence, RSPs proved to be a valuable non-infectious model system to assess biological properties of E, including membrane fusion and antigenic structure (see below: Structure and functions of E – Virus entry and membrane fusion; Antigenic structure of TBEV and virus neutralization).[54-60] Importantly, their particulate nature also makes them an excellent candidate for use as a recombinant vaccine antigen, as shown by mouse immunization and challenge experiments.[61] In these experiments, the immunogenicity of RSPs was compared with soluble E dimers, E rosettes formed by detergent removal after solubilization of the viral membrane, and whole formalin-inactivated purified TBEV. With respect to both the extent of antibody induction and protection from challenge, the RSPs were equivalent to the inactivated virus vaccine. This high immunogenicity is most likely due to the presentation of multiple copies of the native E protein on a large particulate carrier, mimicking its presentation on whole virus particles. Similar conclusions were also derived from a DNA immunization study in mice.[62] Plasmid constructs giving rise to secreted RSPs were superior to those expressing a secreted C-terminally truncated E dimer, or a non-secreted full-length form of E.

Structure and Functions of E

The TBEV E protein has at least two essential functions in the viral life cycle (Fig. 2), consistent with its prominent presentation at the viral surface. It is responsible for interactions with attachment factors and/or entry receptors at the plasma membrane of target cells, and it mediates viral membrane fusion after cellular uptake by receptor-mediated endocytosis. While TBEV membrane fusion has been studied in great detail, the search for viral receptors is still quite elusive, reminiscent of the situation described for flaviviruses in general.[40]

Cell attachment and receptors

Several sets of experiments have provided evidence that TBEV can use negatively charged glycosaminoglycans (GAGs) such as heparan sulfate (HS) as an attachment factor in certain cells.[53,63] Passaging of a virus isolate from ticks in BHK-21 cells resulted in the accumulation of mutations that were distributed over a large part of the upper and lateral surface of E including each of the three domains[63] (Fig. 1). Importantly, these mutations resulted in an increase of positive charges at the viral surface, increasing its affinity for BHK-21 cells. Growth of the mutant viruses, but not the wild type, could be inhibited competitively by heparin, confirming their adaptation and dependence on GAG-binding for entry. The increased affinity for GAGs was associated with a decrease in virulence in a mouse model and may be a general principle for attenuating flaviviruses. A connection between an increased binding to GAGs and attenuation was also observed for viruses of the JEV serocomplex[64-66] and the 17D strain of the live yellow fever vaccine.[67]

The role of GAGs in TBEV entry was investigated in greater detail using mutant CHO cells that are deficient in the synthesis of GAGs.[68] Interestingly, while virus binding to these mutant cells was much lower than that to normal CHO cells, no difference was observed in terms of cell infection. It was therefore suggested that HS is not required for the infection of CHO cells, and that one or more other receptors are required for virus entry into these cells.

Since the structure determination of E, the immunoglobulin -like domain III (Fig. 1) has been hypothesized to be a site of receptor interactions not only for TBEV but for flaviviruses in general.[1,69] This was primarily based on the fact that a number of mutations affecting flavivirus virulence were concentrated in this domain and that the so-called FG loop is enlarged to contain an RGD sequence in some mosquito-borne flaviviruses[70], which is a character-istic ligand-binding motif for members of the integrin family of cell surface receptors.[71] Experiments with recombinant domain III of Langat virus (a close relative of TBEV) have revealed that its addition to cells before infection resulted in a somewhat decreased virus growth, which was interpreted as evidence that DIII is involved in receptor binding.[72] So far, however, there is no information as to possible interaction partners of DIII at the cell surface and further efforts will be necessary to get a more complete picture of TBEV receptor interactions.

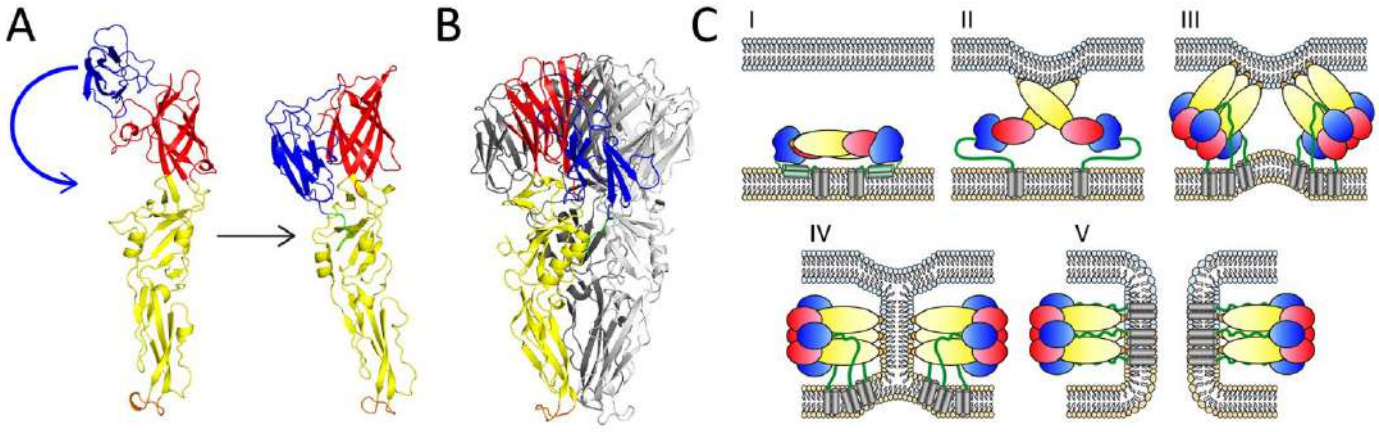
In general, it is believed that flaviviruses may use different receptors in different tissues of the various invertebrate and vertebrate host species involved in natural trans-mission. It was recently shown for several flaviviruses (but not yet for TBEV) that not only the E protein but also lipids of the viral membrane may bind to cellular lipid receptors.[73,74] They normally recognize apoptotic cells and control their removal by phagocytes.[75,76] Hijacking these receptors by viruses to gain access to cells has therefore been designated apoptotic mimicry.[41]

Virus entry and membrane fusion

The presence of acidotropic agents such as NH4Cl or bafilomycin A1 early in infection had a strong inhibitory effect on the replication of TBEV, consistent with virus uptake by receptor-mediated endocytosis and the importance of an acidic endosomal compartment for viral membrane fusion.[29] The acid pH-dependence of TBEV fusion activity was first demonstrated by Guirakhoo et al. 1991[77] and further studied in great detail using a combination of biochemical, structural, mutational, and functional studies.[54,57,59,60,78-83] Chemical cross-linking experiments and sedimentation analyses demonstrated that the exposure to acidic pH caused a quantitative oligomeric rearrangement of metastable E dimers into stable trimers at the virion surface, with a pH threshold of 6.5,[84] suggesting that this dimer-trimer transition provides the energy and drives the fusion of viral and endosomal membranes. Further biochemical studies[85] indicated that the structural conversion of E was a two-step process, in which the acidic pH in endosomes first caused the dissociation of E dimers followed by an irreversible trimerization. Monoclonal antibody studies and mutational analyses provided evidence that the highly conserved sequence element in E, located at the tip of DII and now designated fusion loop (FL), was responsible for interacting with the endosomal target membrane as an initial step in membrane fusion.[55,79]

It was a key finding of these studies that the soluble E dimer (which dissociates into monomers at acidic pH) could be converted into a trimer in the presence of liposomes,[79,86] laying the foundation for the crystallization of this post-fusion conformation and the determination of its atomic structure by X-ray crystallography[87] (Fig. 3). The structure revealed that the folding of the three domains is maintained but that their relative orientation is altered (Fig. 3A,B). Specifically, DIII relocates from its position at the end

Figure 3: Post-fusion structure of E and fusion mechanism



(A) Ribbon diagrams of E monomers in their pre- and post-fusion conformations, revealing the relocation of domain III (indicated by a blue arrow in the pre-fusion conformation).

(B) Ribbon diagram of the trimeric post-fusion structure of TBEV sE [PDB code: 1URZ,[87]].

(C) Schematic of steps involved in flavivirus membrane fusion. Panel I: Metastable E dimers at the viral surface. Panel II: Low-pH-induced dimer dissociation, exposure of the FL and interaction with the endosomal membrane. Panel III: Relocation of domain III and trimer formation. Panel IV: Stem zippering and hemifusion intermediate. Panel V: Final post-fusion structure of E and opening of a fusion pore.

Color code of E as in Fig. 1.

Ribbon diagrams were prepared with PyMOL (Schrödinger LLC).

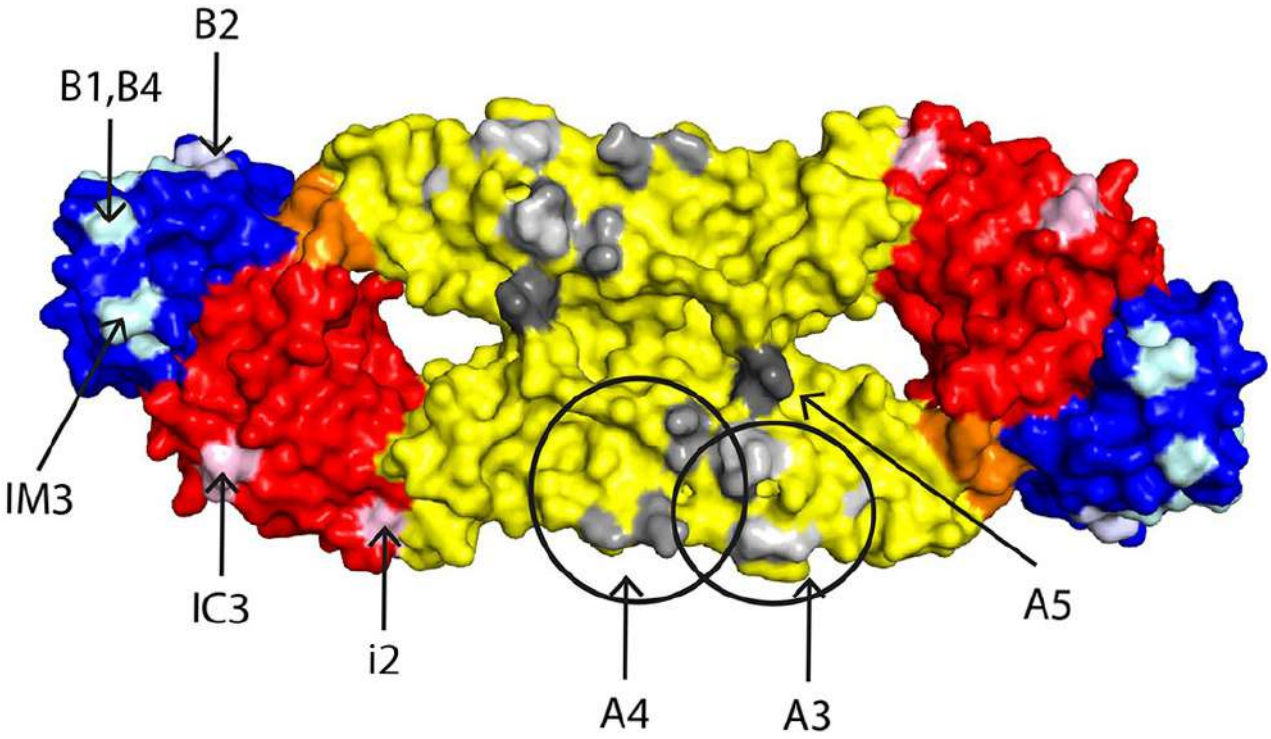
of the dimer to the side of the trimer in such a way that a hairpin-like structure is formed (Fig. 3A), in which the FL and the stem-anchor region of E would be juxtaposed in the full-length E trimer. This structure was reminiscent of the post-fusion structures of class 1 viral fusion proteins such as the influenza virus hemagglutinin and suggested – in combination with studies on fusion intermediates[83] – that the TBEV fusion mechanism consists of several steps as depicted in Fig. 3C. In this process, the acidic-pH-induced dissociation of E dimers leads to the exposure and interaction of the FL with the endosomal membrane, the relocation of DIII and the zippering of the 'stem' along DII in the trimer, thus driving the merger of the two membranes. Mutational analyses provided evidence for a specific molecular interaction at the N-terminal end of the stem and a pocket of DII which appears to be essential for the correct positioning of the stem for the zippering reaction.[60]

An important question in the context of TBEV fusion relates to the molecular switches that sense the acidic pH in endosomes and induce the fusogenic conformational change in E. Because of their pKa near the pH threshold of fusion, histidines have been hypothesized to play such a role in the fusion trigger. There are indeed five histidines (H146, 248, 287, 323, 438) that are absolutely conserved among flavivirus E proteins, suggesting an indispensable structural and/or functional role in the viral life cycles. The use of RSPs (see above) with mutated histidines at these positions allowed the identification of H323 as a key residue for triggering the acidic-pH-induced trimerization of E and concomitant membrane fusion.[57] This residue is involved in intramolecular interactions at the interface between DI and DIII in the E dimer. Its protonation apparently facilitates E dimer dissociation and allows DIII to be released from its original position and to relocate as required for post-fusion trimer formation (Fig. 3). Other conserved histidines were shown to be dispensable for fusion, but they may have critical roles in unrelated low-pH- driven processes of the viral life cycle, such as virus maturation (Fig. 2).

Antigenic structure of TBEV and virus neutralization

Because of its functions in flavivirus attachment and entry as well as membrane fusion in endosomes (see above and Fig. 2,3), the E protein is the major target and inducer of neutralizing antibodies, and all experimental data obtained with TBEV are consistent with this notion. Potently neutralizing and protective antibodies were induced by E solubilized from purified TBEV,[61] confirming the primary role of such antibodies in the induction of a protective immunity.[88] While soluble forms of E and even the isolated DIII were capable of inducing neutralizing antibodies,[61, 89] particulate or aggregated forms (E rosettes) had a much higher specific immunogenicity and would therefore be preferred vaccine antigens.[61]

Figure 4: Binding sites of TBEV E-specific mAbs



Surface representation of the TBEV sE dimer [PDB code: 1SVB,[1]] with the location of amino acids involved in binding sites of neutralizing mAbs. Epitopes are labeled only on one of the two monomers and the mAbs are designated according to references.[92,95]

Color code of E as in Fig. 1.

The figure was prepared with PyMOL (Schrödinger LLC).

Epitopes of TBEV protein E

More precise mapping of epitopes in E and their involvement in virus neutralization became possible with the preparation of TBEV-specific monoclonal antibodies (mAbs).[90-92] By the application of these mAbs for immunochemical analyses and the selection of mAb-resistant virus mutants topological and functional models of epitopes could be defined and epitopes involved in virus neutralization were structurally characterized.[37,58,91-96] The elucidation of the crystal structure of the E dimer then allowed the precise localization of antibody-binding sites (Fig. 4). It became apparent that neutralizing antibodies can be induced by each of the three domains, consistent with a plethora of publications on the antigenic structure of other flaviviruses [reviewed in references[97-99]].

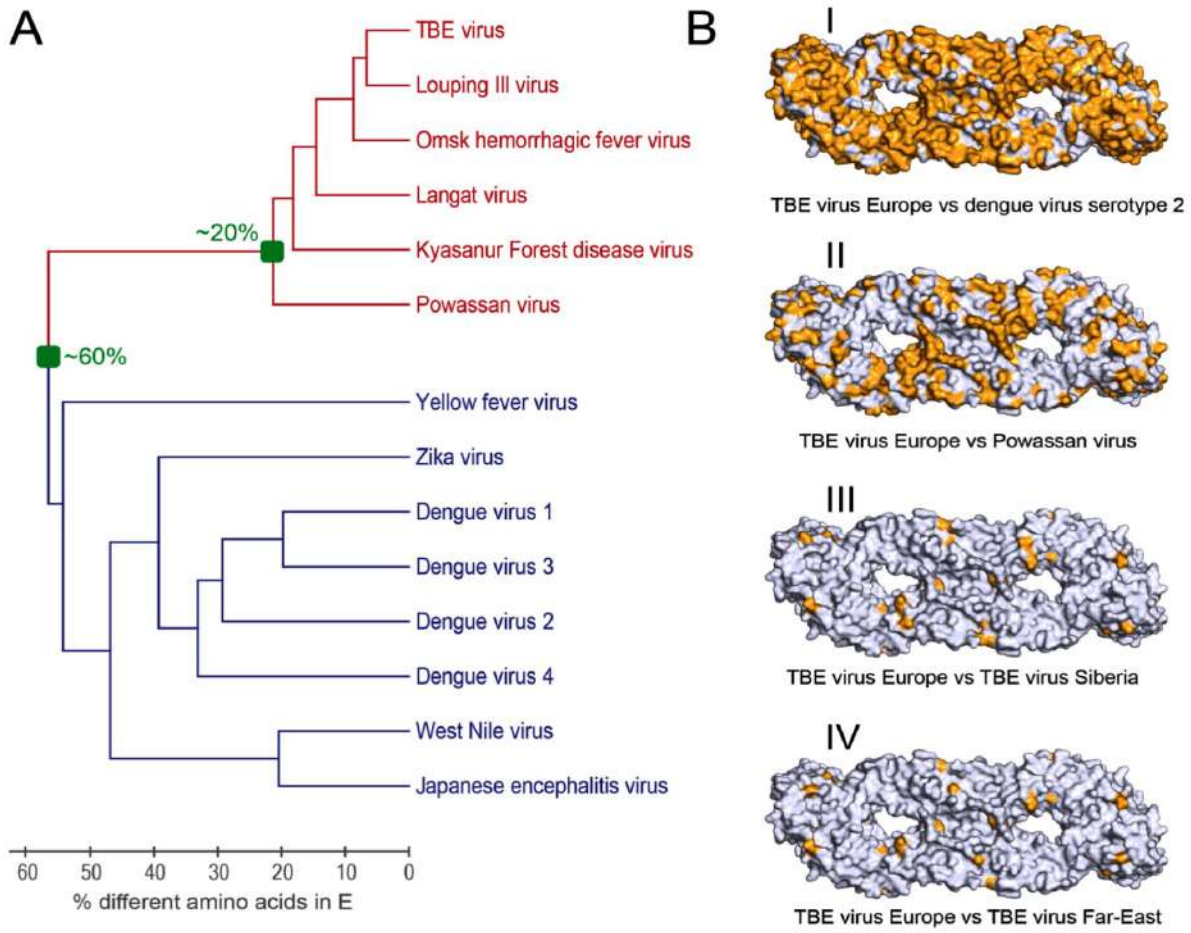
The complexity of the antigenic structure cannot be understood completely on the basis of the isolated E protein. Studies with different soluble and particulate forms of E revealed a strong influence of its quaternary structure and specific display at the surface of virions.[58] The TBEV data are fully consistent with those obtained by high-resolution structural analyses of antibody-E complexes of other flaviviruses [reviewed in references[99,100]].

Taken together, it can be concluded that TBEV, like all other flaviviruses, displays a continuum of antigenic sites at its surface with the potential of inducing neutralizing antibodies. Epitopes of such antibodies have been mapped to individual domains in E or were shown to be more complex and to comprise residues from adjacent domains, from both monomers in the dimer or even adjacent dimers in the herringbone arrangement of E at the viral surface (quaternary epitopes) [reviewed in references[99-101]].

Mechanisms of virus neutralization

The most apparent mechanism of virus neutralization by E-specific antibodies is the blocking of cell attachment, as shown for different flaviviruses.[102] In addition, the inhibition of post-entry processes by antibodies bound to the internalized virus is likely to contribute to virus neutralization.[102] This holds especially true for membrane fusion, which requires substantial relocations of protein domains (see above) that may be impeded by bound antibodies. Insights into such activities were provided by in vitro analyses of TBEV fusion inhibition by E-specific mAbs.[82] Depending on the location of the bound antibody, early and late stages of the fusion process were impaired, by either blocking the initial interaction with the target membrane or

Figure 5: Antigenic relationships of flaviviruses



Dendrogram based on amino acid differences of the TBEV serocomplex (red) and other flaviviruses (blue) (MAFFT Alignment: http://www.ebi.ac.uk/Tools/msa/mafft/).

TBEV (GenBank accession no. U27495), Louping Ill virus (NC_001809), Powassan virus (L06436), Omsk hemorrhagic fever virus (NC_005062), Langat virus (AF253419), Kyasanur Forest disease virus (AY323490), yellow fever virus (AY640589), Zika virus (KJ776791), dengue virus serotype 1 (GQ398255),dengue virus serotype 2 (NC_001474), dengue virus serotype 3 (EU081190),dengue virus serotype 4 (GQ398256), West Nile virus (DQ211652), Japanese encephalitis virus (D90194)

Surface representations of the TBEV sE dimer (strain Neudoerfl, GenBank accession no. U27495, European subtype; PDB code: 1SVB, Rey et al., 1995) in pairwise comparisons with E of other viruses, displaying divergent surface-exposed amino acids in orange. Panel I: dengue virus serotype 2 strain 16681 (NC_001474). Panel II: Powassan virus strain LB (L06436). Panel III: TBEV strain Vasilchenko, Siberian subtype (AF069066). Panel IV: TBEV strain Sofjin, Far Eastern subtype (AB062064).

Panel B was prepared with PyMOL (Schrödinger LLC).

by interfering with the required relocation of DIII and the formation of the post-fusion E trimer (see above).A special case are antibodies directed to the fusion loop at the tip of DII (Figure 1A) which – because of the high conservation of this structural element - are highly cross-reactive with E proteins from all flaviviruses.[56] They react strongly with soluble forms of E and inhibit in vitro liposomal fusion,[82] but they have virtually no neutralizing activity against TBEV. An explanation of this phenomenon is the fact that FL-specific antibodies are unable to react with intact mature TBE virions ('cryptic epitopes')[56] and therefore cannot reach the endosomal compartment where fusion takes place. The cryptic nature of the FL may however differ among flaviviruses, depending on their stability and breathing behavior (see above). As a consequence, broadly flavivirus cross-reactive antibodies may display neutralizing activity against certain viruses only, a feature observed especially with dengue viruses.[35, 103]

Antigenic relationships of TBEV with other flaviviruses

Even the most distantly related flaviviruses have approximately 40% identical amino acids in their E proteins (Fig. 5A). Most of these residues, however, are located inside the protein whereas most of the surface-exposed and

antigenically relevant residues differ among flaviviruses from different serocomplexes. This is visualized in a comparison of such residues in E of TBEV versus that of dengue virus serotype 2 (Fig. 5B, panel I) which shows that almost the whole surface is different, explaining the lack of cross-neutralization between TBEV and flaviviruses of other serocomplexes (Fig. 5A). The only patch of conservation includes the fusion loop, which is cryptic in TBEV and therefore inaccessible for antibodies (see above).

Cross-neutralization is, however, observed within the TBEV serocomplex (Fig. 5A), which also includes Louping Ill, Langat, Omsk hemorrhagic fever, Kyasanur Forest disease, and Powassan viruses. These viruses display a higher degree of conserved patches of amino acids at their surface that is responsible for cross-neutralization. Powassan virus is the most distant relative of TBEV in this serocomplex with approximately 20% sequence divergence in E (Fig. 5A).

TBEV subtypes and strains

Comparison of virus strains from all areas of TBEV endemicity have revealed three major subtypes [European, Siberian, and Far Eastern[101]] which are sometimes also referred to as genotypes.[104] Additional heterogeneity may exist and two further genetic lineages have been described.[104,105] Overall, the amino acid sequence divergence observed in the E proteins of different TBEV subtypes does not exceed 6.9%.[106] This is within the range of natural variation observed with other human-pathogenic flaviviruses (e.g. YFV 5%; WNV 7%). Pairwise comparisons of individual strains from different subtypes show that the differences are relatively small (Fig. 5B). Variation observed within the subtypes is even smaller, and does not exceed 1.8% for the European subtype.

The low degree of antigenic variation is an important aspect of vaccine usage. Experiments with serum samples obtained after vaccination with a European subtype TBE vaccine revealed no differences in the neutralization of European, Siberian or Far Eastern TBEV subtype strains, whereas neutralization of the closely related OHF virus (Fig. 5A), was somewhat reduced.[107] In a related study, a high degree of cross-protection between TBEV subtypes was also observed in mouse challenge experiments after immunization with vaccines based on European or Far-Eastern subtype strains.[105,108] It was therefore concluded that a single vaccine will protect against all TBEV strains circulating in nature, similar to the situation with vaccines against other flaviviruses such as JEV and YFV.

Overall, the degree of cross-neutralization by polyclonal sera within the TBEV serocomplex (and other flavivirus serocomplexes) seems to follow the degree of amino acid conservation in E (Fig. 5A). Observations made with some flaviruses, however, indicate that differences at single amino acids can lead to substantial differences in virus neutralization, presumably due to influences of such mutations on virus envelope dynamics and the accessibility of certain epitopes.[109,110] A similar variation, related to a single amino acid difference in E, was reported in a comparative study of vaccines that use different strains as seed viruses for vaccine production.[111] Differences were found in the induction of antibodies that neutralize circulating strains of TBEV that could be related to a single amino acid difference (N52K) at the hinge region between DI and DII.

Fine specificities of antibody responses to TBEV

The mapping of epitopes in the E protein of TBEV and other flaviviruses with mAbs has provided us with deep insights into antigenic structure and details of antibody interactions with these viruses. In contrast, relatively little is known about the fine specificities of antibodies in polyclonal responses as well as their individual variation after TBEV infections and vaccinations. The issue was addressed by Jarmer et al.[112] who deconstructed human antibody responses after TBEV infection and vaccination using immunoassays with recombinant proteins consisting of individual domains and domain combinations of E. Extensive variation was not only observed with respect to the extent of antibody formation but also with respect to the fine specificities of antibodies produced in the course of immune responses, suggesting that patterns of immunodominance are strongly influenced by individual-specific factors. Importantly, most of the neutralizing activity could be depleted from sera by the dimeric E protein, indicating that complex quaternary epitopes, depending on the herringbone-like arrangement of E dimers at the viral surface (Fig. 1G), play only a minor role in the neutralizing antibody response, both after infection and vaccination.

In humans, it is currently unknown to what extent the fine specificities of antibody responses can be modulated by pre-existing antibodies (against homologous or heterologous flavivirus antigens) when present at the time of infection or vaccination. A mouse immunization study with the recombinant TBEV E protein dimer and passively administered monoclonal antibodies, however, revealed that such influences may be substantial.[113] Mechanistically, the differences observed in antibody responses were related to shielding of epitopes in E by the co-administered mAb and to mAb-induced dissociation of the E dimer, resulting in the exposure of antigenic surfaces that would be cryptic in the native protein. It remains to be seen, whether human antibody responses may be modulated by similar mechanisms and whether the resulting changes in antibody fine specificity and composition can affect virus neutralization.

Perspectives and future research

The era of flavivirus structural biology was initiated by the X-ray structure determination of the TBEV E protein dimer[1] and has now led to unprecedented insights into the organization and molecular changes of flavivirus particles in different phases of the viral life cycle.[114-116] Although a high resolution particle structure of TBEV is now available in its mature form,[26] the structure of immature particles has not yet been determined and will be a topic of future research. The same also holds true for investigations in the complex area of viral receptors. Recent data obtained with other flaviviruses suggest that populations of heterogeneous, partially mature but infectious virus particles may be produced in different hosts and tissues involved in the viral life cycles. Such heterogeneity in combination with the phenomenon of virus breathing is a mechanism that modulates the viral surface and thus increases potential interaction sites with cellular attachment factors.[34,36,117] Particle heterogeneity also promotes the exposure of the viral membrane as a prerequisite for using apoptotic mimicry in virus entry,[41] a mechanism that has yet to be investigated for TBEV. Populations of heterogeneous particles may not only be essential for virus replication in quite distantly related invertebrate and vertebrate hosts, but also modulate antibody responses and epitope recognition.[109,110,118] These are new exciting aspects of flavivirus structure that provide a fertile ground for interesting and important investigations in the future, aiming at a more profound understanding of the complex biology of TBEV as a human pathogen.

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Chapter 3

Transmission / natural cycle

Lidia Chitimia-Dobler, Ute Mackenstedt and Olaf Kahl

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 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 14 genera (hard ticks), the Argasidae with genera (soft ticks), and the Nuttalliellidae, represented by only one species, *Nuttalliella namaqua*.[7-9]

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.

Ixodes is the largest tick genus, with 244 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, *Ixodes persulcatus* 'the taiga tick' in north-eastern Europe and northern Asia, and *Ixodes ovatus* in the forest belt of middle Asia and Japan.

The genus *Dermacentor* has 35 species worldwide.[7] The basis capituli 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 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 capituli. In Europe, TBEV has been *recovered from Haemaphysalis punctata ('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 (Fig. 1). 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 trans-mission 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 2a, 2b, 2c, and 2d) 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 3a and 3b). Nymphs have 4 pairs of legs and spiracles (Figures 4a and 4b). Adult females have 4 pairs of legs and spiracles, a genital aperture, and porose areas on the dorsal surface of the basis capituli (Figures 2a and 2b). 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 2c and 2d).

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.[15] The main vectors of TBEV, *I. ricinus* 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.[15] 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 nearby passing.

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 has also 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]

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]

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*

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] *I. persulcatus* is more restricted to mammal hosts.[28]

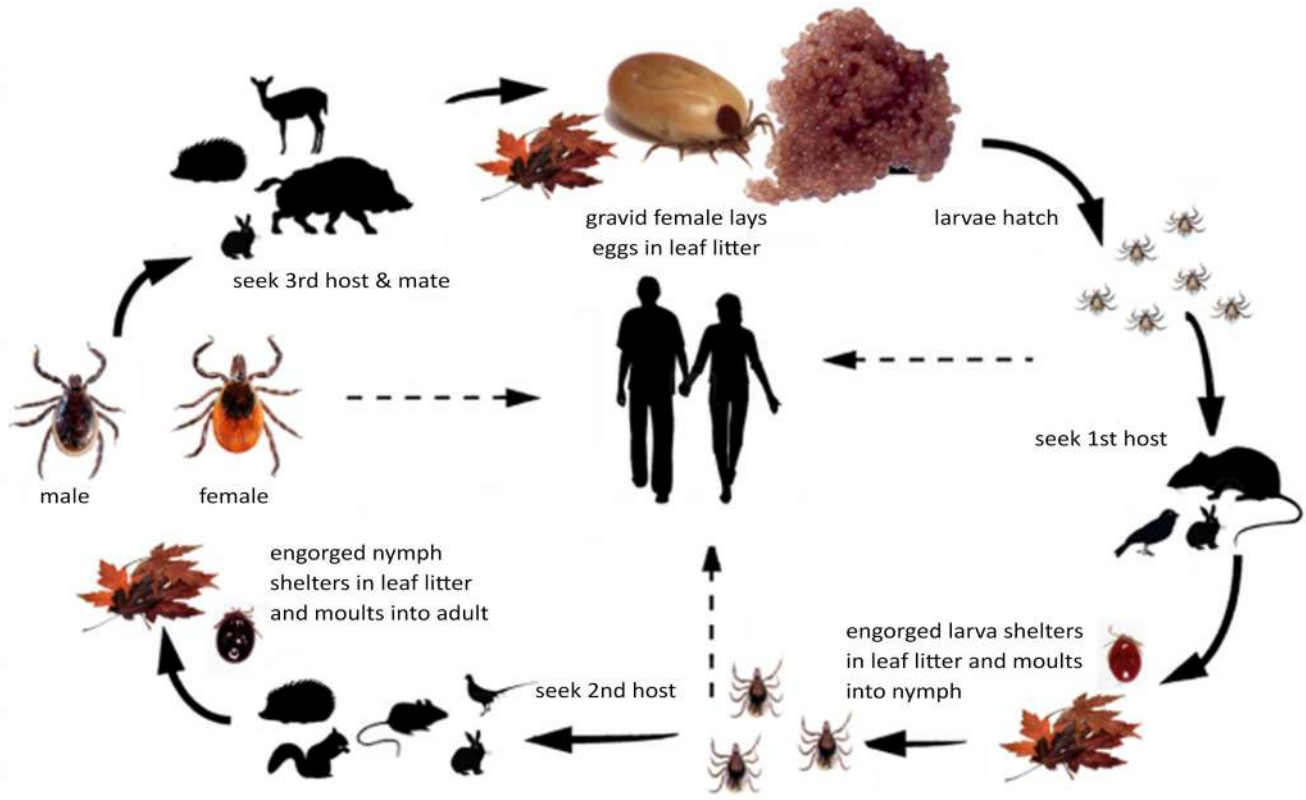
Questing height is also important. Ticks questing on or near the ground are exposed mostly to small animals, while those questing higher in the vegetation are more likely to encounter larger animals. The extent to which different hosts are utilized depends on host behavior and opportunities for contact, such as foraging range, time of day and time spent foraging, habitats visited, and other factors.[15]

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

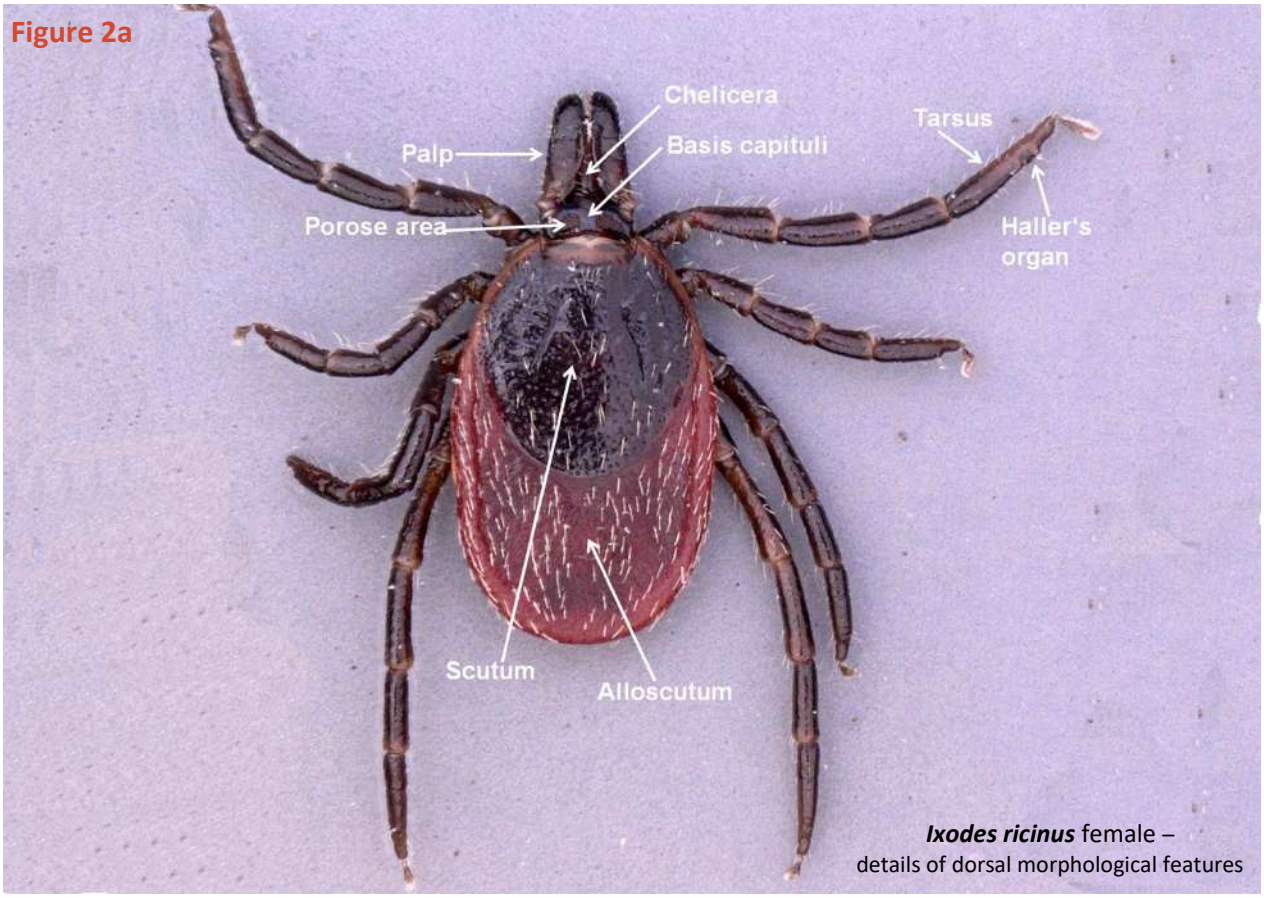
Ticks occur in many terrestrial habitats ranging from cool, arboreal northern forests to hot, arid deserts. Each species, however, has become adapted to the specific types of habitat where it is generally found in highest abundance. All *I. ricinus* postembryonic stages are exophilic and depend entirely 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]

Figure 1



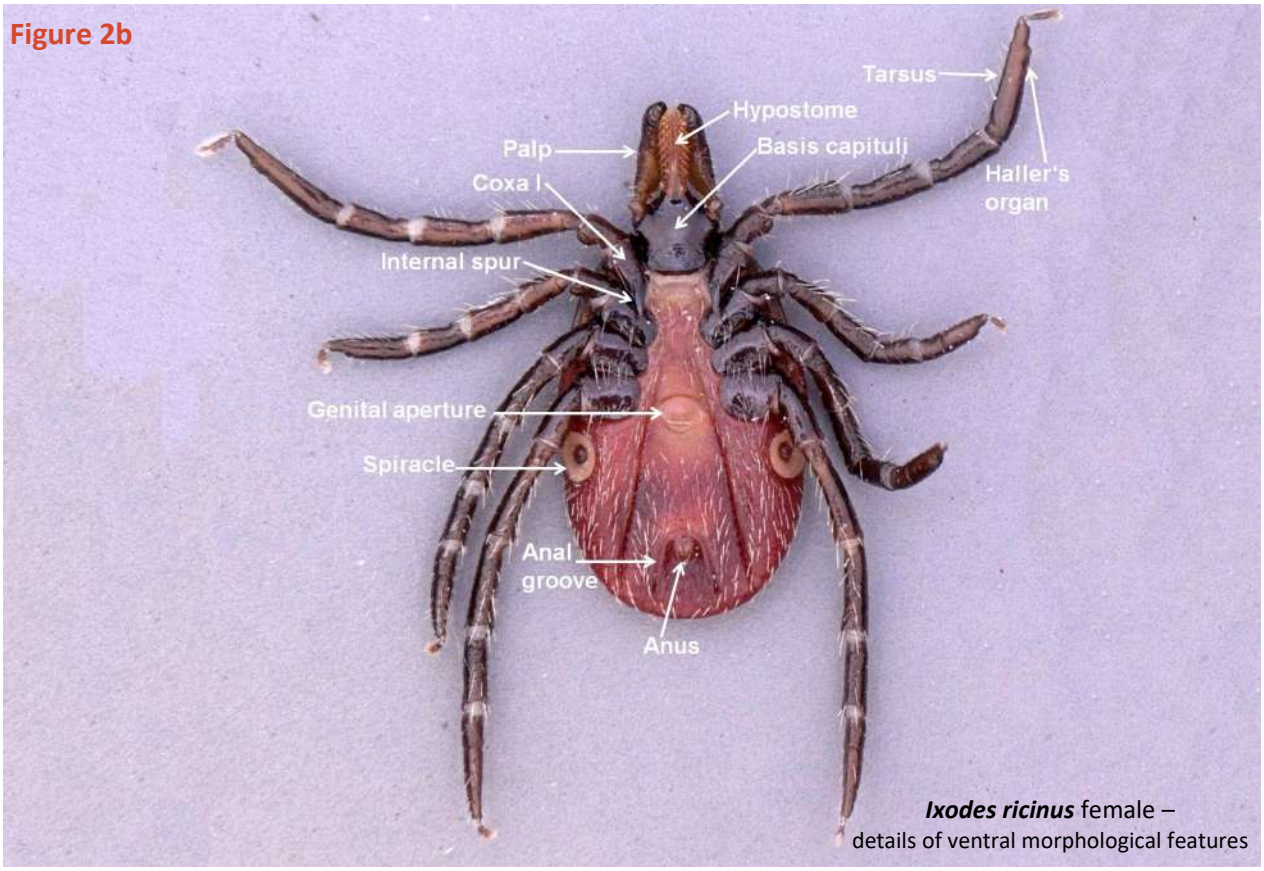
The life-cycle of Ixodes ricinus. The dotted arrows indicate potential transmission to humans. ©Nina Littwin

Figure 2a



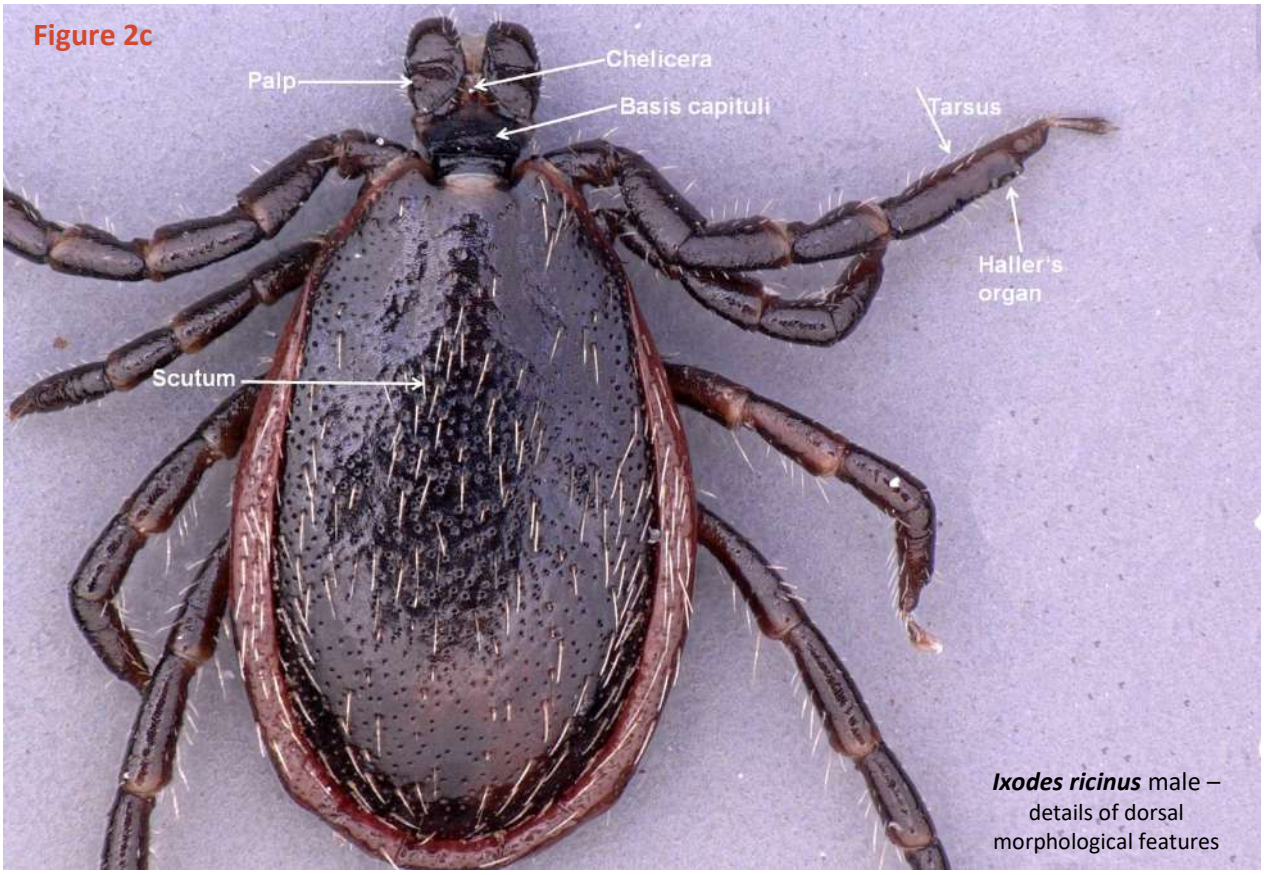
Ixodes ricinus female – details of dorsal morphological features

Figure 2b



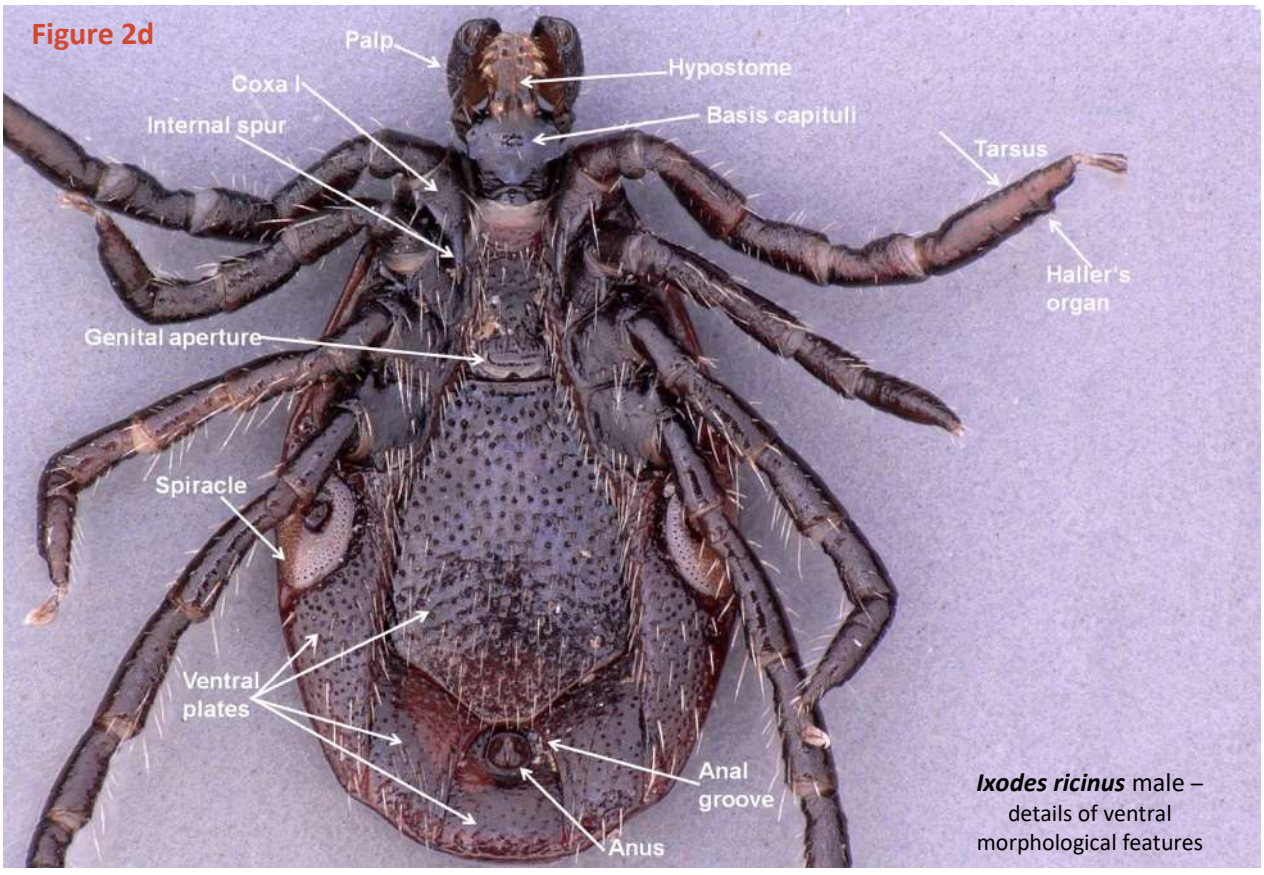
Ixodes ricinus female – details of ventral morphological features

Figure 2c



Ixodes ricinus male – details of dorsal morphological features

Figure 2d



Ixodes ricinus male – details of ventral morphological features

Figure 3a

Ixodes ricinus larva – dorsal view

Figure 3b

Ixodes ricinus larva – ventral view

Figure 4a

Ixodes ricinus nymph – dorsal view

Figure 4b

Ixodes ricinus nymph – ventral view

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 humidities =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]

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]

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 2 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).

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]

Tick species involved in TBEV transmission

Of the 54 species of ixodid ticks known from the Western Palearctic,[47] 8 species from 3 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 to 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 attach to humans.[7] 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.[62] TBEV also has been detected in *Haemaphysalis punctata*.[63,64]

The role of *Dermacentor* ticks (Table 1) in the circulation of TBEV in the environment is unclear and poorly studied.[65,66] *D. reticulatus* appears to be spreading and population density increasing during recent decades.[65-69] 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) in the same area.[70]

Prevalence of TBEV in questing adult *D. reticulatus* ticks from Białowieża Primeval Forest was similar (1.58%)[71] to that in questing *I. ricinus* (1.30%),[72] as was the case in Moldova (adult I*. ricinus* 3.8%, adult *D. reticulatus* 3.9%, but adult *Haemaphysalis punctate* 8.8%).[73] The natural occurrence of TBEV in a *D. reticulatus* tick population has also been proven for Germany during 2016 to 2018 by isolation of several TBEV strains from this tick species in a natural focus.[74]

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.[75–79] Knap and Avsic-Zupanc[80] 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.[79] Waldenström et al.[81] showed a low prevalence (0.5%) in nymphs and larvae feeding on migratory birds in Sweden, while Kazarina et al.[82] 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[83] 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.[84] In the Novosibirsk Region, the prevalence of TBEV in unfed adult *I. persulcatus* was 3.6%, with 0.8% being pathogenic to laboratory mice.[85] 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.).[86] 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.[87]

Vertebrate hosts

The prevalence of antibodies to TBEV in hosts is quite variable.[81] 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,[81,82,88,89] 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.[88] Viremia has been induced experimentally in birds, reaching levels theoretically sufficient to infect feeding ticks.[59] Generally speaking, findings of TBEV in animals, whether indirect or direct, do not mean that 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.[90,91] 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.[105], 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 prevalence 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 prevalence. 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.[91] 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]

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*

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 5 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 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, which were described for the first time as early as 1940.[110] However, only some eggs in the egg 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 history 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 2 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]

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]

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 prevalence 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.

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Chapter 4

Pathogenesis of TBE

with a focus on molecular mechanisms

Andrea Kröger and Anna K. Överby

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.
- The trigger of innate immune and adaptive immune responses, by TBEV is necessary to clear the infection.
- TBEV employs strategies to evade the innate immune response.
- Tools to study TBEV pathogenicity such as mouse knock-out models and reverse genetics are also discussed.

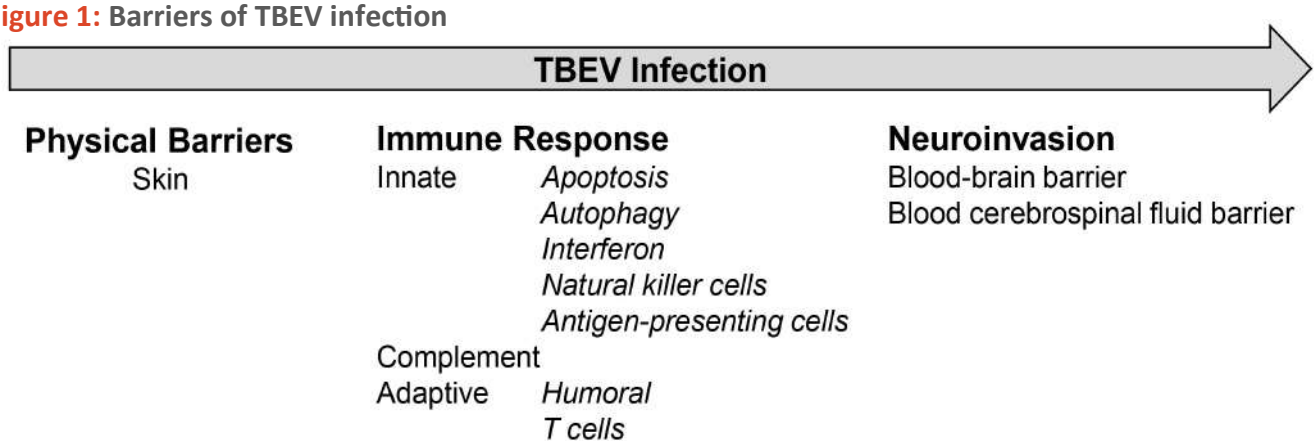
Overcoming the barriers of the host

The host has highly effective defense mechanisms against infections (Fig. 1). The overwhelming majority of infections are normally blocked by physical barriers such as the skin, mucosal membranes, and stomach. However, this first barrier to TBEV is already overcome by the tick through direct injection of the virus into the skin of the host during blood feeding. This allows the first replication phase of the virus locally in the skin. The second barrier is the coordinated innate and adaptive immune response that reacts to infection. The innate immune response includes cell intrinsic defense mechanisms like apoptosis, autophagy, type I interferon (IFN) response, and innate cell-mediated responses, which are then followed by adaptive immune responses with a specific antibody response and stimulation of T cells that limit virus replication and which are involved in pathogenicity. If the virus overcomes the second barrier, it will spread to peripheral organs and cause viremia. The third barrier controls entry of the virus to the central nervous system (CNS), e.g., by the blood-brain barrier (BBB). If overcome, the virus will replicate in neurons and cause encephalitis and meningitis.

Initial infection, viral amplification, and spread

Very early during the tick feeding process TBEV particles are transmitted to the host via tick saliva. Tick saliva acts as a pharmacologically active compound which inhibits pain/itch response, contains anticoagulants, antiplatelet components, vasodilators, and immunomodulators,[1,2] that enhance viral transmission and dissemination.[3] Analysis of skin explants from tick-feeding sites reveals viral antigen in

Figure 1: Barriers of TBEV infection



Host barriers prevent or repel infection by microbes. Anatomical and chemical barriers, cell-intrinsic and cellular-innate immune response, adaptive immunity and other barriers have to be bypassed by invading viruses to establish viral replication, spread and neuroinvasion. TBEV overcome the skin as anatomical barrier by transfer through a tick bite. The complement system as well as innate and adaptive immune response inhibit viral replication and spread. How the virus mediates neuroinvasion is still unknown, but the virus passes through CNS barriers.

neutrophils, monocytes and skin-resident dendritic cells (DC).[4] Although not proven, these cells are likely to serve as a vehicle for transport of the virus to draining lymph nodes. For other flaviviruses it was demonstrated that viral amplification in the lymph nodes results in viremia and spreads to peripheral tissues. The specific target cells for TBEV infection in peripheral tissues are not well defined, but are thought to be subsets of DCs, macrophages and possibly neutrophils.[5]

Neuroinvasion

TBEV is a neurotropic virus and neuropathogenesis depends on the ability of the virus to enter the CNS and propagate. General mechanisms of CNS invasion by neurotropic viruses are breakdown of the BBB, infection of cerebral endothelial cells, virus shedding from choroidal cells, axonal transport through olfactory receptor neurons, and retrograde transport along peripheral nerve axons, or transport by the "Trojan-horse" mechanisms by which virus is transported by infected cells. Although this process has been studied intensively for West Nile virus (WNV) infection[6], it is not known how TBEV reaches the CNS, but breakdown of the blood-brain barrier is unlikely because virus replication is detectable in the brain before BBB disruption.[5,7]

Cellular responses to TBEV and implications for pathogenesis

Cell-intrinsic innate immunity

All cells have the capacity to react to various stresses, such as starvation, temperature extremes, irradiation, and infection. Cell-autonomous protective programs, which are inherent in all cells of the body are termed intrinsic cellular defenses.

Autophagy

Autophagy is a degradation pathway that occurs under stress conditions such as starvation, hypoxia, and infection. It starts with the sequestration of the area of the cytoplasm inside double-membrane vesicles called autophagosomes, which subsequently fuse with lysosomes to form autolysosomes or late endosomes.[8] Dengue virus (DENV) infection promotes the formation of autophagy, which can enhance virus replication and protects cells against other stressors.[9,10] Inhibition of dengue-induced autophagy by pharmacological inhibitors or deficiency of autophagy-related genes (ATG) reduces dengue replication. The importance of autophagy during TBEV replication was shown by stimulation of autophagy which results in significantly increased dose-dependent TBEV production, whereas the inhibition of autophagy showed a dose-dependent decrease of infectious virus.[11]

Apoptosis

Apoptosis is a process of programmed cell death in which cells activate intracellular death pathways.[12] This mechanism occurs in a wide range of human viral infections, including infections of the CNS such as herpes simplex virus (HSV) and cytomegalovirus (CMV) encephalitis.[13,14] In WNV infection of mice, high virus titers in the CNS are associated with the appearance of activated caspase 3 following infection, and apoptosis in neurons occurs in the same areas where viral antigen is present.[15,16] In vitro, TBEV infection causes apoptosis in mouse and human neural cells.[17,18] Although brain-infiltrating CD8+ T cells contribute to the fatal outcome during infection[19] no significant increase of apoptotic cell death was detectable upon infection with Langat virus (LGTV) and TBEV in mice.[5,20] These data are in line with human data, where no prominent signs of neuronal apoptosis were seen in post-mortem brain tissue from patients.[21]

Type I IFN 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.[22] IFNs are 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, and reacts 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.[22]

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

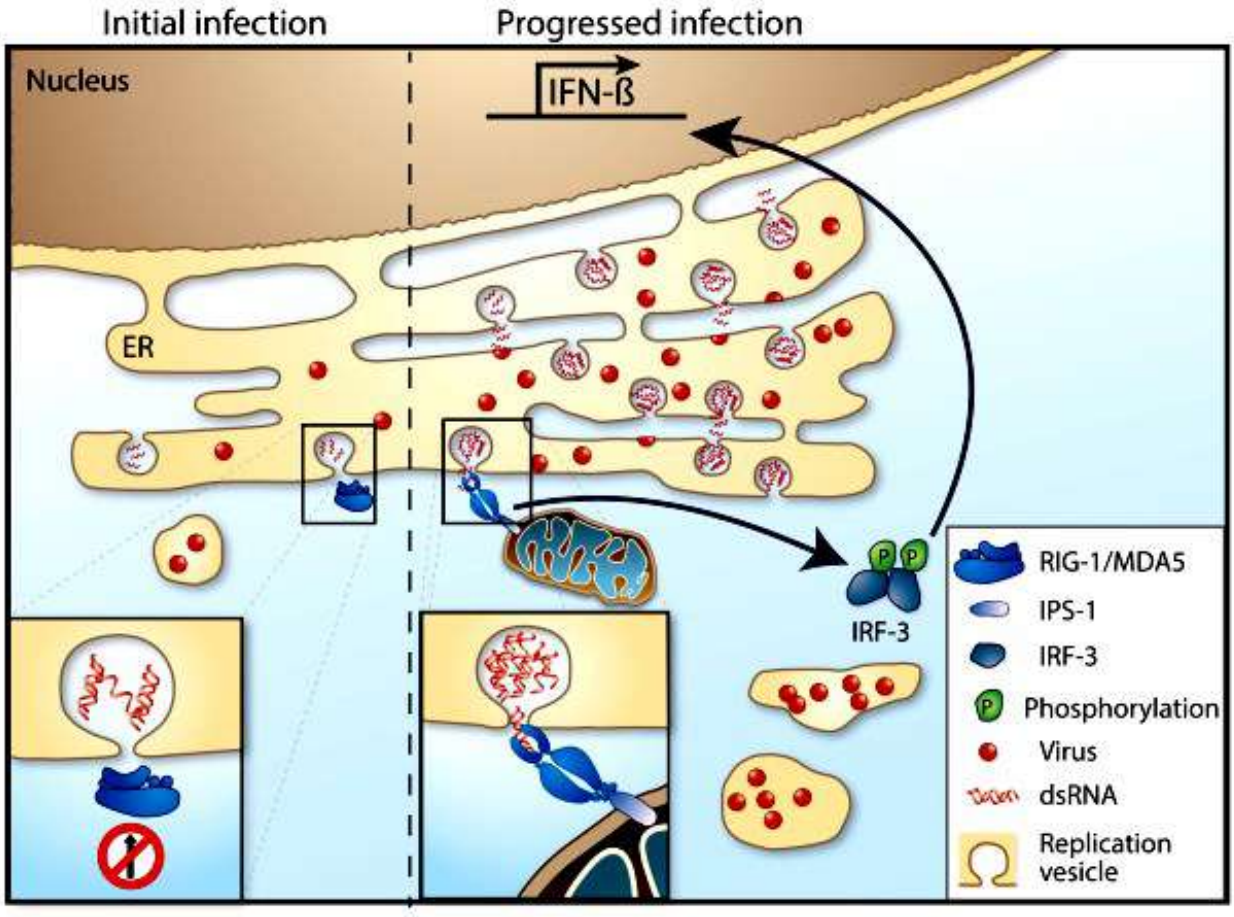
are most likely recognized by TLR3, TLR7, TLR8, or the RLRs RIG-I and melanoma differentiation-associated gene 5, (MDA5), which senses single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA).[23-25]

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,[26] however, the importance of MDA5 as contributing to sensing of TBEV cannot be ruled out as its involvement in sensing other flaviviruses has been demonstrated.[27] 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. IPS-1 is important for IFNβ induction after TBEV infection in mouse embryonic fibroblasts (MEFs); in its absence, no IFNβ was detected.[28] In addition, mice deficient in IPS-1 succumb to LGTV and TBEV infection. These mice showed lower systemic levels of IFNα, resulting in higher viral titers in the periphery and leading to rapid invasion in the CNS.[20] IPS-1 is also important in the local IFN response within the brain, reducing viral load and spread of LGTV,[20,29,30] 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 translocates into the nucleus where it binds to the IFNβ gene promoter to initiate transcription and translation.[31,32] 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.[28]

Very little is known about the importance of TLRs in TBEV infection, and only once the TLR7 has been investigated in

Figure 2: 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 (PRRs) RIG-I and/or MDA5 then trigger signalling through IPS-1, phosphorylated IRF3 dimers are transported into the nucleus and IFN-ß is upregulated.[28,38]

the context of LGTV infection *in vivo*. This report demonstrates that mice deficient in TLR7 have higher viral load in the CNS and lower levels of pro-inflammatory cytokines. Primary neurons did not show a difference in infection rate, but TLR7 deficient neurons induced higher levels of IFNβ[33], indicating that TLR7 is more important for regulating neuroinflammation than type I IFNs.[33]

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. For example, dengue virus has been shown to reduce IFNβ levels by expressing the protease complex NS2B3,[34] possibly by cleaving the adaptor STING.[35] Dengue subtype 1/2/4 NS2A and NS4B and West Nile NS4B protein inhibited TBK1 phosphorylation and IFNβ induction.[36] For TBEV, no specific IFN production antagonists have been identified among the different viral proteins.[28] Instead, TBEV uses 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.[26,28,37,38] 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 (Fig. 2).

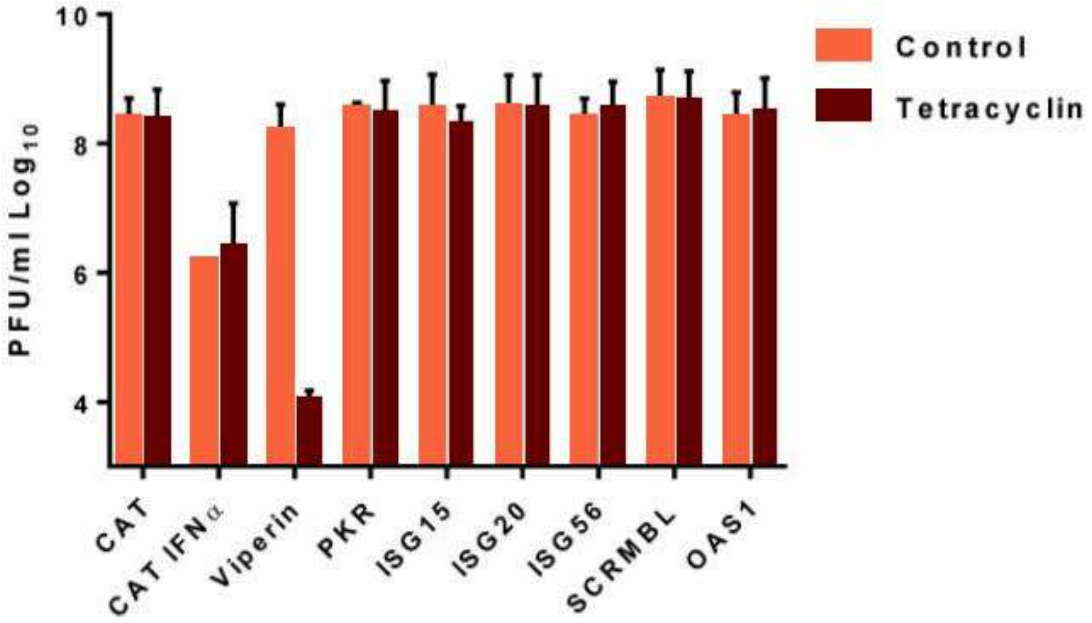
Thus, the virus is produced and released from the cell before IFNβ can trigger an antiviral response in neighboring cells.[28,38]

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.[39] 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 has also shown that IFNAR is important in all cell types; hematopoietic, stroma, neuroectodermal and cells in the periphery.[5]

Most steps in the viral "life" cycle are targeted by 1 or several antiviral proteins encoded by the ISGs. Although several ISGs have been screened against TBEV (Fig. 3), only 2 have been identified to be antivirally active so far; the

Figure 3: Viperin overexpression inhibits European TBEV growth by 4 orders of magnitude



TBEV replication in cells expressing different interferon-stimulated genes (ISG). Cells tetracycline-induced to express different ISGs were used to identify ISGs that inhibit TBEV replication. Cells expressing a reporter gene (CAT) and CAT-expressing cells pretreated with IFNα were used as controls. Virus growth in ISG-induced cells were compared to uninduced cells. Titers of TBEV were measured at 24 hours post-infection and 64 hours after tetracycline induction. The titers shown are mean log10 pfu/mL values from 3 independent experiments; error bars are standard deviations.

rodent tripartite motif (TRIM) protein, TRIM79α, and viperin (virus inhibitory protein, endoplasmic reticulum-associated, IFN-inducible).[40,41] 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 flaviviruses; WNV and Japanese encephalitis virus (JEV), were shown not to be restricted by this protein.[40] 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 seems to depend on the specific virus. For TBEV, viperin selectively targets the positive stranded RNA synthesis. The intracellular location to the ER via viperin's 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.[41,42] Recent studies have identified several viral and cellular interaction partners to viperin.[42-47] 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.[46] The stability of prM, E, NS2A and NS2B are affected by viperin, but only in the presence of NS3.[46] Interestingly, although viperin does not directly interact with the TBEV C protein, viperin expression induces C particle formation and release from virus infected cells and disturbing the assembly process of TBEV.[47] Viperin mediates this effect by interacting and sequestering the cellular protein Golgi brefeldin A-resistant guanine nucleotide exchange factor 1 (GBF1),[47] which is involved in the vesicular trafficking of the secretory pathway[48,49] and is a pro-viral factor for many different viruses.[50-53] 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.[43] This study shows 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 was 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.[43] Although only 2 antiviral proteins have been identified so far, there are likely several others that are involved in the restriction and protection against TBEV and LGTV in vivo. One of the difficulties in identifying antiviral ISGs might be the redundancies seen between different proteins.

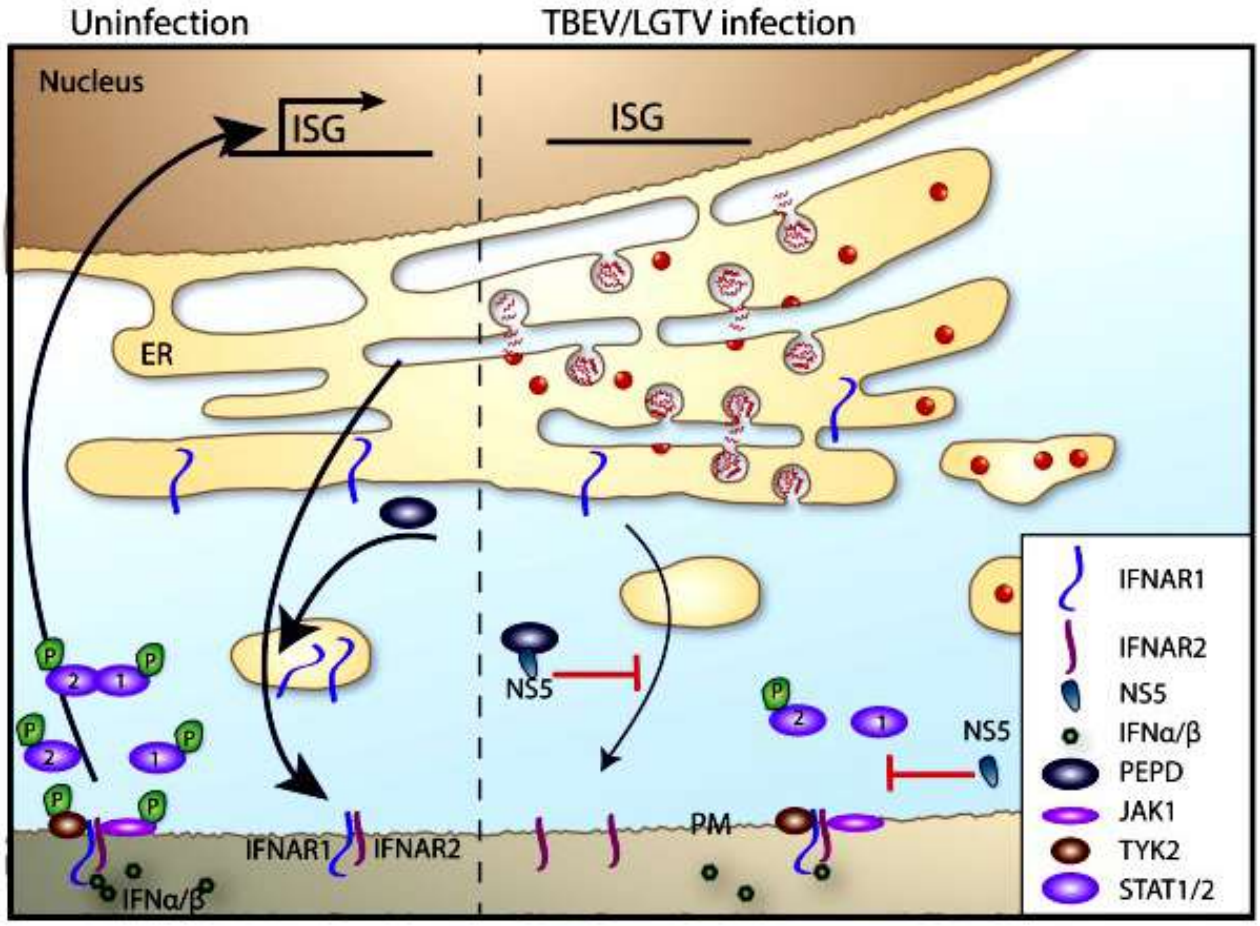
Even though different ISGs can potently restrict TBEV replication if induced before infection,[40,41,54,55] IFN treatment after infection has limited effect *in vitro.*[55] The reason for this is the expression of an IFN antagonist, NS5.[55,56] The NS5 protein of LGTV interferes with the phosphorylation of Jak1 and Tyk2 in response to IFNβ, which leads to failure of STAT1/2 phosphorylation and subsequent ISG expression.[55,56] 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.[56] 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-stimulated gene induction, and IFN-I-dependent viral control (Fig. 4).[57] 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.

Complement

The complement system plays an essential role in the innate immune responses to many pathogens including flaviviruses. There is growing evidence that the complement system participates in the adaptive immune response. More than 30 proteins and protein fragments form a network of soluble and cell surface proteins that recognize and target pathogens. They orchestrate three distinct cascades: the classical pathway, alternative pathway, and lectin pathway. Each complement activation pathway is initiated by a distinct set of recognition molecules and converges at the cleavage of C3 to C3a and C3b. Beyond its lytic capacity, complement protects against viral infections by priming adaptive B and T cell responses, triggering leukocyte chemotaxis through the release of anaphylatoxins (C3a and C5a), and opsonizing viruses for phagocytosis and destruction by macrophages.[58,59]

Stimulation of all complement activation pathways contributes to protection against flaviviruses. For WNV infections enhanced susceptibility was shown for mice deficient in various components of the complement system. Less is known about the complement activation during TBEV infection. Antibody-dependent, complement-mediated cytolysis of infected cells is considered a possible mechanism of protection by NS1 antibodies, since NS1 is expressed on the cell surface.[60] In response to these protective functions, many viral pathogens have evolved

Figure 4: Interferon (IFN) 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.[57] NS5 also interferes with JAK1, TYK2, and STAT1 phosphorylation upon IFNα/β stimulation, thereby inhibiting ISG production.[55,56]

evasion strategies to limit recognition by and activation of the complement cascade. NS1 proteins of different flaviviruses limit complement activation by forming complexes with C1s and C4 to promote cleavage of C4 to C4b. Another mechanism shows direct interaction of NS1 with C4b binding proteins which leads to reduced C4 activity.[58] Although these inhibitory mechanisms are functional in various flavivirus strains, less is known about the role of NS1 protein from TBEV.

Innate and adaptive immune interface

Natural killer (NK) cells

Natural killer (NK) cells are large granular lymphocytes that play an important role in the control of viral infections. NK cells limit viral replication by killing infected cells during early stages of infection. The antiviral response of NK cells includes direct killing of virus–infected cells, which is primarily mediated by perforin and granzyme, as well as the production of several proinflammatory cytokines, including IFN-γ and tumor necrosis factor (TNF).[61] These molecules are components of the innate immune response as they are activated by type I IFNs, but they also play a critical role in immunoregulation during the development of adaptive immunity, thereby bridging innate and adaptive immune responses. Their important role in the host defense against viruses is supported by the finding that humans with complete or partial impairment of NK cell numbers and functions have increased susceptibilities to viral infections, including HSV, varicella zoster virus, CMV, and human papilloma virus.[62]

NK cells have been studied in various flavivirus infections including DENV, WNV, JEV and yellow fever virus (YFV). NK cells have been suggested to affect disease severity and outcome, as well as to contribute to viral control, even though the underlying mechanisms remain unknown.[63-65] The role of NK cells in immunopathology of TBEV infection is largely unknown. Langat or TBEV infection in mice leads to a temporary activation of NK cells during the early phase of infection, followed by suppression,[66] which in later phases of infection was not associated with increased viral replication in splenocytes. Ex vivo infection of whole-blood cells showed activation of NK cells only with low pathogenic TBEV strains while highly pathogenic TBEV inhibits NK cell activation. Decreased expression of perforin and granzyme B was detected in activated CD56dim NK cells of TBEV-infected patients during hospitalization, indicating that cytotoxic granules were released early in NK cell activation and symptom onset, thereby possibly contributing to pathogenesis of infection.[67] Given these ostensibly conflicting results, more investigation is needed to determine the functional role of NK cells in limiting viral replication and in the pathology associated with TBEV infection in different hosts.

Antigen-presenting cells

Effective host defense against infection requires innate and adaptive immune responses working together to mediate clearance of invading pathogens. Dendritic cells (DCs) bridge these 2 arms of immunity. In peripheral tissues, immature DCs recognize RNA virus infection, migrate to local lymphoid tissues, and undergo a process of maturation that involves cytokine production and antigen presentation to activate naïve T cells and shape adaptive immunity.[68] Many flaviviruses including DENV,[69] WNV,[70] and JEV,[71] infect DCs resulting in impaired DC maturation and T cell priming/proliferation and promoting viral pathogenesis. DCs also represent early targets of TBEV infection following the bite from an infected tick,[4] providing the virus with opportunities to manipulate DC functions as a means of evading host immunity. LGTV infection impairs DC maturation by suppression of costimulatory molecules and inhibition of IL-12 production. This immature DC phenotype was associated with an impaired functional capacity to induce T cell proliferation.[72] However, how this is involved in viral pathogenesis is unknown.

Adaptive Immune response to TBEV

Humoral immunity

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.[73] Passive transfer of monoclonal or polyclonal TBEV-specific antibodies protects mice in vivo and protection correlates with in vitro neutralization.[74-77] No infectious virus could be detected in the blood or brain of passively protected mice subsequent to TBEV challenge. 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.[78]

Cellular Immunity

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. However, results from studies in mice lacking B cells or CD4+ T cells during TBEV infections are missing. Nonetheless, mice lacking type I IFN signaling develop a normal antibody response during LGTV infection but are not protected from severe infection.[5,20]

Cytotoxic T lymphocytes (CTL) recognize viral peptides presented on major histocompatibility complex (MHC) class I molecules and eliminate cells producing abnormal or foreign proteins, specifically virus infected cells. CD8+ CTLs control viral replication via distinct mechanisms: non-cytolytically by secretion of IFN-γ or TNFα or cytolytically by cytotoxic proteins like granzyme B and perforin.[79] Long-term immune surveillance effector cells react more quickly against the same virus after a primary infection.

The effects of TBEV infection on T cells are less studied. *Ex vivo* infection of human blood cells leads to an activated phenotype of T cells with low-pathogenic TBEV, whereas the highly pathogenic TBEV suppresses T-cell activation.[80] It is unclear whether T cells are directly infected by TBEV, but no infection of T cells was detectable in highly susceptible IFNAR mice infected by Langat virus,[5] which makes direct infection of T cells unlikely.

Studies in humans showed that CD8 T cells responded strongly to acute TBEV infection and passed through an effector phase, prior to gradual differentiation into memory cells, indicating that TBEV infection induces a robust CD8 T cell response.[81] Comparable studies in mice revealed that the number and activation of T cells in the CNS have no impact in the outcome of infection; both dying and recovering mice showed no difference in number and activation status of T cells upon TBEV infection. However, differences were seen in the specific T cell clones accumulating in the brain.[82]

Besides their role in antiviral response, CD8+ T cells are also believed to contribute to CNS pathogenesis. In brain

autopsy samples from TBEV-diagnosed individuals, inverse topographical correlation of inflammation and TBEV-infected areas has been reported.[83] Inflammatory infiltrates are predominantly composed of T cells and macrophages/microglia. In regions with less infiltration CTL are closely associated with TBEV-infected neurons. These findings suggest that immunologic mechanisms can contribute to nerve cell destruction in human disease. In immune deficient SCID mice or mice lacking CD8 T cells an increased survival upon TBEV infection was shown. Adaptive transfer of CD8+ T cells in SCID mice decreases median survival time. Although these data suggest a contribution of CD8 T cells in pathogenesis, surprisingly, this effect is independent of viral replication in the periphery and the CNS. The pathogenicity of virus strains also seems to influence the effect of CD8 T cells on the outcome of infection. Whereas CD8+ T-cell-deficient SCID mice succumb later from infection with high pathogenic TBEV strains, a survival advantage was shown upon infection with low pathogenic strains.[19]

Although viral infection with LGTV leads to an accumulation of CD4+ and CD8+ T cells in the CNS, no increased numbers of apoptotic cells were detectable.[5,20]

Other data suggest that T cells within the CNS promote survival. In CCR5-deficient mice, an increase of viral replication in the CNS and decreased survival is due to the lack of lymphocyte migration to the CNS. Adaptive transfer of LGTV-specific T cells improved survival outcome. However, whether the protective effect is only mediated by T cells or by the decrease of inflammatory neutrophils in the presence of T cells is not clear.[84] Because TBEV-infected mice also died of encephalitis in the absence of T cells, other cells such as neutrophils could contribute to pathogenic effects of TBEV infection. Further investigation is needed to better understand the processes that control the protective rather than pathogenic CD8+ T cell response during TBEV infection.

Tools to study pathogenesis

Mouse models

Laboratory mice are a useful tool to investigate human diseases, as mice are phylogenetically related to humans and show a striking genomic homology. This is especially true with knockout mice, in which an existing gene is inactivated. Laboratory mice are used to better understand how a similar gene in humans may cause or contribute to disease. The mouse as a model system for studying pathogenesis of TBEV has an advantage compared with other flaviviruses, because mice are susceptible to natural TBEV isolates, and develop encephalitis, whereas other flaviviruses require mouse adaptation to cause disease.[85]

Animal models of TBEV infections have provided insights into the pathogenesis of TBE in humans. In particular, TBEV and LGTV infections of mice enable the identification of host and viral genetic factors that contribute to the outcome of infection, as shown through the studies described elsewhere and in this chapter.

Recently we used C57BL/6 mice to characterize TBEV pathogenesis. Two different strains showing different symptoms are investigated. Namely HB171/11, isolated from questing adult ticks from a natural focus in south Germany[86] and Torö-2003, rescued from a cDNA infectious clone generated from RNA extracts of nymphs collected in the island of Torö, Sweden.[87] Both strains showed highly different symptoms in humans, as HB171/11 leads to mild gastrointestinal and constitutional symptoms without affecting the nervous system. TBE cases in the region of Torö showed relatively mild neurologic disease and few cases of hospitalization. The infection of mice reflects the different course of infection in humans, we observed lower pathogenicity of HB171/11 in comparison to Torö-2003 infections. Torö-2003 replicates faster in the periphery and enters the brain very early during infection. In addition, neurovirulence was lower in HB171/11-infected mice. The mechanism of virulence and neuropathology is still under investigation, although differences in cytokine induction and viral replication in target cells could be involved. In summary, mouse models could be a good tool to contribute to our understanding of pathogenesis of TBEV infection.[88]

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.[89] Since the genome of flaviviruses is positive stranded, they are infectious if introduced into susceptible cells.[90] There are several different approaches to generate infectious virus. One important step is the generation of a complimentary 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.[91]

For TBEV 2 separate approaches were used in the beginning; plasmid-based infectious clones[92] and the PCR-based methods for constructing recombinant virus.[93,94] 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.[95]

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,[96] the function of the membrane protein in virus budding,[97] 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.[96] 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.[98] 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.[99,100] 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).[101-103] The sfRNA has been shown to be critical for WNV induced cytopathic effects[104] and pathogenicity in mice[104], and is involved in viral subversion of type I IFN response by a yet unknown mechanism.[105] The TBEV sfRNA has been shown to specifically interfere with the RNAi system of ticks.[106] 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.[107] 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.[108,109]

However, studies recently showed that the variable region and the poly(A) tract can modulate virulence of the Far Eastern TBEV.[110,111] 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.[112]

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.[87]

Conclusion

Important advances in the identification of molecular and cellular mechanisms of TBEV-induced pathogenesis have been made in recent years. Nevertheless, many questions remain unresolved. The interaction of the virus with the innate and adaptive immunity is not fully understood. Additional questions include: which genes act antivirally to inhibit virus replication in the periphery and in the CNS? Are there cell- and tissue-specific differences? What is the effect of cells of the innate and adaptive immune system in antiviral defense and which factors influence neuroinvasion and neuropathogenesis? And, last but not least, how can CNS infections be prevented or treated?

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Chapter 5

TBE in adults

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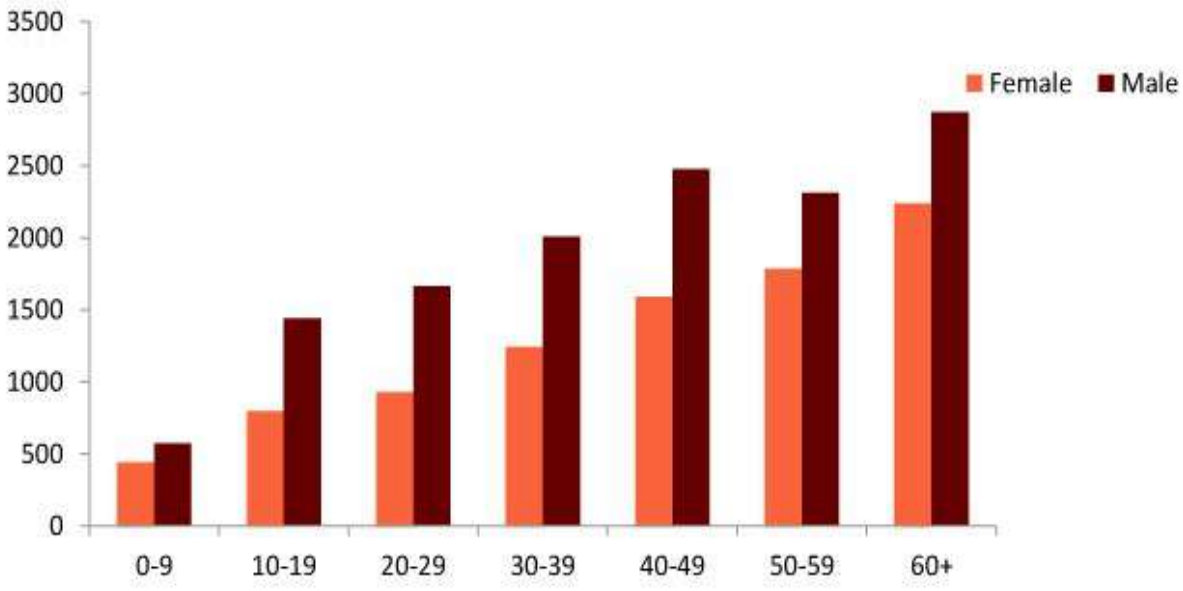
Key Points:

- TBE is the most important tick-borne arbovirus disease of humans. Epidemiological data indicate a trend towards an increasing severity with higher age.
- A number of possible genetic and non-genetic risk factors have been identified, which might have an impact on the manifestation and severity of human disease.
- Different TBEV strains seem to cause differing clinical courses of disease. While the TBE-Eu mainly causes a biphasic course, the clinical course of TBEV-FE and TBEV-Sib are mainly monophasic.
- The diagnosis of TBE is based on serological tests.
- So far there is no effective treatment of TBEV infections.

Introduction

The tick-borne encephalitis virus (TBEV) causes serious infections of the brain, the myelon, and / or the meninges. Initial symptoms include headache and fever. Severe forms of tick-borne encephalitis (TBE) progress to a loss of consciousness, coma, and even death (Fig. 1).[1,2] Overall, TBE is associated with a high burden of disease and often disabling long-term sequelae. From an epidemiological perspective all age groups are at risk for TBE; however, the individual risk depends on age, sex, occupation, leisure activity profile, and the local environmental presence of TBEV. Within the European Union (EU), TBE became a notifiable disease in 2012, and in 2014, 2,057 cases were reported to the European Centers for Disease Control and Prevention (ECDC), 1,986 with a confirmed TBEV infection based on ECDC diagnostic criteria (0.42 cases per 100,000 population).[3] The highest rates were documented in the Baltic states and TBE was predominantly reported in males ≥45 years of age. The majority of cases occurred in males in all age groups, based on aggregated data from 2000–2010 (n=22,378); however, in Lithuania, Latvia, and Estonia in the ≥60 years group, female cases predominated. The ECDC report showed that most TBE cases were reported to public health authorities between June and October.[3]

Figure 1: TBE by age and gender



TBE cases by age group and gender reported in 16 EU/EFTA countries (2000-2010; n=22,378) Source ECDC (TBE technical report).[3]

Predisposition factors and risk factors

Risk factors – age, comorbidities

The personal risk for TBE in non-immune persons depends on the probability of exposure to TBEV. Exposure is usually the result of close contact with the environment in which ticks are found. Forest workers, professional hunters, and landscape gardeners are examples for occupational groups with a relatively high work-related risk of acquiring a TBE infection.

However, the most endangered groups for severe clinical manifestation are elderly people, with studies showing that TBE tends to be more severe in the elderly.[4] This may be due to their social habits and leisure activities, e.g., collecting mushrooms, hiking, or other activities in the forests, that lead to a higher probability of exposure to ticks and therefore for TBEV infection.

Epidemiological data from different European countries demonstrate that the incidence of TBE is higher in elderly people than in younger age groups. In some countries (e.g., Germany, Austria, Sweden, Lithuania) more than 50% of TBE patients are ≥50 years of age. Long-term data (1993–2008) from a single center in Białystok/Poland showed that among 710 hospitalized patients with TBE, 235 patients (33%) were ≥50 years old. The clinical manifestation of TBE depends on the virulence of the pathogen and individual factors of the host. The most important host risk factors are age, comorbidities, and notably immunosuppression. In detail, the latter encompass autoimmune diseases such as rheumatoid arthritis, psoriasis, vasculitis, encephalomyelitis disseminata, solid organ recipients, or oncological diseases requiring treatment with biologicals, steroids, chemo-therapeutic agents, or other immunomodulating sub-stances. The case fatality rate for TBE is exceedingly high in these patient groups. A recently published cluster of TBEV in the organ transplant recipients underscores the role of host immune suppression and fatal outcomes.[5]

Another factor that may result in a more severe clinical picture of TBE is the relatively rare occurrence of coinfection with other tick-borne pathogens like *Borrelia burgdorferi*, *Anaplasma phagocytopilum*, *Rickettsia* spp. or *Listeria monocytogenes*.[6] According to Pikelj et al., other predictors for severe courses of TBE infection are early alteration of consciousness (Glasgow Coma Scale [GCS] score <7), development of limb paralysis together with respiratory insufficiency within 24–48 hours from the beginning of the second phase of the disease, and pleocytosis >300 cells.[7] According to Mickiene et al., a monophasic course of the disease is also a predictive factor.[8] Late onset of specific anti-TBEV IgM antibodies in cerebrospinal fluid (CSF) is linked with the severity of acute encephalitis symptoms. Thus, it can be concluded that a fatal outcome of TBE may be a consequence of coexisting risk factors, such as old age and chronic underlying diseases. Special efforts should be made to vaccinate elderly people (>50 years of age), who constitute the main group at risk for development of severe courses of TBE and long-term sequelae.[6]

Host genetic risk factors

As mentioned above, clinical and epidemiological data indicate that human susceptibility to clinical TBEV infection greatly varies according to age, gender, and ethnicity. The potential role of a genetic background for TBE was investigated in mouse models, where different mouse strains differ greatly in susceptibility, virus replication rate, and characteristics of the immune/inflammatory response.[9] Most of the genetic factors analyzed are part of the innate immune response of the mammalian host to TBEV. A selection of genetic predispositions is discussed in the following section in the context of TBE.[10]

C-C chemokine receptor type 5 (CCR5)

The CCR5 plays a key role in leukocyte migration and attraction. In human immunodeficiency virus (HIV) infections, the CCR5Δ32 mutation is important for the invasion of CD4 cells by HIV particles with a CCR5 tropism. Based on these findings, the CCR5 entry inhibitor, mavaviroc, was successfully introduced into clinical use in 2007. In mouse models for flaviral infections, it had been demonstrated that homozygote CCR5-deficient (-/-) mice died in almost 100% of all infections with West Nile virus (WNV)[11]; whereas CCR5 (-/+) heterozygote mice, and homozygote mice with a wildtype CCR5 receptor, had a significantly lower mortality rate after WNV infection. These data from animal studies were later confirmed in a cohort study from North America during a WNV outbreak. In conclusion, the CCR5Δ32 mutation is a strong predictor for a severe clinical course of WNV infections in the human host. 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.[12] In adult patients with TBE, 2.3% (n=129) of patients were homozygous for CCR5Δ32. Control patients with aseptic meningitis (n=76), and a second healthy control group (n=134), included no patients with a homozygote CCR5Δ32 mutation. A second study with adult and pediatric TBE patients[13] enrolled 246 patients in total (n=117 pediatric, n=129 adult). There were 2 control groups: 1 with 79 patients with aseptic meningitis and a second with 135 healthy individuals. Patients with TBE were stratified into subgroups on the basis of clinical parameters and severity of disease. It was shown that, in the TBE patients, a homozygote CCR5Δ32 mutation was detected with significantly greater frequency than in the control

populations, but there was no correlation between disease severity and CCR5Δ32 mutational status. Mutations in another C-C chemokine receptor, CCR2, are linked to a slower progression of HIV infections; however, any association between CCR2 and TBE has not yet been studied.

Toll-like receptor 3

Toll-like receptor 3 (TLR 3) is an intracellular pattern recognition receptor (PRR), active within the cell and located to a lesser degree at the cell surface, which recognizes double-stranded ribonucleic acid (dsRNA), thus making it an important factor in the innate response to most viruses.[14,15] Activation of TLR3 results in activation of the NFκB proinflammatory transcription factor pathway, and synthesis of type 1 and type 3 interferon (IFN).[14,16] TLR3 -dependent signaling has been described in different cell types, including epithelial and endothelial cells, fibroblasts, different populations of mononuclear leukocytes, as well as neurons and glial cells.[14,16–19] TLR3 is present in different types of glial cells within the central nervous system (CNS) and its expression is upregulated during CNS inflammation. However, some viruses seem to suppress or bypass TLR3 signaling, at least in some types of cells. For example, herpes simplex virus type 1 (HSV1) induces only a limited response in human neurons expressing TLR3, with no type 1 IFN synthesis.[19]

Data from WNV infections suggest that there is a link between genetic risk factors and severe clinical courses; however, findings are, in part, inconsistent. WNV entry into the CNS depends on the expression of TLR3 as an important cofactor.[21] Knockout of TLR3 results in higher peripheral viral load, reduced CNS viral load, and inflammation of the brain.[21] The detrimental effects of TLR3 stimulation depend on the activation of tumor necrosis factor-α (TNF-α) receptor 1, suggesting that TNF-α is a downstream mediator of TLR3-induced blood–brain barrier permeability.[21] However, knockout of TLR3 reduces the inflammatory response and thus neuronal damage in the CNS, irrespective of the local viral load.[21]

For TBEV, the exact mechanism of entry to the CNS is not known.[22–24] It is speculated that, very similar to WNV, TBEV enters the CNS after an intense peripheral inflammatory response disrupts the blood–brain barrier, or via olfactory neurons.[25] Investigating the clinical relevance of these findings from animal studies, a clinical study analyzed the frequency of 2 TLR3 mutations in 128 TBE patients from Lithuania,[26] presumably infected with the European subtype of TBEV (TBEV-EU). There were 2 control groups: 1 consisted of 77 patients with aseptic meningitis and the second comprised 135 healthy individuals from the same region. Results indicated that fully-functioning TLR3 is a risk factor for TBE. These findings were confirmed by a large cohort study from Lithuania[13] and a trial from Russia.[27] In the Lithuanian cohort study, which enrolled adult and pediatric TBE cases, the TLR3 polymorphism, rs3775291, was found to be less prevalent in the combined adult/ pediatric TBE cohort than in the healthy control group.[13] In the Russian analyses, which investigated 137 non-vaccinated TBE patients for the presence of the rs3775291 polymorphism compared with a healthy control group (n=239), it was concluded that a functioning, wild type TLR3 seems to be a risk factor for TBE.

2'-5' Oligoadenylate synthetase (IFN-induced oligoadenylate synthetase 2 (OAS2) and IFN-induced oligoadenylate synthetase 3 (OAS3))

2'-5' Oligoadenylate synthetase is an IFN-induced antiviral protein that catalyzes oligomerization of ATP into 2',5'-linked oligoadenylate (2-5A), which in turn activates latent RNase L. Activated RNase L is able to degrade viral RNA, particularly RNA from neurotropic viruses.[19] The human OAS gene family comprises 4 genes, of which 3: OAS1, OAS2, and OAS3 encode functional OAS, which by alternative splicing may be expressed in 8 different isoforms: 5 of OAS1 (p42, p44, p46, p48, and p52), 2 of OAS2 (p69 and p71), and a single OAS3 isoform (p100). OASL encodes 2 isoforms of the OASL protein (p30, p59) which lacks OAS enzymatic activity. OAS1 and OAS2 synthesize mainly 2-5A oligomers and OAS3 mainly (but probably not exclusively) dimers, which do not activate RNase L, but may initiate alternative antiviral pathways. Particular isoforms of OAS1 and OAS2 differ in their activity and intracellular distribution.[28] Constitutive OAS activity is individually variable and strongly correlated between close relatives, suggesting that genetic factors determine activity. In theory, single nucleotide polymorphisms (SNPs) in all the genes of the OAS complex could contribute to the phenotypic variability, as well as different isoforms of OAS proteins, contributing to the overall activity in vivo. Several findings from other viral infections point to an important role of OAS genes in the anti-flaviviral immune response. For example, in patients with dengue shock syndrome, transcription levels of OAS3 in peripheral blood mononuclear cells are significantly lower than in patients with the less severe form of the disease.[30] The very initial stage of infection with TBEV depends on virus replication in the Langerhans cells, macrophages, and neutrophils in the skin at the site of tick feeding.[31] It is worthy of speculation that the innate antiviral response, including OAS activity, could influence the outcome of the infection at an early stage, protecting some individuals from clinically overt disease even before an adaptive immune response and seroconversion, very similar to the suggestion by Lim et al. in the context of WNV infection.[28,31] There are few clinical data regarding TBE and mutations in the OAS genes. In a study by Kindberg et al., rs1077471 distribution was not significantly different between healthy controls and TBE

patients from Lithuania, although there was a non-significant tendency towards a more frequent homozygosity for the mutant allele in meningoencephalitis patients (7% in TBE group vs. 11% in non-TBE group) than in healthy individuals (3%).[26] Barkhash et al. studied 23 SNPs within the OAS gene cluster in a group of patients from Novosibirsk, presumably infected with the Siberian TBEV subtype (TBEV-Sib), and identified 3 SNPs in the OAS2 gene (rs1293762, rs15895, and rs1732778) and 2 SNPs in OAS3 gene (rs2285932, rs2072136). There were significant differences in allele and haplotype frequency of these SNPs between patients with mild TBEV infection (uncomplicated meningitis and febrile disease) and with encephalomeningits or myelitis. However, in contrast with other studies,[26,28] no SNPs in the remaining genes of the OAS cluster (OAS1 and OASL) were associated with the severity of infection. The authors of the Russian study analyzed, in a second step[27], the frequency of such OAS 'TBE risk SNPs' in different ethnic populations within the territory of the Russian Federation. The authors found that the frequency of the aforementioned SNPs correlated with the probability of exposure to TBEV. Very low SNP frequencies were detected in Altaians, Khakasses, Tuvinians, and Shorians, groups that have a high exposure risk for TBEV in their native habitats. In conclusion, these 'TBE risk SNPs' may even serve as selection factors in these ethnic groups.

IL-28 and IL-10 polymorphisms

The IL-28B polymorphism has been used in the era before the 'directly acting antivirals' (DAA) revolution to predict a sustained virological response (SVR) in patients chronically infected with the hepatitis C virus (HCV). The IL- 28B polymorphism (rs12979860) is associated with an improved SVR in response to an antiviral HCV regimen based on pegylated IFN, proteinase-inhibitors, and optional ribavirin.[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, 132 non-vaccinated patients with TBE were compared with a regional control group comprising 221 healthy individuals. The results indicated that the IL-28B polymorphism (rs8103142, rs12980275) and the IL-10 polymorphism (rs1800872) are genetic risk factors for TBE and in particular for severe TBE disease.[33]

CD209 – (ICAM)-3-grabbing non-integrin (DC-SIGN)

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 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. It is coded by CD209 gene on the chromosome.[19] 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.[34] Dendritic cells in the skin and gut probably play an important role in the early stages of TBEV infection. These antigen-presenting cells act as a source of pro-inflammatory and antiviral mediators and as initiators of a specific immune response. However, this cell type is susceptible to TBEV as well and facilitates the initial spread of TBEV from the primary site of infection to remote regions of the mammalian host.[31] A clinical study from Russia enrolling 136 non-vaccinated TBE patients showed a correlation between the presence of 2 SNPs (rs4804803, rs2287886) in the promotor region of the CD209 gene and severity of TBE disease course.[34] The studied patient population was stratified into different clinical syndromes–isolated febrile illness (n=35), meningitis (n=61), and severe CNS manifestation such as meningo-encephalitis (n=40). The control group comprised 263 healthy individuals from the same Siberian region. The Russian TBE cases were presumably infected with the TBEV-Sib. However, it should be noted that, to date, there has been no study investigating CD209 polymorphisms in European TBE patients infected with the western subtype of TBEV.

Clinical course

Pathogenesis – preclinical phase

After a tick bite, the released TBEV in the skin replicates subcutaneously and is, in this early stage, limited to the skin. Dendritic cells of the skin, the Langerhans cells, bind with antigens and subsequently induce an immune response by producing proinflammatory cytokines. After the initial replication in the skin, TBEV replicates in the lymph nodes and lymphatic system, leading to viremia. DCs and macrophages are crucially involved in TBEV replication, and may contribute to the spread to uninfected cells, thereby serving as an important source of local virus replication before viremia occurs.[35,36]

Time frames of the clinical presentation and definitions

The incubation period is difficult to assess in many TBE cases, because tick bites often remain unnoticed. There are few data and only rough estimates about the percentage of infected individuals who develop symptomatic disease.[2] Published data suggest an average incubation period of 7–

10 days (range, 4–28 days) after a bite of an infected tick.[8,37] In a recent Polish study a median incubation period as long as 22 days (range, 4-34 days) has been reported[38] However, in a case of food-borne TBEV transmission, the incubation period may be even shorter and depends upon the number of viable virus particles ingested with food. 3-4 days have been reported by Hudopisk et al.[39] and by Dumpis et al.[40] in a recent outbreak of alimentary TBE in Austria, the length of the incubation period ranged between 9 and 14 days.[41]

Symptoms of TBEV infection usually appear in a 2-phase course. In order to harmonize the use of terminology, TBEV infection is categorized into a first phase, which progresses to a second (neurological) phase of the disease. Sometimes, the disease progression is terminated after the first phase; this clinical pattern is termed 'abortive'. Monophasic disease expresses only 1 phase of the disease with neurological symptoms. The typical course of the infection exhibits the aforementioned 2 phases and is called 'biphasic'. The TBEV-Sib might even cause a chronic disease.[38,39]

Dynamics of TBE – first phase and second phase

During the viremic phase, extra-neural tissues, including the reticuloendothelial system (spleen, liver, and bone marrow), are infected and release TBEV. Viremia lasts for several days and facilitates the invasion of the CNS.[40] The outcome at this stage depends on the initial immune response at the entry site and the subsequent peripheral immune response. Impaired clearance of viremia, and consequent entry of TBEV to the CNS, are the result of a limited humoral response in the early course of TBEV infection.[25] Flu-like symptoms develop during the initial viremic phase of the illness, including fever, headache, fatigue, myalgia, anorexia, nausea, and vomiting. However, fever and headache are the chief complaints of patients presenting to health services. This initial phase lasts for 2–4 days (range 1–8 days).[41,42]

Overall, approximately 30% of all infected individuals remain free of clinical signs and symptoms, and 30–50% of all symptomatic TBE patients experience only the initial phase; however, epidemiological data show wide variability. An estimated 30% of patients with TBE who have gone through the initial phase will develop a second phase with CNS involvement. The asymptomatic period between the first and second phase of symptoms lasts for 8 (1–20) days.[41,43] Abortive disease is diagnosed if there is only an initial phase. The second phase of the disease may involve the CNS with symptoms of meningitis, meningoencephalitis, meningoencephalomyelitis, encephaloradiculitis, or even mixed forms. A biphasic course is observed in 74–85% of TBE patients infected with TBEV-EU.[42] Up to 46% of patients experiencing the second phase of TBE develop long-term sequelae. Interestingly, Kaiser et al. report that patients with encephalomyelitis often present with only 1 phase (i.e., absence of prodromal phase).[41] Infections with the TBEV-Sib and the Far Eastern subtype of TBEV (TBEV-FE) are predominantly monophasic. Only a small remainder shows a biphasic or even chronic pattern (8–21%).[44] An abortive form of TBEV infection presenting exclusively with febrile temperatures, without CNS involvement, has been reported in some European studies.[45] This abortive pattern seems to be a rare clinical manifestation, estimated to account for 1.8% of all TBEV infections in Europe.[46] In Russia, however, these abortive forms represent up to 50% of all clinical presentations of TBE.[39] The exact ratio of abortive versus CNS forms of TBE still remains unknown and depends on a variety of pathogen-related and individual host factors.[8]

Seroconversion without any obvious clinical signs is common and well documented, especially in populations that are highly exposed to TBEV and ticks.[47] In a study from Sweden,[48] 25% of infected individuals developed CNS disease. This proportion seems to be lower with the TBEV-Sib and TBEV-FE subtypes, where 70–95% of infections are reported to remain without any symptoms.[49]

Clinical presentations of the neurological (second) phase

The exact mechanism by which TBEV crosses the blood–brain barrier and invades the CNS remains unclear. There are 4 proposed mechanisms: I. Cytokine mediated (targeting the endothelium) whereby cytokines modulate endothelial cell permeability, disrupt the blood–brain barrier, and lead to the passage of the TBEV into CNS; II. Trojan horse hypothesis, in which immune cells migrate into CNS and establish an infection of the neural cells, endothelial cells, or choroid plexus epithelial cells, with budding of TBEV into the parenchymal compartment;[22,23,50,51] III. Digestive tract infections from epithelial cells to DCs; and IV. Infection via olfactory epithelium and olfactory neurons.

The second stage of TBE begins with an increasing body temperature. This second febrile phase is characterized by temperatures 1–2°C higher than peak body temperatures in the first phase, frequently exceeding 40°C.[41] The further course of acute TBE can be classified as mild, moderate, or severe depending on the affected parts of the CNS, such as meninges (meningitis), brain (encephalitis), cranial, or spinal nerves (meningoencephalomyelitis). The specific clinical symptoms in the second stage of TBE result from the affinity of the virus for distinct CNS regions, producing additional clinical symptoms like chorea,[52] parkinsonism, mutism, nystagmus, and others. Encephalitic symptoms are classified as mild to severe.

Meningitis presents with headache, nausea, vomiting, vertigo, and neck stiffness. Signs of meningeal irritation

(neck stiffness, Brudziński, Kernig's signs) have a low clinical sensitivity and could even be absent, leaving headache and febrile temperatures as the only symptoms. In a study from Poland, 10% of the TBE patients with CSF pleocytosis were without objective meningeal symptoms.[53,54] In pediatric TBE patients, fever without neurological symptoms is more often the chief complaint compared with adult patients.

Meningoencephalitis is observed in adults in 50% of TBE cases.[55,56] Symptoms are cerebellar signs and typically include ataxia. The most common neurological symptom is altered mental state, ranging from somnolence to coma with 12% of TBE patients in this phase exhibiting a GCS score below 7.[41] Disorientation, excitation, seizures, and confusion are also observed, as well as hyperkinesia of limbs and facial muscles, cranial nerve involvement with paresis of facial and ocular nerves, cerebellar ataxia, and autonomic disturbances of the bladder and intestines. Spinal nerve paralysis has been documented in 11-15% of patients. Depending on the extent of the CNS affection, meningoencephalitis can be moderate or severe. Severe myalgia in the extremities sometimes precedes the development of paresis. Involvement of the cranial nerve nuclei and motor neurons of the spinal cord causes flaccid paralysis of neck and upper extremity muscles (Photo 1)

Patients with meningoencephalomyelitis may experience paresis of the arms, back, and legs, with the upper extremities affected more often than the lower extremities. Bilateral paresis is a rare symptom (Photo 1 and 2). Involvement of the medulla oblongata and the central parts of the brainstem (bulbar) is associated with the poor prognosis[41] (Photo 3). Occasionally, TBE can be associated with autonomic dysfunction including reduced heart rate variability and tachycardia.

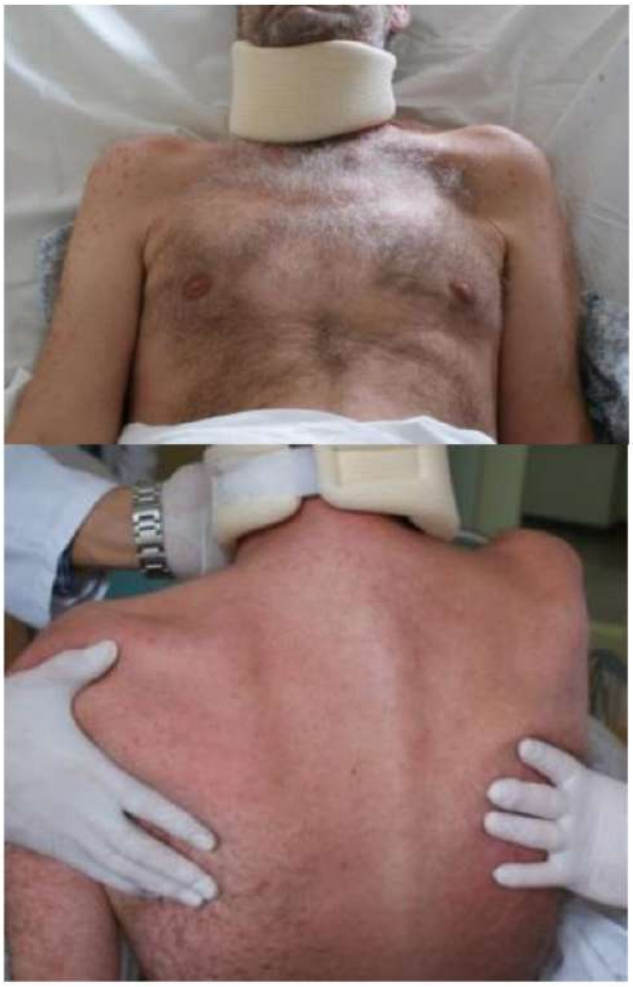
Flaccid paralysis arises, very similar to that seen in poliomyelitis, due to the viral preference for the anterior horn of the cervical spinal cord. In contrast to poliomyelitis, mono-, para-, or tetraparesis develops in 5–10% of patients. Paralysis of respiratory muscles may also occur, necessitating ventilatory support. Cranial nerve involvement is associated mainly with ocular, facial, and pharyngeal motor functions. Hearing defects may also occur. Brainstem involvement (particularly of the medulla oblongata) can lead to bulbar syndrome, with the risks of sudden respiratory and circulatory failure.[6,7,41,42,57]

Chronic TBE – chronic TBEV infection

Some peculiarities in the course of TBE are observed in Western Siberia. The onset of illness is more often gradual than acute, with a prodromal phase including fever, headache, anorexia, nausea, vomiting, and photophobia. These symptoms are followed by a stiff neck, sensorial changes, visual disturbances, and variable neurological dysfunctions, including paresis, paralysis, sensory loss, and convulsions. In fatal cases, death occurs within the first week after onset. The case-fatality rate is approximately 20%, compared with 1–2% for the European form. However, these findings may be biased by the different types of medical treatment available in Western and Eastern Europe. It is supposed that, in contrast to the European form, the disease caused by TBEV-FE is more severe in children than in adults. Neurological sequelae occur in 30–80% of survivors, especially residual flaccid paralysis of the shoulder girdle and arms. Overall, there is little information available on the virulence of the recently described TBEV-Sib with respect to the course of disease in humans. The few systematic and unsystematic data accumulated over the past 20 years show that TBE is a chronic disease in 1–1.7% of cases. Chronic TBE affects mainly working-age people and children, often leading to their debilitation.[38] The frequency of the chronic form is described as approximately 1–1.8% of patients.[58]

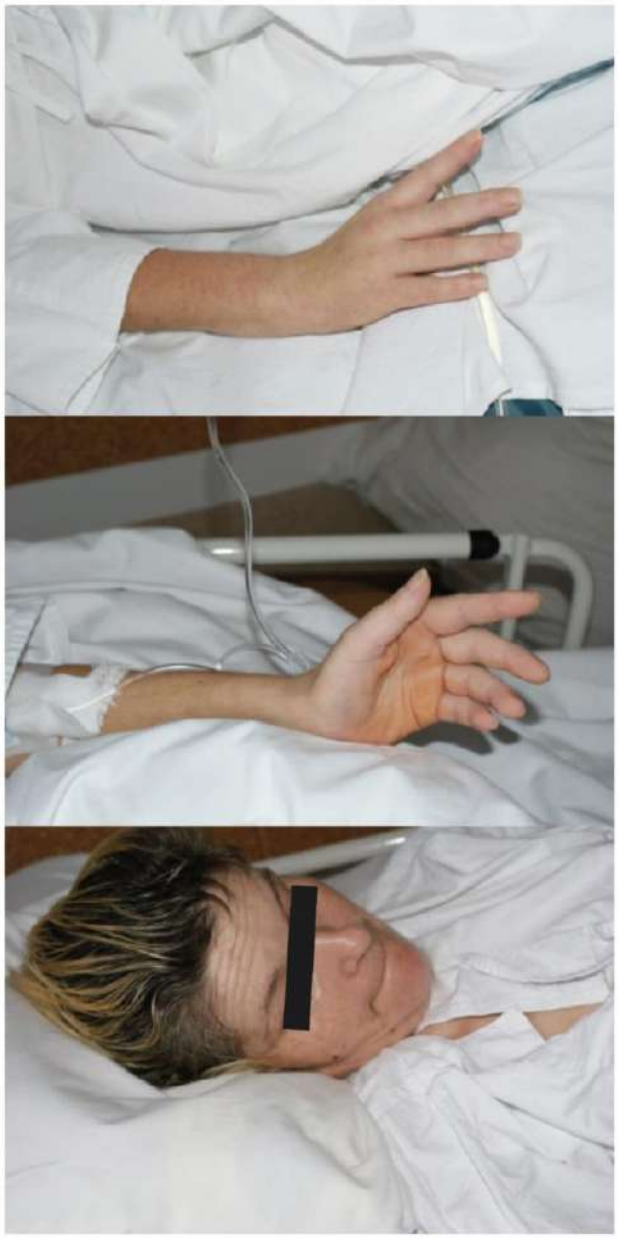
Hyperkinetic syndrome is the main manifestation of chronic TBE (86%), which presents as myoclonic hyperkinesia in

Photo 1.



Bilateral flaccid paralysis of the arms, shoulders, and the levator muscles of the head.

Photo 2.



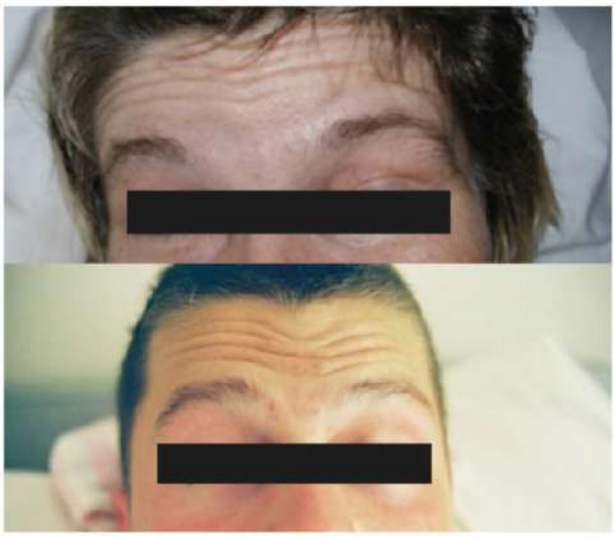
Movements are limited to the hands, due to bilateral flaccid paralysis of the arms, shoulders, and the levator muscles of the head

paralyzed muscles, myoclonus of oral muscles, myoclonus of the oral, shoulder girdle, and abdominal wall muscles with Parkinson tremor, and spontaneous progressive form of chronic TBE (from myoclonic hyperkinesia in the arm to typical Kozevnikoff epilepsy).

Laboratory findings

Compared with other forms of viral meningitis, white blood cell counts in the CSF are low in TBE (median 60/µL, range 5–1200/µL).[59] The CSF albumin is moderately increased; indeed, in some TBE cases, an increased albumin concentration may be the only pathological finding in the CSF.[56] Increased CSF-to-serum-albumin ratio indicates an impaired blood–brain barrier, significant disruption of which can be observed in up to 60% of patients with TBE.[59] Initially there is a predominance of polynuclear cells (granulocytes) in the CSF cell count; however, the immune response switches within a few days towards an increased lymphocytic cell count (Figures 2–4). In serum samples, patients with TBE display only moderate markers of inflammation. For example, CRP is marginally altered (median 3 mg/dL; range 1–60 mg/dL).[41] TBE-specific IgM and IgG antibodies are already present in CSF samples at the time when patients are admitted to hospitals because of CNS symptoms.[41] Meningeal signs can be absent in TBE patients with CSF pleocytosis. For microbiological confirmation of TBE please see chapter 12.

Photo 3.

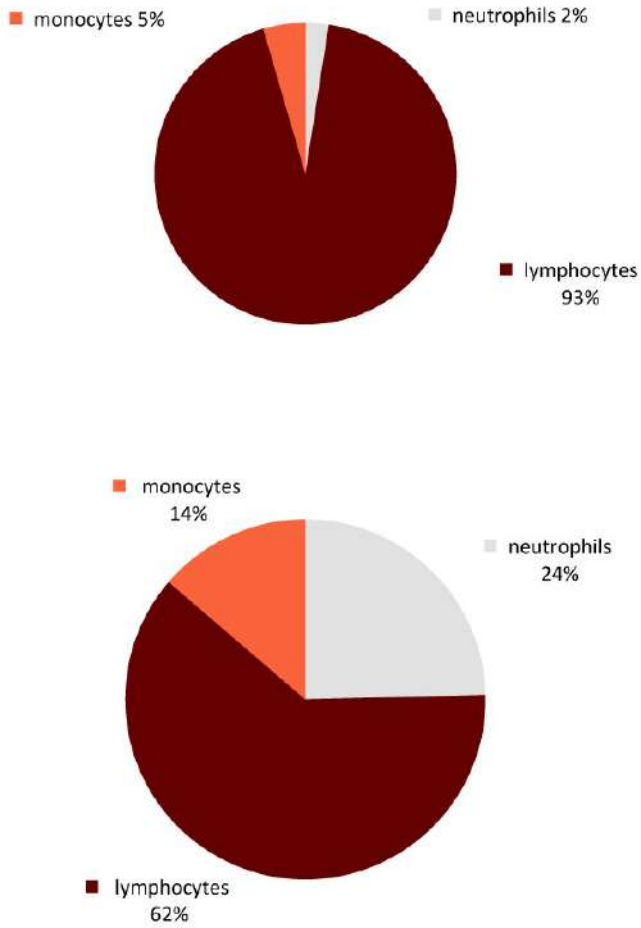


TBE with affection of the brain stem.

Neuroimaging

Magnetic resonance imaging (MRI) is the standard for the evaluation of patients with any type of encephalitis, including TBE. However, only about 18% of patients with TBE have abnormalities on cranial MRI (cMRI) examination. These abnormalities are mainly located in the thalamus, the cerebellum, the brainstem, and the nucleus caudatus (Figures 5–7).[57,60,61] MRI findings are bilateral or unilateral. The most characteristic MRI finding is the bilaterally-increased signal intensity in T2-weighted images and in fluid-attenuated inversion recovery (FLAIR) images within the basal ganglia or thalamus. The localization of the MRI lesions corresponds with neuropathological findings. The cerebellum, brainstem, cerebral cortex, and spinal cord are further brain structures that may be clinically affected; however, pathological MRI enhancements are rarely seen in these locations. There is usually no restricted diffusion on

Figure 2: Pleocytosis in CSF



Pleocytosis in CSF; Percentage of cells seen in pleocytosis at the onset of symptoms, and follow–up (Zajkowska, unpublished data).

diffusion-weighted imaging (DWI) in patients with TBE. Sometimes single-voxel 1H-MRI spectroscopy may show pathological patterns, indicating lactate or lipid peaks or other metabolic alterations in the otherwise unremarkably appearing basal ganglia or thalami. TBE patients with meningoencephalitis and unfavorable prognosis are more likely to present with MRI lesions; however, anecdotal reports show that even some patients with normal cMRI scans may die from TBE. It is difficult to correlate MRI results with clinical findings or even outcomes in TBE patients. However, there is one prospective cohort study from Germany that enrolled 111 TBE patients from 2004 to 2014. All patients were evaluated by high-resolution MRI. Clinical symptoms were scored using the modified RANKIN Scale at admission and at defined follow-up dates. The results indicate a strong correlation between meningo-encephalomyelitis documented on MRI and a poor outcome. Further risk factors for a worse outcome were age, male gender, and preexisting diabetes mellitus.[61] Cranial computed tomography (CCT) is usually negative and not recommended for the diagnosis of encephalitic brain lesions. In some rare occasions CCT may show symmetric or asymmetric bilateral hypodensities of the thalami and/or basal ganglia.[62–65] Single photon emission computed tomography (SPECT) is a highly sensitive functional imaging method for the detection of cerebral perfusion abnormalities. A SPECT study from Sweden including adult patients with TBE (n=73), and patients with other forms of meningoencephalitis (n=56), showed a decrease in regional cerebral blood flow (rCBF). However, these findings were not significantly related to the clinical course or the outcome of TBE.[42,66] Thus, the significance of this reduced rCBF remains unclear. Another pilot study using 18F-FDG PET/CT in 10 patients with TBE showed that glucose hypometabolism was present in 7 out of 10 patients with TBE, reflecting neuronal dysfunction in areas prone to TBEV infiltration and responsible for the development of clinical signs and symptoms.[67]

EEG

In the acute stage of CNS inflammation, electro-encephalograms (EEGs) show pathological patterns. In a study by Lindquist, there were abnormal EEG findings in 77% of patients with TBE.[57] An abnormal EEG correlates with the severity of the clinical condition of the TBE patient; however, there is no link between the degree of EEG pathology and clinical condition. In most cases, an initially abnormal EEG normalizes within a few weeks. The EEG may be of prognostic value when there is a persistence of pathological patterns or the appearance of new irregularities.[68,69]

Prognosis, long-term sequelae and Postencephalitic syndrome (PES)

The case fatality rate (CFR) from infection with TBEV-EU is reported to range between 1% and 2%.[2,41] In lethal cases, death occurs within 5–10 days after the onset of neurological symptoms in the context of diffuse brain edema and bulbar involvement.

There are several retrospective and prospective studies from different countries regarding long-term morbidity of TBE patients (Table 1). Overall, there is a high proportion of patients with persistent post-TBE symptoms of different severity. However, differences in patient selection, age-specific exposure, access to medical care, and selective reporting of more severe cases may result in bias in these data and may explain, in part, the discrepancies between different TBEV subtypes.[8,43,56,70–74]

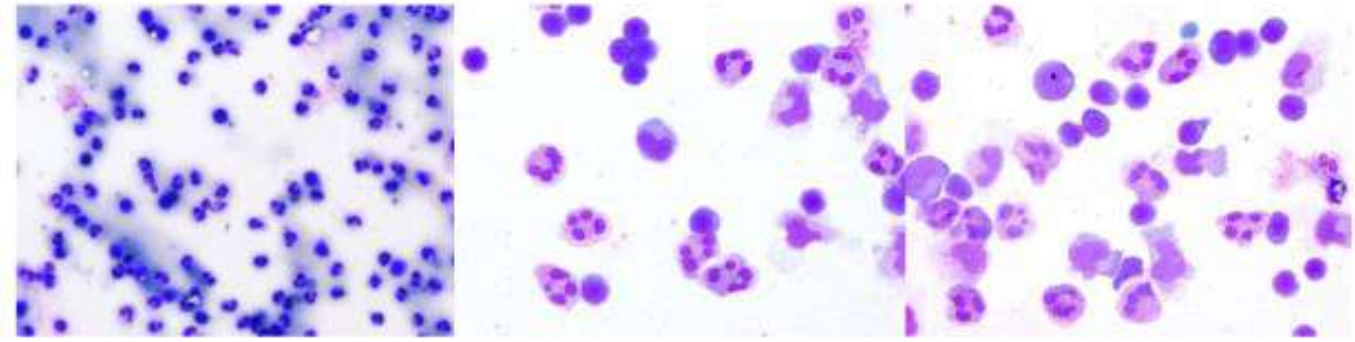
PES is a condition that includes residual (behavioral) changes following the recovery from viral or bacterial

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%**

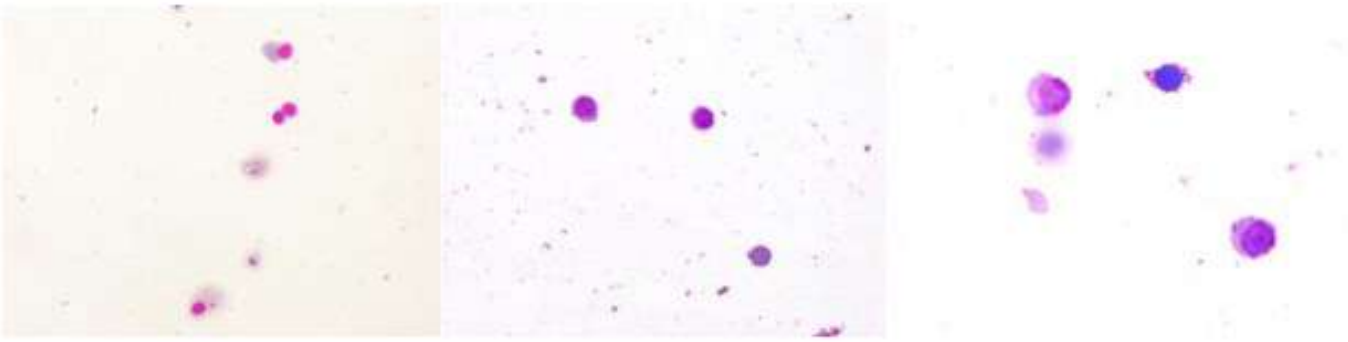
encephalitis according to the international classification for diseases. The symptoms are nonspecific and, in contrast to organic disorders, are often reversible. There may be a variety of residual neurological dysfunctions, such as paralysis, deafness, or aphasia. Initially, doubts were raised whether PES after TBE exists; however, a recent study specifically assessed the incidence and characteristics of PES after TBE. Between 1995 and 2008, 124 patients aged 16–76 years were followed for >3 years. Of these, 60 patients (48%) had no symptoms of PES; 15 patients (12%) had symptoms that were mild and of short duration; and the remaining 49 patients (40%) developed PES lasting for 3–18 months. In 15/49 patients (12%) PES was severe. The main characteristics of PES were psychiatric symptoms, balance and movement disorders, headache, general malaise, and reduced working ability.[41] Kaiser et al. followed patients

Figure 3: Evaluating pleocytosis in TBE (early)



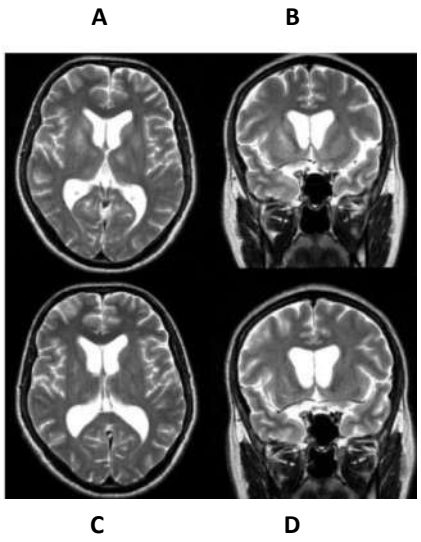
First evaluation of pleocytosis in TBE. The cell preparations cerebrospinal fluid of patients with TBE observed a plurality of cells. In all microscopic views there are cells that occur singly or in small clusters, neutrophils with different numbers of lobes nuclear and clearly visible large monocytes. (1 x 100; 1 x 400; 1 x 400.)

Figure 4: Evaluating pleocytosis in TBE (later)



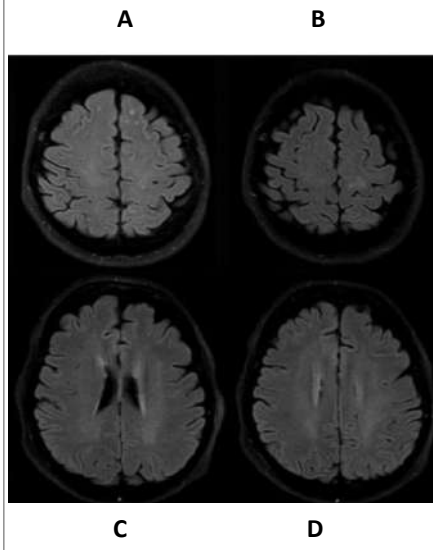
During recovery, after acute phase, control LP. x 100, single lymphocytes, some monocytes, lack of granulocytes x 200 x 400.

Figure 5: MRI visualization of TBE-related abnormalities



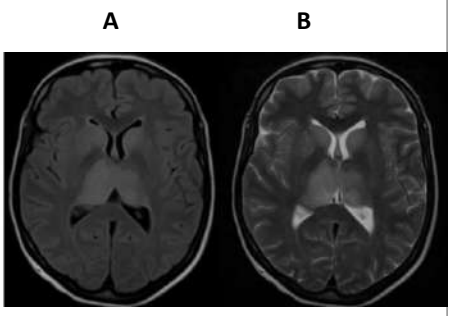
Axial (A) and coronal (B) T2-weighted MRI images show high signal intensity in the basal ganglia and thalami. The second scans (C, D) obtained several months later, show partial resolution of the lesions. Patient with chorea presentation.

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.

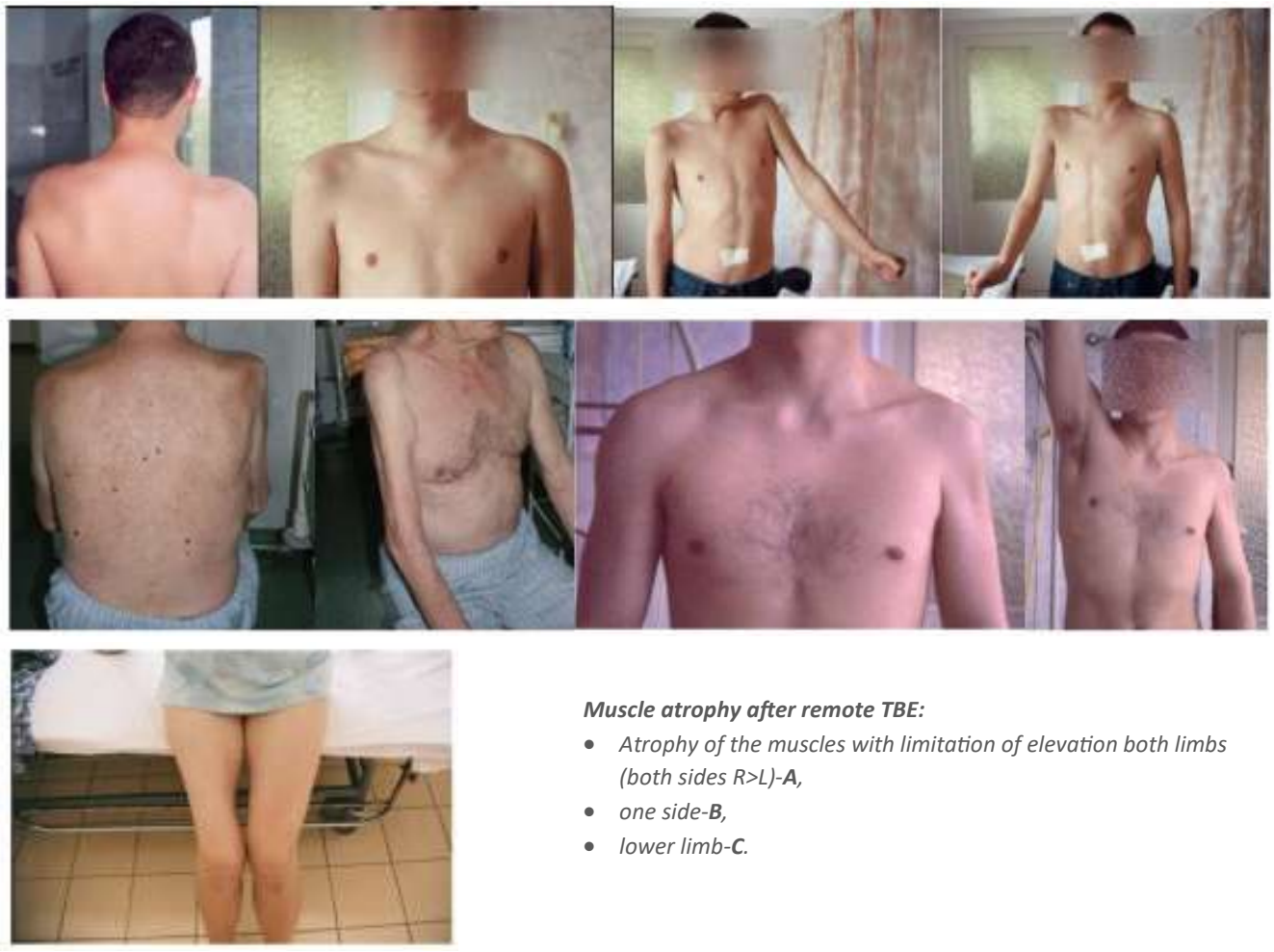
with meningoencephalomyelitis for 10 years.[41,43,73] There were 57 patients with complete follow-up data, of whom 19% recovered, 51% had moderate/severe sequelae, and 30% died. The most substantial improvements were seen in the first 12 months after acute TBE. These follow-up results indicate that the chance for clinical improvement reaches a maximum in the first 12 months after acute TBE and decreases substantially after the first 3 years. The severity of acute TBE correlates with long-term prognosis. Patients who died during the 10-year follow-up had a significantly higher deficit sum than those who survived. By contrast, patients with complete recovery within 5 years had the lowest initial deficit measured in a standardized scoring system. Mechanical ventilation was required in 30 patients with TBE in the acute phase of the disease–14 patients died during the follow-up period (7 within the first year). Respiratory symptoms resolved completely in 14 patients with TBE. Overall, there is a correlation of disease severity and prognosis. Patients with ataxia, impaired consciousness, double vision, urinary retention, or mild paresis of only 1 extremity had the best prognosis. However, TBE patients with tetraparesis and concurrent respiratory paralysis, dysphagia, or dysarthria were among those with the highest risk for a fatal outcome.

Post-mortem examinations of deceased TBE patients and animal studies provided some explanations and insights into the neuropathological mechanisms of the disease.[75,76] Viral infection of neurons causes cell lysis. There is only a limited chance for improvement of muscle paresis, because neurons have restricted regenerative capabilities. TBEV has a high affinity for cranial nerve nuclei, the cells of the anterior horn of the spinal cord, the Purkinje cells in the cerebellum, and cellular components of the thalamus. Clinical improvements achieved in patients with paresis are linked to physical exercise increasing the muscular strength of neighboring muscle groups and, to a lesser extent, to learning effects in the context of neuronal plasticity. However, if cellular damage is multisegmental, the resulting neuronal muscle atrophy has little or no chance for regeneration (Photo 4).

Treatment

Treatment is mainly supportive and symptomatic. No specific antiviral therapy is currently available and approved for TBEV infections. Some antiviral agents, specific

Photo 4.



Muscle atrophy after remote TBE:

- *Atrophy of the muscles with limitation of elevation both limbs (both sides R>L)-**A**,*
- *one side-**B**,*
- *lower limb-**C**.*

immunoglobulins, and other potentially protective substances are under investigation for their anti-TBEV efficacy in vitro and clinically[77–80]; however, a detailed review of these 'pipeline' agents is beyond the scope of this chapter. If there are clinical signs and symptoms such as status epilepticus, severe meningoencephalitis, encephalitis, and myelitis, the patient should be admitted to an NICU (neurological intensive care unit) for further monitoring and treatment. In a large study of 709 patients with TBE in Germany, 12% of patients required intensive care and 5% required assisted ventilation.[43] Maintenance of an adequate cerebral perfusion and prevention of secondary complications are the main objectives of treatment. Correct positioning, deep analgosedation, and osmotherapy (mannitol, hypertonic saline) can be considered, but only for 1–2 days and provided exclusively as boluses. However, the use of mannitol did not affect the outcome in terms of survival. In the case of an increasing intracerebral pressure and a decreasing cerebral perfusion, therapeutic hypothermia might be considered. High fever is associated with increased metabolic consumption. Antipyretics, or other physical measures like cooling blankets, or infusion of cooled fluids, should be employed to reduce body temperature effectively. The use of steroids is still a matter of debate and cannot be recommended based on current evidence.[8,72] Dehydration increases the risk for cerebral infarction. Severe TBE is often 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 salt-wasting syndrome, and reduced sodium supplementation. Mental and behavioral disturbances, delirium, and psychotic signs and symptoms may justify treatment with neuroleptics. For seizures, the administration of benzodiazepines is recommended. 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. Rehabilitation should be introduced as soon as possible, as early introduction of rehabilitation therapy is essential to protect muscle atrophy.

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Chapter 6

TBE in children

Malin Veje and Mikael Sundin

Key Points

- Most cases of TBE in childhood will present similarly as in adults. However, a more diffuse clinical picture is seen especially in preschool children.
- Laboratory evaluation may show elevated blood inflammatory indices, but cerebrospinal fluid analysis and anti-TBEV serology are needed for establishing the diagnosis.
- There is no specific treatment for TBE; supportive care needs to be provided based on the individual clinical course.
- The mortality in pediatric TBE is very low but severe courses have been reported in a fraction of the children.
- Long-term somatic residua exist, but are uncommon (2%) in childhood TBE. Yet, long-term symptoms and neurodevelopmental/cognitive deficits are seen in 10–40% of infected children.
- Protective immunity can be elicited in children by TBE vaccines as of 1 year of age.

Children, ticks, and TBE

Compared with tick-borne encephalitis (TBE) in adults, childhood TBE has been described as a rare disease, particularly in preschool children.[1–4] This is puzzling, as children appear to be perfect prey for ticks, primarily because of a high level of exposure and the short climbing distance to a proper bite site. The low incidence of pediatric TBE cases becomes even more puzzling as Borrelia infections are well documented in children.[5–7] Additionally, Borrelia infections have been reported to be up to 5 times more common in preschool children than in older children and adults.[5] The discrepancy between a high tick exposure and a low TBE incidence leads to the suspicion that childhood TBE is underdiagnosed.

Awareness of TBE varies greatly. In countries with a high TBE burden, such as Estonia, the pediatric cohort accounts for a large proportion of all cases, as was the case also in Austria in the pre-vaccination era.[8] The general TBE immunization policy in Austria has increased awareness of the disease.[9] The Baltic states have advocated for proper immunization strategies.[10] The general knowledge of TBE is likely higher in the high-endemic areas and as a consequence fewer cases are possibly eluded.

In addition, some childhood TBE cases are probably overlooked because of the non-specific clinical picture (discussed in more detail below).[5,6,11] Younger children also seem to be less frequently considered for TBE,[5] but the disease can be seen in children as young as only a few months of age.[12–15] The idea that pediatric TBE could be underdiagnosed was further substantiated by a high incidence, the highest in years, in a prospective study of neurologic complaints at an emergency ward in Stockholm, Sweden,[6] demonstrating the adage: "he who seeks will find."

To summarize, children evidently get tick bites and they do contract TBE. The disease itself, the child's attributes (e.g., age, physical activity level, etc.) and parental as well as medical community awareness may influence the number of children who are diagnosed.

Children's clinical course of TBE

Acute phase or nonspecific phase

The onset and acute phases of TBE in children have been found similar in part to the clinical picture seen in adulthood, but there are also differences reported. Tick-bites have been recalled in 48–75% of childhood TBE cases.[3,5,16-18] Approximately 70% have had a biphasic clinical course, i.e., a flu-like prodrome followed by a short asymptomatic period and thereafter a varying degree of meningitis to meningoencephalomyelitis, as reported in prospective and retrospective studies,[1,3,16,17] others have reported considerably fewer biphasic courses, certainly among preschool-age children.[5,6] A large Polish retrospective study from 2020 compared 68 pediatric to 601 adult TBE cases, and concluded that the disease was milder in children.[19] However, the comparison was potentially biased as there were no standardized inclusion criteria.

That younger individuals may have a vague/nonspecific clinical presentation and a generally milder clinical course is

well established,[2,3,5,20] but this may also denote that childhood TBE manifests differently in children versus adults and that the condition may be underdiagnosed.[5,6] This notion was further emphasized by Meyer et al, who reported a case series of TBE appearing as fever without localized symptoms.[11]

In the majority of reports on pediatric TBE, fever is present in virtually all cases at diagnosis.[1,3,16,17] However, both retrospective data from a fairly large cohort[5] and prospective data from a study with broad inclusion criteria,[6] 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.[1–3,5,6,16,17,21] Meningeal signs are prevalent findings, noted in >80% of infected children,[1,3,16-18] but here as well, young children have a less-pronounced clinical presentation.[5]The clinical picture of pediatric TBE is classified as meningitis in 63–79% of cases, meningoencephalitis in 21–38%, and meningoencephalo-myelitis in 0–4%.[1,16,21] Other findings in childhood TBE are tremor, impaired general appearance, somnolence, lymphadenopathy, apatheia, hyperesthesia, and confusion/ cognitive dysfunction.[1,3,5,6,16,17,21,22] Though uncommon, some children present with seizures and hemipareses.[1,5,17] A recent retrospective Lithuanian study on TBE in children noted a higher proportion of milder disease (i.e.) meningitis in children aged 1–8 years compared with those aged 9–17 years, who more often suffered from meningoencephalitis or meningoencephalomyelitis.[18] Again, the comparison was potentially biased as there were no standardized inclusion criteria.

Detection of specific anti-TBE virus (TBEV) antibodies, as described in other chapters, is required to establish a diagnosis. Some cases require testing of both acute and convalescent sera, as antibodies may be absent in the initial phase.[5,6] Although serologies are reported as diagnostic, they are of little help at the first clinical assessment in the acute phase. Instead, the clinical presentation corroborated with routine laboratory evaluation has to guide the clinician. Nonspecific inflammatory signs, i.e., leukocytosis, elevated C-reactive protein (CRP), and elevated erythrocyte sedimentation rate (ESR) are reported in many children with TBE.[1–3,5,16,17] Worth noting is that many adults with TBE present with less pronounced blood inflammatory indices.[3,5] Laboratory evaluation for children with suspected TBE should include lumbar puncture. The most common cerebrospinal fluid (CSF) finding is pleocytosis with a mononuclear preponderance.[1–3,5,16,17,22] Additionally, some children present with elevated CSF protein/albumin levels. However, this is more commonly observed in adults than in children,[3,5] suggesting a more restricted encephalitic presentation in childhood TBE. This can also be concluded from the lower proportions of meningoencephalitis and meningoencephalomyelitis observed in children compared with adults, as noted above.

Laboratory evaluation for children with suspected TBE should include lumbar puncture, as cerebrospinal fluid pleocytosis with a mononuclear preponderance has been described.[1–3,5,16,17,22] Additionally, some children have presented with elevated cerebrospinal fluid protein/ albumin levels. However, this has been more common in adults than in children,[3,5] suggesting a more restricted encephalitic presentation in childhood TBE. This can also be concluded from the lower frequencies of meningo-encephalitis and meningoencephalomyelitis observed in children than in adults, as noted above.

Electroencephalographic (EEG) examinations in the acute phase of childhood TBE can help confirm the diagnosis. The EEG abnormalities seen include mild to moderate, generalized, slowing background activity, but also sharp waves in contrast, though seldom generalized spike wave activity.[2,22] 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.[2,22-25] 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.[22,24] In a recent 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.[24] 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.

To conclude, the clinical picture of TBE in childhood bears similarity to the disease in adults. However, some pediatric patients, more likely the younger ones, may not present as ‘expected’. Fever, headache, and vomiting are common. Children tend to more commonly present with symptoms and findings of meningitis, with increased blood inflammatory indices. Anti-TBEV serology and cerebrospinal fluid analyses are essential in establishing the diagnosis. EEG and MRI can strengthen the diagnostics.

Short-term consequences

As in adults, most tick bites from TBEV-carrying ticks do not result in clinical cases. Nevertheless, childhood TBE is associated with severe disease in some of those with clinical infection, as described above. This was concluded by Fritsch et al., who demonstrated that children required a median of 18 days of care in pediatric hospital wards.[1] Others have reported median hospital stays ranging 5–13 days.[2,3,6,16,17]

A large proportion of children still have symptoms but do not require medical attention at discharge,[21,25] which contrasts with children with some other CNS infections.[26] 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.[27]

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.[2,3,7,28,30]

That pediatric TBE has been associated with severe disease courses can be further supported by reported rates of admission into intensive care units, ranging from 5% to 22% of TBE cases.[1,17,28] Compared with adults, fatal cases of TBE are reported only infrequently.[5,28-31]

Long-term consequences

While the occurrence of long-term neurologic and neuropsychological sequelae in adults after TBE infection is well-established,[2,31] the literature is inconsistent when it comes to the risk for long-term residua of childhood TBE. For many years, but also recently, some studies have concluded that pediatric TBE has a more favorable outcome.[16,17,21]

However, defining the complications of TBE is important. Only determining the gross neurologic status and 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.[22]

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.[21] 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.[32] 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.[27]

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 3 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.[34] 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.[35] 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%).[17]

Long-term sequelae of a somatic nature are less frequently reported in childhood TBE. However, such cases occur and should not be forgotten. 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.[13,17,23,28] 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.[2,4,13,16,21,23,28] 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,[1,13,17,22,23] 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.[29]

To conclude, pediatric TBE carries a high risk for subjective sequelae, which to some extent can be objectively assessed by using structured questionnaires and inter-views.[26,28,32] A Swiss review on sleep-related symptoms concluded that 73.9% of children suffer from fatigue at long-term follow up (≥12 months) after TBE.[36] The early findings by Schmolck et al.[22] that TBE in childhood can be associated with neurodevelopmental/cognitive difficulties have now been verified.[17,27,34] As summarized in a recent review by Dr. 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.[37]

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 (for example, to explain why the child may not function as usual, to initiate educational support, to start medication for attention deficits, etc.).

Immune response against TBE in children

Children, from the age of 1 year, as well as adults, can elicit protective immunity to TBEV (i.e., response to the viral E protein) by immunization with the two TBE vaccines available in the EU. These vaccines are based on the European TBEV strains Neudörfl (FSME-IMMUN® Junior) and K23 (Encepur® Children).[38] (For more details, see Chapter 14). 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%).[39] 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.[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.[40] However, 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.[40,41,43]

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.[44] 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.

The differences in clinical appearance of TBE between children and adults could stem from the immune response to the TBEV. In adults, polymorphisms and alterations in immune receptor genes (such as *CCR5, TLR3*, and *CD209*) have been reported to play a role in predisposing individuals to infection and/or severity of TBE.[47-49] However, Engman et al. reported that the 32-basepair deletion in the chemokine receptor 5 gene (*CCR5*Δ32), which impacts adult TBE, was neither more frequent in children with TBE nor did it have any association with the clinical course.[27] The lack of an effect on clinical TBE course by *CCR5*Δ32 in children was later confirmed by Mickiene et al. Yet, the later and larger study by Mickiene et al. demonstrated that *CCR5*Δ32 predisposed children for TBE.[48]

Autoantibodies have been detected in children with TBE at a low frequency. The occurrence of these antibodies did not contrast to those with neuroborreliosis and had no association with the clinical course.[27] The first case of Anti-NMDAR antibodies in a child after TBE was recently published.[49] The role of autoantibodies in pediatric TBE pathogenesis needs to be further elucidated.

In a study of both children and adults, Palus et al. reported a significant global pro-inflammatory cytokine balance in patients with higher serum interleukin (IL)-12:IL-4 and IL-12:IL-10 ratios versus controls. Also, novel and mechanistically interesting biomarkers like hepatocyte growth factor and vascular endothelial growth factors were increased in patients with TBE.[50] The significance of immune reactions in pediatric TBE has also been reported by Fowler et al. They found that development of sequelae in pediatric TBE could be related to the grade of inflammation (i.e., cytokines) rather than direct neuronal damage. High concentrations of cytokines (interferon-γ, IL-6, and IL-8) in the CSF might be associated with a risk of incomplete recovery.[51] In a recent publication from the same group, the CSF IL-6:IL-10 ratio was found to be significantly higher in a cohort of 37 children with TBE, compared with pediatric neuroborreliosis cases and healthy controls.[52] Another recent study of children with TBE indicates a relative abundance of CD4+ T cells intrathecally.[53] But, as stated in Chapter 9, the complexity of the immune response to TBEV has not yet been fully understood.

To conclude, the available TBE vaccines based on the Neudörfl and K23 strains, respectively, are safe and provide a protective immunity in most children. The natural long-term immunity after childhood TBE must be further investigated. Evidence suggests that the immune reactions to the TBEV serve as a key player in the clinical course, including risk for residual symptoms and sequelae, in childhood TBE.

Concluding remarks

All children deserve the best chance to reach their full potential. Such a chance includes a life without TBE-related sequelae. Childhood TBE may be associated with death, with considerable acute disease severity, prolonged convalescence, and long-term residua. Hence, advocating for immunization against TBE in children, even for the smallest ones, and proper neurodevelopmental follow-up after cases of TBE infection cannot be regarded as controversial. Despite being infrequent, disabling neurologic injuries exist after pediatric TBE and, together with the emerging evidence of altered cognitive functioning, action clearly is required–both from the medical community and from the health authorities in TBE endemic regions.

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Chapter 7

TBE in special situations

Gerhard Dobler and Igor Stoma

Key Points

- TBE often takes a severe clinical course in immuno-suppressed patients.
- In transplant patients TBE usually takes a fatal course.
- TBE vaccination in immuno-suppressed patients can be non-effective.
- TBE in pregnancy has rarely been reported; from recent cases there is no evidence of transplacental infection of the offspring.
- The alimentary route of infection of TBE is still common in some European countries resulting in a high clinical manifestation index.
- TBEV can be infectious in milk and milk products for up to 14 days under optimal environmental conditions.
- TBE is an important travel-related disease. Increasing numbers of non-endemic countries report imported cases.
- Imported TBE cases in non-endemic areas pose challenges regarding the diagnosis of TBE.

TBE in immuno-suppressed patients

Changes in modern treatment regimens in hematology, oncology and autoimmune diseases significantly improved both quality of life as well as survival rates for numerous diseases. Modern approaches including hematopoietic stem cell transplantation (HSCT), solid organ transplantation, mono-clonal antibodies and target therapy are becoming more accessible, and thus the number of people living with immune-suppression continues to grow. It is well documented that there is a higher risk for this population for (severe) infections and this includes infections with the TBEV.

Currently, there are only few published cases of TBE in immunocompromised hosts, how-ever, these show common patterns. Two fatal cases of patients treated with the anti-CD20 monoclonal antibody rituximab have been reported. The disease course in both cases was extremely fulminant with severe neurological symptoms and damage, and in both cases delayed antibody formation was observed.[1] Two additional cases of rituximab-treated patients developing severe TBE were published by Steininger et al., indicating that TBE is a previously unrecognized severe infectious complication of rituximab therapy.[2] In a recent case (Dobler, personal observation), a 22-year-old male patient suffering from non-Hodgkin lymphoma treated with rituximab developed clinically severe TBE one year after the end of a successful therapy. According to the treating physician the clinical course was life-threatening with fever and severe encephalitis. Finally, the patient survived with several months of convalescence and persistent neurological sequelae.

Expectedly, the inability to generate an antibody response renders rituximab-treated patients susceptible to TBE and it also impedes laboratory diagnosis. It is reported that in patients under rituximab therapy the antibody response is deficient for up to 6 months, making vaccination of these patients a challenge. The above case of a young patient who developed life-threatening TBE one year after rituximab medication was stopped shows that rituximab-induced B-cell response/antibody-deficiency may even last up to one year. Therefore, with patients receiving rituximab, information should be stressed on the importance of protecting themselves from tick bites and unpasteurized milk. There are no general recommendations to vaccinate patients against TBE before rituximab therapy. To obtain a good level of protection, repeated vaccine doses over time are needed, and this may not be possible in patients with an acute diagnosis of cancer. An accelerated schedule, with three doses on days 0, 7 and 21, has been used in some centers for patients with rheumatic diseases before initiating rituximab and can be recommended if the clinical situation permits.[2] Still, the resulting protection rate remains unknown.

In a recent report an immunosuppressed patient in Italy had persistent viremia associated to the erythrocyte fraction of the blood as well as shedding of the virus in the urine for more than six weeks, while receiving chemotherapy for relapsing blastic plasmacytoid dendritic cell neoplasm.[3] Another dramatic case of TBE in a 12-year-old patient was published by Chmelik et al., where the immunosuppressive treatment regimen including dexamethasone and etoposide resulted in viral replication and fatal outcome.[4] It has also been reported that thymectomized patients showed a delayed humoral immune response to TBE virus.[5]

Table 1: Compilation of three patients with TBE after solid organ transplantation

Patient No.	Organ	Immuno-suppressive treat-ment	Incubation	Symptoms	Duration	Outcome
1	liver	steroids, tacrolimus	17 days	fever, meningitis, encephalitis, tetra-plegia	69 days	fatal
2	kidney	steroids, tacrolimus, mycophenolate mo-fetil	22 days	fever, meningitis, encephalitis, brain bleeding	36 days	fatal
3	kidney	steroids, tacrolimus, mycophenolate mo-fetil	49 days	fever, meningitis, encephalitis	83 days	fatal

Lipowski et al. recently described three patients who had received solid organ transplants from a single (undiagnosed viremic) donor (2 received a kidney, and 1 received a liver) and all organ recipients developed TBE-encephalitis 17–49 days after transplantation with fatal outcomes.[6] The incubation period ranged from 17 to 51 days and thus was longer than what is seen in natural infections. The difference of 27 days between the two recipients of kidneys might result from the amount of virus in the respective donor organ. The presence of TBE virus was confirmed by real time PCR in all recipients and their donors, and direct sequencing of amplification products showed the presence of the same viral strain.

All three patients died. It remains unclear whether the differences in the clinical courses in the three patients were due to the non-natural transmission, the immune-suppression or both. Only one of the three patients showed the typical features of TBE in the cerebrospinal fluid, pleocytosis and increased protein concentration.

In another case (Dobler, personal observation), a 55-year-old male patient with a complete primary 3 dose vaccination against TBE several followed by one booster dose several years before a liver transplantation, but was not boosted for 7 years, developed a fatal form of TBE presenting with fever, encephalitis and tetraplegia. No information on the incubation period or on the immunosuppressive therapy was available. The patient died after 5 days of mechanical ventilation with severe symptoms of encephalomyelitis. A tick in his garden, which adjoined a known natural TBEV focus, had infected the patient. This case gives evidence that the “natural route” of TBE-infection may result in severe and fatal disease in transplant recipients.

In a published study including 31 heart-transplant recipients, seroconversion rates and post-vaccination antibody titers were markedly reduced in comparison to the control group, and these findings served as evidence for recommending other protective measures against TBE virus infection (clothing, avoiding high-risk areas for travel) in these patients.[7] This study also reported the safety of TBE-vaccination in the above-mentioned cohort of immune-suppressed patients.

In summary, based on clinical cases published, TBE appears to be more severe in immunocompromised patients with prolonged viral shedding and a higher risk for a fatal outcome, while standard vaccination and vaccination schedules appear to be less effective.

Vaccination against TBE for HSCT patients at risk, i.e. those living in or travelling to endemic areas, can be performed starting at 6–12 months after transplantation; however, due to the lack of data this cannot be recommended as a routine procedure.[8] The assessment of the immunogenicity of TBE-vaccine in patients with rheumatoid arthritis treated with tumor necrosis factor-inhibitors (TNFi) and/or methotrexate (MTX) was recently carried out by Hertzell et al. In this study, individuals < 60 years of age were given three doses of vaccine at month 0, 1, 12; individuals > 60 years old received an additional priming dose at month 3, i.e. a total of four doses, while TBE neutralizing antibodies were assessed by a rapid fluorescent focus inhibition test. The results reveal an insufficient antibody response one month after a complete schedule of three or four doses, compared to healthy age- and gender-matched controls.[9]

In another study 29 HIV-infected patients were vaccinated against TBE. The vaccination schedule was modified by the inclusion of a fourth dose according to the schedule 0-1-2-9 months.[35] The immune response depended on the CD4 counts of the vaccinees at the time of vaccination. With this schedule 85% of the vaccinated persons achieved protective antibody titers. The titers persisted at least for one year after the third vaccine dose.

In summary, based on a few published clinical cases, there are individual reports of patients with severe immuno-suppression (solid organ transplantation) who developed TBE via the infected organ or by tick bite. All known TBE cases in transplant patients showed a fatal course.

The incubation period in organ-transplant patients was longer than reported in tick-infected TBE patients with no underlying disease. The ICH does not show the typical findings of TBE-infection in cerebrospinal fluid. In one patient with immuno-suppressive treatment for non-Hodgkin lymphoma about 1 year earlier, a life-threatening form of severe TBE was observed. It is, however, unclear whether the immuno-suppression was the reason for the severe form.

Therefore, in immunosuppressed patients with a risk of TBE infection the immunogenicity of TBE vaccination should be tested by neutralization test. Vaccinated ICH-patients who had received 3 primary TBE vaccine doses before immunosuppressive therapy was started and with continuing risk of TBE infection should be tested after the immuno-suppression was stopped and they should be re-vaccinated in case no neutralizing antibodies are detected.

TBE in pregnancy

Pregnancy is another situation with (physiological) immunosuppression (for review see Koutis et al., 2014[32]) and it may also result in more severe forms of TBE. Although TBE is endemic throughout most of Europe and Asia with high incidence rates in some regions, there are only few data on TBE during pregnancy and its effect on the human offspring. The only available reference is one report on the occurrence of three TBE cases during pregnancy in the former German Democratic Republic.[10] Three pregnant females developed TBE after drinking contaminated milk and developed a clinically overt TBE.

Two of the three cases described developed TBE during the early phase of pregnancy (week 8 to 10 of gestation). The third woman showed first clinical signs of TBE in week 24 of gestation. Diagnosis at that time was confirmed using a neutralization test; however, no detailed information on the diagnostic confirmation (e.g. fourfold titer increase etc.) is given. Two of the three females showed a severe form of TBE (myelitis, encephalomyelitis). One pregnant female showed only a febrile course of the disease. All three women survived without neurological sequelae. The outcome in the offspring of the two pregnant females with TBE early during pregnancy was unfortunate (see Table 2). No serological information is provided from any of the three neonates.

In a few more cases described in the Czech Republic and in Austria in the 1950s and early 1960s no specific serological TBE diagnosis could be made, but the diagnosis was made on the basis of clinical symptoms and the epidemiological situation.[34] However, in all cases the newborns showed neither any signs of infection nor did they develop any clinically overt neurological acute or persistent symptoms.

In 2018 two additional cases of TBE during pregnancy were brought to the attention of the author of this chapter (manuscript in preparation). One woman was in week 20 of gestation when she developed a very severe form of TBE requiring mechanical ventilation for several days. She gave birth to a healthy child. Serological testing at the time of birth and 3 and 6 months later as well as virologic testing of mother and baby showed that antibodies of the mother were diaplacentally transferred to the baby and could be detected at birth; however, the infant never developed IgM as evidence of active infection and IgG antibodies significantly decreased during the first months of life.

In the case of a twin pregnancy in Sweden reported in 2018 (manuscript in preparation) the mother developed severe clinical TBE (encephalitis). Symptoms started in week 30 of gestation. The mother gave birth to twins at term. Both infants did not show any serological evidence or signs of active TBE infection.

Table 2: Summary of three TBE cases during pregnancy during a milk-borne outbreak of TBE in the former German Democratic Republic in 1961

Clinical course of mother	Outcome of mother	Age of gestation	Neutralization test	Outcome of newborn
fever	healthy	week 8	positive	pre-term (gestational week not provided) with intracranial bleeding
myelitis	healthy	week 10	positive	birth at week 40 of gestation with intra-cranial bleeding
meningoencephalomyelitis	healthy	week 24	positive	birth at week 40 of a healthy newborn

In conclusion there are few reports on TBE in pregnancy. The two cases of TBE in the late second trimester from 2018 show that a diaplacental infection could not be detected.

This observation is confirmed by a case early in the 1960s where the infection in week 24 of gestation resulted in a healthy child with no evidence of TBE or any neurological symptoms.

There are two early reports from an outbreak in 1961 where two pregnant females were infected early during the 1st trimester. Both gave birth to children (one of them pre-term) with brain bleeding. The infection in these cases was via contaminated milk, however, which might modulate the clinical course of TBE. Further-more, it is unclear whether the neurological symptoms resulted from TBE infection or from possible other causes.

Some other less well-documented TBE cases during pregnancy resulted in healthy neonates with no evidence of infection or neurological symptoms.

These cases also show that pregnancy may be associated with a more severe course of TBE, which may result from the immunological situation during pregnancy, where there is a kind of physiological immunosuppression.

Alimentary TBE

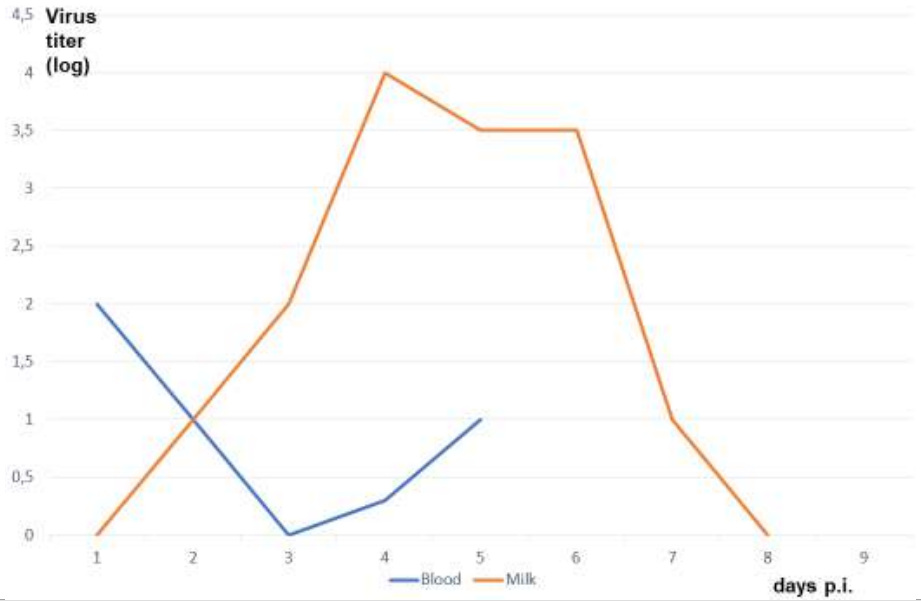
It has been known for a long time that TBE can be transmitted by contaminated milk. In fact, the first larger outbreak of TBE (in 1953) in Europe was milk-borne, described in Roznov, Czech Republic with more than 600 human cases.[11] At that time TBEV-transmission by milk was more important than transmission by ticks and TBE was named "Biphasic milk fever". With increasing industrialization in milk production and dairy production the alimentary route of infection of TBE was more and more forgotten in industrialized European countries.

However, in the more agriculturally-based countries of eastern Europe this means of transmission was still present, although only a small proportion of patients became infected this way. During the last few decades there has been an increasing trend back to "natural production methods" of foods. With this tendency, milk-borne TBE outbreaks now are reported even from industrialized countries like Austria and Germany.[12] However, there have been numerous milk-borne outbreaks in different European countries during the last decades.[13] In Slovakia it is estimated that up to 20% of all TBE cases are transmitted by the alimentary route.[14] In many of the milk-borne outbreaks it was reported that the manifestation index of clinical disease by the oral route was almost 100%. Experimentally, it was already shown in the 1960s that ruminants actively discharged TBEV in their milk when infected with the TBEV. There is a delay between the occurrence of viremia (first) and the shedding of TBEV to the milk (second).

In experimentally-infected goats the concentration of shed TBEV is higher than the concentration of the virus during viremia in the animal, which implies an active replication and shedding of TBE virus in the mammary glands.[15] Goats discharge the highest amount of TBEV, followed by sheep and then cows.[16,17] Nevertheless, single cases and small outbreaks caused by cow milk have been observed.[18]

So far, it is unclear whether the milk of a breast-feeding woman can also transmit the TBEV to the infant. There is one such case, where there is a high suspicion that milk of the mother might have infected the infant[19]: the infant developed TBE on the 10th day of life. Two days later the mother also suffered from TBE. The infant had only been fed with the milk of the mother. Although it is possible in principle that the child had been infected transplacentally,

Figure 1: TBEV titers in milk and blood in a goat after subcutaneous experimental infection



(modified after [15])

the course of the disease more likely suggests a milk-transmitted infection with a short incubation period, while the mother had been infected by a tick bite. Therefore, breast-feeding females should be cautious when being exposed to ticks in TBE-endemic regions.

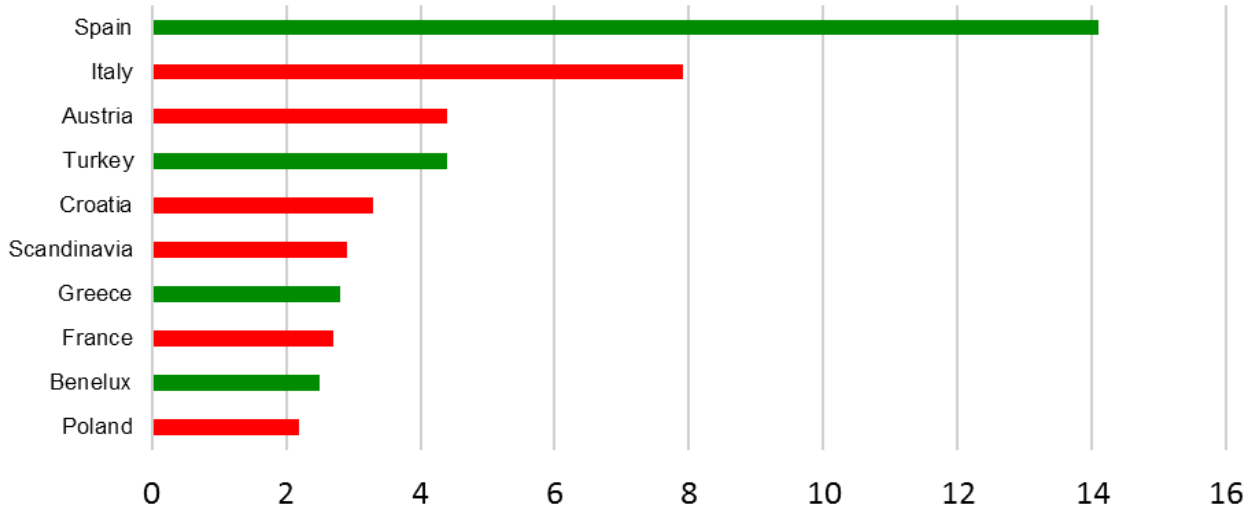
There is evidence that TBEV is stable in milk and cheese for up to 14 days, depending on environmental conditions.[20,21] At 4°C TBEV might remain viable up to 14 days, while at room temperature virus titers decrease after some days.[20] There is evidence that the adsorption of TBEV in the human gut takes place in the duodenum,[34] and moreover, TBEV seems to be protected by milk proteins during the stomach passage with its acid conditions, finally infecting duodenal cells.

TBE as travel risk

TBE is endemic in some of the most popular holiday destinations in Europe. In six of the 10 most visited countries TBE is endemic at least in some areas (Fig. 2), including Austria and Scandinavia. In Germany about 2 to 6% of the annual TBE cases are acquired abroad (Robert Koch Institut Jahrbuch 2006 - 2017) (Fig. 3). Countries where Germans acquire TBE outside their own territory are ranked by frequency in Fig. 4.

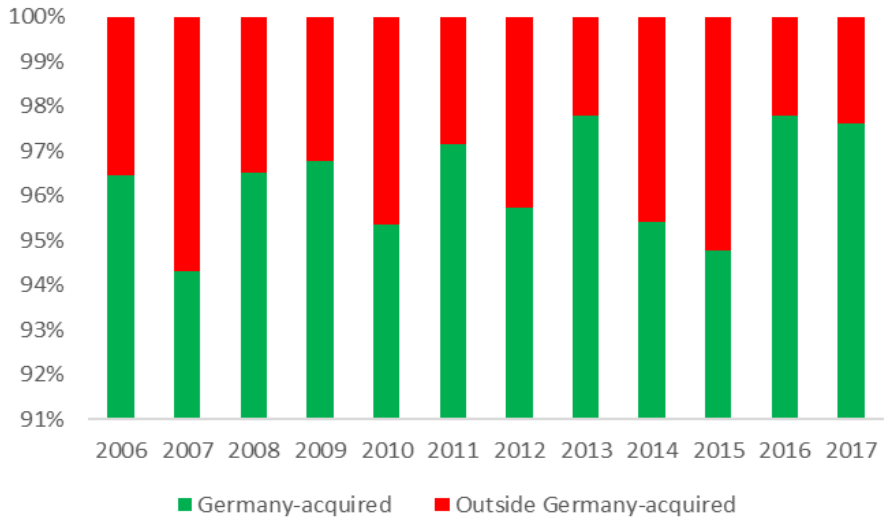
According to Süss[22] the risk of infection with TBE after a tick bite in endemic areas varies according to the respective human activity from 1:200 to < 1:1000. Estimating the number of reported TBE cases proportional to the number

Figure 2: Travel targets/100 travelers in German travelers for 2017



(modified after Zukunft Aktuell 276, 39. Jg., 07.02.2018, green bars indicate "TBEV not known to circulate", red bars indicate TBEV-endemic countries. Taken from: https://www.stiftungfuerzukunftsfragen.de/newsletter-forschung-aktuell/276.html).

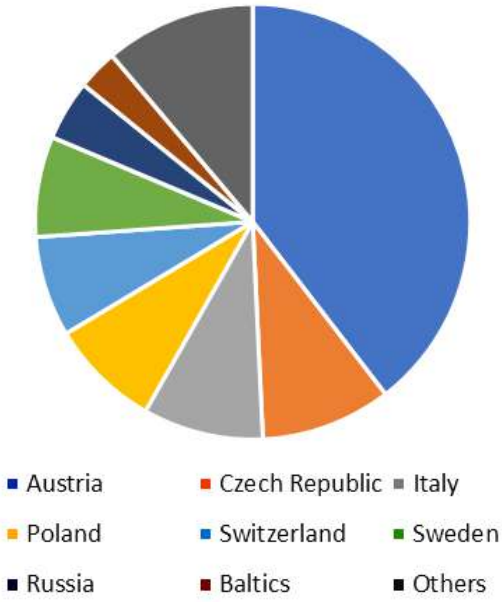
Figure 3: Percentage of travel-associated TBE cases in German patients



Compilation of data from the Epidemiologisches Jahrbuch 2007 to 2018, RKI).

Note that the X-axis starts at 91%.

Figure 4: Origin of travel-imported TBE cases in German patients



(according to the Epidemiologische Bulletin 2007 to 2017; RKI)

of visitors to TBE endemic areas in Europe, there is an estimated risk of infection of 1:77,000 to 1:200,000 travels in TBE endemic areas.[23] In an Austrian study the risk of TBE infection was calculated at 1:10,000 for a four week stay in Austria.[26] The risk depends on the time of travel (e.g. summer vs. winter), the duration of the travel and the risk activities during traveling.[24] The real number of travel-associated TBE infections is underreported for many different reasons, most importantly lack of awareness and under-diagnosing in non-endemic areas.[25,26]

Travel-related cases in non-endemic countries have been reported during recent years from Israel, the Netherlands, Australia, United States and England. The Australian patient travelled by car from Moscow to Novosibirsk with ample opportunities for exposure in nature although it was unclear whether he was infected by a tick bite or by the alimentary route. He developed a generalized infection with drowsiness, fatigue and lower limb myalgia.[27] Two Germans from Baden-Württemberg, father and son, acquired their TBE infections during a travel rest by drinking goat milk and eating goat cheese in Zwiefalten, southwestern Germany.[13] The infection of the son was diagnosed several days later when back again in London, UK, where he was employed. The infection of the father was only diagnosed in Germany after the diagnosis of the son was available. French physicians reported a number of TBE cases acquired outside of the country, mainly on the other side of the Rhine River in the Black Forest in Germany. Single travel-related TBE cases have also been reported from Austria, Russia, Czech Republic and Sweden.[28]

While recently the first autochthonous TBE cases were reported from the Netherlands,[29] the greater proportion of TBE cases diagnosed in the Netherlands are still imported cases in travellers. TBE infections are imported to the Netherlands mainly from Germany and from Austria.[30,31] In2019 the first autochthonous cases of TBE in the United Kingdom were reported in travelers coming back from the UK to Germany.[36]

These examples show the importance of endemic holiday areas for the importation of TBE into non-endemic areas and the importance of the travel history in patients with encephalitis in order not to miss TBE in patients with CNS-infection.

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Chapter 8

TBE in animals

Martin Pfeffer, Hannah M. Schmuck and Michael Leschnik

Key Points

- TBE can cause clinical symptomatic disease in dogs and horses
- Diagnosis of TBEV infection in animals is similar to diagnosis in humans
- Animals can be used as sentinels for human exposure

Introduction

While tick-borne encephalitis (TBE) is well documented as a public health threat, the veterinary aspects of this zoonotic disease are little noticed. 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 milk 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 TBE 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 (see chapter 7). 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 or the TBEV-harboring tick.

In this chapter, we cover what is known in animal species (dogs and horses) that develop disease with strikingly similar clinical symptoms as humans. Then, we describe the animal species which readily become infected with TBEV, without developing any kind of illness, but which serve as a source of the infection for humans via the alimentary route (domestic ruminants). We then focus on other animal species that could be used either as sentinels for natural TBE foci: primarily game animals (such as cervids and wild boar), which provide easy access to sampling; or which are known to be reservoir hosts to the virus (small mammals). In particular, it is the population and infection dynamics of the latter that are suspected to be the main drivers of TBEV prevalence in ticks and, consequently, of human TBE incidence.

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] As 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 ixodid ticks; 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]

Course of disease

Despite frequent TBE infection in dogs, most dogs do not develop any clinical signs. 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 in dogs. 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 (Fig. 1).[10,16,17]

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

Table 1: Serosurveillance studies for TBEV and TBEV antibodies in dogs

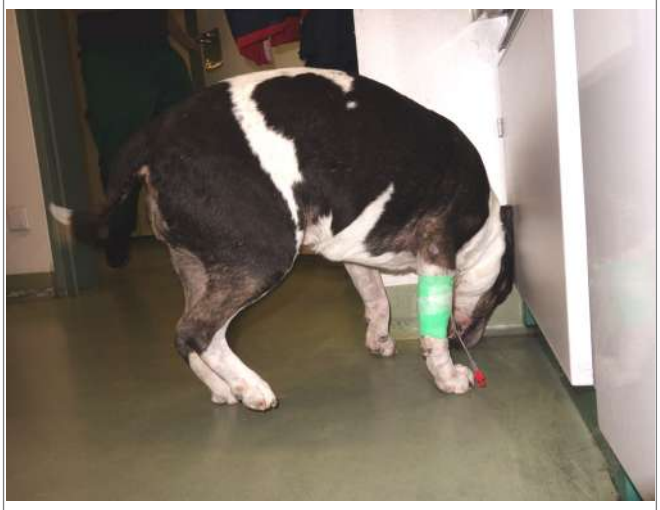
Year	Country	Number of dogs	Clinical signs	Virus detection	Reference	Results
1988–1991	Sweden	255	not observed	n.d.	104	18 seropositive
1993–1998	Germany	~ 1000 dogs	not observed	n.d.	105	2%–31% ELISA seropositive
1994–1995	Japan	10 sentinel dogs each year	not observed	3 virus isolates	106	high Ab-titers upon seroconversion ELISA & SNT
1997–1998	Czech Republic	151 dogs	in 3 dogs	n.d.	10	HIT
1999	Austria	552 dogs	in 57 seropositive dogs	n.d.	14	133 seropositive (24.1%, ELISA), 110 confirmed by SNT (19.9%)
1998–2003	Norway	317 dogs	not observed	n.d.	12	52 seropositive (16.4%) 2 different ELISA
2002	Germany	54 healthy and 56 ill dogs	in 56 dogs, not further specified	n.d.	7	17/54 positive 30/56 positive
2005–2006	Denmark	125 dogs	not observed	n.d.	9	30% ELISA, 4.8% SNT
2009	Belgium	960 dogs	not observed	n.d.	8	0.1% positive (ELISA, HIT & SNT)
2011	Austria	90 dogs	not observed	n.d.	3	repeated testing within one year: 9.8%–13.4% seropositive (ELISA)
2011–2012	Czech Republic	159 dogs	in 7/20 viremic dogs	by PCR	11	11.3% seropositive dogs, viremic dogs 12.6% (ELISA)
2011–2012	Finland	148 dogs	not observed	n.d.	13	6%–40% seropositive dogs (2 ITF ELISA)
2012–2014	Germany	331 healthy dogs	not observed	n.d.	6	2.1% seropositive dogs (ELISA & SNT)
2013—2015	Spain	815 healthy dogs	Not observed	n.d.	114	1.7% seropositive dogs (ELISA & SNT)

n.d. = not determined, SNT = serum neutralization test, Ab = antibodies, HIT = hemagglutination inhibition test, IFT = immunofluorescence test

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.

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 (Fig. 2).[10,16-19]

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.[17,19]

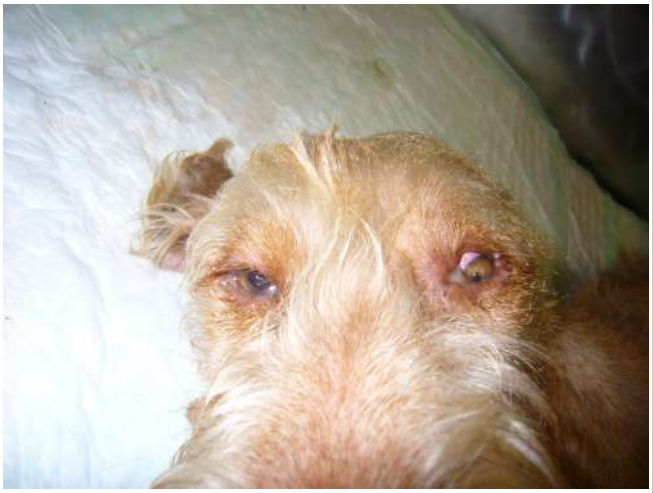
Neurological symptoms like paresis (8–38%), vocalization due to painful perception of active and passive back movement (21–66%), and deficits of the cranial nerves (16–50%) (Fig. 3) develop within a few hours thereafter.[17,19,20]

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.[21,22] 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, Fig. 4), and facial palsy, are observed.

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.

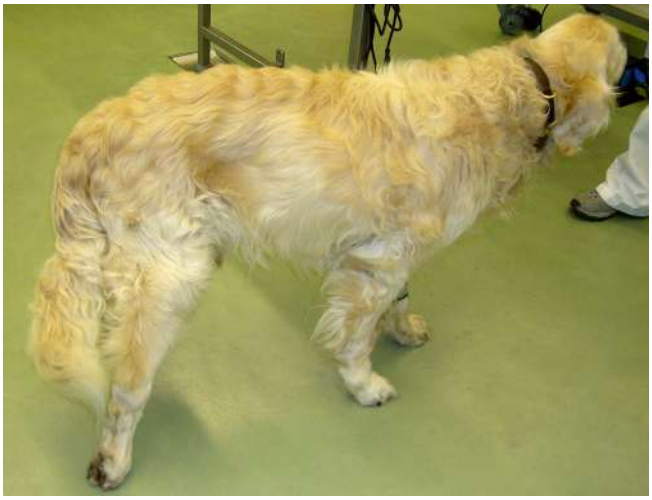
Brainstem symptoms like arhythmical breathing pattern may be present in comatose dogs, especially in severe cases with guarded prognosis (see Video — https://id-ea.org/tbe/wp-content/uploads/2017/08/VIDEO_TBE_breathing-dog.mp4)

Video: Comatose dog of Figure 3 with arhythmical breathing indicative of brain stem lesion

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 (Fig. 5).[10,17,19,20]

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.[14,20,21] (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 been supposed very unlikely to detect the virus in the blood or in the cerebrospinal fluid (CSF). In 1 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 incubation period, as reported in humans, remained unclear. Virus detection in the CSF has been achieved only in isolated cases within the first 3 days of disease.[19] 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.[17]

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.[7,17,18,20] Cross-reactivity to Louping ill virus, West Nile virus, and Usutu virus should be taken into consideration in endemic areas.[10,23] 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.

Table 2: Case reports and case series of TBE in dogs

Year	Country	Dog breed	Clinical symptoms	Reference	Antigen detection	Antibody response	Confirmation	Outcome
1960	Sweden	1 dog	n.d.	107	n.d.	yes	Antibody response	
1970	Switzerland	1 Landseer	behavioral changes, fever, tremor, paresis, seizures	108	yes	n.d.	virus isolation	died
1993	Switzerland	2 Rottweiler, 1 Greyhound, 1 Husky, 1 Golden Retriever	ataxie, tetraparesis, fever, grand mal seizures	20	n.d.	IgM in CSF in 2 dogs	IHC	all 5 dogs were euthanized
1994 -1997	Austria	3 Husky, 1 Terrier-Mix, 1 Rottweiler, 1 Irish Setter, 1 Bastard, 1 Pekingese	tremor, ataxia, hyperesthesia, hem-tetraplegia, pisthotonus, anisocoria, miosis, nystagmus	25	n.d.	n.d.	IHC, pathohistological changes	all 8 dogs died or were euthanized
1998	Germany	1 Rottweiler, 1 Newfoundland dog	fever, hyperesthesia, seizures, opisthotonus, facial nerve paresis, strabismus	109	n.d.	yes (both dogs)	antibody response	one fully recovered, one partially recovered
1996-1998	Germany	2 Rottweiler, 1 Newfoundland dog*, 1 Boxer	ataxia, fever, weakness, tetraparesis, cranial nerve deficits, seizures	18	n.d.	yes	antibody response (SNT in 2 dogs)	2 euthanized, 2 fully recovered
2001	Czech Republic	3 Rottweiler, 1 Fila Brasiliero, 1 Dachshound	behavioral changes, ataxia, tetraplegia, hyperesthesia,	10	n.d.	yes	antibody response	1 asymptomatic, 1 partially recovered, 3 fully recovered
2001-2002	Sweden	1 Riesenschnauzer	behavioural changes, fever, ataxia, tetraplegia	110	n.d.	yes	antibody response	
2006	Sweden	1 dog	ataxia, tremor	111	n.d.	yes	antibody response	
2007	Sweden	2 dogs	fever, ataxia, tremor, hyperesthesia	112	n.d.	Yes	antibody response	full recovery after one year
2008	Austria	8 dogs: including 1 Rottweiler, 2 Husky	ataxia, grand mal seizures, hyperesthesia, fever, compulsive walking, blindness	17	negative from csf in 2 dogs	yes	2 IHC, 6 antibody responses	2 dies, 6 fully recovered
2009	Italy	1 bastard	ataxia, weakness, hyperesthesia	113	n.d.	n.d.	PCR and IHC	euthanized
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	
2012-1014	Switzerland	12 dogs: including 2 Labrador, 1 Rottweiler, 1 Husky, 1 New-foundland dog	behavioral changes, ataxia, seizures, paresis, cranial nerve deficits, hyperesthesia	24	n.d.	yes in 11 dogs	Antibody response, IHC in 5 dogs	6 euthanized, 6 fully recovered

**one dog was also published in a previous paper ; IHC = immunohistochemistry, n.d. = not determined; PCR = polymerase chain reaction; SNT = serum neutralization test*

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.

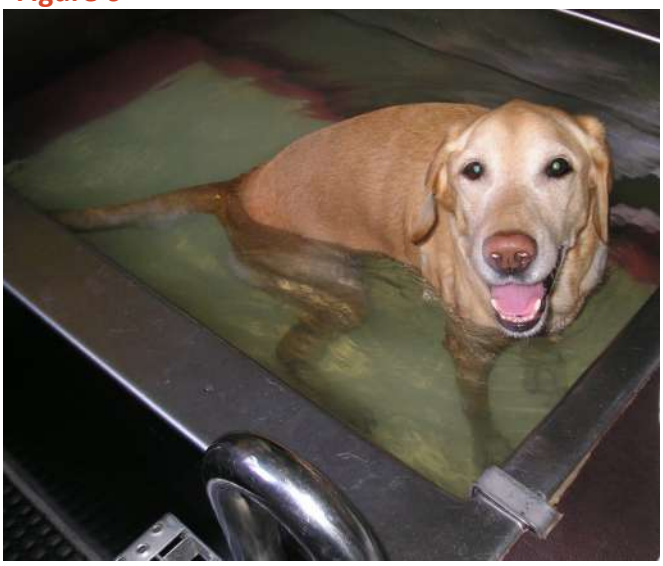
Treatment

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 is necessary in the case of seizures. Steroid use is controversial, as immune-suppression 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 (Fig. 6) as early as possible has been shown to improve the general outcome and shorten the time of rehabilitation.[19,20]

Prevention

There is no licensed anti-TBE vaccine for dogs, although dogs develop detectable antibody titers after vaccination with a human vaccine.[27] 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]

Figure 6



An old Labrador Retriever during rehabilitation. Water training over months improved muscle strength and coordination.

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.[21]

Horses

Although the first clinical case of laboratory-confirmed TBE in a horse was published more than 35 years ago,[28] our knowledge about the impact of TBEV in horse populations is scarce. To the best of our knowledge, there are 4 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;[29] 1 out of 3 diseased animals from a study in Germany had to be euthanized;[30] and again in Germany, some years later, an infected animal had to be euthanized.[31]

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[29,32] to 2.9% in central Germany,[30] and 5.2% and 23.4% in southern Germany[31,33] to 0 of 40 horses investigated in Hungary[34] or 0 of 2349 horses from the Czech Republic.[35] Cross-reactivity to other flavivirus may influence these results.[35,36] 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.[37,38] However, in 2015, a five-month-old lamb in Bavaria displayed symptoms of a neurological disorder, and after euthanasia, TBEV infection was subsequently diagnosed.[39] 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.[40] 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.[41] In the canton of Ticino, with no history of TBE, SNT-positive goats were found in 10 out 37 flocks (14.6% out of 662 sera).[42]

Even if the viremia is shorter than 1 week, the 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.[43] 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.[44] However, alimentary TBEV infection with clinical TBE occurred recently in Germany as a result of consumption of fresh raw goat milk.[45] 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%).[46] 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.[47] 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.[23,27,48] Exposure to TBEV seems not to result uniformly in seroconversion of the entire flock of animals.[49,50] 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.[51]

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.[52] 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.[53] However, clinical or pathological signs that raise suspicions of an overt TBEV infection have never been described for roe deer. 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.[54-61] 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.[59]

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.[62] As Belgium is considered to be traditionally free of autochthonous TBE,[2,63,64] 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.[65] 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.[66]

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.[67] 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.[68] One single case report describes the pathological and immunohistological findings in a mouflon (*Ovis ammon musimon*) with marked encephalitis due to TBEV.[69] 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.[70] 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.[71] 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 sero-prevalence or a single sero-reactive serum sample only.[72,73] However, the latter report found every third fox in South-Western Germany to have antibodies against TBEV.[73] 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.[74] 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.[73,75]

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. 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.[69] 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.[77] An Italian study found roe deer density to have a better predictive value for a model explaining the increasing human TBE incidence than red deer density.[78]

Small mammals

Small mammals have an essential role in the maintenance of TBE foci in 2 ways. Firstly, rodents and, to a lesser extent, shrews are the main hosts for Ixodes larvae. Without this first blood meal, a tick population would die out over time. They are also 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 re-infections of ticks via transovarial transmission, i.e., the transfer of TBEV from a female tick to her eggs, although this is negligible for the epidemiology of the virus. 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.[79] 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.[80]

Recent publications have reviewed the prevalence of either viral ribonucleic acid (RNA) or specific antibodies against TBEV in rodents in various countries.[81-83] 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),[84] 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%.[85] Studies from Hungary identified TBEV-RNA in 4.2%[86] and TBEV-specific anti-bodies in 5.19% and 4.93% of the tested small rodents.[87] Recently, TBEV-positive bank voles (and ticks) were found in a forest within the city borders of Moscow, Russia.[88] Experimentally infected common voles (*Microtus arvalis*) harbored infectious TBEV for at least 3 months.[85] Viral RNA could be found in the brain tissue of experimentally infected bank voles for up to 168 days.[89] 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.[90] Seroprevalence in Microtus rodents were found to be 4% in Poland.[91] 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.[92,93] Other individuals of this monkey group sero-converted without showing clinical signs.[94] 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.[95] 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.[96] Subsequent studies from Estonia (0.4% positive nymphs[97]), Switzerland (0.27% TBE viral RNA positive[98]), Latvia (14%[99]), Germany (no TBE virus found in almost 2500 *Ixodes ricinus* ticks collected from birds[84]) and Slovakia[100] (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 detectable virus or even moderate viremia after infection.[101] 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.[102]

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 10: 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 mice (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.[23,27,103]

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. 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, while birds seem to play a role in long-distance transportation of TBEV-infected ticks and thus the geographic spread, small mammals, in particular rodents, are the key players in maintaining a TBE focus in nature.

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Chapter 9

Immunology of TBEV infection

Sara Gredmark-Russ and Renata Varnaite

Key Points

- Tick-borne encephalitis (TBE) is a viral infectious disease of the central nervous system caused by the tick-borne encephalitis virus (TBEV).
- TBE is usually a biphasic disease and in humans the virus can only be detected during the first (unspecific) phase of the disease.
- Pathogenesis of TBE is not well understood, but both direct viral effects and immune-mediated tissue damage of the central nervous system may contribute to the natural course of TBE.
- The effect of TBEV on the innate immune system has mainly been studied *in vitro* and in mouse models.
- Characterization of human immune responses to TBEV is primarily conducted in peripheral blood and cerebrospinal fluid, due to the inaccessibility of brain tissue for sample collection.
- Natural killer (NK) cells and T cells are activated during the second (meningoencephalitic) phase of TBE. The potential involvement of other cell types has not been examined to date.
- Immune cells from peripheral blood, in particular neutrophils, T cells, B cells and NK cells, infiltrate into the cerebrospinal fluid of TBE patients.

Introduction

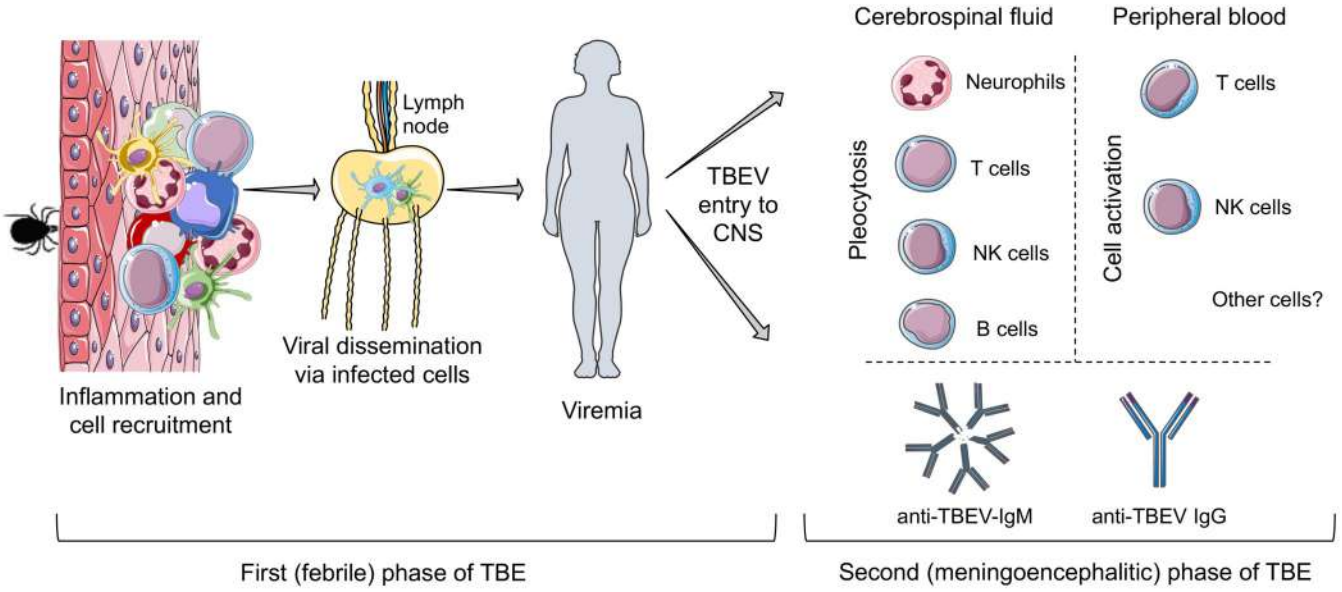
The immune system is a complex network of organs and processes within the host which protects from the invasion of pathogenic microorganisms. This network consists of an enormous variety of cells and molecules with specialized functions and is generally divided into innate and adaptive immunity. The innate immune system provides the first line of defense in infection and acts broadly against various pathogens, whereas the adaptive immune system generates a highly specialized response to individual pathogens. Adaptive immunity is also capable of "striking" harder upon secondary exposure to the same pathogen due to its ability to generate immunological memory. Importantly, the innate and adaptive immune systems function as allies to produce a more efficient total response than either one alone.

Tick-borne encephalitis (TBE) is a viral infectious disease of the central nervous system (CNS) caused by tick-borne encephalitis virus (TBEV). It is usually a biphasic disease manifesting with influenza-like febrile illness during the first (viremic/febrile) phase followed by a second (meningo-encephalitic) phase with neurological symptoms of different severity, ranging from meningitis to severe meningoencephalitis (as reviewed in[1]). The first phase of TBE is challenging to study in humans, as infected individuals rarely seek medical attention. Therefore, sampling from blood or tissues from humans to study TBE is mostly possible during the second phase of disease. Interestingly, upon the emergence of neurological symptoms, the virus can no longer be detected in peripheral blood and cerebrospinal fluid (CSF).[2] Whether as of this time the virus persists in other locations in the body (e.g. the brain parenchyma) has yet to be investigated.

The pathogenesis of TBE is also not completely understood. It may be attributed to either the direct viral cytolytic effects or the immune cell-mediated tissue damage, or both. TBEV viral proteins and immune cell infiltrates have been detected in neuronal tissues from fatal TBE cases supporting both mechanisms of pathogenesis – at least in such cases.[3,4]

In this chapter of the book, we aim to summarize the current understanding of the immune system responses to TBEV infection (Fig. 1). First, we discuss the initial stages of TBE development including host barriers, viral spread, mechanisms of TBEV entry into the CNS and innate immune responses, most of which are delineated from *in vitro* or mouse models. We later review the adaptive immune system responses to TBEV infection, both humoral and cellular, from studies conducted primarily on human peripheral blood and CSF compartments.

Figure 1: TBE disease progression from TBEV-infected tick bite to the development of the second (meningoencephalitic) phase of TBE.



Upon a tick bite, TBEV is transmitted into the skin where it infects local cells including fibroblasts and antigen presenting cells. This creates an inflammatory environment at the bite site leading to immune cell infiltration. Infected antigen presenting cells are believed to migrate to the draining lymph nodes and contribute to virus dissemination. Virus dissemination leads to viremia (presence of virus in the circulation), however it is not yet known at what point during the disease the virus reaches the central nervous system. Upon the development of the second phase of TBE involving neurological symptoms, patients usually present with immune cell infiltration into the cerebrospinal fluid (CSF), a process referred to as pleocytosis. Cells infiltrating the CNS include neutrophils, T cells, NK cells and B cells. Meanwhile, T cells and NK cells in peripheral blood are activated and respond to virus infection with their effector functions. To date, other cell types activated in peripheral blood in human TBE have not been investigated for activation status and phenotype. Anti-TBEV IgM and IgG antibodies are detected in serum during the second phase of TBE. This figure does not represent the complete mechanism of TBEV spread and TBE development. Figures were adapted from Servier Medical Art (http://smart.servier.com/).

Throughout this chapter, we also highlight the observed correlations between human immune responses and clinical TBE disease outcomes.

TBE disease progression

Barriers and local transmission of TBEV

Skin is one of the first physical barriers of the host that prevents the entry of pathogenic microorganisms. However, it is not purely a physical barrier, it is also equipped with many specialized immune cells, such as macrophages, mast cells, dendritic cell (DC) subsets, T cell subsets, and natural killer T cells ready to respond to any threatening micro-organism.[5] TBEV is mainly transferred to humans through the bite of infected ticks. Thus, the skin is the primary site of viral transmission from the tick's saliva to the host. Virus transmission from the tick is facilitated by ''saliva-activated transmission" factors within the tick's saliva which contains components that interfere with the immune response, including factors that block and modulate inflame-mation, haemostasis, innate and acquired immunity, and wound healing.[6]

Already within the first hour of feeding, a stronger inflammatory microenvironment with increased cell recruitment is created at the TBEV-infected tick feeding site, as compared to uninfected tick feeding sites.[7]

After TBEV is transmitted, the cells residing in the skin tissue are exposed to the virus. Tick-feeding experiments in mice show that dendritic cells (DCs), mononuclear phagocytes and fibroblasts are the main cells to be infected by TBEV in the skin.[7,8] Tick saliva further modulates TBEV infection of DCs by increasing their susceptibility to the virus and decreasing their ability to release inflammatory cytokines.[9] Mononuclear phagocytes and DCs are believed to be involved in viral dissemination of TBEV, as these cells can migrate from the skin to the draining lymph nodes.

Viral dissemination and entry into the CNS

Systemic virus infection – viremia, is a common cause of febrile flu-like symptoms manifesting due to the immune response to a virus. It is therefore assumed that the first

phase of TBE involving febrile symptoms is the result of immune responses to the systemic infection with TBEV. During this phase, TBEV RNA can be detected in human blood samples.[10,11] As soon as anti-TBEV antibodies are detectable in the blood, viral RNA can usually no longer be found in blood or CSF samples.[10,11] TBEV RNA has been detected in urine samples during the second (meningo-encephalitic) phase of the disease, and persistent viremia has been described in immunosuppressed patients.[12,13]

The exact route of TBEV entry into the CNS is unknown. TBEV antigen is found in brain tissue in autopsies from fatal cases of TBE, and the virus is selectively localized in the neurons.[3] As for other, more well-studied neurotropic flaviviruses in this context, e.g. West-Nile virus (WNV) and Japanese Encephalitis virus (JEV), different ways of viral entry have been suggested, that may be dependent on blood-brain barrier (BBB) breakdown, passive diffusion of virus, or via infected-leukocytic trafficking.[14] An additional mechanism that has been suggested is transneural invasion of virus into the CNS, via either peripheral somatic or the olfactory nerves.[14] TBEV can infect various cells from the central nervous system *in vitro*, including brain microvascular endothelial cells in a BBB model.[15-18] However, BBB breakdown does not seem to be a prerequisite for TBEV entry into the brain. *In vitro* studies show that the virus can cross the BBB via a transcellular pathway without altering the BBB integrity.[18] Additionally, in a rodent TBE model BBB breakdown is primarily a result of cytokine release by the infected cells and BBB breakdown is not required for TBEV entry into the brain.[19]

Innate immune system and TBE

The innate immune system

The primary function of the innate immune system of the host is to prevent the entry of and colonization by pathogenic microorganisms, and if entry occurs, to limit the infection. All cells in the body, though to a varying extent, are “trained” to recognize and respond if such penetration occurs. The innate immune cell recognition of pathogens takes advantage of pattern recognition receptors (PRRs). PRRs detect pathogen-associated molecular patterns (PAMPs) to initiate protective immune responses and subsequent elimination of the “invaders”.[20] Different classes of PRRs are involved in detection of viral infections, such as Toll-like Receptors (TLRs), cytoplasmic protein retinoic acid–inducible gene 1 (RIG-I) and structurally related melanoma differentiation-associated gene 5 (MDA5).[21] Via different signalling pathways these molecules induce antiviral responses upon sensing viral PAMPs from different cellular compartments.[21] Endosomal TLRs, for example, including TLR3, TLR7, TLR8 and TLR9, recognize viral nucleic acids, including double-stranded RNA, single-stranded RNA, and DNA.[22] Upon ligand recognition, TLRs trigger the production of type I interferons (IFN) and inflammatory cytokines/chemokines to activate antiviral defense mechanisms and to initiate adaptive immune responses.[22]

Type I IFN can be produced by most cell types and its receptors are widely distributed on the cell surface.[23] Binding of IFNs produced by infected cells to the IFN receptor complex on the surrounding cells results in the expression of interferon-stimulated genes (ISG). ISGs have been shown to modulate/inhibit viral replication by inducing an antiviral state.[24] In addition to interferons, cytokines and chemokines are also very important secreted factors of the immune response to pathogens.[25] They orchestrate many processes during infection by controlling immune cell trafficking and determining the nature of the downstream immune responses. Important cells of the innate immune system are dendritic cells (DCs), phagocytes (neutrophils, monocytes and macrophages), cells releasing inflammatory mediators (basophils, mast cells and eosinophils) and the NK cells.

As the innate immune system is activated during the early stages of infection, it is difficult to study its role in TBEV-infection in humans. TBE patients are usually admitted to hospital very late during the infection, already after the adaptive immune system responses are initiated. Therefore, the majority of research on TBEV and the innate immunity are performed in mouse and *in vitro* models, also taking advantage of the natural attenuated Langat virus that belongs to the TBEV serocomplex.

Pattern recognition receptor signalling and type I interferon response to TBEV

As for other viral infections, animal models and *in vitro* experiments demonstrate that type I IFN has a protective role against TBEV-infection. IFN-receptor-deficient mice infected with TBEV or Langat virus develop severe clinical symptoms and succumb to the infection, most likely due to unrestrained systemic viremia, and local inflammation induced by viral replication in the brain.[26] Further experiments have suggested that interferon-beta promoter stimulator 1 (IPS-1), a downstream adaptor for MDA5 and RIG-1-like receptor signalling is important in controlling TBEV and Langat virus infection in mice.[27] Knockout of IPS-1 leads to increased viral replication, release of inflammatory cytokines and immune cell infiltration in the CNS of Langat infected mice.

In vitro experiments demonstrate that astrocytes initiate a very early type I IFN antiviral response upon TBEV-infection thereby limiting viral replication and spread.[28] RIG-1-like

receptors are upregulated together with various ISGs in human neuronal derived cell lines by TBEV-infection.[29] A number of ISGs have been shown to specifically target TBEV -infection. The Tripartite motif (TRIM) 79α protein restricts TBEV and Langat virus replication by mediating lysosome-dependent degradation of the NS5 protein.[30] TRIM79α was also important for eliciting antiviral activity of IFNβ for inhibition of TBEV replication. Viperin (virus-inhibitory protein, endoplasmatic-associated, interferon inducible) has also been shown to restrict TBEV replication by proteasome -dependent degradation of NS3 viral protein, and reduced the stability of other TBEV proteins (prM, E, NS2A and NS2B) in the presence of NS3.[31] Studies of polymorphisms in innate immune genes support the importance of innate immunity in TBE, as 5 different single nucleotide polymorphisms (SNPs) in the interferon-induced antiviral proteins oligoadenylate synthetase 2 (OAS2) and 3 (OAS3) have been suggested to be associated with clinical TBE infection.[32]

Even though TLR signalling has been studied to some extent in other flavivirus infections[33], the role of TLR in TBEV infection is not clear. A functional TLR3 receptor was suggested to be a risk factor for clinical TBE infection in adults, but not in children.[34-36] TLR7 signalling has also been shown to have a role in controlling the replication of Langat virus as TLR7-deficiency in mice increases the virus burden in the CNS. However, increased viral burden does not seem to influence the level of neuropathogenesis.[37] The mechanism for the increased viral replication in the neurons of these TLR7-deficient mice is not clear. However, lower levels of pro-inflammatory cytokines and chemokines is observed.

Innate response and its antagonism by TBEV

Many viruses induce activation of PRR and subsequent IFN signalling within hours of viral infection. Similarly to other viruses, TBEV may also have many mechanisms to interfere with or evade the innate immunity. TBEV as a single-stranded RNA (ssRNA) virus produces double-stranded RNA (dsRNA) intermediates during replication. One of the earliest immune evasion strategies by TBEV is to hide its dsRNA from the cytoplasmic PRRs within the host cells by rearranging internal cell membranes.[38] Inaccessibility of dsRNA for cytoplasmic PRRs delays the activation of interferon regulatory factor 3 (IRF-3), a key transcriptional regulator of type I IFN response. This results in a subsequent 24h delay of IFN production giving an opportunity for TBEV to replicate unhindered.[38]

Effective and early IFN responses are critical during viral infection, thus active antagonism of host proteins involved in IFN responses is another common viral mechanism of evasion. Viruses often use their own proteins to directly interact with and inhibit IFN signalling molecules. Studies on Langat virus have shown that viral nonstructural protein 5 (NS5) is an IFN antagonist and inhibits the JAK-STAT signal transduction pathway by blocking the phosphorylation of STAT1, STAT2, Tyk2 and Jak1.[39] Similarly, TBEV NS5 protein was also found to block the phosphorylation of STAT1 by binding to the host membrane protein scribble (hScrib) resulting in inhibition of downstream IFN signalling.[40] Another known target of TBEV NS5 is host protein prolidase (PEPD). Interaction of NS5 with PEPD is associated with decreased surface expression of type I IFN receptor subunit IFNAR1 resulting in reduced ISG expression.[41] These findings highlight an important role of TBEV NS5 protein as a strong antagonist of type I IFN response.

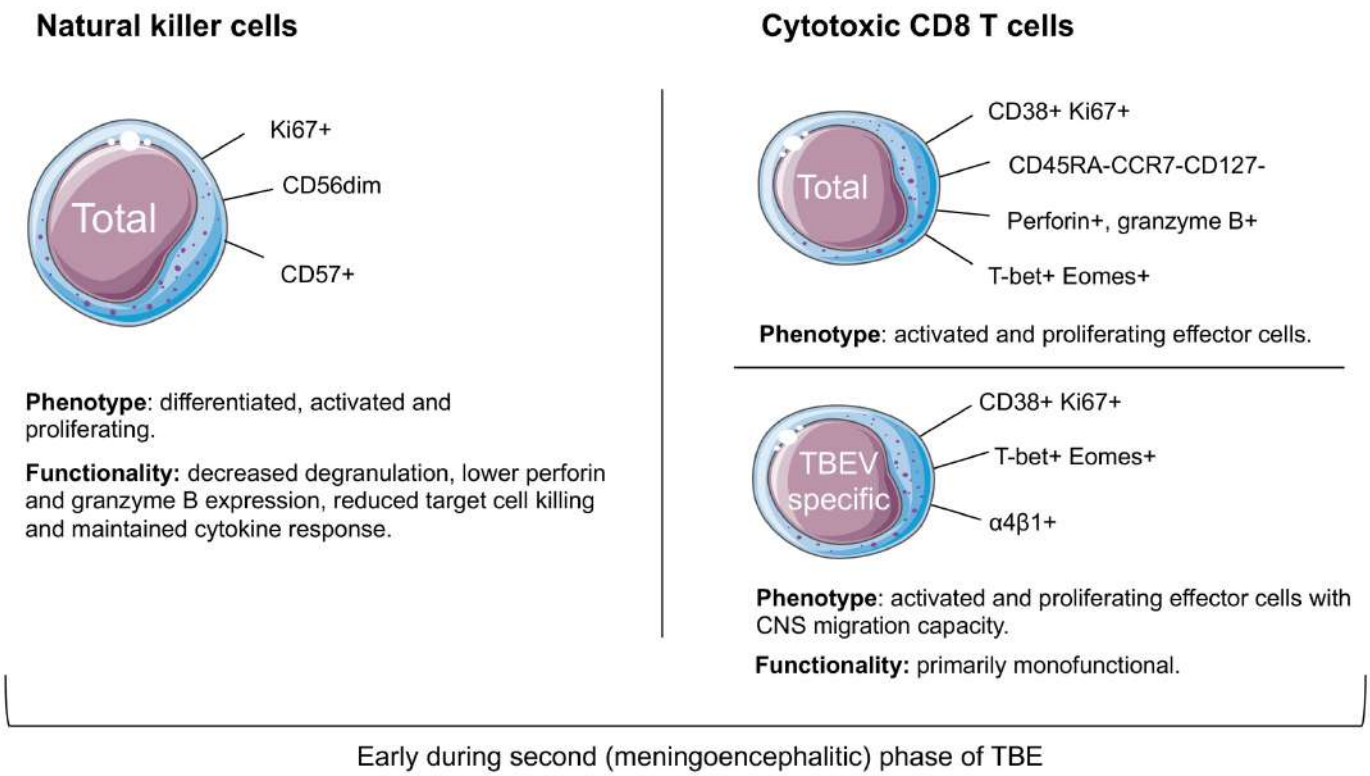
Innate cellular responses in circulation during TBE

NK cells

Natural killer (NK) cells are cytotoxic innate lymphoid cells that are an important part of the immune response against viruses and tumor cells. NK cells represent a distinct population of lymphocytes that lack CD3 and express CD56. The two main NK cell populations in peripheral blood of healthy individuals are the cytotoxic $CD56^{dim}CD16^{+}$ NK cells, and the less cytotoxic $CD56^{bright}CD16^{-}$ NK cells which produce larger amounts of cytokines upon activation.[42] The ability of NK cells to distinguish between normal and infected cells is partly dependent on the surface MHC class I expression levels. In addition, NK cells express multiple activating and inhibitory receptors, and the state of NK activation or tolerance is dependent on a balance of the engagement of these receptors.[43] NK cell cytotoxicity is mediated via three main pathways: 1) cell lysis of infected cells using perforin- and granzyme, 2) Fas ligand–mediated induction of apoptosis, 3) antibody-dependent cellular cytotoxicity, NK cells also produce cytokines and chemokines for communication with surrounding cells, thereby also bridging the innate and adaptive immune response.[44]

In patients suffering from TBE, NK cells are present in both peripheral blood and the CSF with higher percentages of NK cells residing in the blood.[45] Even though the virus is not detected during the second phase of TBE, increased levels of cytokines that either activate or are produced by NK cells are detected in blood.[46] In addition, NK cells are shown to be activated at early time points during the second phase of TBE (Fig. 2). The TBEV-induced NK cell activation was predominantly seen in more differentiated NK cells ($CD57^{+}CD56^{dim}$). The activated NK cells had less expression of perforin, granzyme B, and Bcl-2, suggesting that the cells

Figure 2. Cellular responses to human TBEV-infection in the peripheral blood compartment during the second phase of TBE.



Natural killer cells and CD8 T cell phenotype and activation status in the peripheral blood of recently hospitalised TBE patients during second (meningoencephalitic) phase are presented. Figure compiled from studies.[46,60,61]
Figures were adapted from Servier Medical Art (http://smart.servier.com/).

have already responded to target cells. In addition, $CD56^{dim}$ NK cells had a decreased responsiveness to target cells *ex vivo*, but recovered their functional capacity during the convalescent phase of TBE. In contrast to the decreased response to target cells, the NK cells could respond to cytokine stimulation *ex vivo* throughout the infection. Interestingly, the characteristics of NK cell responses in TBE infection are different from those of other human viral infections. The release of cytotoxic granules early in NK cell activation may contribute to the pathogenesis in TBEV-infection.

Neutrophils

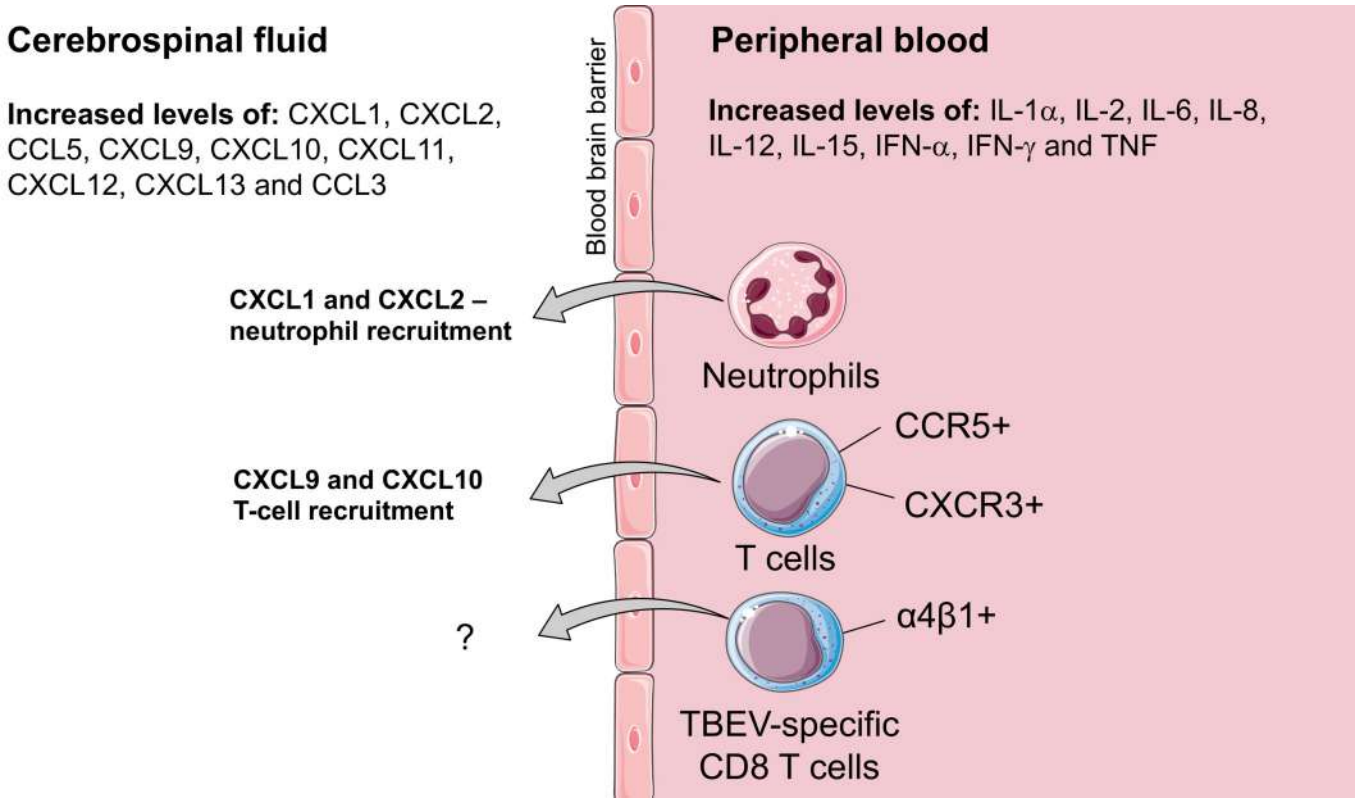
Neutrophils contribute to the inflammatory response and have phagocytic activity early during innate immune responses to viral infections. They are attracted to the bite site during tick feeding experiments and can also be infected by TBEV.[8] Neutrophils are also present at high levels in human CSF early after TBE onset, slowly decreasing over time.[47] However, despite decreasing numbers, neutrophil counts in CSF are higher during the convalescent phase in patients with persistent neurological symptoms.[48] In TBE patients concentrations of chemokines signalling through CXCR1 and CXCR2 receptors are upregulated in the CSF suggesting a potential mechanism for neutrophil infiltration.[48]

In a mouse model using Langat virus, neutrophils have been suggested to mediate brain injury. Increased levels of neutrophils in the CNS were observed during late infection time points in CCR5 deficient mice, together with high levels of neutrophil attracting chemokines CXCL1 and CXCL2, higher viral load and increased apoptosis in the brain tissue. Depletion of neutrophils reversed this phenotype (Fig. 3).[49]

Adaptive immune system and TBE

The adaptive immune system recognizes and selectively eliminates specific foreign microorganisms and toxic

Figure 3. **Expression of chemokines, cytokines and other signalling molecules in the cerebrospinal fluid (CSF) and peripheral blood, as well as their role in immune cells recruitment to the central nervous system in TBE.**



Increased levels of many signalling molecules are observed in CSF and blood, however, information on their exact role in TBE is not well understood. Increased levels of CXCL1 and CXCL2 in mouse TBEV infection may explain neutrophil infiltration into the CNS. Higher levels of CXCL9 and CXCL10 in CSF compared to blood may be responsible for the selective recruitment of T cells expressing CCR5 and CXCR3. Integrin role in TBE is underexplored, but one study shows that almost all TBEV-specific CD8 T cells in peripheral blood express α4 and β1 integrins suggesting their capacity to infiltrate the central nervous system. However, it has not yet been possible to isolate and characterise the phenotype and function of TBEV-specific T cells in the CSF. Compiled from studies.[46,48,49,61,74,75,80-84]
Figures were adapted from Servier Medical Art (http://smart.servier.com/).

molecules, i.e. antigens. The adaptive immune system displays characteristic attributes including antigen specificity, immunologic memory, self-tolerance and non-self-discrimination. The key cells of adaptive immunity are the T and B lymphocytes, which express antigen-specific receptors on their cell surface. Adaptive immunity can be divided into humoral and cell-mediated.

Antigen presenting cells and antigen presentation during TBE

Dendritic cells (DCs) act as an important bridge between the innate and adaptive immune systems. DCs express many types of PRRs, thus enabling DCs to respond to various pathogens by the recognition of PAMPs.[50] Antigen uptake and the engagement of PRRs induce processes of chemokine receptor switching, upregulation of co-stimulatory molecules and cytokine secretion. DCs become activated and mature after PRR stimulation resulting in their migration from tissues to lymph nodes where they can initiate T cell responses.

In order to specifically recognise a given antigen, naïve antigen-inexperienced T cells require antigen presentation in the form of a peptide by antigen-presenting cells (APC) via Histocompatibility Complex (MHC) molecules on their surface. APCs include DCs, monocytes/macrophages, Langerhans cells and B cells. DCs are the most potent antigen-presenting cells and are professional inducers of T cell responses. MHC-peptide complexes on DCs bind to the T-cell receptor (TCR) providing the first signal of activation. The secondary signal of co-stimulatory molecule engagement is required for full activation of T cells. The CD28 is a major co-stimulatory molecule on T cells, and it facilitates T cell activation upon binding to CD80 and CD86 on DCs.[51] DCs are also producers of type I IFN that have multiple functions in adaptive immunity, such as T cell proliferation, CD8 T cell activation, B cell isotype switching and differentiation into plasma cells.[50]

Many flaviviruses, including Langat virus, can infect DCs *in vitro*.[52] Infection of DCs results in impaired DC maturation and subsequently decreased T cell priming/proliferation. However, when mouse dendritic cells were infected with 2 different strains of TBEV, DC maturation was instead induced, as measured by co-stimulatory molecule and MHC class II upregulation on the cell surface.[53] Tick-feeding experiments on mice also showed that DCs and monocytes are locally infected by TBEV at the bite site, therefore potentially contributing to subsequent viral spread and the initiation of the adaptive immune responses.[8]

B lymphocytes and antibody responses during TBE

B cells carry a large variety of immunoglobulin (Ig) surface receptors that can directly recognize antigens and are responsible for specific antibody production. Naive B cells are activated after primary antigen encounter and initially produce antigen-specific IgM, and later IgG. Activated B cells later differentiate into plasma and memory B cells. Plasma cells are responsible for antibody secretion during the immune response, whereas memory B cells are responsible for the recall responses during repeated infection with the same pathogen.[54]

Many viral infections and vaccines give rise to long-lasting protective immunity consisting of pathogen-specific antibodies and memory B cells. TBEV infection also elicits an efficient B cell response. During the first (viremic) phase of TBE, anti-TBEV antibodies are generally not detected.[11] However, anti-TBEV IgM- and IgG-antibodies appear in serum during the second phase of the disease.[2] During the second phase of TBE, virus is rarely present in human serum, therefore detection of viral RNA using PCR is not optimal for TBE diagnosis. For this reason, diagnosis of TBE is primarily based on serology, i.e. presence of TBEV-specific IgM and IgG- antibodies in serum and CSF of patients.[2]

A number of studies have attempted to correlate humoral responses to TBE infection with clinical outcome. High anti-TBEV IgM antibody levels were detected early during TBE, decreasing over time in both serum and CSF, whereas IgG antibodies were detected later than IgM, peaked in the six-week convalescent samples and persisted for more than a year.[55] The persistence of serum and CSF antibodies did not correlate to the disease severity, but patients with low levels of IgM antibodies in CSF during the early second phase of TBE, and patients with low TBEV-neutralizing antibody levels in serum suffered from a more severe disease.[55,56] In addition, a more recent study found a higher concentration of anti-TBEV IgG antibodies in serum of patients with a milder disease as compared to those with a severe TBE.[57] These studies may support a link between humoral immunity and TBE clinical outcome.

Mouse studies provide additional data that suggest that B cells contribute to the outcome of TBEV infection. Increase of CD19 mRNA levels in brain tissue of infected mice coincides with high levels of TBEV-neutralizing antibodies.[58] These mice are also less susceptible to TBEV than mice producing low levels of neutralizing antibodies.[58] However, the mice more susceptible to TBEV also exhibited strong cytokine/chemokine mRNA production in the brain, suggesting that other immunopathological mechanisms are involved in the disease outcome.

Even though the antibody response during TBE has been studied to some extent, the cellular aspects of B cell response, including phenotype and activation status, as well as the overall B cell role in TBE pathogenesis remain to be investigated.

T lymphocyte responses during TBE

T cells are characterized by the expression of the cell surface marker CD3, which forms a complex with the T cell specific receptors. Conventionally, the T cells are divided into two groups with different immune functions: T helper (CD4) and cytotoxic (CD8) cells, based on their surface expression of either CD4 or CD8 markers. Activated CD4 T cells secrete various cytokines that orchestrate the immune response by activating B cells, CD8 T cells, macrophages and other cells of the immune system. CD4 cells are restricted to recognizing peptides presented on MHC class II molecules on APCs, whereas the CD8 cells recognize peptides presented on MHC class I molecules. CD8 T cells have a cytolytic ability to kill infected host cells. The killing is mediated by the release of cytolytic proteins like perforin and granzyme. Most CD8 T cells are also efficient cytokine producers.

Adequate T cell responses, both CD4 and CD8, are important during viral infections. The effector CD8 T cells contribute to the clearance of the infection and provide long-lasting immunity. CD4 T cells have a central “helper” role to assist and activate B cells and CD8 T cells. After the naïve T cells encounter an antigen they differentiate into effector T cells, with the majority of the effector cells dying off after clearance of the infection, with only a small pool of cells remaining as memory cells.[59] Memory cells can respond rapidly upon re-exposure to the same infectious agent.[59] Different subsets of memory cells can be defined based on their phenotypic markers, with the central memory cells homing to secondary lymphoid organs and effector memory cells mostly found in non-lymphoid organs.[59]

T cell activation and phenotype during TBE

T cell activation and phenotype was investigated longitudinally in TBE patients during the second phase of the disease, from the time of hospitalization up to the convalescent period. Peripheral blood T cells were found to be activated (as determined by Ki67 and CD38 co-expression), with the activation peaking at one week after hospitalization (Fig. 2).[60] In contrast to CD8 T cells, CD4 T cells showed only low levels of activation at this time of infection.[60] Activated CD8 T cells had increased expression of perforin and granzyme B and passed through an effector phase prior to differentiation into memory cells. TBEV-specific CD8 T cells were further shown to be mainly monofunctional in response to TBEV-peptide stimulation early after hospitalization, but became more polyfunctional in the convalescent phase.[60] Additionally, TBEV-specific CD8 T cells express higher levels of α4- and β1-integrins than the bulk CD8 T cells, which may indicate their ability to migrate into the CNS.[61]

These data indicate that the primary CD8 T cell response to TBEV infection occurs during the second phase of TBE, as the peak of activation of CD8 T cells along with the occurrence of TBEV-specific CD8 T cells take place at about one week into the second phase of TBE.[60,61] In a yellow fever vaccine-based infectious model the peak of CD8 T cell response was observed at day 15 after immunization.[62] This may suggest a slight delay of CD8 T cell activation during TBEV infection as compared to the yellow fever vaccine-based infectious model. However, without access to patient samples during the first phase of TBE it is difficult to explain the exact kinetics of T cell responses.

Role of T cells in TBE pathogenesis

Even though T cells participate in the immune response to TBEV, the role of these cells in the outcome of TBE is not clear. One study suggests that T cell infiltration into the CNS during TBEV infection might contribute to a favourable disease outcome.[49] *In vivo* studies of CCR5-deficient mice infected with Langat virus, show a delayed influx of CD4 and CD8 T cells into the CNS, increased viral replication and decreased survival of these mice, suggesting a protective role of T cells.[49] Other conflicting studies suggest immunopathological rather than protective role of CD8 T cells in TBE. Brain tissue biopsies from fatal TBE cases show cytotoxic T cell infiltration in close proximity of TBEV-infected neurons.[4] In addition, CD8 deficient mice have a prolonged survival as compared to immunocompetent mice during TBEV infection, and this effect is independent of viral load in the periphery or the brain.[63] This immunopathology is primarily mediated by CD8 T cells and not CD4 T cells, as shown by shorter time of survival of immunodeficient SCID mice receiving CD8 T cells, whereas adoptive transfer of CD4 T cells increases the survival time.[63] Yet, other mouse studies that compared mice challenged with TBEV that died or recovered, did not detect any differences in T cells numbers in the brains of the two groups, even if the cell numbers were increased in both groups as compared to uninfected mice.[64] Interestingly, T cell receptor antigen specificity might determine the severity of TBEV-infection in mice, as T cell clones that express certain TCRs accumulate in the brains of mice dying from TBEV.[65] These conflicting studies highlight the need for further research to understand the role of T cells in the context of TBE pathogenesis.

T cell antigen specificity in TBEV infection and vaccination

Antigen-specific T cells can be detected by artificially generated and fluorescently labelled peptide-MHC complexes.[66,67] Antigen-specific T cells recognize the peptides presented on these complexes and bind to them. This binding can then be detected using flow cytometry. This is an invaluable tool to study virus-specific cells in patients including TBEV-infection.

In total, seven TBEV-specific peptides have been identified, and all of them are located in nonstructural (NS) proteins of the virus.[60,61] The majority of previously identified CD8 T cell viral peptides in other flaviviral infections, such as YFV and DENV, are also derived from NS proteins.[68-70]

Immunodominant regions of viral proteins can be determined by stimulating cells with peptides based on the full viral protein sequences. Antigen specific cells upon binding to such peptides might initiate cytokine release (like IL-2) which can be measured for each peptide. Such studies on CD4 T cells after TBEV infection and vaccination identified immunodominant regions of structural viral proteins.[71,72] Both vaccinated and infected individuals responded to similar regions of TBEV structural proteins, even if the response was higher in the vaccinated cohort. Another study showed that, full recombinant structural TBEV proteins trigger CD4 T cells, but not CD8 T cells in TBE-vaccinated individuals.[73] Therefore, CD4 T cell responses seem to be skewed toward recognition of structural proteins, whereas CD8 T cell responses are skewed toward recognition of NS proteins.

Inflammation during TBE

Inflammation upon acute infections is an important part of the immune response essential for the elimination of pathogens. On the other hand, excessive inflammation may be harmful to the host. Many cells contribute to the inflammatory processes during infections to produce different cytokines, chemokines and growth factors. During TBEV infection there is both a systemic inflammatory response in the peripheral tissues, as well as a localised

inflammation in the central nervous system (Fig. 3). Numerous studies investigated the levels of cytokines and chemokines in serum and CSF of TBE patients, yet there are limited data on the role of inflammation in the pathogenesis of TBE.

Early during the second phase of TBE, significantly increased levels of cytokines, such as IL-1α, IL-2, IL-6, IL-8, IL-12, IL-15, IFN-α, IFN-γ and TNF can be detected in patient serum samples.[46,74,75] The levels of these cytokines decline over time. Increased levels of growth factors, such as hepatocyte growth factor and vascular endothelial growth factor, as well as increased serum levels of matrix metallopeptidase-9 (MMP-9) have also been found in the sera during second phase of TBE.[75,76] Increased levels of MMP-9 highlight the presence of local inflammation within the CNS in TBE patients, as increase of MMP-9 is associated with brain tissue damage.[77] A polymorphism in MMP-9 gene (rs17576 SNP), which affects the function of this protein,[78] was also found to predispose TBE patients with this SNP to develop CNS damage.[79]

Chemokines are a type of cytokines that mediate immune cell recruitment and activation in inflamed tissues. Chemokine gradient also determines the direction for the immune cell movement in and out of tissues.[25] In the context of TBE patients, increased CXCL9 and CXCL10 levels in the CSF were shown to create a chemokine gradient between the CSF and serum, potentially resulting in the recruitment of CXCR3 receptor expressing T cells into the CNS.[80-82] Even if a gradient between the CSF and serum could not be confirmed for CCL5 (RANTES), CXCL11, CXCL12, CXCL13, and CCL3, the concentration of these chemokines is also increased in the CSF of TBE patients.[81,83,84] The level of CCL5 in CSF is correlated with pleocytosis, and activated CD4 T cells in CSF also expressed a high level of CCR5 (receptor for CCL5), further indicating that CCL5 acts as a chemoattractant to recruit cells into the CNS of TBE patients.[84] The neutrophil chemoattractant CXCL1 and CXCL2 has also been shown to be increased in CSF early during TBE.[48]

In addition to chemokines, the levels of other cytokines in the CSF of TBE patients were assessed and correlated with clinical TBE outcome. A significantly increased concentration of IFN-γ, IL-4, IL-6 and IL-8 was found in the CSF of children who developed sequelae after TBE, as compared to the children who did not.[85] In adults, low levels of IL-10 in the CSF later during the second phase of TBE (day 7-18) correlated with a more severe disease.[86]

CNS immune responses during TBE

The mechanisms underlying TBE pathogenesis in the CNS in humans are still largely unknown and under-explored. Relatively low mortality of TBE patients and inaccessibility of brain tissue samples are the main challenges in describing the immune mechanisms taking place in the CNS in humans. Therefore, most of the research on immune cell subsets within the CNS is performed on CSF, a fluid that is separated from peripheral circulation via the BBB and is in direct contact with the brain and spinal cord. Even though cellular constitution of CSF reflects which cell types selectively migrate through the BBB from peripheral blood during CNS infections, it does not necessarily fully represent the composition of the immune cells within infected brain tissue. However, selective migration of certain cell types may contribute to defining the mechanisms for pathogen clearance and immunopathogenesis and may also predict TBE disease outcome.

Pathogenesis in the CNS during TBEV infection may be attributed to direct viral effect and immune-mediated tissue damage, both of which are supported by the detection of TBEV viral proteins and immune cell infiltrates in neuronal tissues from cases of fatal TBE.[3,4] The mechanism for virus passage through the BBB into the brain is not yet defined, as discussed more in detail under the section “Viral dissemination and entry into the CNS”. Neurons are believed to be the primary targets for TBEV in the CNS,[3,87] but other brain cells are also infected *in vitro*.[15-17]

Immune cell infiltration into the CSF, defined as pleocytosis, is a common event during CNS infections. Early during the second (meningoencephalitic) phase of TBE, CSF contains a higher proportion of neutrophils, whereas mononuclear cells steadily increase overtime to become the dominant cell type.[47] Importantly, immune cells such as CD4 and CD8 T cells, NK cells and B cells are also present in the CSF of TBE patients, with T cell frequencies being higher than in blood, indicating selective migration of these cells through the BBB.[45] Most previous studies on CNS infiltration of T cells, however, were performed in human neuroinflammatory diseases and in animal models for autoimmune and viral infections, including HIV.[88-92]

In general, virus-specific effector T cells are recruited to the CNS during infections by chemokines and integrins.[88-90,92] To date, the exact mechanism for the recruitment of T cells (including TBEV-specific T cells) into the CNS during TBE is not clear, however certain chemokines and chemokine receptors were suggested to be involved. For example, infiltrating CCR5 and CXCR3-expressing T cells seem to have a role in TBE in humans (Fig. 3). Chemokine CXCL10 (ligand for CXCR3) and CCL5 (ligand for CCR5) levels in the CSF of TBE patients are increased together with higher CCR5 expression on infiltrated CD4 T cells as compared to blood.[80,81,84] Interestingly, a mutation in CCR5 is associated with a more severe course of the disease.[36,93]

In mouse models, TBEV infection induces CCL5 expression accompanied by increased immune cell infiltration into the

CNS.[94] Blocking of CCL5 reduced cell infiltration and extended the survival of mice after TBEV infection. *In vitro* TBEV infection of human glioblastoma cell lines and primary astrocytes by TBEV demonstrated that increased CCL5 expression is mediated by the viral TBEV protein NS5.[94,95]

An integrin role in T cell CNS recruitment during TBE is discussed in a recent study on TBEV-specific CD8 T cell and their expression of α4-integrin and β1-integrin.[61] Almost all of the TBEV-specific CD8 T cells from peripheral blood express α4-integrin and β1-integrin early during second phase of TBE (1 and 3 weeks), whereas the bulk CD8 T cells expressed lower levels of integrins. Expression of α4β1 is associated with the ability to infiltrate tissues and cross the BBB.[88-91] The same study, however, did not detect higher CXCR3 expression on TBEV-specific CD8 T cells as compared to bulk CD8 T cells. This may be due to the majority of TBEV-specific CD8 T cells residing in the CSF during patient sampling or by the possibility that CXCR3 is not crucial for CD8 T cell migration across the BBB in TBEV-infection. Further investigations on the mechanism for T cell migration into the CNS during TBE are required in order to explain the local CNS pathogenesis of this disease.

Host factors and TBE disease

As for most human infections, the clinical outcome of TBE is extremely variable, ranging from asymptomatic to lethal. A more severe TBE is associated with increased age, severity of symptoms during the first (febrile) phase, low neutralizing antibody titers at onset and low early CSF IgM response (as reviewed in[1]). The risk of developing TBE after exposure to the virus may also vary between individuals. For instance, an epidemiological study in Sweden measured seroprevalence for TBEV in an endemic area and found that only 25% of individuals who were seropositive for TBEV developed clinical TBE, suggesting that only 25% of naturally infected persons may develop disease, while 75% of the infections are non-symptomatic.[96]

Clinical appearance and the progression of TBE may also be related to host genetic factors. Studies on TBE in this context have thus far not been able to correlate susceptibility to TBE or disease severity to one single host genetic factor, but a few candidates have been suggested including *CCR5Δ32 polymorphism,*[93] *a functional* TLR3 receptor,[34-36] 5 different SNPs in the interferon-induced antiviral proteins oligoadenylate synthetase 2 (OAS2) and 3 (OAS3),[32] 2 SNPs in the promoter region of CD209 (encoding dendritic cell-specific intercellular adhesion molecule (ICAM)-3 grabbing non-integrin (DC-SIGN)) expressed on the surface of dendritic cells,[97] and SNPs in interleukin 28B (IL28B) and interleukin 10 (IL10).[98] In a more recent study, the rs17576 SNP in the MMP-9 gene predisposed TBE patients for CNS damage.[79]

Conclusions

TBE is a complex and rather understudied disease in the context of human immune system responses. *In vitro* experiments, animal models, as well as research in humans have greatly contributed to describing TBEV infection and defining the mechanism of TBE disease progression, however, many aspects of it remain to be investigated further.

It is clear that TBEV is a potent inducer of innate immunity, but at the same time the virus is capable of antagonising certain pathways of innate immune responses. Adaptive immune system responses are also initiated during TBE as reflected by anti-TBEV antibody presence in serum, as well as NK and T cell activation in peripheral blood of TBE patients. Local pathogenesis in the central nervous system in TBE may be attributed to both direct viral effects and immune mediated tissue damage, but the exact mechanism is unclear. More research is needed in order to fully understand the development of TBE in order to create effective and specific therapeutic strategies.

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Chapter 10

Diagnosis

Gerhard Dobler

Key Points

- TBE appears with non-characteristic clinical symptoms, which cannot be distinguished from other forms of viral 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 clinic 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 from either 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 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

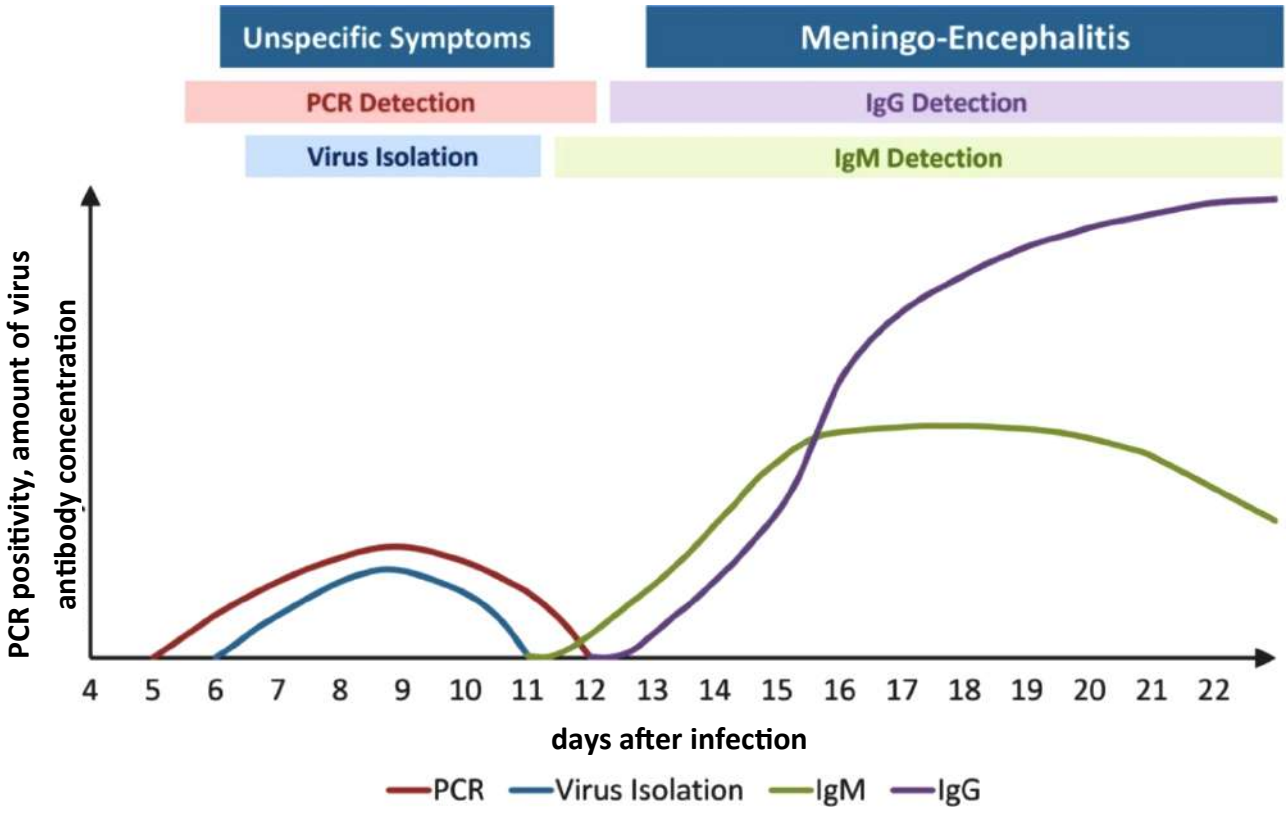

Table 1: Detection of TBEV by RT-PCR in patient samples according to stage of infection[4]

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 (C) 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 amounts of antigen used. In general, ELISAs exhibit high levels of sensitivity but only moderate specificity due to cross-reactivity with antibodies against dengue virus (caused by infections) or yellow fever virus (caused by vaccinations) and other flaviviruses.

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

Figure 2: Schematic diagram of the course of specific anti-TBE antibodies in primary or secondary flavivirus infection

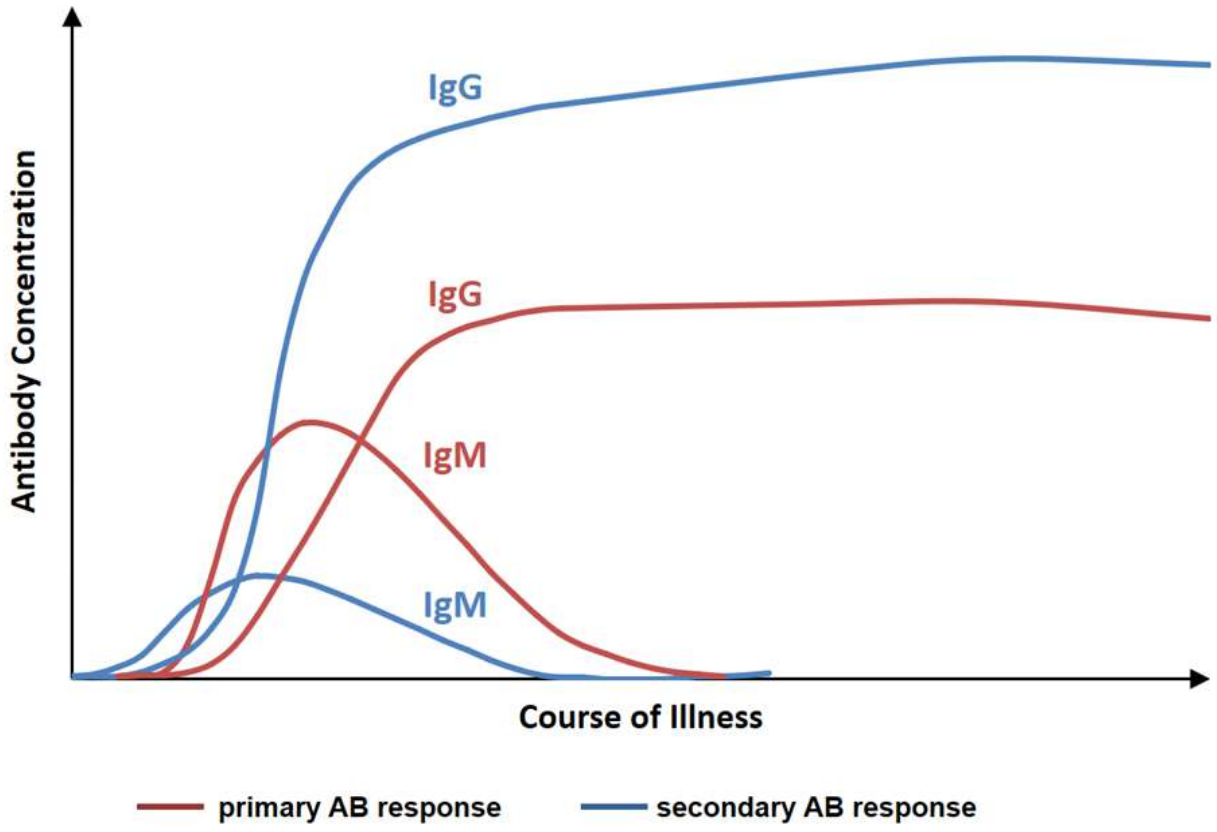

Table 2: Possible serologic constellations, their possible interpretation, and steps necessary for confirmation of TBE infection

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

can confound interpretation of whether an infection is recent or not. In TBEV infections, persistent IgM from a past 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

$$\text{IgG index} = \frac{\dfrac{\text{OD TBE-IgG (CSF)}}{\text{OD TBE-IgG (SER)}}}{\dfrac{\text{Total IgG (CSF)}}{\text{Total IgG (SER)}}} > 2$$

$$\text{IgG index} = \frac{\dfrac{\text{OD TBE-IgG (CSF)}}{\text{OD TBE-IgG (SER)}}}{\dfrac{\text{Albumin (CSF)}}{\text{Albumin (SER)}}} > 2$$

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 besides 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 be 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 cannot be distinguished anymore. 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 wrong diagnosis.

These cross-reactions are also important for defining an 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.

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Chapter 11

General epidemiology of TBE

Gerhard Dobler and Sergey Tkachev

Key Points

- Tick-borne encephalitis virus (TBEV) exists in natural foci, which are areas where TBEV is circulating among its vectors (ticks of different species and genera) and reservoir hosts (usually rodents and small mammals).
- Based on phylogenetic studies, four TBEV subtypes (Far-Eastern, Siberian, European, Baikalian) and two putative subtypes (Himalayan and "178-79" group) are known. Within each subtype, some genetic lineages are described.
- The European subtype (TBEV-EU) (formerly known also as the "Western subtype") of TBEV is prevalent in Europe, but it was also isolated in Western and Eastern Siberia in Russia and South Korea.
- The Far-Eastern subtype (TBEV-FE) was preferably found in the territory of the far-eastern part of Eurasia, but some strains were isolated in other regions of Eurasia.
- The Siberian (TBEV-SIB) subtype is the most common and has been found in almost all TBEV habitat areas.
- The Baikalian subtype is prevalent around Lake Baikal and was isolated several times from ticks and rodents.
- In addition to the four TBEV subtypes, one single isolate of TBEV (178-79) and two genetic sequences (Himalayan) supposed to be new TBEV subtypes were described in Eastern Siberia and China.
- The data on TBEV seroprevalence in humans and animals can serve as an indication for the presence or absence of TBEV in studied area.

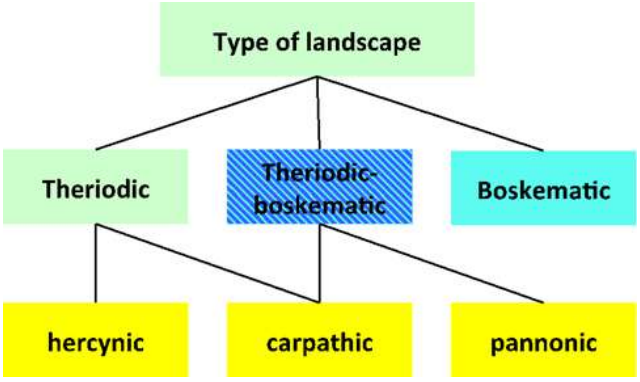
The natural focus

In the early 1920s, reports surfaced concerning a severe form of brain disease in woodcutters, topographers, road construction workers, and residents of newly founded villages in the Taiga forest in the far eastern region of the former Soviet Union. The severity of the disease was such that in 1937 an expedition was organized to detect the origin of this unusual disease. During this first Taiga expedition to identify the etiology of a newly occurring form of encephalitis, Zil'ber et al.[1] showed that the etiologic agent of this disease seemed to be a filterable pathogen that was transmitted by ticks of the genera *Ixodes* and *Dermacentor*. In at least 2 more expeditions to study the transmission of this disease (later named Russian Spring Summer Encephalitis, and currently known as tick-borne encephalitis [TBE]) Pavlovsky recognized that it was associated with specific types of landscape, and from this observation he developed his theory on the nature of human diseases.[2]

In his theory, Pavlovsky describes a natural focus ("Nidus") of a disease as an area where specific climate, vegetation, soil, and favorable microclimatic conditions exist, so that vectors, donors, and recipients of infection find favorable conditions to exist. In this respect a natural focus of disease is related to a specific geographical landscape. According to this theory, humans acquire a zoonosis with natural foci only if they are in the territory of the natural focus in a definite season of the year and if they are attacked as prey by hungry vectors or come into contact with the animal reservoir (via hunting), which have already acquired the infection as carriers or donors of the respective agent.

During the last century a number of scientists, especially from Russia and the Czech Republic, studied in detail the landscapes that are associated with the occurrence of TBE. Rosicky[3] and Blaskovic[4] defined landscape types of TBE natural foci (Fig. 1).

Figure 1: Different landscape types of TBE natural foci (according to Rosicky[3] and Blaskovic et al.[4])



According to this classification, a theriodic focus is a focus in a forest with game animals as the main vertebrate hosts for adult ticks. A boskematic focus is a focus where meadows dominate and where farm animals are the main vertebrate hosts for adult tick stages. The theriodic-boskematic form is a mix of the two, having both types of landscape.

Another classification was made by Blaskovic et al.,[4] who categorized the natural foci according to their main geographic location into Hercynian foci (located mainly in the Central German Uplands), Carpathian foci (located in the far southeastern part of Europe), and Pannonian foci (located at the western part of the Hungarian Danube lowlands). Similarly, Korenberg et al.[5] made a classification according to the main geographic type (and not so much landscape type) for the TBE foci in Eurasia (Fig. 2).

By these classifications, the European TBE foci are located in the Central European–Mediterranean TBE focus region according to Korenberg et al.[5] The classification developed by Rosicky[3] indicates the European TBE foci are mainly of the theriodic type, while Eastern European countries have the mixed type or rarely also the boskematic type. Overall, these classifications may be helpful in getting an impression of the focus type in the landscape, but they are not very helpful for describing a TBE natural focus in detail. Also, so far, no clear associations have been identified between genetic profiles or phenotypic characteristics of TBEV strains and their respective focus types.

The natural cycle

As described above, a natural focus is an area where the ecological conditions allow the presence and transmission of a pathogen. In the case of TBEV, a natural focus is an area where TBEV is circulating among its vectors (ticks of different species and genera) and vertebrates (usually rodents and small mammals, which support the transmission of the TBEV). Details of these transmission cycles and the animal species involved are described in Chapter 3. However, at the moment it is not clear which ecological structures and requirements are needed to establish and maintain a TBE natural focus. A sufficient number of ticks that are infected or might be susceptible to infection must be present. Also, a sufficient number of susceptible small mammals to support virus transmission is required. There must also be an adequate number of larger animals to support the developmental cycle of the nymphs and adult stages of the tick vectors, as these are rarely found on rodents. The virus itself is transmitted via viremic vertebrates or via co-feeding of TBEV-infected ticks together with non-infected ticks, with the latter transmission mechanism being more effective. However, so far, no proof exists as to the actual importance of any of these mechanisms in the field.

A number of models on natural foci of TBEV are now available, but fieldwork is missing. In the early 1960s Austrian researchers were studying TBE foci in Austria.[6] According to the authors' data and estimates, focus size was 60,000 m^2 with an estimated 2 million larvae and about 500,000 nymphs in the focus. They estimated that between 500 and 1500 nymphs (0.1% to 0.3%) are infected at any time in the year and may infect 15 to 30 rodents out of an estimated total number of 700 rodents in the focus. They found a total of 4 small mammal species with a clear dominance of *Apodemus spp*. (*Apodemus flavicollis* > *Apodemus sylvaticus* > *Myodes glareolus* > *Microtus agrestis*). The focus was highly fragmented into old forests, young forests, and meadows that existed within the forests.

Nosek et al.[7] described the structure of TBE natural foci in the Czech Republic. Their work showed that a focus is maintained by a number of so-called microfoci. The size of the natural focus is not given. The authors estimate that per 10,000 m^2 (1 ha) the number of ticks ranges from 15,000 to 50,000 nymphs. A microfocus is defined as a structure in the focus area where virus transmission is continuously active and therefore the virus can be generally detected. The rate of positive ticks in the microfocus is approximately 0.5% to 1% in nymphs and up to 5% in adult ticks.

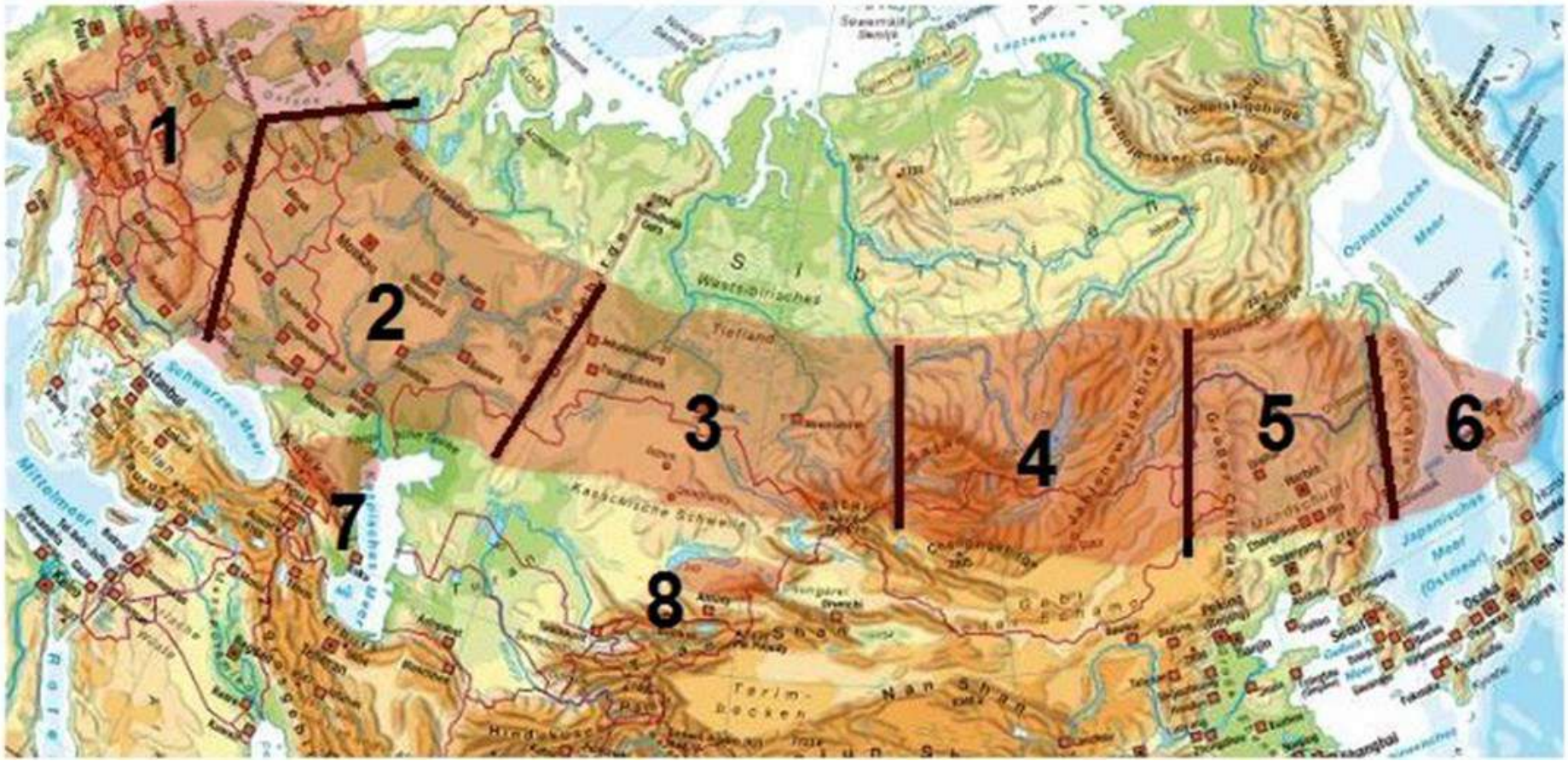
In a recent study over 4 years in a TBE focus in Hungary, the authors reported that an area of 36 ha (3,600,000 m^2) was screened and that only in an area of 0.49 ha (4900 m^2) sero-positive rodents were detected.[8] They found TBEV in a total of 3 tick pools (2 pools of *Ixodes ricinus* and 1 pool of *Haemaphysalis concinna*) out of 7247 sampled ticks (0.05%). Of note, in an area around 170 m away from the focus but in the same natural focus area, no TBEV was detected among 2369 sampled ticks. This description supports our own observation on TBE natural foci in southeastern Germany[9] that a TBE natural focus has a size of about 5000 to 10,000 m^2. The main ecological structure, which can be identified as important in the focus, is the ecotone between forest and meadow. More data must be collected in the field to get a clear picture of the ecological structure that is required for the development and maintenance of a TBE natural focus.

The phylogeny and phylo-geography of TBEV

According to phylogenetic studies at least 3 and possibly 6 subtypes of the TBEV can be genetically distinguished by molecular technologies. At present, 3 subtypes of TBEV—the European (western) subtype (TBEV- EU), the Siberian subtype (TBEV-SIB), and the Far-Eastern subtype (TBEV-FE)—are recognized. Russian virologists have claimed 2 new subtypes, strain 178-19 and strain 886-84, both isolated in the Lake Baikal region in Siberia.[10] Also, a new putative

Figure 2: Eurasian TBE focus regions classified after Korenberg et al.[5]

(1) Central European-Mediterranean; (2) Eastern European; (3) Western Siberian; (4) Central Siberian-Trans-Baikalian; (5) Khingan-Amur; (6) Pacific; (7) Krim-Caucasian; (8) Kazakh-Central Asian



TBEV Himalayan subtype was claimed in China.[84] The European subtype differs by 4% to 6% from the other 2 subtypes (amino acid sequence). The Siberian and Far-Eastern subtypes also differ by 4% to 6% in amino acid sequence from each other.

Phylogenetic analysis shows that the TBEV group separated from the other flaviviruses about 30,000 years ago in Central Africa. From there, the tick-borne flavivirus ancestors migrated east and arrived in central Siberia about 7,500 years ago. The virus ancestor then divided into a western branch and an eastern branch. The eastern branch developed into the Siberian and Far-Eastern subtypes plus also into potentially 2 newly identified subtypes. This evolutionary development took about 3,000 years. The western branch spread to Central Europe and further evolved on the British Isles into Louping ill virus and on the Iberian Peninsula into the Spanish sheep encephalitis virus.[11]

In Western Europe, TBEV-EU is prevalent. However, in the Baltic countries and in parts of Finland, the Siberian and Far-Eastern subtype virus strains have been isolated and identified. So far, it is not clear whether the Siberian subtype in particular moves in a western direction. However, identification of virus strains in Siberia shows that a few of the strains circulating in Siberia belong to the European and Far-Eastern subtypes. According to results from Russian investigators, the Siberian subtype invaded the Baltic countries only recently, coincidentally with the construction of the Trans-Siberian Highway and the Trans-Siberian Railway.[12] Also, the European subtype has been detected in South Korea and also in Siberia.[13,14] Improved understanding of the phylogeography of these strains will require additional studies.

European subtype

The European subtype (formerly known as the “Western subtype”) of TBEV is prevalent in Europe. However, the distribution ranges from France and The Netherlands at its western limit of distribution to South Korea, the easternmost region where TBEV-EU has been detected so far.[9,13,15] While only TBEV-EU is found in Central Europe, more than 80% of identified strains in the Baltics belong to the European subtype. In Western and Eastern Siberia, only a low percentage (<10%) of the identified TBEV strains is characterized as European subtype. As noted, some other TBEV-EU strains have been identified and isolated in South Korea.[13,16,17]

According to phylogenetic data, TBEV-EU is the youngest of all TBEV subtypes.[11] These data indicate that about 3,000 years ago the European strain diverged from the ancestor virus and migrated westwards. Some evidence suggests that the TBEV strains in Central Europe originated in the Czech Republic. From there the virus migrated about 350 years ago to Germany.[18] Several waves of spreading and migration seem to have occurred. In Germany intensive studies on particular TBE foci show that in each TBE focus, a particular and clearly identifiable virus strain is prevalent. The TBEV strains seem to be stable in their E gene sequences for decades as shown in Finland (Kumlinge strain) and in Austria (Zillertal strain).[9] However, no clear pattern of viral spread exists that can be correlated to landscapes or to human activities to explain the introduction of the Siberian and Far-Eastern subtypes in the Baltic region. Analysis of the E genes of TBEVs from different strains shows a kind of geographic clustering e.g. in Scandinavia, Germany, the Czech Republic or the Slovak Republic (Slovakia). But there are also some strains that are genetically related to strains from greater distances, e.g. German strains that are similar to Russian or Scandinavian strains. It is unclear at the moment whether these genetic relationships are due to missing link strains. A clear classification of European strains into genetic clusters or branches is still missing and awaits the analysis of more strains from different parts of Europe.

The phylogenetic analysis of TBEV-EU is unclear and confusing. For about 3,000 years, when the European strain branched off from the ancestor virus and migrated westward, TBEV-EU appears to have remained mono-phylogenetic. All currently known strains from Central Europe separated only about 300 to 400 years ago.[11] In contrast to the Siberian subtype, the European subtype shows a parallel evolution. All currently known strains seem to originate from a single genetic clade. In contrast, the Siberian subtype shows a more consecutive genetic evolution. Only recently, a TBEV strain from The Netherlands was shown to have a distant genomic relationship to all other TBEV-EU strains. While TBEV-EU has also been identified and isolated outside Europe, the phylogenetic connection between European strains and the Siberian and Korean strains is as yet unclear.

A number of phenotypic characterizations have demonstrated TBEV strains of differing pathogenicity, which are circulating in nature. The TBEV strain MucAr HB171/11 shows low neuropathogenicity and neuro-invasiveness in a mouse model.[9] A Czech strain, ts263, is a temperature-sensitive strain that does not grow at 40°C and also exhibits non-neuro-invasiveness.[19]

In addition, TBEV-EU is mainly associated with the biphasic form of TBE. So far, no chronic forms of disease caused by TBEV-EU have been reported. The clinical picture of infection ranges from subclinical to febrile disease to CNS symptoms with severe and persisting neurological sequelae in up to 10% of human cases. The fatality rate of infections with TBEV-EU ranges from 1% to 2%. Acute fatal cases have been rare since a fast-acting treatment of brain edema was introduced. Disease sequelae and fatal cases are mainly

seen in elderly patients. The fatalities often result from super-infections (e.g. pneumonia) relating to the neurological sequelae (e.g. paralysis of breathing muscles); therefore these conditions must be named as indirect causes of fatalities due to TBE.

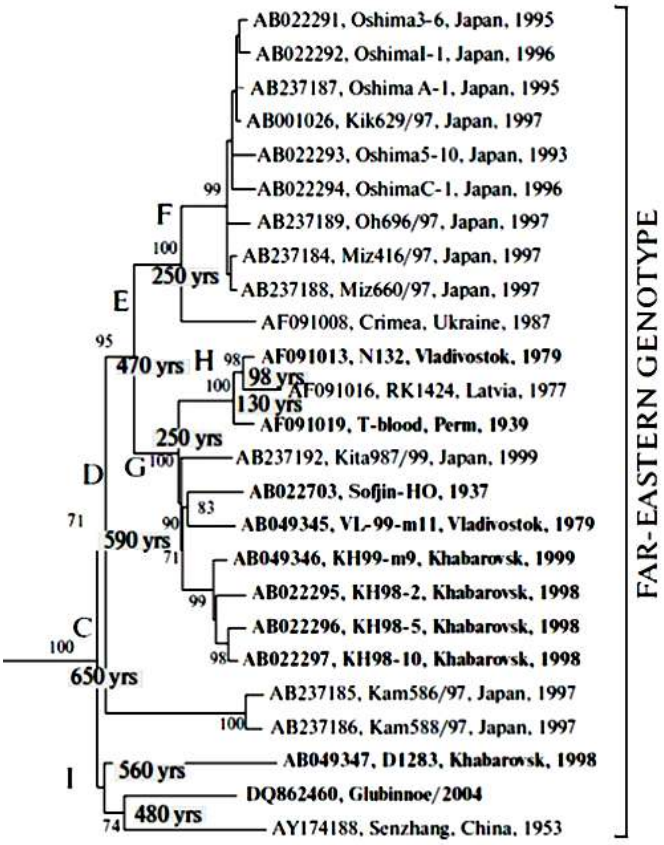
Far-Eastern subtype

The TBEV-FE viral subtype can be primarily found in the territory of the far-eastern part of Eurasia.[20–27] However, this subtype was detected in other regions of Eurasia, including the Baltic countries, the Crimean Peninsula, the Republic of Moldova, the Republic of Belarus, and the territories of Komi Republic, Republic of Bashkortostan, Ural Mountains, Siberia, and the European part of Russia.[10,28–32] In some territories, TBEV-FE has been more prevalent in urban and suburban areas.[33,34] Also, TBEV-FE can cause different forms of disease, from subclinical to acute.[35,36]

Within this subtype at least 4 separate groups (lineages) of TBEV have been described (Fig. 3). The first group consists of TBEV strains similar to the Sofjin strain, which was isolated in the Khabarovsk region of Russia in 1937 from a patient's brain (Zil'ber, 1939)[1] and includes strains from far eastern Russia, Japan, China, Latvia, and the European part of Russia.[26,27] The group of strains similar to the Oshima strains isolated in Japan on Hokkaido Island forms a separate cluster on phylogenetic dendrograms that is significantly different from the Sofjin strains group[20–22] and includes TBEV strains from Japan, China, and the Crimean peninsula.[26,27] The third group consists of the Chinese Senzhang strain, which was isolated from a patient's brain in 1953;[24] the MGJ-01 strain, which was obtained from a patient's blood serum and used in China for the production of vaccines and immunobiologic drugs;[37] and other strains from far eastern Russia. In addition, the fourth group formed by TBEV-FE strains from Japan (Kam586/97(AB237185), Kam588/97(AB237186)) has been described.[27] The time of divergence among different TBEV-FE clusters within the Far-Eastern subtype was estimated at approximately 470 to 650 years ago (Fig. 3).

Also, within TBEV-EU some unique virus variants have been described. In 1999, in the southeast of the Novosibirsk region of Western Siberia, Russia, cases of hemorrhagic forms of TBE with fatal outcomes were reported.[38] Previously, infections resulting in a hemorrhagic disease had not been described for TBEV, although other tick-borne flaviviruses such as Omsk hemorrhagic fever virus and Kyasanur forest disease virus may cause blood-clotting (see section 6 below). The sequencing of the E gene fragment of 6 samples (Figure 3) shows that these TBEV variants corresponded to TBEV-FE, and a number of observed nucleotide substitutions (and amino acid substitutions in the corresponding E protein fragment) were not previously described. Thus, the appearance of new variants of highly

Figure 3: The fragment of the TBEV dendrogram corresponding to TBEV-FE strains[27]



pathogenic, atypical TBEV can be evidence of the continuing evolution of this virus group.

In 2004, the TBEV Glubinnoe/2004 strain was isolated from the brain of a deceased patient in the Primorsky region of far eastern Russia. The sequencing of its genome demonstrated that this TBEV variant corresponds to TBEV-FE, but has 53 or 57 substitutions in polyprotein amino acid sequence compared with Far-Eastern strains 205 (DQ989336)[39] or Sofjin-HO (AB062064),[40] respectively, and 14 of these substitutions are unique and have not been described previously.[41] Researchers also found that Glubinnoe/2004 has a high level of production of infectious viral particles during the early stages of infection in cell cultures as compared with other Far-Eastern 205 strains.[41]

Siberian subtype

The TBEV-SIB subtype is the most common TBEV and has been found almost everywhere in TBEV habitat areas. Thus, it has been detected in most parts of Russia, including the central and northwestern regions, Ural Mountains, Western and Eastern Siberia, the Far East, etc.,[10,12,28,42–44] as well as in Mongolia,[45] Kazakhstan and Kyrgyzstan,[46–49] Finland and the Baltic countries,[12,50] Ukraine,[28,49] and the Balkan peninsula.[49]

TBEV-SIB is believed to be the most genetically heterogeneous, with a nucleotide substitution level about 5.4% within the subtype.[51] At first, based on the analysis of E protein sequences at amino acid positions 234 and 431, two genetic lineages were defined: one lineage including Zausaev strain (AF527415) was characterized by H234/ A431, whereas strains of the second lineage including Vasilchenko strain (AF069066) revealed Q234/ T431.[52,53] Later, the "Baltic lineage"[50,54–56] and "European topovariant"[57] of TBEV-Sib were described. Also, the heterogenicity of TBEV-Sib was demonstrated by molecular hybridization of nucleic acids with 2 subgenotype-specific probes (designated as 3a and 3b) differentiating lineages/ subgenotypes "Vasilchenko" and "Zausaev" of Siberian subtype (Fig. 4).[10] The Zausaev and Vasilchenko lineages were found in various regions of Eurasia at different ratios, and moreover, some TBEV strains of Siberian subtype could not be attributed to any of these lineages.

Baikalian subtype

In addition to the 3 primary and accepted TBEV subtypes, 2 groups of TBEV strains supposed to be new TBEV subtypes were described. At this time, the members of now accepted fourth prototype strain 886-84 (EF469662, KJ633033) subtype have been found only in the Republic of Buryatia, in the Irkutsk and Chita regions of Eastern Siberia and in northern Mongolia (Fig. 5).[10,21,51] This subtype is also now named "Baikalian subtype" and about 20 TBEV strains have been identified and genetically characterized.[10,49,51] These strains (called the "886-84 group") form an independent cluster on the TBEV dendrogram (see Chapter 2) and have no close homology with any strains of the 3 original subtypes. Within the group, high homology (more than 98%) of nucleotide sequences was observed while the genetic differences with other subtypes were shown to be greater than 12%.[51]

TBEV strains of the Baikalian subtype were isolated from ticks and small mammals collected in the Irkutsk region, Buryat Republic, and Transbaikalia in 1984-1990 indicating their ecological connection with all elements of transmission chain. Despite the fact that these strains were isolated over 20 years ago, their circulation probably continues in natural foci. Thus, 2 TBEV strains similar to the reference strain of the Baikalian subtype were described recently in the territory of Transbaikalia from a taiga tick (in 1999) and 1 strain from Myodes rutilus (in 2010).[58,59] Also, in 2010, a report was published on a case of fatal meningoencephalitis in Mongolia caused by a TBEV isolate having a high degree of homology in the E gene fragment (98.5%) with strains of the 886-84 group.[60] The case was

Figure 4: Correlation and distribution of TBEV genotype 3 subgenotypes throughout the whole sampling area and Eastern Siberia. Altogether, 197 strains were typed using oligonucleotide probes[10]

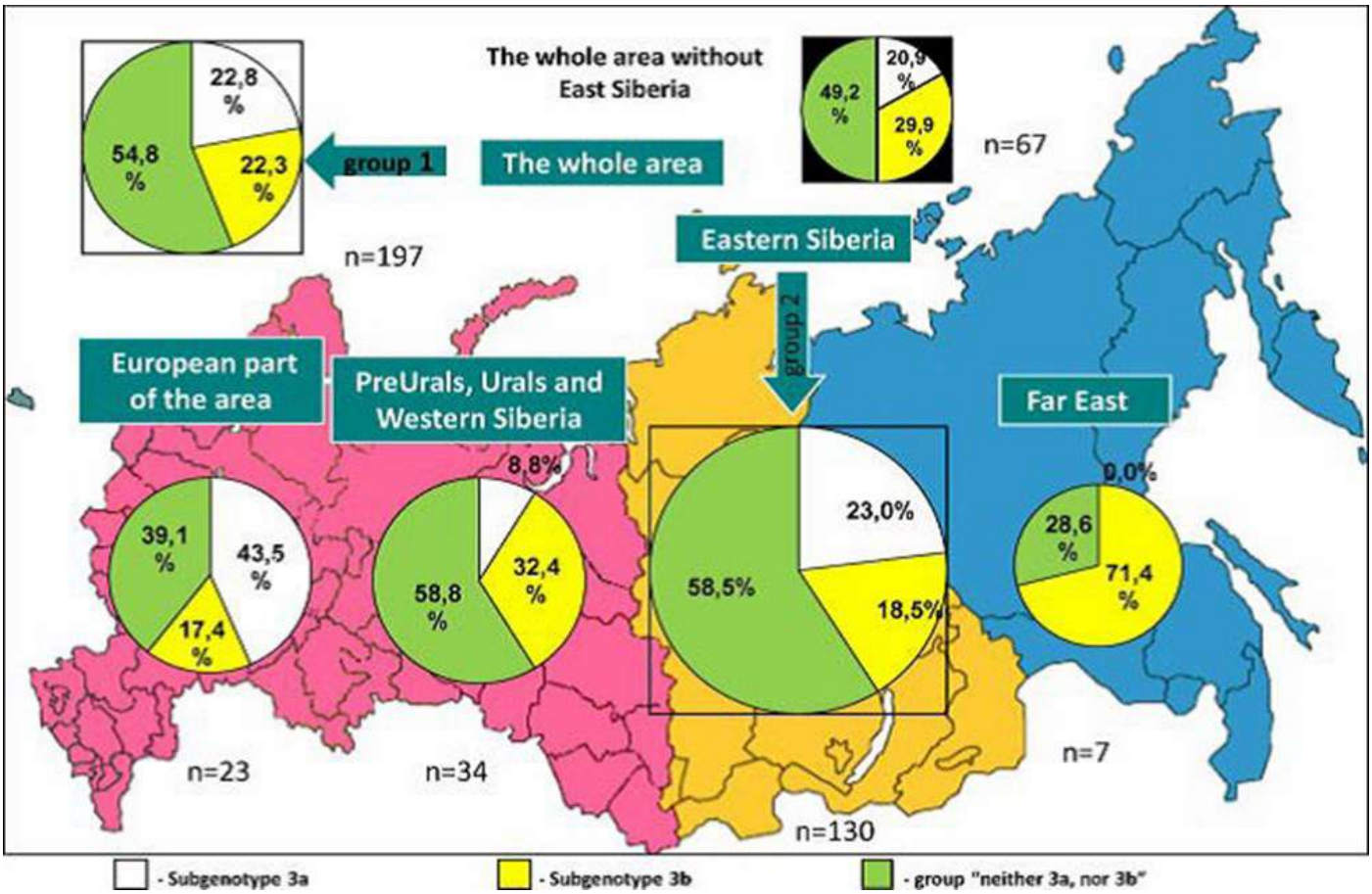


Figure 5: Habitat area of TBEV group "886-84" strains

described in Bulganskiy province, bordering to the south with foci where TBEV group 886-84 strains had been isolated previously. The patient was hospitalized with meningoencephalitis on the 11th day after a tick bite and then died that same day. The presence of TBEV RNA in macromyelon samples, in the core and the *meninx vasculosa*, demonstrated the multilevel localization of lesions and was typical of the most severe forms of acute TBE that result in death or disability.[60]

The analysis of complete amino acid sequences of polyprotein from some strains confirmed that it is a "mixture" of sequences common for the 3 genotypes. Twenty-nine unique substitutions were detected that could probably be genotype-specific for group 886 members.[51] The studies of biological properties demonstrated that group 886 strains have a wide spectrum of antigenic properties, hemagglutination and neutralizing activities, high virulence, and thermotolerance.

Other putative subtypes

Besides the now four accepted subtypes there are two genetically distant groups of viruses, which show high genetic distance to all known TBE virus strains. One virus was isolated only once. The prototype strain which is named "strain 178-79" (EF469661) and was isolated in 1979 from a tick pool of *Ixodes persulcatus.*[10] The single available isolate and genome sequence show 10 to 16% difference to other TBEV subtypes on nucleotide level and 3 to 6% difference on amino acid level.[10]

Chinese researchers reported on another new TBEV subtype.[84] Two TBEV sequences were detected in two specimens of *Marmota himalayana*, collected in the Haixi prefecture at an altitude of 2,994m in the Qinghai-Tibet Plateau in China. So far, no virus isolates are reported. Only the sequence of the complete genome and of the viral polyprotein have been available. According to these data, the virus differs in 16 to 18% on nucleotide level and in 6 to 8% on amino acid level from all other TBEV subtypes. According to a phylogenetic analysis the putative new subtype diverged earlier from the Far-eastern subtype than the Siberian subtype.

Seroepidemiology in humans

From the start of the use of antibody testing in this field, the prevalence rates of antibodies against TBEV (and other pathogens) were used to estimate the burden of infection as well as the burden of disease in human populations. Although these rates depend on a number of different factors (such as a person's age, profession, leisure activities, place of living, interest in nature/outdoor activities, degree of protection measures, knowledge about disease and transmission, and vaccination status, as well as presence of cross-reacting viruses, assay technology used, etc.), the data

at least serve as a rough indication for the presence or absence of TBE in an area.

In determining TBE seroprevalence rates, studies in the normal population have to be distinguished from studies and their results in highly exposed professionals such as woodcutters, farmers, or hunters. In European countries, the available seroprevalence rates in different countries in the normal population range from 0% to 39%. However, the highest of these values are usually found in special

Table 1: Seroprevalence of anti-TBE antibodies in normal populations of different European countries

Country	Prevalence (%)	Literature
Bornholm (Denmark)	1.4	Kristiansen[17]
Estonia	0-5	Vasilenko et al.[72]
Archipel (Finland)	5	Han et al.[73]
Lithuania	3	Juceviciene et al.[74]
Norway	2.4	Skapaas et al.[75]
Poland (North)	4.8-6.5	Anonymous 1983
Czech Republic	15-28	Gresikova 1988[76]
Switzerland	0.5-5.0	Matile et al. 1979[77]
Hunchun (China)	10.9	Satz 2006[78]

geographic conditions, for example 39% on Finnish islands in the Baltic Sea. Usually the seroprevalence rates in European populations range from 0% to 5% (Table 1).

While other studies on the prevalence rates in high-risk populations resulted in similar rates, some also indicated more extreme values under special conditions, e.g. >30% to 40% in some groups of forest workers in Poland (Table 2).

These data showed that the risk of acquiring TBE infection might be high, both in an exposed general population and in a high-risk population. However, many of these studies were conducted before the introduction of vaccines. Therefore, awareness of the disease among the general population in rural areas was low and personal protection measures usually were not applied. This might be one reason why in some areas the seroprevalence rates in the normal population might be in a similar range as seen in highly exposed groups.

Seroepidemiology in animals

Humans are not natural hosts of the TBEV. Therefore, the seroprevalence rates in humans usually give an incomplete picture of TBEV epidemiology. During the past few decades, a number of studies have been undertaken to study the seroprevalence rates in different species of wild and domestic animals. The seroprevalence rates of particular animals can document the presence of a transmission cycle.

Table 2: Seroprevalence of anti-TBE antibodies in high-risk populations of different European countries

Country	Risk group	Prevalence (%)	Literature
Bornholm (Denmark)	Forest worker	16	Kristiansen[71]
Germany	Forest worker	5.6-7.2	Satz[78]
Alsace (France)	Forest worker	8	Collard et al.[79]
Poland (North)	Forest worker	20-40	Satz[78]
Switzerland	Forest worker	4.7	Matile et al.[77]
Hungary	Forest worker	3.3	Molnar[80]

These data may also help with understanding the intensity of transmission in the natural cycle. In addition, they may document the role of particular animals in virus transmission and in the maintenance of the TBE transmission cycle. Recently, data on the prevalence of antibodies and virus were tested in wild and domestic animals to identify species that might be used as surrogates for detection of endemic areas.

The role of particular mice and voles, *Apodemus flavicollis* and *Myodes glareolus*, respectively, as primary vertebrate hosts for the virus in the transmission cycle was demonstrated in a number of isolations of virus strains in TBE natural foci and through experimental infections.[61–63] Also, *Apodemus sylvaticus* seems to support the transmission cycle as evidenced by high seroprevalence rates in Switzerland.[64] In a recent study, Achazi et al.[65] detected TBEV using molecular techniques in 6 rodent species in Germany: *Apodemus agrarius*, *Apodemus flavicollis*, *Apodemus sylvaticus*, *Microtus arvalis*, *Microtus agrestis*, and *Myodes glareolus*. The seroprevalence rates in rodents of different areas ranged from 0% to 72% (Table 3).

While the role of mice (Muridae) and voles (Cricetidae) for TBEV transmission seems clear, the importance of Insectivora is still not finally clarified. Different studies show that hedgehogs (Erinaceidae) are highly infested with ticks. Kozuch et al.[62] detected up to 50% seroprevalence rates in hedgehogs in a study in Slovakia, and they could isolate a strain of TBEV from the hedgehog. Even less clear is the role of shrews (Soricidae). However, TBEV was isolated from a brain of a common shrew, *Sorex araneus*.[66] According to early studies, the common mole (*Talpa europaea*) produces high viremia and therefore may act as a maintenance host in the natural transmission cycle. Systematic seroprevalence data on TBE antibodies in insectivores are not available.

In addition, seroprevalence studies in foxes and correlations with human TBE are limited. One study on TBEV seroprevalence in foxes from different areas in Germany found prevalence rates from 0% in Brandenburg to 10% in the Odenwald and Taunus region (a known endemic area of low activity) to 35% in the Black Forest area, a highly

Table 3: Seroprevalence of anti-TBE antibodies in wild animals in different European countries

Country	Vertebrate	Prevalence (%)	Literature
Bornholm Archipel(Denmark)	Deer	83	Freundt[69]
Aland Archipel (Finland)	Rodents	0.5	Han et al.[81]
Austria	Yellow-necked mouse	47.9	Labuda et al.[82]
Austria	Bank voles	29.4	Labuda et al.[70]
Slovakia	Deer	35.3	Labuda et al.[70]
Slovakia	Boar	36.8	Labuda et al.[70]
Slovakia	Rodents	14	Labuda et al.[70]
Czech Republic	Rodents	14.6	Gresikova et. al.[83]

endemic region for TBE.[67] Also a number of game animals have been tested as indicator animals for TBEV circulation.

These studies, in Germany but also in other European countries (e.g. Denmark), showed high seroprevalence rates against TBEV. Studies in Germany showed the seroprevalence rate in red deer and reindeer in the former German Democratic Republic was up to 72% positive.[68] A similar rate of 83% was reported in a study from the Danish island of Bornholm, also in the red deer population.[69] A study in red deer from Slovakia showed lower antibody rates of 35%.[70]

In natural transmission cycles of the boskematic type, the testing of antibody rates in farm animals may give good evidence of TBEV transmission and also of the risk of alimentary TBEV transmission. Therefore, a number a seroprevalence studies in cows, sheep, and goats from different countries are also available. In most available studies, these data show that the seroprevalence rate is around 5%. There are some exceptions in Germany. In the former German Democratic Republic, an antibody prevalence rate of 60% in cows was reported.[68] A recent study in several federal states of Germany revealed seroprevalence rates of 0% to 43% in goats and sheep.[85] The patchy distribution of high antibody rates in these animals correlated only in part with the presence of human TBE disease.

Other tick-borne mammalian flaviviruses

The International Committee on the Taxonomy of Viruses (ICTV) lists in the genus Flavivirus a total of eight tick-borne mammalian flavivirus (TBMF) species. They distinguish single virus species according to several characteristics:

- Nucleotide and deduced amino acid sequence data.
- Antigenic characteristics.
- Geographic association.
- Vector association.
- Host association.
- Disease association.
- Ecological characteristics.

However, this actual species description no longer includes many of the known and ecologically different TBMF, as no virus subtypes or strains below species level are listed. However, there is a number of flaviviruses with specific names often found in literature, which cause severe human and animal disease. The known subtypes of TBMF are listed in Table 4 including some features regarding their geographical distribution and epidemiology. All viruses listed are genetically closely related to the viruses of the TBEV complex. Therefore besides their medical and veterinary importance they also play a role regarding the diagnosis of flavivirus diseases due to cross-reactivity of antibodies with TBEV antibodies in areas of overlapping geographical distribution. For some of the viruses (Omsk hemorrhagic fever, Louping ill virus Kyasanur Forest disease virus) in laboratory tests the neutralizing cross-reaction of TBEV vaccine-induced antibodies was shown. However, no data are available on the field effectiveness of TBEV vaccines against these viruses.

Table 4: Viruses and virus subtypes of the tick-borne mammalian flavivirus complex of the tick-borne flavivirus group

Virus	Virus type/-subtype	Clinical symptoms in humans/in animals	Geographical distribution	Vector
Louping ill virus	Louping ill virus	Meningoencephalitis Louping ill in sheep	British Islands; possibly Norway	*Ixodes ricinus*
	Turkish sheep encephalitis virus	No human disease known; encephalitis in sheep	Turkey	Unknown
	Greek goat encephalitis virus	No human disease known; encephalitis is goats	Northern Greece	*Ixodes ricinus*
	Spanish sheep encephalitis virus	No human disease known; encephalitis in sheep	Spain	Unknown
	Spanish goat encephalitis virus	No human disease known	Northern Spain	Unknown
	Negishi virus	Meningoencephalitis	Japan	*Ixodes ricinus; Ixodes persulcatus*
Omsk hemorrhagic fever virus	Omsk hemorrhagic fever virus	Hemorrhagic fever	Western Siberia	*Ixodes apronophorus, Dermacentor* spp.
Kyasanur Forest virus	Kyasanur Forest virus	Hemorrhagic fever	Southwestern India; possibly China	*Haemaphysalis* spp.
	Alkhumra virus	Hemorrhagic fever	Arabian Peninsula; Egypt	*Ornithodoros* spp.
Powassan virus	Powassan virus	Meningoencephalitis	Northern America; Far east of Russia	*Ixodes* spp.; *Dermacentor* spp. (?)
	Deer tick virus	Meningoencephalitis	East coast of Northern America	*Ixodes scapularis; Dermacentor andersoni*
Langat virus	Langat virus	Meningoencephalitis in severely immuno-compromised patients	Malaysia to Central Siberia	*Haemaphysalis* spp.

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Chapter 12a

TBE-epidemiology by country - an overview

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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 forested belts of Northern 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 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, east, west, even south and to higher altitudes to areas that were previously believed to be free of the virus.

Burden of disease and case definition

To date, tick-borne encephalitis virus (TBEV) foci have been identified in Europe, Siberia, far-eastern Russia, northern China, South Korea, and Japan. Up to 12,000 tick-borne encephalitis (TBE) cases are identified annually from countries where the disease is reportable. Mortality rates between 0.2% to 20% are reported, depending on region and perhaps on viral subtype.[3] Severe long-term sequelae of TBE are well described both in children and in adults (see Chapters 5 and 6).

Because TBEV is present in reservoir animals in nature, eliminating or eradicating the disease is impossible. Thus, TBE is an important concern for the individual who becomes infected, but the disease 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, 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[35]. 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[2] (Table 1).

As the ECDC case definition and reporting have not been implemented around the globe and not even throughout Europe, data on the burden of disease from 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/meningoencephalitis, not necessarily associated with hospitalization.”[6]

In addition to the use of different case definitions and case classifications, there is a lack of implementation of routine diagnostics in patients with encephalitis particularly with regard to detecting TBE. This is exemplified by the Polish experience: between 2004 and 2008, only 39% of the country’s hospitals had access to TBEV-serology. Therefore,

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.*

a pilot project of enhanced surveillance for TBE was implemented in 2009.[7] Testing for TBE in patients with signs of meningitis or encephalitis in the entire country doubled in 2009 compared with previous years, and 38 new 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.

Finally, vaccine uptake may substantially modify the number of cases in a TBE risk area, as exemplified again by Austria, where in the last decade less than 100 cases are reported annually; this number was up to 700 cases annually before the introduction of a vaccination program. TBE vaccine uptake in Austria is around 84%. Neighboring countries with lower vaccine uptake continue to have increasing TBE case numbers.[1] The following figures show countries with their respective current vaccination recommendations and vaccine reimbursement policies (Fig. 1, Table 2).

Until 2018, only Austria has a national universal vaccination recommendation for the whole population, established a long time ago. Switzerland is the only other country that followed the same pathway, in February 2019 the entire country – except the cantons of Geneva and Ticino – is now defined as a TBE risk area by the Federal Office of Public Health and Vaccines Technical Committee). TBE vaccination is recommended for all persons in Switzerland (=6 years), who are tick-exposed and either live in a risk area or stay there temporarily; for children between 1 and 5 years, the situation is to be individually assessed. The entire Swiss population has a potential risk of exposure, depending on individual activity and mobility.

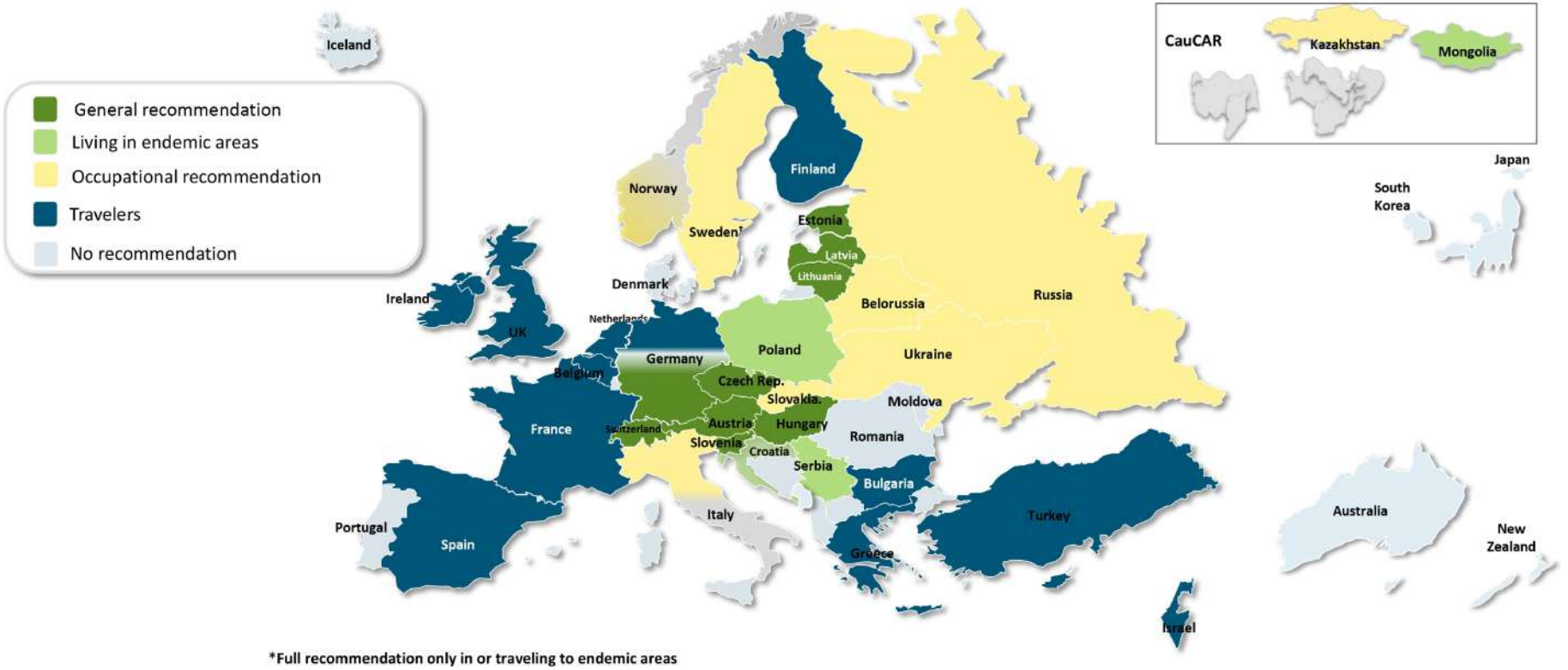
In 2019, National Institute of Health Institute of Slovenia decided to partially fund the vaccination against TBE for children 3 years old and adults 49 year with three doses of the TBE vaccine (primary vaccination or booster). Previously unvaccinated adults 49 years old and children 3 years old, will be included in the vaccination program every year, thus gradually increasing the protection of the Slovenian population against TBE. (See chapter 12b Slovenia).

Recommendations in other countries, if they exist at all, are linked to certain conditions, e.g. predefined risk areas, age, or possible occupational exposure (Fig. 1 and Table 2).

Overall, TBE surveillance in Europe is more sporadic than systematic, and TBE cases are likely underreported. For 2020, 24 EU/EEA countries reported 3,817 cases of tick-borne encephalitis, whereas the sum of all TBE cases outlined in the following country chapters results in 5,429 cases (Table 3).[42] In the end, the real burden of disease from TBE remains unknown and the identification of TBEV endemic areas is far from being complete. With only inconsistent and incomplete scientific databases available, it is fair to conclude that the true TBEV disease burden is significantly underestimated.[3,8]

In this article we take the ECDC definition of TBE as a baseline (Table 1), requiring 1) clinically apparent disease of

Figure 1: TBE vaccination recommendations



General recommendation - recommendation for vaccination of all age groups
Living in endemic areas - recommendation for residents in endemic areas
Occupational recommendation - limited recommendation for specific areas or outdoor activities, vaccination targets individuals in the most severely affected cohorts (e.g. forestry workers)
Travelers - recommendation only for travelers

Table 2: Country-specific recommendations and reimbursement for TBE vaccination (as of 2021)

Country	Notifiable disease/ mandatory reporting system	Recommendation	Population	Reimbursed	Reimbursement type	Reimbursement details
Armenia	+	No recommendation		N	No reimbursement	No TBE vaccine registered
Australia		No recommendation		N	No reimbursement	No TBE vaccine registered
Austria[1,2,3,4]	+	All		Y	Partial reimbursement for all Austrians being vaccinated, and full reimbursement for special vacc. groups (eg, army, farmers)	Partial reimbursement for all Austrians being vaccinated, and full reimbursement for special vacc. groups (eg, army, farmers)
Azerbaijan	-	No recommendation		N	No reimbursement	No TBE vaccine registered
Belarus[3,5]	+	High Risk				TBE vaccine Moscow and Encepur available on the market
Belgium[3]	-	Travelers	Recommendation by the tropical institute for travelers to endemic regions and potential exposure (for travelers abroad to endemic areas)	N	No reimbursement	
Bulgaria[1,2,3,4]	-	Travelers	Recommendation for travelers to endemic regions	N	No reimbursement	No TBE vaccine registered
Croatia[1,2,3,4]	+	At Risk + High Risk	Only recommended for residents in endemic areas and those visiting endemic areas (for recreation); forestry workers in the Koprivnica-Križevci region	N	Non-governmental reimbursement	Both registered, but only Austrian currently available
Czech Republic[1,2,3,4,6]	+	All	Vaccination is recommended to all people permanently or temporarily residing in endemic areas with prevalence of TBE	Y	Non-governmental reimbursement	Contribution from preventive funds of health insurance companies only (approx. at the level of price of 1 dose of vaccine)
Denmark[2,3]	-					
Estonia[1,2,3,4]	+	All	All individuals aged >1 y; Recommended for travelers visiting endemic areas	Y	Non- governmental reimbursement	No vaccine reimbursement is available for the general population; Free for risk groups (foresters, irrigators, military personnel) – vaccination is covered by the employer; both vaccines registered
Finland[1,2,3,4]	+	Travelers	Travelers to endemic areas			
France[1,2,3,4]		Travelers	Travelers to endemic areas		No reimbursement	

Table 2: continuation

Country	Notifiable disease/ mandatory reporting system	Recommendation	Population	Reimbursed	Reimbursement type	Reimbursement details
Germany[1,2,3,4]	+	All	All individuals aged >1 y tick-exposed in RKI-defined (Robert Koch-Institut), TBE-risk areas, either they travel, live, and/or work there	Y	Reimb. for all those who live in, work in, or travel to RKI-defined 'TBE-risk areas'	Reimb. for all those who live in, work in, or travel to RKI-defined 'TBE-risk areas' (often even reimb. by insurances for travel to foreign endemic areas)
Georgia	+	No recommendation		N	No reimbursement	No TBE vaccine registered
Greece[2,3]	+	Travelers	Travelers to endemic areas			
Hungary[1,2,3,4]	+	All	Recommended for everyone	Y	Partial governmental reimbursement	Free for residents of highly endemic areas; **Mandatory** for people with extensive exposure to ticks in rural areas (e.g., forestry workers and farmers since 1998, hikers and campers); German vaccine is reimbursed (25% of cost covered by National Health Insurance); both vaccines on the market
Iceland[7]	-	No recommendation				
Ireland[8]	+	Travelers	A vaccination is the best way to prevent TBE for people living, working, or travelling in risk countries. You may consider having the TBE vaccination if: You are living in or planning to move to a risk country. Your work puts you at risk of TBE (for example, if you are a farmer, forestry worker or soldier). You are planning to travel to a risk area during late spring or summer and will be taking part in activities that put you at risk, such as camping, hiking, or bird watching.	N	No reimbursement	None
Israel[9,10]	-	Travelers	Recommendation for travelers to the endemic area	Y	Partial governmental reimbursement	Reimbursed: TBE is not registered in Israel yet, and this is only OOP in traveler clinics and MOH clinics
Italy[3,11]	-	High Risk	TBE vaccination is recommended to high- risk population groups (foresters, scouts, persons with hobbies or leisure activities potentially leading to tick exposure) in Veneto and in Trentino Alto Adige			
Kazakhstan[3]	+	High Risk	Adults occupational (forest workers and soldiers)	Y	Partial governmental reimbursement	TBE vaccine Moscow is available on the market; FSME-immun is not registered
Kyrgyzstan	-	No recommendation		Y	No reimbursement	No TBE vaccine registered

Table 2: continuation

Country	Notifiable disease/ mandatory reporting system	Recommendation	Population	Reimbursed	Reimbursement type	Reimbursement details
Latvia[1,2,5,6]	+	All	For children and adolescents living in endemic areas; strongly recommended for adults	Y	Partial governmental and private reimbursement	For children and adolescents living in endemic areas; for orphans/children without parental care - **free of charge**. **50%** reimbursement for children (0-2y); **25%** for pregnant women and women 42 days after delivery . **Mandatory** for high-risk groups and/or individuals expecting to have high occupational exposure (e.g. forest workers and military personnel for whom vaccination is paid by employers); both vaccines registered.
Lithuania[1,2,5,6]	+	All	Recommended > 1 y of age; Recommended for travelers and severely-affected cohorts	N	Non- governmental reimbursement	Some employers provide vaccination (e.g., forest workers); both vaccines registered
Luxembourg						
Malta[5]	-					
Mongolia[20]	+	At Risk + High Risk	Adults occupational; residents and for tourists in provinces (Northern Mongolia- Selenge and Bulgan aimags)	Y	Partial governmental and private reimbursement	TBE vaccine Moscow and Chinese
Netherlands[5,22]	-	Travelers	Persons with high-risk occupations in endemic areas; persons who go camping and hiking in nature reserves for longer than 2 days in the endemic regions of the Baltics, former Soviet Union, Kazakhstan, Mongolia, and Japan in the active tick season Persons who go camping and hiking in nature reserves for (cumulatively) longer than 4 weeks in the endemic areas of Central and Northern Europe in the active tick season	N	No reimbursement	
Norway[5]	+					
Poland[1,2,5,6]	+	At Risk + High Risk	Recommended for residents of endemic areas, particularly for military personnel, border guards, fire fighters, farmers, and tourists	Y	Non- governmental reimbursement	TBE vaccination is not universally reimbursed; **Mandatory** for forestry workers (since 1994) – reimbursed by the employer; both vaccines registered
Portugal[5]	-					
Romania[1,2,3,4]	+	No recommendation	No national TBE vaccination recommendations	N	No reimbursement	No national TBE vaccination policy; both vaccines registered

Table 2: continuation

Country	Notifiable disease/ mandatory reporting system	Recommendation	Population	Reimbursed	Reimbursement type	Reimbursement details
Russia[3,5]	+	High Risk	Recommendations in the second part of NIP for endemic regions	Y	Partial governmental and private reimbursement	3 locally produced Russian TBE vaccines, FSME-IMMUN and Encepur are available on the market
Serbia[14]	-	At Risk + High Risk	All aged >1 years of age residing or staying temporarily in endemic areas	N	No reimbursement	Vaccines are not registered
Slovakia[1,2,3,4]	+	High Risk	Recommendation implemented only for high-risk occupational groups: forestry workers, farmers, surveyors, geologists, mountain hut and cableway staff, police officers, military personnel, and railway workers	Y	Non- governmental reimbursement	Implemented only for high-risk occupational groups; **Mandatory** for staff working in TBE testing laboratories; One private health insurance company (DOVERA) provide reimbursement of 3rd dose and second private health insurance company (UNION) provide reimbursement of 50% of each dose; both vaccines are available
Slovenia[1,2,3,4,15]	+	All	Recommended for people living in or traveling to highly endemic areas, including children aged >1 y	Y	Partial governmental and private reimbursement	National TBE vaccination policy and recommendation implemented only for high- risk groups. **Mandatory** for high-risk workers; **Mandatory** for students at high risk, e.g. forestry, wood processing (reimbursed within compulsory health insurance); both vaccines on the market Since March 2019- primary series are reimbursed for two cohorts – children 3 years of age and adults 45-50 yrs of age
Spain[2,3,16]	-	Travelers	Travelers to endemic areas	N	No reimbursement	Not applicable
Sweden[17,18]	+	High Risk	The regional recommendation of Stockholm County is to vaccinate (3+1); no national TBE vaccination recommendations in different endemic settings or at different ages Travelers who intend to spend time in the outdoors, especially in the Stockholm archipelago, might want to get vaccinated	N	No reimbursement	No reimbursement is offered for TBE-vaccine
Switzerland[4,19,20,21,22]	+	At Risk + High Risk	Adults and children >6 years of age residing or staying temporarily in endemic areas	Y	Full governmental reimbursement	Reimbursed for Swiss citizens (except region Genf and Tessin), for adults and children >6 years of age residing or staying frequently in endemic areas

Table 2: continuation

Country	Notifiable disease/ mandatory reporting system	Recommendation	Population	Reimbursed	Reimbursement type	Reimbursement details
Tajikistan	-	No recommendation		N	No reimbursement	No TBE vaccine registered
Turkey[3,23]	-	Travelers	Individuals with high- risk activities (camping or working in farm and forest lands, adventurous journeys) and living in endemic countries	N	No reimbursement	
UK[24]	-	Travelers	Limited to travelers to high risk areas	N	No reimbursement	
Ukraine[3,5]	+	High Risk		N	No reimbursement	TBE vaccine Moscow and Encepur available on the market

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Table 3: TBE cases by year and country (source: data provided by the authors of Chapter 9b, 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
Albania																																
Austria [1]	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
Belarus [2]										23	61	18	53	44	46	108	82	66	88	91	108	122	109	119	77	141	142	135	171	108	108	
Belgium [3-6,*]																							2	3	1	1	3	2	0	3	2	2
Bosnia and Herzegovina [7]											1									2				5						0	n.a.	n.a.
Bulgaria [2]																			2	0	0	1	0	0	2	0	1	0	1	2	1	0
China																														0	0	0
Croatia [7-9]	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	4	23
Czech Republic [10]	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	854	587	710
Denmark [2]				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
Estonia [11]	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
Finland [12]		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	148	125
France [2]	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	24	68	31	31
Germany [13-15]	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
Greece [16]																								1	1						n.a.	n.a.
Hungary [2]	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
Italy [2]	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	24	40	37	55	14	40
Japan [18]			1																							1	2	1	0	0	0	0

**Autochthonous cases only. For travel-related cases, see country chapter.*

Table 3: TBE cases by year and country (source: data provided by the authors of Chapter 9b, 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
Kazakhstan[2]	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
Kyrgyzstan																														n.a.	n.a.	n.a.
Latvia[19]	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	211	210	249	240
Lithuania[20]	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
Moldova																														0	n.a.	n.a.
Mongolia[2]															5	6	52	12	8	9	13	6	15	7	40	52	62	32	19	20	5	8
Netherlands[21-23,*]																							0	0	0	2	1	2	2	5	2	2
Norway[24]								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
Poland[25]	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	159	210	445
Romania[2,26]																		8	4	3	3	3	1							0	n.a.	n.a.
Russia[27-29]	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	989	1015	1969
Serbia[2,30]														1	6	1						4			4	1	5	13		0	n.a.	n.a.
Slovakia[31]	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
Slovenia[32,33]	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	126
South Korea																															n.a.	n.a.
Sweden[34]	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	465
Switzerland[35]	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	391
Tunisia																															0	n.a.
Ukraine[36,37]												12	28	4	8	7	4	7	8	3	10	3	3	6	3	6	4	5	2	2	n.a.	n.a.
UK[38,39]																														2	0	2

**Autochthonous cases only. For travel-related cases, see country chapter.*

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the central nervous system plus 2) at the same time a valid virological or serological documentation of current infection of the patient. However, in some countries "fever cases only", if severe enough to be hospitalized and if laboratory confirmed are also counted and reported as TBE cases. Reported numbers of TBE cases by country and by year are summarized in Table 3.

2. TBE risk areas

While the challenges of detecting TBE cases in Europe have been described above, here we look at the collection of data to identify TBEV-endemic areas. Again, several methods are employed in different countries for epidemiological mapping:[9]

1. testing of ticks and animal reservoirs for the presence of TBEV (especially by molecular diagnostic techniques);
2. seroprevalence studies of populations exposed to ticks; and
3. description of clinical cases with verifiable tracking of the place where the infection was acquired.

Each of these methods gives only a part of the complete picture. Some countries report the geographic prevalence of TBE based on the incidence of human cases only. However, this type of information does not give a clear picture on TBE endemic areas because often the exact place of TBE infection cannot be determined with certainty. Thus some TBE cases are "lost" for surveillance and reporting for the location where the infection was acquired. Overall, data on TBEV distribution are incomplete, heterogeneous between the different countries, and sometimes even inconsistent for the same country.

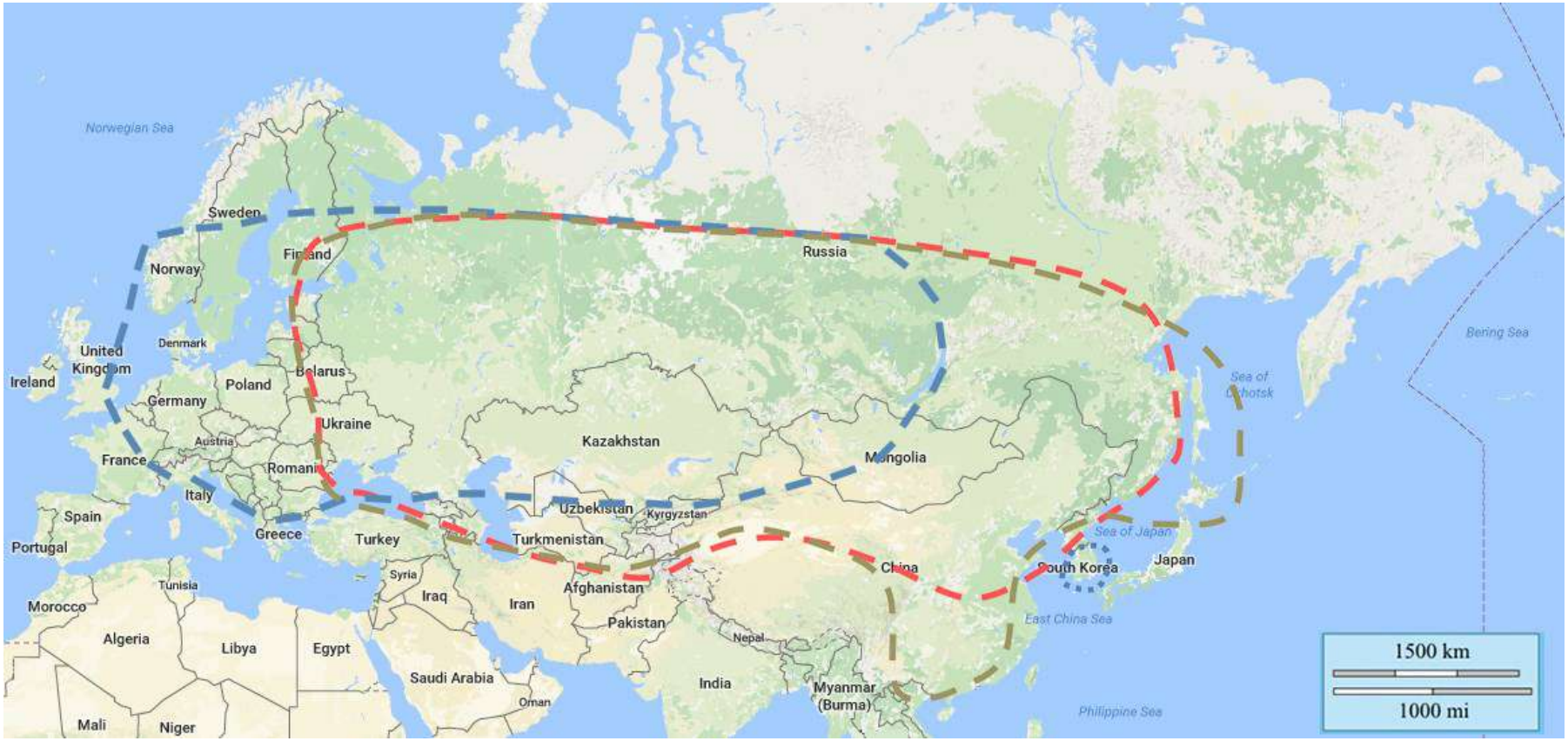
In the end, estimating the risk of infection by the TBEV in a specific (endemic or non-endemic) area is impossible for several reasons.

1. The epidemiology of TBE is the result of a complex interaction between reservoir animals, birds, ticks, plants, climate, weather, and human behavior (including vaccine uptake; see Chapters 3 and 13 for details). These variables change annually and unpredictably resulting in great annual differences in case numbers, as is well demonstrated in Chapter 12b (country-specific summaries) as well as in Table 2 above.
2. 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.
3. TBEV exists in microfoci, i.e. the virus often is detectable in small areas only, whereas the surrounding areas are TBEV-free.

Considering these points, the prevalence of TBEV in ticks obviously can vary considerably, even within 1 country or 1 area within an endemic region. 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). Or: a highly TBE endemic area may have a very low population size, so no TBE cases or only low incidence numbers of TBE cases are identified. Thus, the area may appear to be TBE-free whereas hikers in the area may have a high risk.

Finland for example is the eighth-largest country in Europe

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)

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 30 on the island of Åland. Recommendations per municipality are based on human incidence numbers exclusively and do not consider those many municipalities where there are only few people living.[40]

With this in mind, the TBE-incidence of a country alone is not an adequate measure for the individual risk to acquire TBE. Moreover, to date there is no commonly accepted definition to characterize "TBE risk areas". To address this problem in a transparent and scientific way the country surveys listed in Chapter 12b of THE TBE BOOK are based on a proposal by ECDC[10] for assessing the risk for arbovirus infections in general.

- The key point from this[10] 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.
- A **predisposed area** is a risk area where existing conditions might facilitate the transmission of an to humans, but the respective pathogen has not been detected.
- An **imperiled area** is a risk area where the pathogen has been detected in vectors, or transmission of the pathogen to animals or humans has been detected indirectly (by serology).
- 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[10] 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 by no means a risk assessment, but rather a way to grade available evidence.

In South Korea, TBEV has been detected in ticks, but no single human case has been identified to date. In Japan, only 1 case had been confirmed by 1993, and 4 other human TBE cases were identified between 2016 and 2018. In The Netherlands 3 autochthonous TBE cases were identified in 2017 and a total of three TBE foci have been identified so far.[11,12] In Belgium, circulation of the virus in wild life has been documented, and the possibly first two autochthonous human TBE disease cases were reported.[36, 37] Recently, circulation of the TBEV in wild life as well as one possible indigenous human TBE case has been reported from the UK.[38,39] With this in mind, it remains unknown if travelers to South Korea have any risk for TBE if exposed to ticks in this country, whereas clearly, at least some parts of The Netherlands, Belgium, Japan and the UK are now TBEV-affected areas at least, and they may become endemic in case universal testing is applied.

Physicians, travelers, or the public in general may refer to the respective country in Chapter 12b of "The TBE Book" to see the number of reported cases by year, the local vaccine uptake (as available), and other key information to judge on possible risks – bearing in mind the limitations, and the accuracy of surveillance in the given country, and reporting mentioned herein.

It is the task of local authorities to define "TBE-areas" and decide on recommendations for vaccination either for all persons living in an area or for special risk groups. As the epidemiology of TBE changes annually it has to continuously be re-evaluated.

3. Areas without confirmed TBE risk

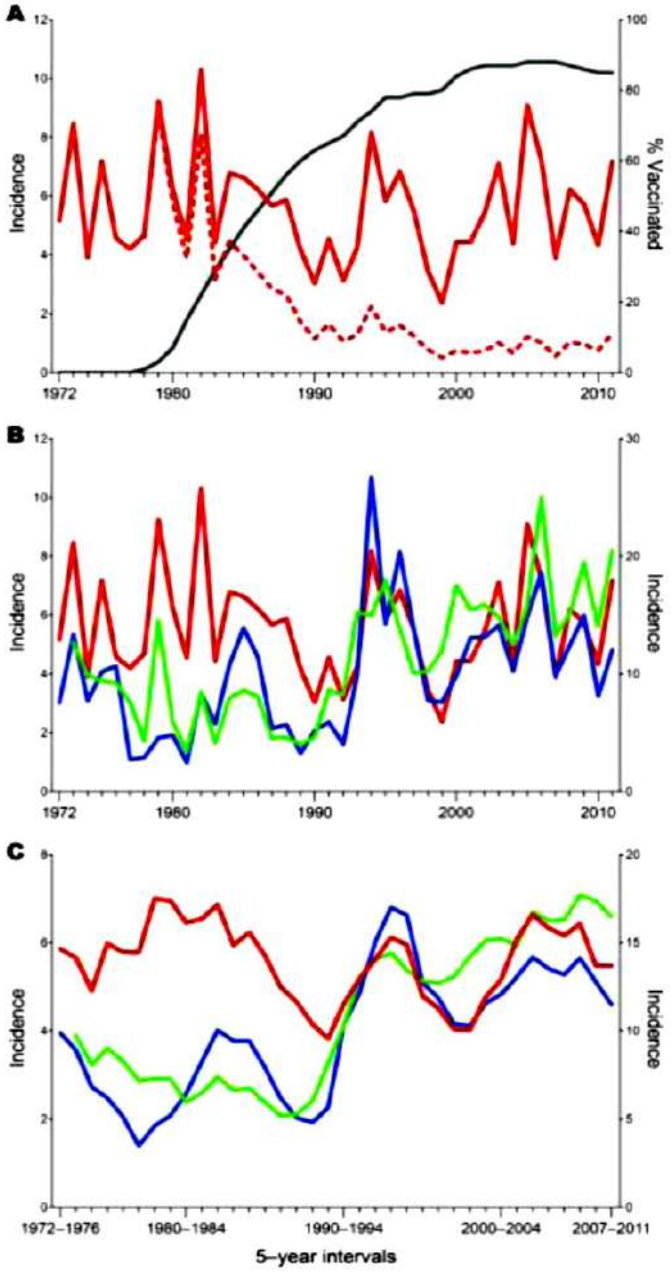
Lack of circulation of the TBEV in Eurasia has been confirmed for Spain, Portugal, The Republic of Ireland, Luxembourg, Kosovo, Macedonia, Montenegro, Greece, and Turkey as well as for some other parts of the continent.

First reports of TBEV seropositivity for TBEV in Spain were published in animals in Extremadura in 2003 and in Andalusia in 2014, and neutralizing antibodies against TBEV were recently detected in a horse on the island of Mallorca (off the eastern coast of Spain).[13,14] Nonetheless these data are difficult to interpret as they could be due to cross-reactivity with IgG directed against closely related viruses of the same serogroup. Indeed, the louping ill virus, a member of the TBEV serocomplex, has already been detected in ticks and livestock in Spain.

An investigation in Turkish blood donors in Zonguldak in the Black Sea region has shown one TBEV positive sample by NT (PRNT).[15] However, cross-reactions in commercial serological tests as well as by PRNT cannot be entirely ruled out. Recently IgM but no IgG antibodies to the TBEV were detected by ELISA in five children in Turkey.[16] This constellation is highly implausible and indicates possibly nonspecific cross reactions as well. More data on TBE are required in order to confirm the existence of the TBEV in Turkey.

Eltari reported a total of 82 TBE cases from 1983 to 1990 in

Figure 4: Tick-borne encephalitis (TBE) incidence rates, 1972–2011, central Europe



A. Total population (red dashed line) and non-vaccinated population (red solid line) in Austria. The black line represents the increasing coverage of vaccination, which started in 1978.
B. Comparative representation of TBE incidences in Austria (red line), Czech Republic (green line), and Slovenia (blue line). The incidence scale for Slovenia (right y-axis) differs from that of Austria and the Czech Republic (left y-axis).
C. Sliding-window representation of TBE incidence in Austria (red line), Czech Republic (green line), and Slovenia (blue line) in means of 5-year intervals. The incidence scale for Slovenia (right y-axis) differs from that of Austria and the Czech Republic (left y-axis).[1]

Albania. No further data are available after 1990.[43,44] There is only one report from Bosnia mentioning TBE cases.[45]

In a serological study in Northern Greece serum samples from 921 apparently healthy individuals were investigated for the presence of TBEV antibodies. According to the authors two percent of the general population was found to be TBE-seropositive in their test system.[17] However as TBEV is not endemic in Greece these findings may well result from cross-reactivity to Greek Goat Encephalitis Virus. Nonetheless two imported TBE cases confirmed by neutralization test were reported to TESSy-ECDC, one in 2014 and a second in 2016.[18]

Recent data definitely confirmed the presence of TBEV in Northern Italy (see country chapter). Older data show up 14 human TBE cases and two virus isolations between 1975 and 2004 in the Toscana region however, no additional TBE case has been reported ever since. Still, a seroprevalence study in hunters and wild boar breeders in Turin Province and in particular in the Susa valley showed an about 5% seroprevalence by ELISA and amongst low-risk individuals seroprevalence was below 2%.[19]

In Afghanistan, a study showed 23.4% seroprevalence and 20 human cases of IgM positivity; however, cross-reaction with Royal Farm virus cannot be excluded.[46]

In Georgia, 7% of acute febrile patients showed TBEV seropositivity.[47]

Most recently a study investigated the possible circulation of TBEV in Northern Iran, where climatic conditions, presence of Ixodes ticks, and variability of mammalian hosts might contribute to TBEV establishment. Anti-TBEV IgG antibody positive ELISA results were reported[41]; however, no confirmatory test was done, and cross-reactivity among flaviviruses is highly plausible.

Within the Central Asian countries there are reports of TBE in Kazakhstan and Kyrgyzstan (see country chapters), the only other single report without any further details is Turkmenistan.[48]

4. TBEV subtype and vector distribution

Three main TBEV subtypes have been described based on their main distribution pattern and sequence similarity: the European virus (previously CEE virus, Central European encephalitis virus; **TBEV-EU**), the Far Eastern virus (previously RSSE virus; **TBEV-FE**), and the Siberian virus (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**)[19] (details see chapter 11). So far, it is unclear whether the recently detected strain "Sallandse" from The Netherlands forms an 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. TBEV-BLK was found in East Siberia near Lake Baikal and in Northern Mongolia, and TBEV-HIM was recently isolated in wild rodent (*Marmota himalayana*) in the Qinghai-Tibet Plateau in China.[20]

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, and the European part of Russia.

All 3 main TBEV subtypes are present in Estonia and Latvia.[21,22] 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.[23,24] The TBEV-SIB has been detected in Bosnia as well.[23]

TBEV-EU foci have been reported from South Korea, approximately 7000 km away from the European range of the TBEV-EU subtype circulation.[25] 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.[23,26]

TBEV-FE foci have not only been reported from Crimea, about 3000 km away from the known TBEV-FE circulation area[27] but also from the Republic of Moldova between 2010 and 2011.

Geographical circulation of the TBEV subtypes, unusual TBEV subtype foci, and various carrier vectors are described in more detail in Chapters 3 and 13.

5. Trends in TBE epidemiology

A characteristic feature of TBE is that the incidence of the disease in risk areas can vary significantly from year to year. In addition to short-term fluctuations, there are also longer-range undulations of incidence rates in intervals of >5 years, which have been analyzed in detail for Austria, the Czech Republic, and Slovenia[1] (see Fig. 4). Except for the strong overall upsurge of TBE cases in the Czech Republic and Slovenia starting around 1992 (but not in Austria, as a result of vaccination), the long-range incidence curves for 1990–2011 are remarkably similar for all 3 countries, suggesting that the causes for the increase in TBE cases are the same but not yet identified.

A similar fluctuation over time has been recognized in Estonia, a country with one of the highest overall TBE incidence in Europe. Looking into more detail for the years 2005 till 2017 case numbers are fluctuating between 6.2 and 18.6, and when comparing different counties, mean incidence (2005–2017) vary between 5.2 and 52.8 (see Chapter 12b, Estonia).

Again, as noted above, the epidemiology of TBE is a "moving target." Current changes include an increase in geographical distribution of TBE-risk areas as well as an overall increase of reported TBE cases (Table 2). In recent years new TBE foci have been reported from altitudes up to 2100 meters above sea level.[29-31,49] New endemic zones in previously unaffected alpine regions in western Austria[32] 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.[33] Within the last couple of years, 4 TBE cases (2 proven and 2 suspected ones) have been identified in UK and Scotland (for more details, see country chapter UK). A remarkable increase in annual disease numbers over the last couple of years is seen in Central European countries, i.e., Austria (most common in unvaccinated subjects), the Czech Republic, Germany, Sweden and Switzerland (see Table 3).

It appears that areas with TBEV endemicity as well as the total number of reported TBE cases have increased over the last several decades. Comparing the periods from 1976 to 1989 and 1990 to 2009, the average increase in TBE infections among humans in central and western European countries was 317.8% in Europe including Russia and it was 193.2% in Europe excluding Russia.[34]

Various factors may explain 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 reservoir animals), and/or technological factors (advanced diagnostics, increased medical awareness).

There is increasing research interest in habitat suitability modelling to define universal environmental characteristics of TBEV foci, to predict suitable conditions where potentially human TBEV infections may occur.[50-52]

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.

The country reports in Chapter 12b provide standardized information, as available on:

- 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

Chapter 12c provides a risk map for 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.

Acknowledgement: We thank all authors and the co-editors of THE TBE BOOK let 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 12b for providing their timely reports.

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Chapter 12b

TBE by country – country data

Gerhard Dobler, Wilhelm Erber, Michael Bröker, Lidia Chitimia-Dobler and Heinz-Josef Schmitt

While TBE is listed as a "communicable disease" in the EU since 2012, each country implements reporting nationally with own resources and methods.

In most instances, reporting of TBE cases is based on passive surveillance and thus largely depend on disease awareness with physicians and on availability, cost, and use of serological tests for TBE diagnosis.

Systematic, regular use of TBE serology in the appropriate clinical setting is in place in few countries only, despite the fact that it would be a necessary prerequisite to gain reliable data on the true incidence of TBE in a given country.

The scientific appropriate methodology would be to document that >80% of subjects with meningitis/encephalitis are tested for TBE in an endemic region.

As this is not accomplished anywhere, the data shown for individual countries in Chapter 12b are 1) not necessarily comparable to each other and 2) underestimate the real burden of disease and indicate just the minimum number of cases.

Chapter 12b

TBE in Austria

Karin Stiasny and Judith H. Aberle

E-CDC risk status: endemic (data as of end 2022)

History and current situation

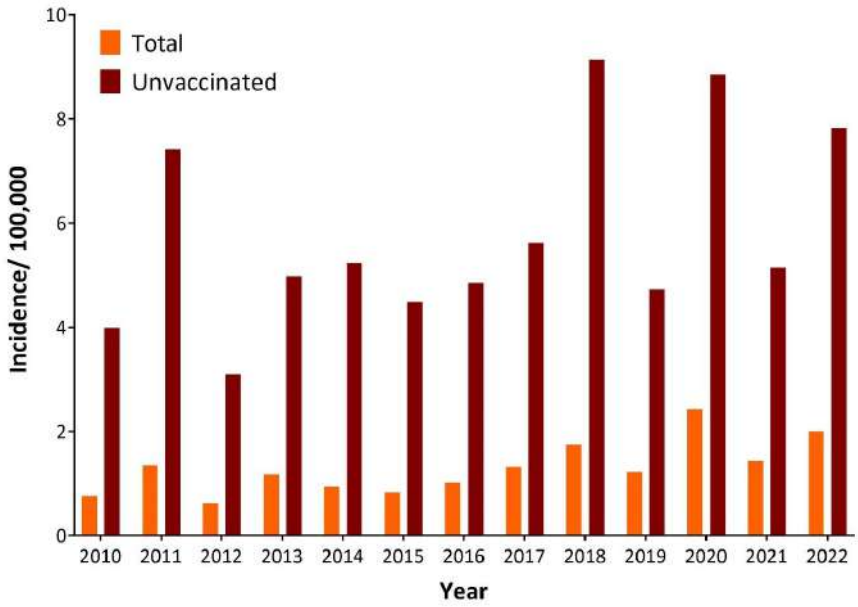
Since 1972, the documentation of human cases of tick-borne encephalitis (TBE) in Austria has been performed by the Center for Virology, Medical University of Vienna, which acts as the National Reference Laboratory for TBE and other flavivirus infections. Only hospitalized patients with a recent tick-borne 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 2022 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] 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)[4] [Gerhard Dobler, personal communication].

Mapping of the most likely sites of human infection has been performed by the National Reference Laboratory since 1972 through the use of questionnaires sent to TBE patients with confirmed laboratory diagnosis.[5] These data are shown in Figure 3.

Figure 1: Incidence of TBE in Austria in total and unvaccinated population, 2010–2022



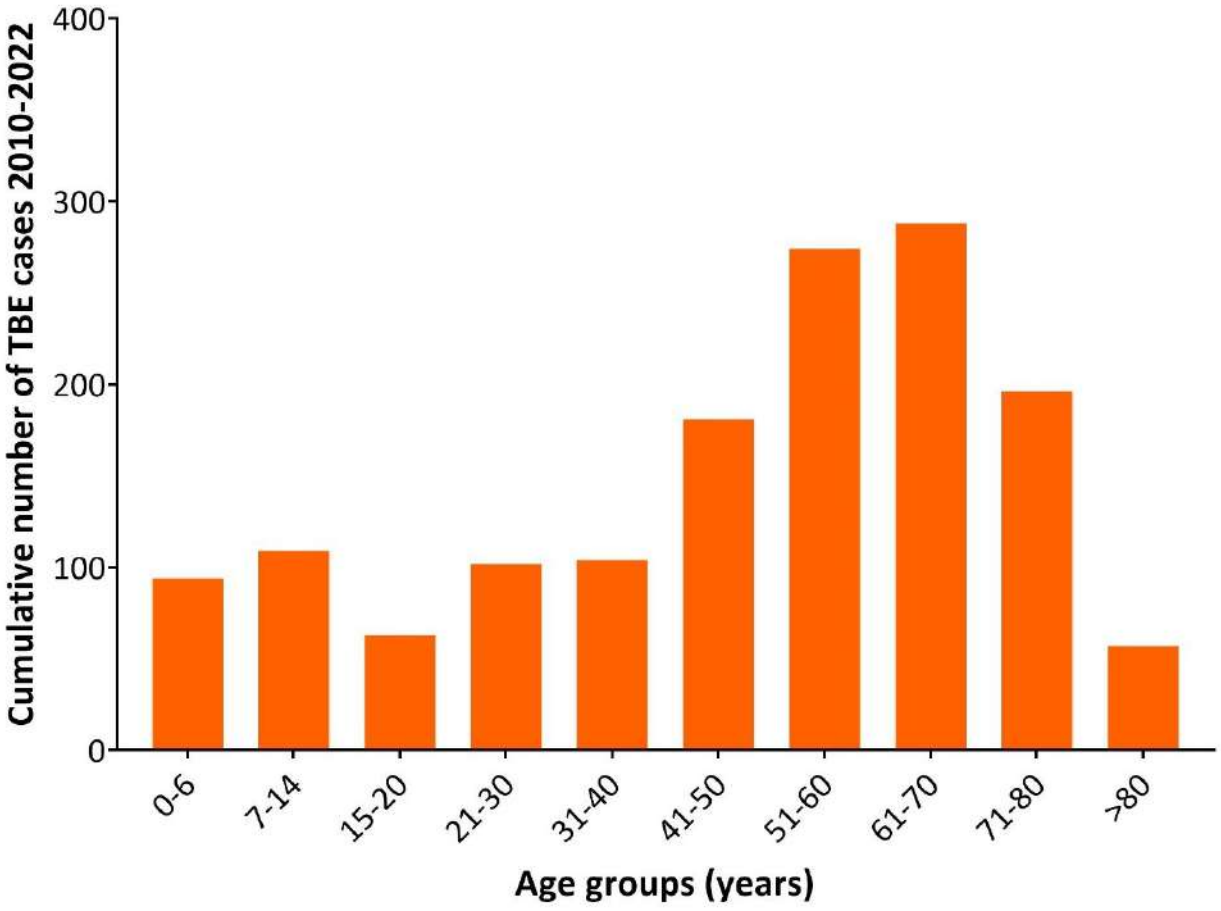
Orange columns: TBE incidence in the total population
***Magenta** columns: TBE incidence in the unvaccinated population (based only on patients with a documented status of "no vaccination"). Population data were obtained from the Austrian Statistical Office ("Statistik Austria", https://www.statistik.at/) and vaccination coverage data from "The TBE Book 4th Edition".*[7]

Source Data: Appendix—Figure 1

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 – have become established.[5] 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.[5] In parallel, the incidences in the northeastern part of the country (comprising regions with relatively low altitudes) declined,[5] 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.[5]

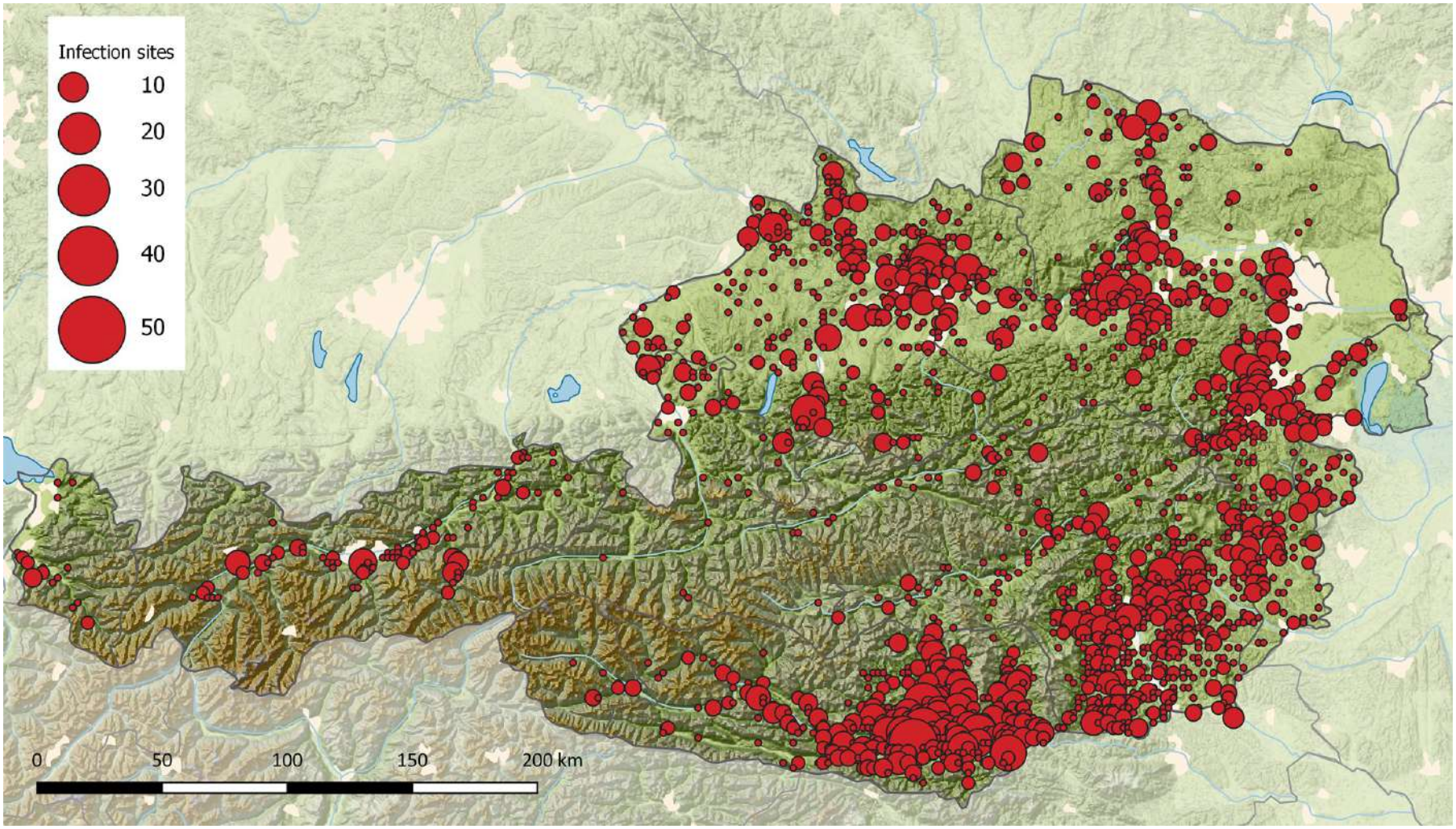
The causes for establishment of new endemic regions in Austria as well as the decline of TBE in other parts of the country are unknown. Surprisingly, these changes are not paralleled by similar alterations in the incidence of borreliosis, which is transmitted by the same ticks as TBEV but remained relatively constant over time in all parts of Austria.[6] 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 2: Age distribution of TBE in Austria, 2010–2022



Source Data: Appendix—Figure 2

Figure 3: Sites of TBEV infection in Austria, 1972–2022



Red circles: Cumulative infection sites of TBE patients for the period from 1972 to 2022

Infection sites were geocoded and processed for spatial mapping by QGIS (https://www.qgis.org/). Spatially close sites were aggregated using a 2 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 using 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,[8] http://www.marine-geo.org/tools/GMRTMapTool/].

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	2.00	7.82

Source data: Figure 2

Cumulative number of cases by age (Austria)

Age	0-6	7-14	15-20	21-30	31-40	41-50	51-60	61-70	71-80	>80
All	94	109	63	102	104	181	274	288	196	57

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Chapter 12b

TBE in Belarus

Volha Kniazeva, Wilhelm Erber and Tamara Vuković-Janković

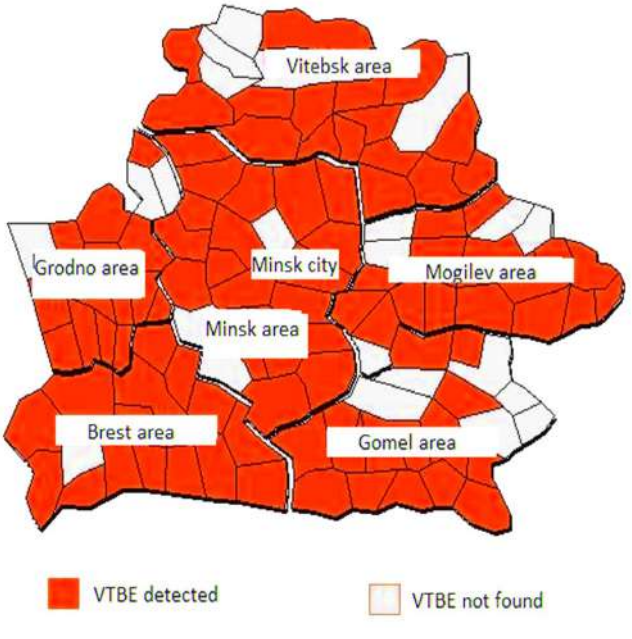
E-CDC risk status: endemic (data as of end 2022)

History and current situation

Belarus is a landlocked country of eastern Europe with a population of 9.4 million, of which 78.4% reside in urban areas bordered by Lithuania and Latvia to the north west, by Russia to the north and east, by Ukraine to the south, and by Poland to the west. The country of Belarus is divided into six administrative districts (Brest, Gomel, Grodno, Minsk, Mogilev, Vitebsk regions) each centered around a major city (Minsk). Much of the country consists of flat lowlands separated by low-level topped hills and uplands; the highest point is Dzyarzhynskaya Hill, being only 1135 feet (346 meters) above sea level. Over half of the surface area of Belarus lies below 660 feet (200 meters), and about 40% of the country is forested. The most common tick species in Belarus are *Ixodes ricinus* and *Dermacentor reticulatus*.[1,2,3]

Almost the entire territory of Belarus is believed to be endemic for tick-borne encephalitis virus (TBEV), with the Central European subtype, also known as TBEV-EU (Figure 1). In all, 96 counties (i.e., 71.5% of all administrative districts) are considered to be risk areas for tick-borne encephalitis (TBE).[1] The most intensive natural foci have been found in the western part of the country Tick-borne encephalitis virus circulation is detected in 15 out of 16 administrative territories of Brest region, among which 5 districts are defined as endemic (where the disease has been formed and maintained for a long period of time): Berezovsky, Ivatsevichy, Kamenetsky, Malorita and Pruzhany districts, and most of administrative territories of Grodno region.[4]

Figure 1: Administrative territories of the Republic of Belarus where circulation of tick-borne encephalitis virus (TBEV/VTBE) causal agents were identified, 1998–2007[5]



To determine whether or not changes in the TBEV infection rates in ticks followed a trend over time, joinpoint regression was estimated for annual percentage of infected ticks group by using the Joinpoint Trend Analysis Software, Version 4.5.0.1 (Statistical Research and Applications Branch, National Cancer Institute; https://surveillance.cancer.gov/joinpoint/). Same analysis was performed for TBE incidence of population of Belarus.

In brief, by using the TBE incidence rate per 100,000 and population of Belarus data for TBE and annual percentage of infected ticks rate data for TBEV in the transmitters as inputs, this method identifies the year(s) when a trend change occurs. One can therefore calculate the annual percentage change (APC) in rates between trend-change points, and also estimate the average annual percentage change (AAPC) in the whole period studied (Figure 2).[6,7]

To estimate the APC, the following model was used:

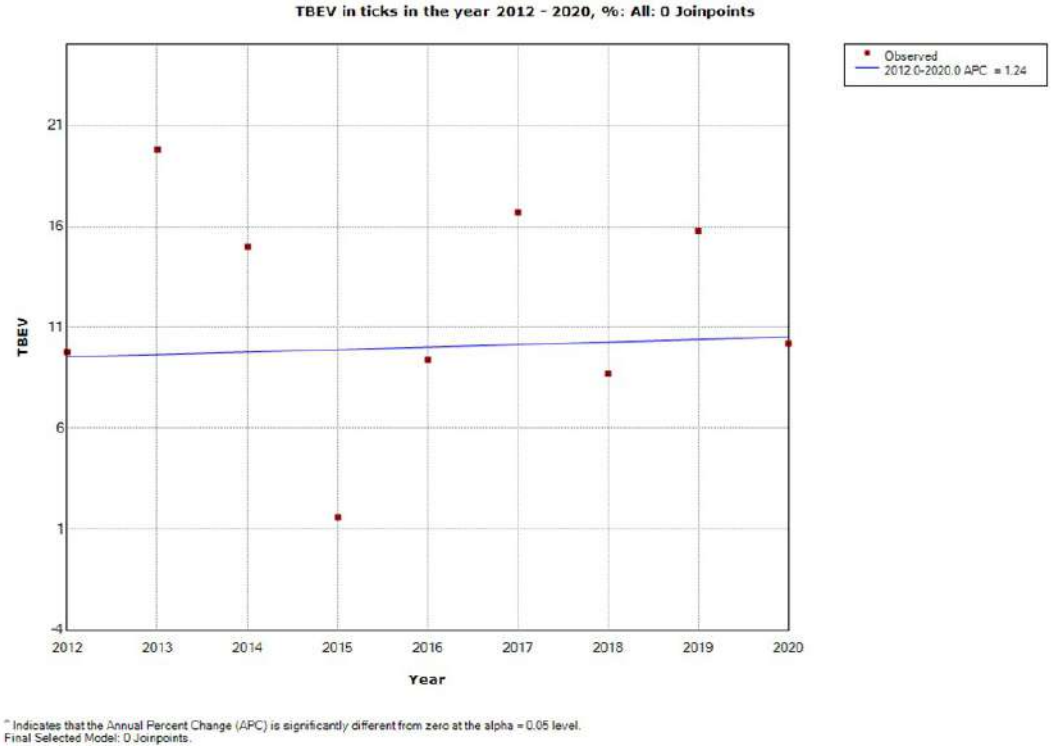
$$\log(Y_x) = b_0 + b_1 x, \text{ where } log\,(Y_x)$$, where log (Yx) is the natural logarithm of the rate in year x.

Then, the APC from year x to year x + 1 is:

$$APC = \frac{e^{b_0+b_1(x+1)} - e^{b_0+b_1 x}}{e^{b_0+b_1 x}} \times 100 = (e^{b_1} - 1) \times 100$$

When there are no join points (i.e., no changes in trend), APC is constant, so it equals the AAPC.[2,3]

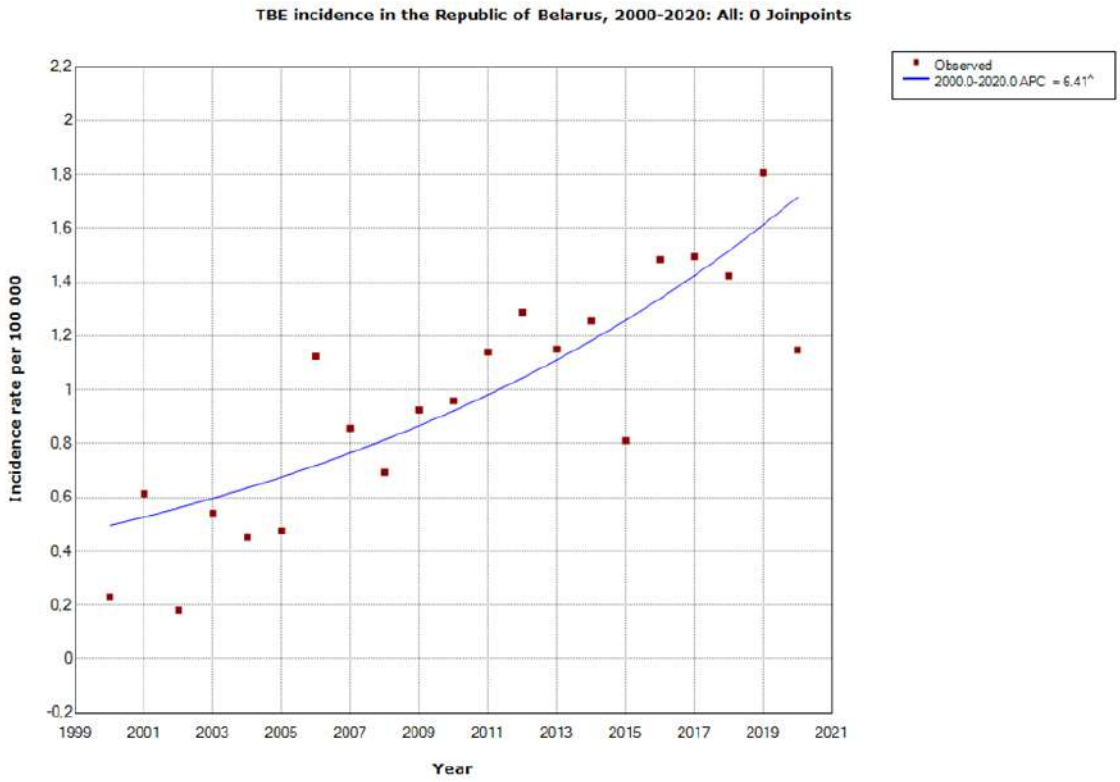
Figure 2: Joinpoint regression model of TBEV in ticks in Belarus, 2012-2020



Source Data: Appendix—Figure 2

In the decades from 2000 to 2020, the number of registered human TBE cases ranged from 18 in 2002 (incidence rate, 0.2 per 100,000) to 171 in 2019 (1.8 per 100,000). Overall, 1912 cases were registered in that period, which corresponds to a mean annual case number of 91. Figure 3 displays the increasing trend of TBE incidence by 6.41% every year that was significantly different from zero at a = 0.05 (Figure 3).[8]

Figure 3



Source Data: Appendix—Figure 3

Given the presence of high numbers of TBEV-infected ticks, the number of reported cases appears to be low and the true burden of TBE is likely underestimated.

Children aged 7–14 years represented 10%–15% of the total number of TBE cases.[5]

Two alimentary outbreaks have been reported, one in 2006 and one in 2007, with a total number of 16 persons infected.[5]

Overview of TBE in Belarus

Table 1: Virus, vector, transmission of TBE in Belarus	
Viral subtypes, distribution	Central European subtype (TBEV-EU) has been detected in almost the entire country.[5]
Reservoir animals	Information not available
Infected tick species (%)	In Belarus the main vectors for TBE are *Ixodes Ricinus Dermacentor reticulatus.*[5] Since 2005, surveillance of TBE in ticks started. A medium direct correlation was established ($r = 0.7$ with $P \leq 0.05$) between the incidence rate of tick-borne encephalitis and the natural foci intensity rate.
Dairy product transmission	Cases of alimentary TBE 2006–2007: 16 cases reported due to the consumption of raw goat milk.[5]

Table 2: TBE reporting and vaccine prevention in Belarus	
Mandatory TBE reporting	Registration of people with tick bites seeking medical advice and/or primary diagnosis of TBE according to clinical signs and epidemiological anamnesis. From counties, reports are sent to higher healthcare organizations.
Other TBE surveillance	No information available
Special clinical features	Biphasic disease
Available vaccines	EnceVir, Tick-E-Vak (Клещ-Э-Вак), TBE-vaccine Moscow
Vaccination recommendations and reimbursement	Recommended for high-risk population living in endemic areas
Vaccine uptake by age group/ risk group/general population	Information not available
Name, address/website of TBE National Reference Center	Republican Centre of Hygiene, Epidemiology and Public Health (Ministry of Health) of Belarus http://rcheph.by/en/

Age and gender distribution of TBE in Belarus: No data available

TBEV-isolation in Belarus: No data available

Appendix

Source data: Figure 2 — Prevalence of TBEV in ticks in Belarus in year 2012–2020, %

Year	Observed	Modeled Crude Rate
2012	9.8	9.55
2013	19.8	9.66
2014	15	9.78
2015	1.6	9.9
2016	9.4	10.03
2017	16.7	10.15
2018	8.7	10.28
2019	15.8	10.4
2020	10.2	10.53

Source data: Figure 2 — Annual Percent Change (APC) TBEV in ticks, 2012–2020

Segment	Lower Endpoint	Upper Endpoint	APC	Lower CI	Upper CI	Test Statistic (t)	Prob > |t|
1	2012	2020	1.2	-20.7	29.2	0.1	0.9
*Indicates that Annual Percent Change (APC) at significantly different from zero at a=0.05							

Source data: Figure 3 — Annual Percent Change (APC)

Segment	Lower Endpoint	Upper Endpoint	APC	Lower CI	Upper CI	Test Statistic (t)	Prob > |t|
1	2000	2020	6.4^	4.1	8.8	5.9	0
*Indicates that Annual Percent Change (APC) at significantly different from zero at a=0.05							

Source data: Figure 3 — TBE case numbers and incidence in Belarus in year 2000–2022

Year	Number of cases	Incidence/10^5
2000	23	0.2
2001	61	0.64
2002	18	0.2
2003	53	0.5
2004	44	0.4
2005	46	0.5
2006	108	1.1
2007	82	0.8
2008	66	0.7
2009	88	0.9
2010	91	1
2011	108	1.1
2012	122	1.3
2013	109	1.2
2014	119	1.3
2015	77	0.8
2016	141	1.5
2017	142	1.5
2018	135	1.4
2019	171	1.8
2020	108	1.1
2021	108	1.17
2022	No data	

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Citation:
Kniazeva V, Erber W, Janković TV. TBE in Belarus. Chapter 12b. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 6th ed. Singapore: Global Health Press; 2023. doi:10.33442/26613980_12b2-6

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Chapter 12b

TBE in Belgium

Marjan Van Esbroeck, Tinne Lernout, Vanessa Suin and Steven Van Gucht

E-CDC risk status: affected (data as of end 2022)

History and current situation

In 2018, the two first human tick-borne encephalitis (TBE) cases with possible/probable autochthonous infection were diagnosed at the National Reference Centre (NRC) of Arbovirus (The Institute of Tropical Medicine, Antwerp, Belgium). Every year, some imported cases of TBE are also detected, infected in other European countries such as Germany,[1] Scandinavia, Austria, Kyrgyzstan or Slovenia[2] and Russia.

Several seroprevalence/prevalence studies in sentinel animals and ticks have been performed in Sciensano. Seropositive dogs, cattle, roe deer, and wild boar have been found in Belgium.[3-7] Up till now, no positive tick has been detected in Belgium (on a total of ca. 1,600 ticks tested).[8]

1. Serum samples of Belgian dogs were obtained from 3 diagnostic laboratories in Northern Belgium (n=688) and Southern Belgium (n=192). All samples were taken by local veterinary surgeons between March 15, 2009 and June 22, 2009. ELISA-positive and borderline samples were subjected to a tick-borne encephalitis virus (TBEV) seroneutralization test. One dog was confirmed TBEV seropositive but the clinical history of the seropositive dog could not explain beyond doubt where and when TBEV infection was acquired.

2. Based on a targeted, risk-based sampling design, serological screening was performed on Belgian cattle (n=650), selected from the 2010 Belgian national cattle surveillance serum bank. All samples were subjected to a seroneutralization test. Seventeen bovines were seropositive and 6 had borderline results. The overall bovine seroprevalence in the targeted area was estimated between 2.61% and 4.29%. This confirmed for the first time the presence of TBE foci in wild animals in Belgium.

3. Roe deer sera collected between 2008 and 2013 (n=190) in Flanders were examined for antibodies against TBEV using a seroneutralization test. Seroprevalence was 5.1%.

4. As part of a Flemish wildlife surveillance in 2013, a serological screening was performed on sera from wild boar (*Sus scrofa*; n=238) in order to detect TBEV- specific antibodies by using a seroneutralization test. Ten wild boars were found to be TBEV-seropositive (2.9% of tested wild boars). This study demonstrated the presence of TBEV-specific antibodies in wild boar and highlighted potential TBEV- foci in Flanders.

The above studies in animals suggest that TBEV has been circulating for at least several years in Belgium (at a low level), and infections in humans were expected to occur. In 2018, two human cases of TBE were reported, in people that were possibly/probably infected in Belgium. However, since they also traveled abroad during the incubation period, the autochthonous origin could not be confirmed. In 2019, a seroprevalence study among 195 forestry workers in Flanders revealed that none had antibodies showing evidence of an acquired infection.[9]

Three confirmed autochthonous cases have been diagnosed in Belgium during summer 2020. The patients had been exposed in geographically separate regions of the country, two of which were adjacent to an area with known TBEV seropositivity in animals.[10] Two travel-associated TBE cases were diagnosed in 2021. One after travel to Austria while the other after travel to the Czech Republic.

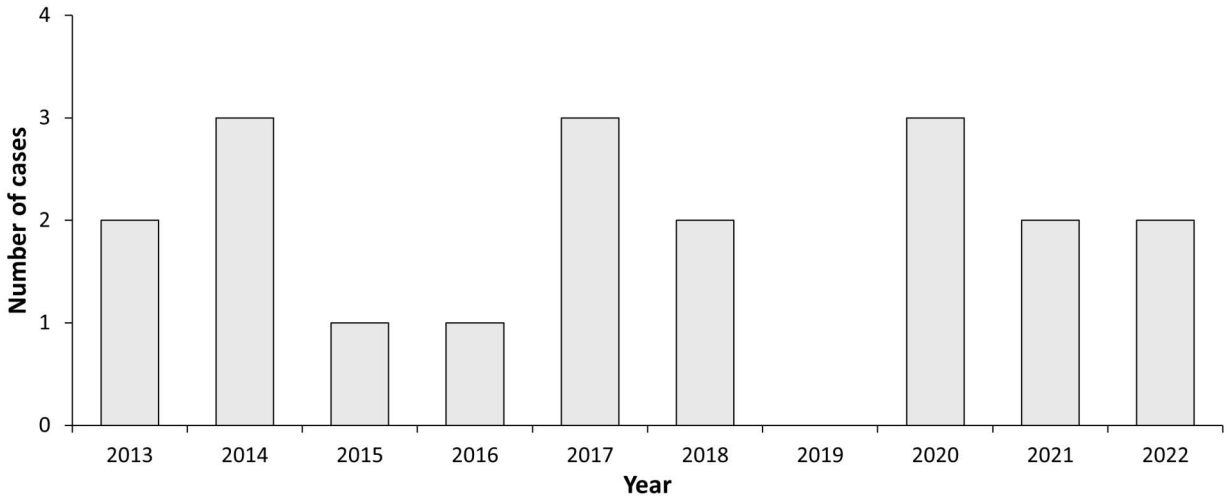
There were also two travel-associated TBE cases diagnosed in 2022. One after travel to Sweden and the other after travel to Slovenia. No autochthonous cases were reported in 2021 and 2022.

Based on the current epidemiological findings, Belgium is classified as an affected country for TBE.

Table 1: Virus, vector, transmission of TBE in Belgium	
Viral subtypes, distribution	No information available in humans yet. No virus-positive animals or ticks have been reported to date.
Reservoir animals	Rodents: To date, no rodents seropositive for TBEV have been found in Belgium (Study realized by Sciensano in 2014, not published). Seropositive cattle, roe deer, and wild boar have been identified (cattle in the Wallonia region and wild boar in Flanders).[3-7]
Infected tick species (%)	No positive ticks have been detected (Sciensano project in 2018 – RNA detection, Institute of Tropical Medicine (ITM) project in 2018 – RNA detection).[8]
Dairy product transmission	No information available

Table 2: TBE reporting and vaccine prevention in Belgium	
Mandatory TBE reporting	None
Other TBE surveillance	1. A national reference center (NRC) for TBE has been established since 2011. This center performs laboratory confirmation in suspected human cases and reports to Sciensano. a. From 2011 to 2015: Sciensano, Brussels, Belgium b. From 2016 to 2020: ITM, Antwerp, Belgium c. From 2021 to 2025: ITM, Antwerpen, Belgium 2. Human surveillance via NRC a. From 2011 to 2015: Sciensano, Brussels, Belgium b. From 2016 to 2020: ITM, Antwerpen, Belgium c. From 2021 to 2025: ITM, Antwerpen, Belgium 3. Animal surveillance (2011 to present): Sciensano, Brussels, Belgium.[3-7] 4. Tick surveillance (2018 to present): ITM, Antwerp, Belgium and Sciensano, Brussels, Belgium. Case definition as described in https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:262:0001:0057:EN:PDF. Confirmed case: symptoms of TBE and immunoglobulin M (IgM) and/or ribonucleic acid-positive.
Special clinical features	No information available
Available vaccines	FSME-IMMUN (purchased from Baxter by Pfizer in 2014)
Vaccination recommendations and reimbursement	In 2019, the Belgian Superior Health Council published recommendations for 3 different epidemiological situations. In the current situation (sporadic cases possible), vaccination is only recommended for travelers to endemic regions doing outdoor activities in forested areas (such as hiking, camping, mushroom picking, etc.) during the tick season (spring, summer and autumn), and for people handling TBEV in a laboratory setting.[11]
Vaccine uptake by age group/risk group/general population	No data available
Name, address/website of TBE NRC	ITM, Antwerpen, Belgium, www.itg.be

Figure 1: Burden of TBE in Belgium over time



Source Data: Appendix—Figure 1

Appendix

Source data: Figure 1

Year	Number of cases
2013	2
2014	3
2015	1
2016	1
2017	3

Year	Number of cases
2018	2
2019	0
2020	3
2021	2
2022	2

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TBE in Bosnia and Herzegovina

Wilhelm Erber and Tamara Vuković-Janković

E-CDC risk status: affected (very limited data available)

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]

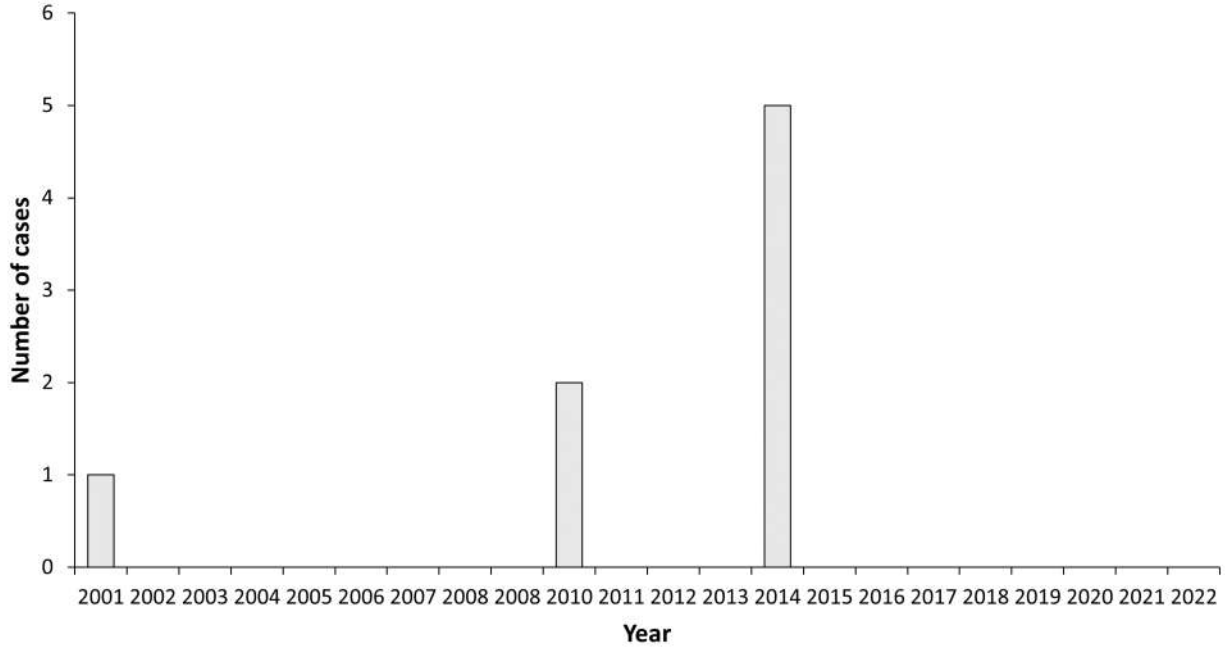
The only official TBE case report data so far are from the Centralized Information System for Infectious Diseases ([CISID] – WHO: incidence of tick-borne encephalitis) where 1 case was reported in 2001, and 2 cases were reported in 2010, and additionally 5 cases of alimentary outbreak were reported in 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	

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Chapter 12b

TBE in Bulgaria

Iva Christova

E-CDC risk status: endemic (lack of consistent testing and reporting)

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 resulted in the isolation of 8 TBEV strains among 6,849 ticks investigated.[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 case in 2021.

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 either with tick bites or consumption of unpasteurized milk.

Overview of TBE in Bulgaria

Table 1: Virus, vector, transmission of 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

Table 2: TBE reporting and vaccine prevention in Bulgaria	
Mandatory TBE reporting	TBE reporting is mandatory since 2014. Both physicians and laboratory report. EU case definitions for confirmed, probable, and possible TBE case are accepted.
Other TBE surveillance	No
Special clinical features	Biphasic disease
Available vaccines	No information available
Vaccination recommendations and reimbursement	No
Vaccine uptake by age group / risk group / general population	No information available
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

Case reporting is sporadic, and thus incidences cannot be calculated. Here only microbiologically confirmed cases are mentioned.

Case numbers by year are listed in the appendix.

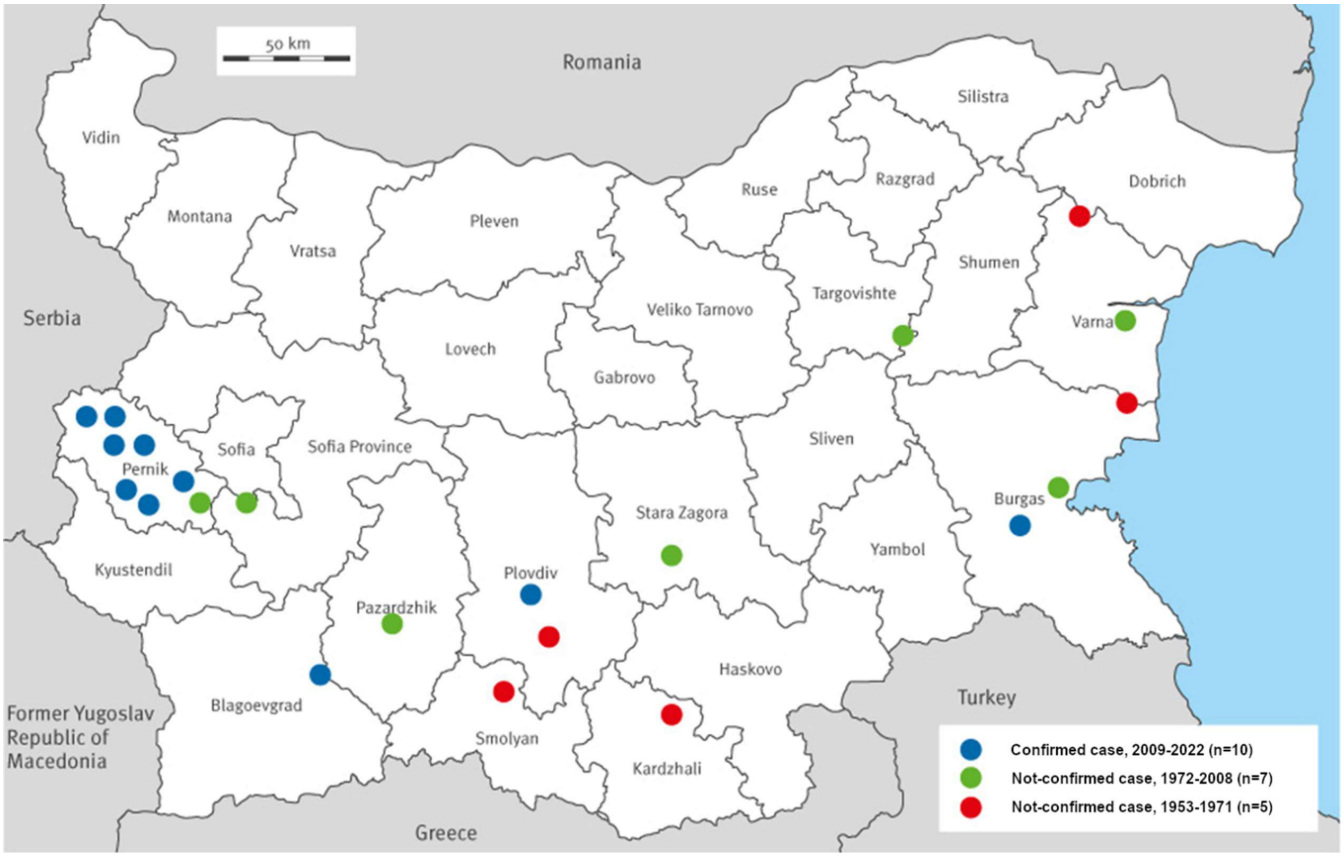
n.c. = not calculable

Source Data: Appendix—Figure 1

Figure 2: 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 3: Place of residence of reported TBE cases in Bulgaria, 1953–2022



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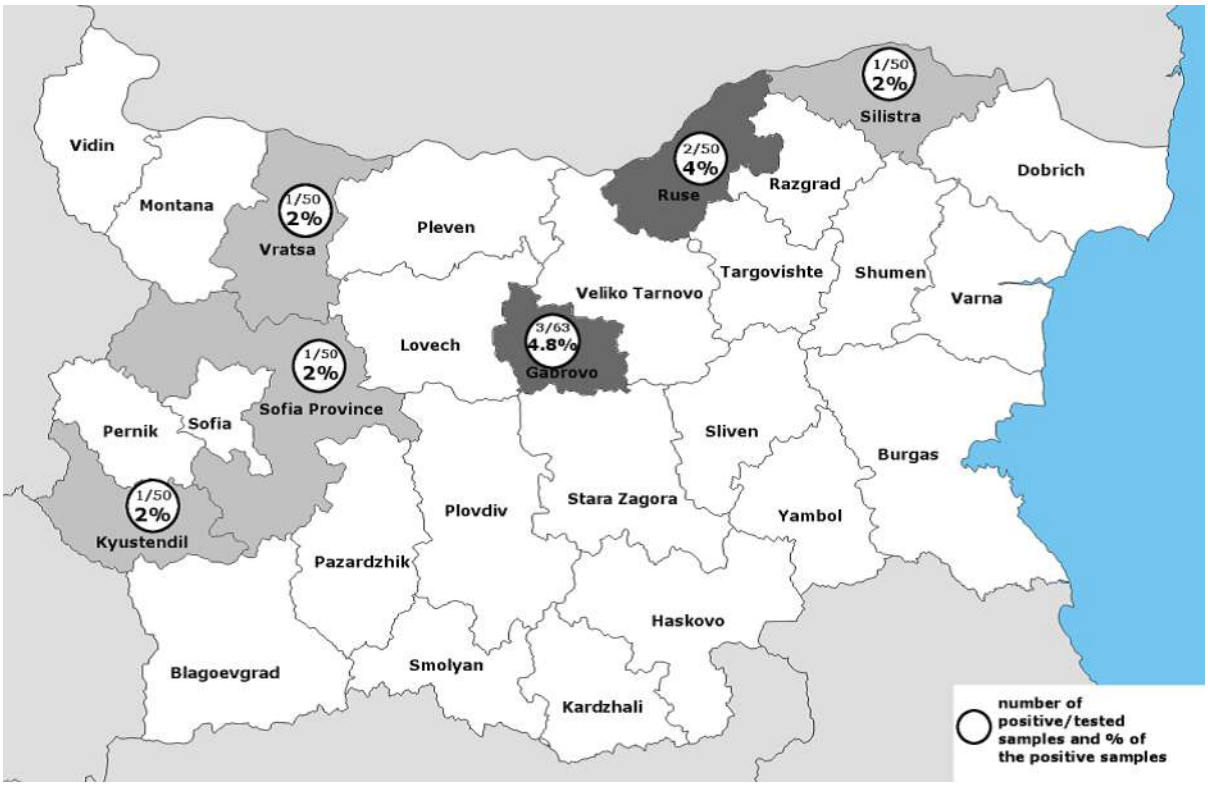
Christova I. TBE in Bulgaria. Chapter 12b. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 6th ed. Singapore: Global Health Press; 2023. doi:10.33442/26613980_12b5-6

Figure 4: 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.

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Chapter 12b

TBE in China

Yang Junfeng and Heinz-Josef Schmitt

E-CDC risk status: endemic (no new data available as of May 2023)

History and current situation

The first TBE patients in China were reported in 1943, and the TBEV was isolated from the brain tissues of 2 patients in 1944 by Japanese military scientists,[1] and from patients and ticks (*Ixodes persulcatus* and *Haemaphysalis concinna*) in 1952 by Chinese researchers.[2] The Far Eastern viral subtype (TBEV-FE) is the endemic subtype that has been isolated from all 3 known natural foci (northeastern China, western China, and southwestern China).[14] Recently a new "Himalayan subtype" of the TBEV (TBEV-HIM) was isolated from wild rodent *Marmoata himalayana* in the Qinghai-Tibet Plateau.[15] One recent report suggests that the TBEV-SIB is prevalent in the Uygur region (North West China).[13] The main vector of the TBEV in China is *I. persulcatus*.[3] Epidemiological modelling indicates that the TBEV may occur even widely all over China (Figure 3).[4] Likely, the disease is often missed by clinicians due to a lack of the availability of specific diagnostic assays.[16]

Serological research has demonstrated that there are high numbers of human TBEV-infections in the 3 foci mentioned above. However, TBE patients are mainly reported from northeastern China, including Inner Mongolia Autonomous Region (Daxing'an Mountains), Heilongjiang Province (Xiaoxing'an Mountains), and Jilin Province (Changbai Mountains). In a recent report 803 cases including 4 deaths were reported from the Jilin Province with most cases from the Changbai Mountains and neighboring areas.[17] Interestingly, 61.5% of patients were farmers and this is different from previous reports where soldiers and forest workers made up the majority of patients. The most recent publication of the Chinese CDC[18] reports 3,364 TBE cases in China between 2007 and 2018 (incidence 0.09 – 0.44/10^5). Overall only 14%–84% of cases were laboratory confirmed. Given the extremely high percentage of TBE-infected ticks as well as the high seroprevalence in humans (Table 1), TBE numbers may be hugely underreported. Patients also were reported from another important epidemic area: the Tianshan Mountains and Altai Mountains of the Xinjiang Autonomous Region.[4]

Despite the small geographic distribution, the whole belt that connects the 3 above-mentioned foci is considered to be at risk (E-CDC status: predisposed) for occurrence of TBE if the virus is imported, including a few densely populated regions such as Beijing, Shaanxi, and Sichuan provinces, where the environment could be suitable for circulation of TBEV (see Figure 3). In addition, cases may be missed in regions with lower TBE incidences due to low rates of serological testing and lack of awareness among both physicians and the general population.[15]

The incidence of TBE decreased in China during the 1980s, but has been rising in recent years, as noted by disease control and prevention authorities and local hospitals.[4] TBE patients were mainly forest workers before the 1980s, however, it has been reported that changes in the occupation / type of "exposure risk" occurred among TBE patients since the 1980s 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 endemic forest areas.[5]

Table 1: Virus, vector, transmission of TBE in China

Viral subtypes, distribution	Far Eastern TBEV subtype (TBEV-FE)
Reservoir animals	Mice and insectivorous animals; migratory birds; lagomorphs, goats[6]
Infected tick species (%)	*I. persulcatus*; however, TBEV has also been isolated from *Haemaphysalis concinna*, *Haemaphysalis japonica*, *Dermacentor silvarum*, and *Ixodes ovatus*[5]
Infection rate among the ticks	13.0%–14.3%, 0.79%–6.45%, and 0%–37.5% in northeastern China; 14.3%–47.7% in northwestern China; 8.3% in southwestern China[4]
Dairy product transmission	Not known
Serological infection rate in healthy people	19.7% in southwestern China,[6] 35.4% in northwestern China,[7] 0%–10.9%,[8] 0%–9.8%,[9] 7.6% in northeastern China[10]

Table 2: TBE reporting and vaccine prevention in China	
Mandatory TBE reporting	Heilongjiang Province only
Other TBE Surveillance	No
Special clinical features	Biphasic disease not reported in China Different symptoms among patients with different disease severities In the early 1950s, case fatality rate (CFR) of TBE in the northeastern forest areas was over 25%, but since the 1980s it has decreased to around 8%.[1,2,11] 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.[2] Recently, the complications of TBE over a 10-year period reported to be 16.6% (90/542.)[12]
Available vaccines	TaiSenBao produced in China with Sen-Zhang strain as seed strain in primary hamster kidney (PHK) cells
Vaccination recommendations and reimbursement	No
Vaccine uptake by age group/risk group/general population	No information available, estimated to be low
CFR	25% in 1950s, and decreased to <10% after 1980s[1,2,11]
Name, address/website of TBE National Reference Center	Chinese Center for Disease Prevention and Control: http://ivdc.chinacdc.cn/

Figure 1: Reported TBE cases in China 2007–2018[18]

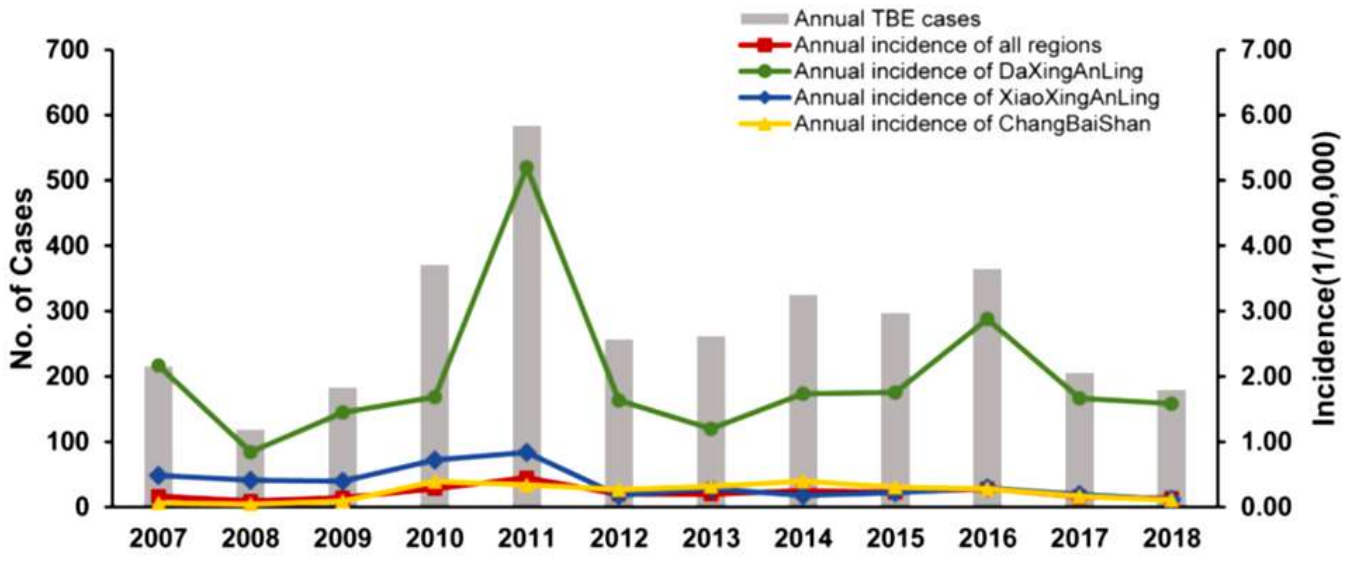


Figure 2: Gender-related distribution of TBE cases in China, 2007–2018[18]

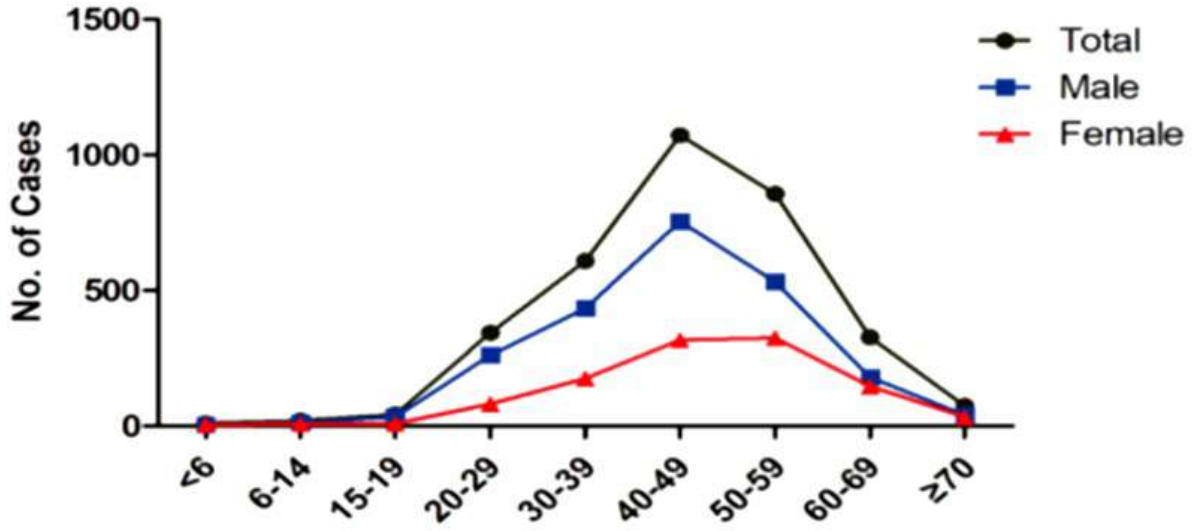


Figure 3: Distribution of the occupation of TBE patients over time in China, 2007–2018[18]

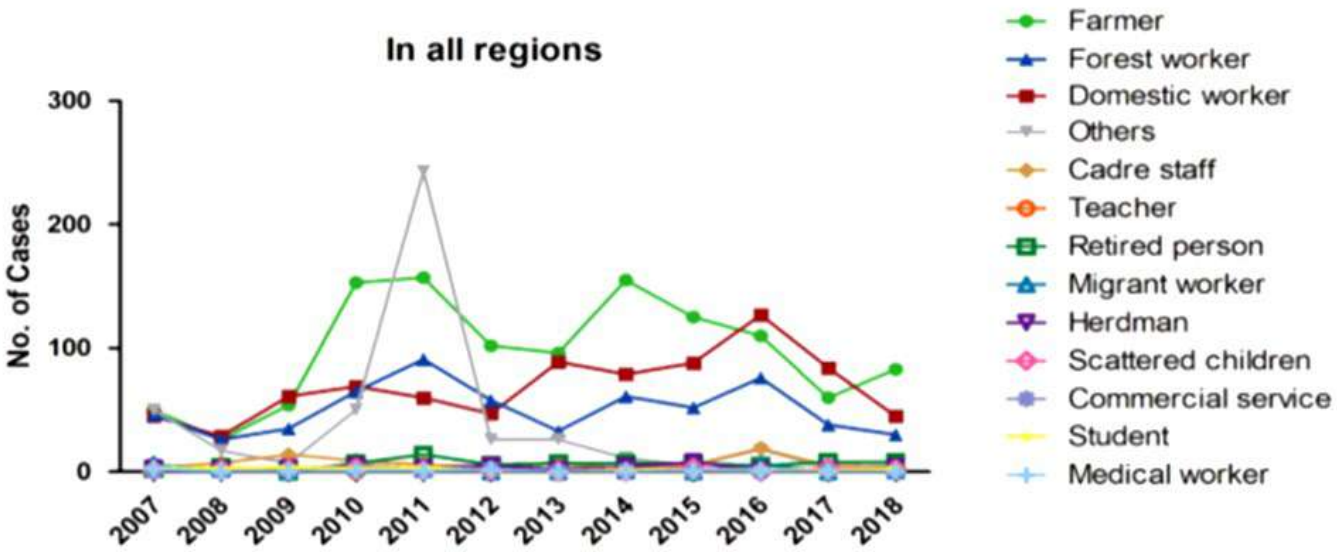
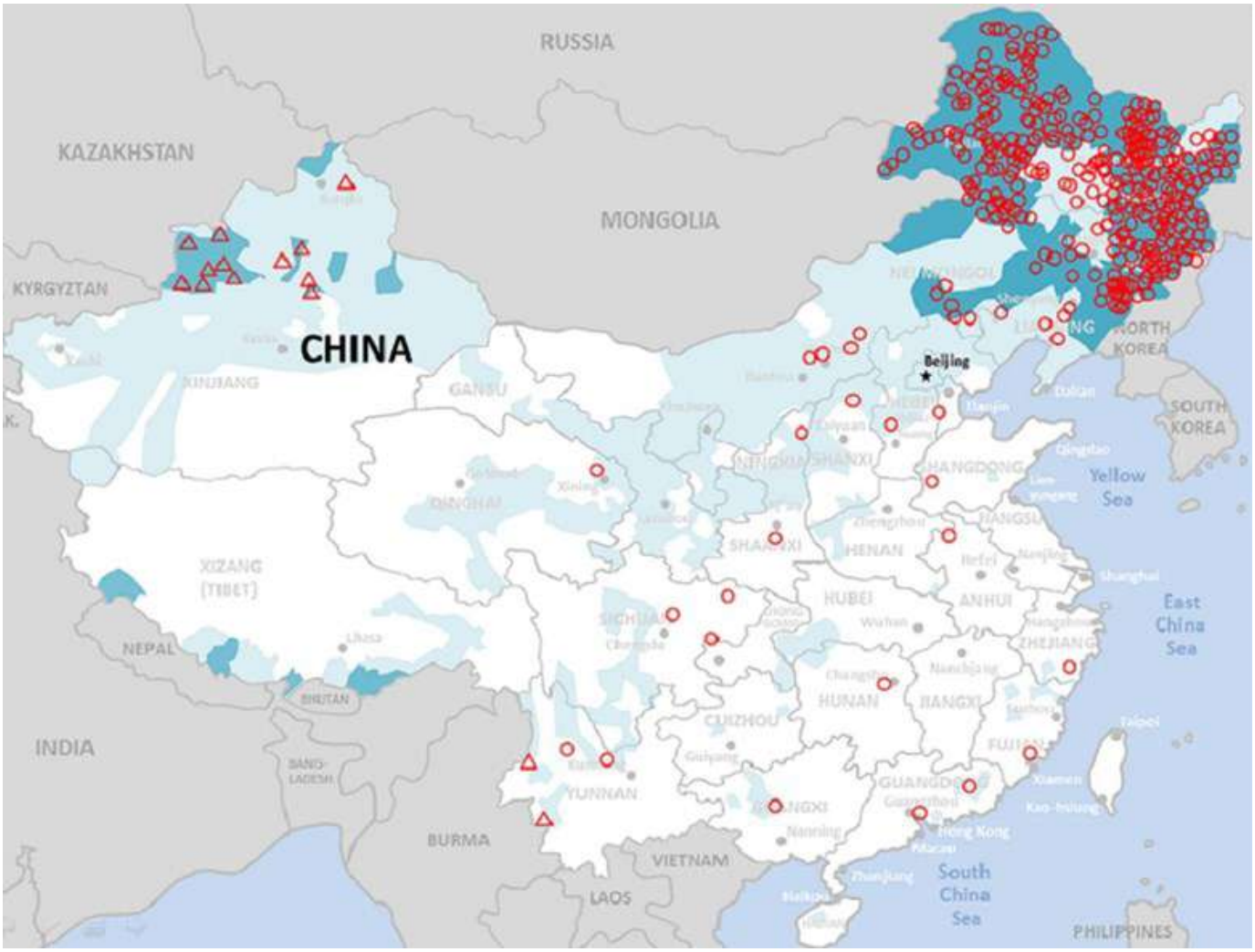


Figure 3. Geographic distribution of TBE in China as reported in published literature[4,5]



○ *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 criteria as published by Sun et al. 2017*[4]

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TBE in Croatia

Wilhelm Erber and Tamara Vuković-Janković

E-CDC risk status: endemic (data as of end 2022)

History and current situation

Even though TBE has been a notifiable disease in Croatia since 2007, there are no or only limited data available on the occurring tick species in the endemic areas, on the prevalence of TBE virus (TBEV) in ticks, its distribution in Croatia, and its genetic characteristics. Reporting of human cases also is very scarce. The Central European subtype of virus (TBEV-EU) appears to be present in Croatia.

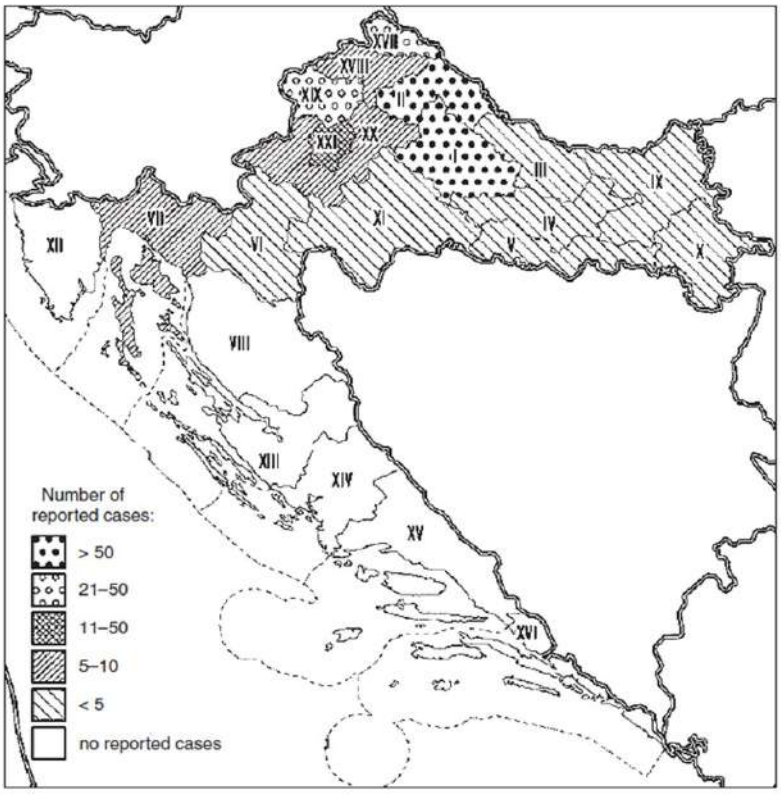
Natural foci of TBE have been found so far in the continental area in the northwestern region (between the Sava River and the Drava River, near Zagreb, Varaždin, Bjelovar, Koprivnica, Križevci, and Vinkovci – with an area around Slavonski Brod) and in the northeastern region (across a small area on the western outskirts of Osijek city).

Recently, 2 new natural foci have emerged in the central mountainous region, south of the Sava River. Cases are reported only sporadically in the Adriatic coastal region.[1]

A recent study found a prevalence of TBEV similar to other European countries (0.1%–5%) in ticks removed from red foxes in Varazdin County and Zagreb County (in the vicinity of Medvednica mountain), both well known as TBE areas. Furthermore, a viral prevalence of 1.1% (95% CI: 0.3%–3.0%) has been found in red deer (*Cervus elaphus*) from 2 areas in northeastern Croatia (Vukovar-Srijem County and Osijek-Baranja County). The latest human TBE cases from these 2 counties were recorded in 2009 (1 case) and 2010 (1 case), respectively.[2]

An average of 20 human cases of TBE is reported each year (minimum 11, maximum 45)[3,4] i.e., 0.26–1.05 cases/100,000 persons. The majority of cases were registered in the Koprivnica-Križevci County (average annual incidence 5.2/100,000), Međimurska County (5/100,000), and Bjelovar-Bilogora County (4.3/100,000). The average incidence rate in the city of Zagreb, within the observed period, was 0.2/100,000 (16 cases registered). In 2015, the first outbreak of TBE after consumption of raw goat milk was reported in 7 out of 10 exposed persons.[5]

Figure 1: Geographical distribution of TBE by counties of the Republic of Croatia (1999–2008)[3]

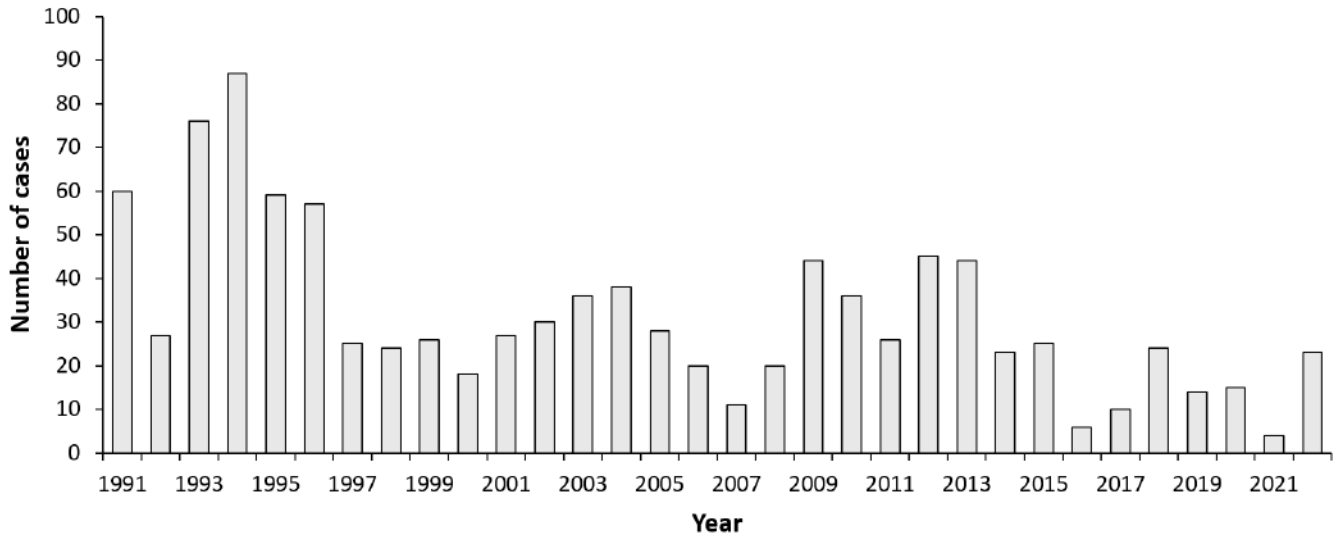


Overview of TBE in Croatia

Table 1: Virus, vector, transmission of TBE in Croatia	
Viral subtypes, distribution	European subtype (TBEV-EU)[2,3]
Reservoir animals	Rodents
Infected tick species (%)	Information not available
Dairy product transmission	In 2015, a small outbreak of TBE affecting 7 people from the region of Bjelovar after consuming fresh goat's milk and cheeses.[5] In 2019, 5 patients were reported to have consumed raw goat milk from the same farm in the Gorski Kotar region.[6]

Table 2: TBE reporting and vaccine prevention in Croatia	
Mandatory TBE reporting	TBE has been an obligatory reportable disease in Croatia since 2007[1]
Other TBE surveillance	Not applicable
Special clinical features	Information not available
Available vaccines	FSME IMMUN[7]
Vaccination recommendations and reimbursement	Only recommended for residents in endemic areas and those visiting endemic areas (for recreation), as well as forestry workers in the Koprivnica-Križevci region[1]
Vaccine uptake by age group/ risk group/general population	Year / Number of vaccinated individuals in Zagreb[8] 2010 / 670 2011 / 678 2012 / 781 2013 / 577 2014 / 415
Name, address/website of TBE National Reference Center	National Institute of Public Health of Croatia https://www.hzjz.hr/en/

Figure 2: Burden of TBE in Croatia over time[8-9]



Source Data: Appendix—Figure 2

Age and gender distribution of TBE in Croatia: no available data

Appendix

Source data: Figure 2

Year	Number of cases	Incidence / 10^5
1991	60	1.4
1992	27	0.6
1993	76	1.8
1994	87	2.1
1995	59	1.4
1996	57	1.4
1997	25	0.6
1998	24	0.6
1999	26	0.6
2000	18	0.4
2001	27	0.6
2002	30	0.7
2003	36	0.9
2004	38	0.9
2005	28	0.7
2006	20	0.5
2007	11	0.3
2008	20	0.5
2009	44	1.1
2010	36	0.9
2011	26	0.6
2012	45	1.1
2013	44	1.1
2014	23	0.5
2015	25	0.6
2016	6	0.1
2017	10	0.2
2018	24	0.6
2019	14	0.3
2020	15	0.4
2021	4	0.1
2022	23	0.6

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Chapter 12b

TBE in the Czech Republic

Petr Pazdiora

E-CDC risk status: endemic (data as of end 2022)

History and current situation

The TBE virus (TBEV) was first isolated in the Czech Republic by Czech scientists 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 TBE virus. 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 TBE virus 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 TBE virus.

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 occupational transmission has decreased over the last years (in 2022: 20 cases, i.e., 2.8% among foresters and farmers). Numbers of imported cases from abroad are very low with only 1 case (0.2%) in 2021 and 5 cases (0.7%) in 2022. 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.

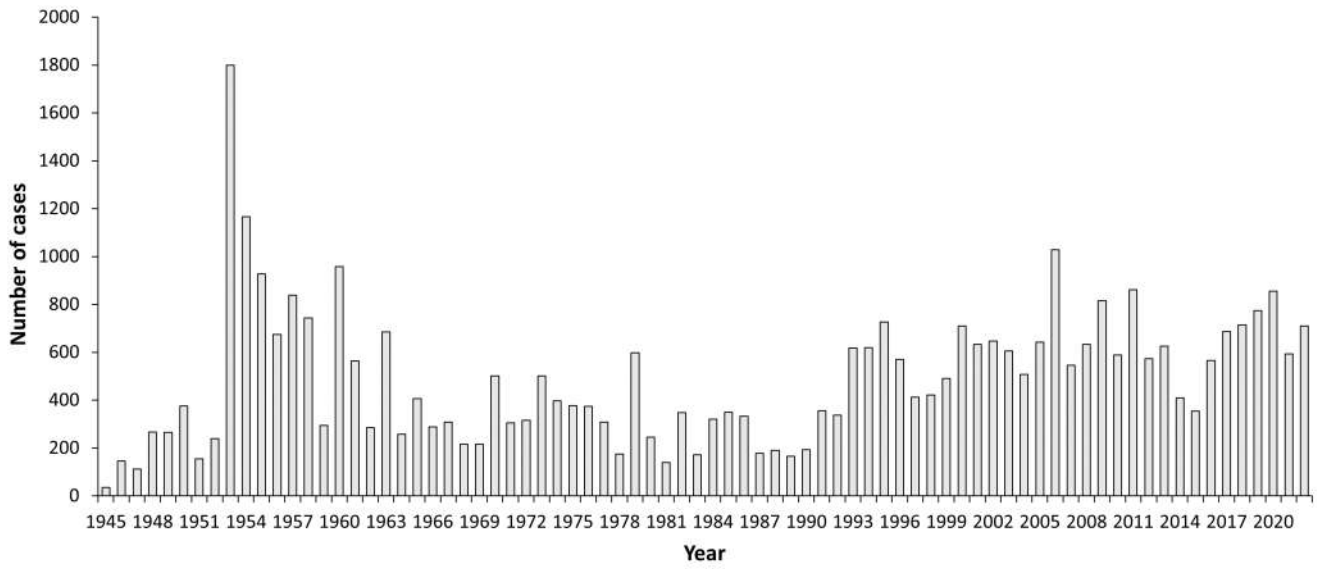
Vaccine uptake is very low, the highest rate is reached in the age group of 18–24-year-olds, the lowest among children younger than 4 years; however, there is no central vaccination registry. Data from 8 international telephone surveys in 2009, 2013, 2015, 2018, 2019, 2020, 2021, and 2022 which covered 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 and 38%, respectively. Unpublished data from some Czech regions indicate that vaccine uptake with ≥3 doses is even lower.

Table 1: Virus, vector, transmission of TBE in the Czech Republic

Viral subtypes, distribution	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]
Indicator animals	*Capreolus capreolus, Cervus elaphus, Sus scrofa, Canis lupus, Oeservis ammon, Bos taurus, Capra aegagrus hircus*[15]
Infected tick species (%)	1970–2022: 156/127,579 (0.122%)[16]
Dairy product transmission	Rare: 1997–2008: 0.9%[11]; 1993–2019: 3.4%[18]; 2022: 0.4%[14] Children and adolescents (1993–2019): 6.8%[17]

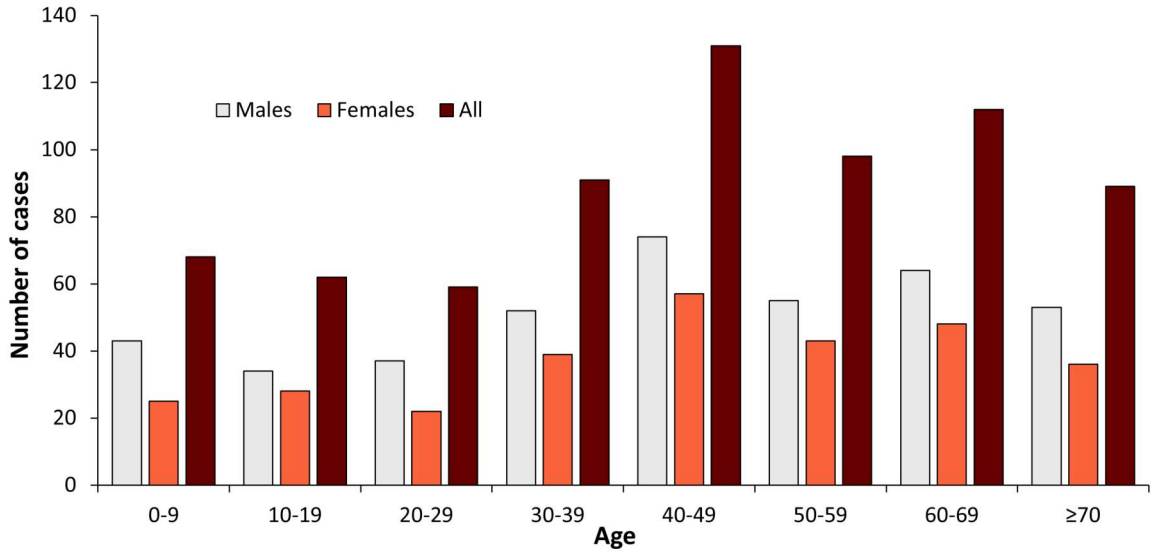
Table 2: TBE reporting and vaccine prevention in the Czech Republic	
Mandatory TBE reporting	Each case of TBE is reported by the diagnosing physicians to the respective public health authorities after obtaining positive findings from biological specimens. Only confirmed cases on the basis of clinical and lab criteria are reported.[1]
Other TBE surveillance	No information available
Special clinical features	Biphasic disease: 1994–1997: 80%[15] Children and adolescents (1993–2012): 58%[10] Risk groups: no information available % with sequelae: children and adolescents (1993–2012): 3%[10] Mortality: case fatality rate (1960–2019): 0.79%[17]; (1970–2008): 0.55%[12]; (2018–2022): 0.55%[14] Children and adolescents (1960–2019): 0.2%[17]
Available vaccines	FSME-IMMUN (Baxter, Pfizer) since 1990. Encepur (Bavarian Nordic) since 1996. Doses sold: No information available
Vaccination recommendations and reimbursement	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 by age group/risk group/ general population	No valid nationwide information available, results from telephone surveys in 2009, 2013, 2015 2018, 2019, 2020, 2021 and 2022 indicate a vaccine uptake in the general population of 16, 23, 24, 25, 29, 33, 33 and 38%[2-9]
Name, address/website of TBE National Reference Center	National Reference Laboratory for arboviruses, Public Health Institute of Ostrava, Partyzánské nám. 7, 702 00 Ostrava https://zuova.cz/Home/Page/NRL-arboviry[16]

Figure 1: Burden of TBE in the Czech Republic over time[14]



Source Data: Appendix—Figure 1

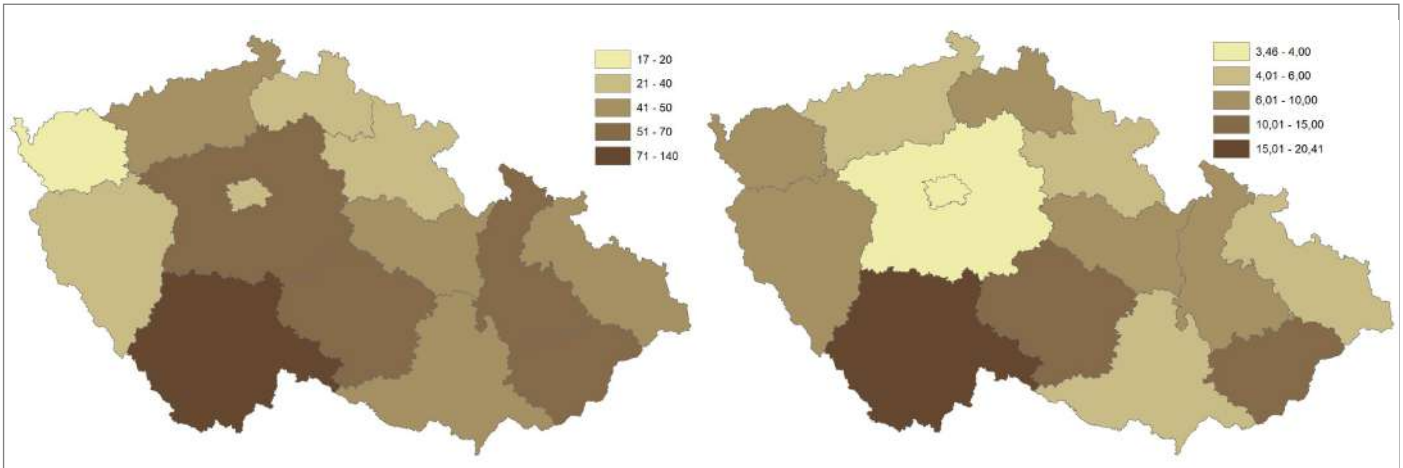
Figure 2: Age and gender distribution of TBE in the Czech Republic (2022)[14]



Source Data: Appendix—Figure 2

Figure 3a: Probable TBE cases transmitted in individual regions of the Czech Republic (2022)

Figure 3b: Incidences (per 100,000) of TBE in the individual regions of the Czech Republic (2022)



Regional data according to cases and viral isolation from ticks are not available.
The first map shows the numbers of probable TBE cases and the second the incidences of TBE by region.

Note: Readers may also wish to review the accompanying chapter for Slovakia, given the geographic proximity and national history of these countries. Author's note: Evidence of reported cases in Czechoslovakia cover the period 1945–1992; cases have been tracked independently in Slovakia since 1993.

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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
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
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

Year	Number of cases	Incidence/ 10^5
1984	320	3.16
1985	350	3.44
1986	333	3.22
1987	178	4.81
1988	191	1.84
1989	166	1.6
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
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
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

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

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Chapter 12b

TBE in Denmark

Anders Fomsgaard

E-CDC risk status: endemic (data as of end 2022)

History and current situation

Since the 1950s, tick-borne encephalitis (TBE) has been known to be endemic in Denmark, but only on the island of Bornholm. Bornholm is situated east of mainland Denmark, south of Sweden (Fig. 3) and has a different fauna and flora from the rest of Denmark. Bornholm has about 45,000 inhabitants, but about 500,000 tourists visit the island every year.

Freundt carried out a serosurvey 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 rediscovered 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%) got permanent sequelae. In addition, 32 forest workers on Bornholm were tested in 2000, and 20% had IgG antibodies but never 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 (Fig. 1).

In 2009, we succeeded in identifying a TBEV microfocus in a small forested area, Tokkekøb Hegn on Zealand just north of Copenhagen, which had a severe TBE case reported.[6] A forest worker was infected in his backyard in Tokkekøb. The location is a small, open grass field bordering a lake and with a deer path. The patient had not been traveling. The patient described a similar case of encephalitis in 2008, when another man working in the forest kindergarten just 500m away from the forest worker got tick bites at the same spot. Both subjects had a typical biphasic disease and TBE was diagnosed.[6] Both experienced persistent neurological sequelae, paralysis of one arm (both patients) and neuropsychiatric complications (one patient).

TBEV European (Western) sub-type (TBEV-E) was identified in 2009 in *I. ricinus* tick adults and nymphs from the location identified by both patients (the "smoking gun principle").[6]

In July and September 2011, TBEV-Eu was again identified (endemic) in adults and nymphs at Tokkekøb, and TBEV isolated (isolates T2 and T3). The virus sequence grouped with isolates from Sweden-Norway, whereas one Bornholm TBEV from 2012 grouped into a different subclade from South and Central Bohemia.[7]

A recent (2018) sequenced TBEV isolate from Bornholm (lake Rubinsøen) grouped with TBEV from Switzerland and Finland.[10] Whereas some TBEV microfoci may contain TBEV unchanged for decades (Finland), other foci in Denmark may merely provide permissive conditions for random and repeated TBEV introductions from various geographical locations. 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–September 2010) and 96 hospitalized encephalitis patients after tick bites (2007–2009), who were negative for *Borrelia,* all tested negative for TBE antibodies.[7]

This supports a limited TBEV introduction into the new microfocus. Serological testing of roe deer "sentinels"[8] and computer predictions[9] suggest TBEV outside Bornholm. But no other TBE cases have occurred in Denmark outside Bornholm from 2009–2017, and recent flaggings for ticks (September, October 2016 and June, July 2017) from the Tokkekøb microfocus were negative for TBEV.[11] Yearly flaggings will continue, but we believe that the activity of the Tokkekøb microfocus has ended. All this changed by the hot summer in 2018 where 3 cases of TBE occurred, of which two were infected outside Bornholm: in Jutland (north of Esbjerg) and Fuen (near Faaborg), respectively. Moreover, the clinical manifestation of one of these was atypical, showing meningoradiculoneuritis rather than encephalitis.[12]

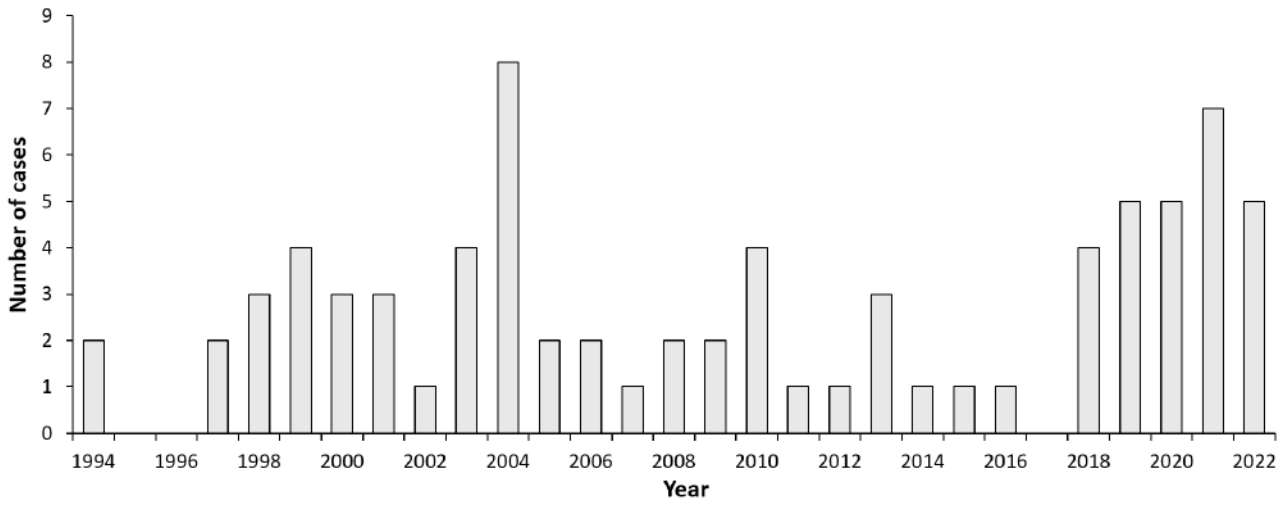
In June–July 2019 four cases (one case appeared during the publication of the three) were hospitalized, infected in the same wood area Tisvilde Hegn in Northern Zealand, bordering a playground. A new micro-focus was identified with a very high prevalence of 8% and only in nymphs. Whole genome sequencing showed clustering with a TBEV from Norway.[13]

In 2022, Denmark had 5 hospitalized cases of typical TBE diagnosed at Statens Serum Institut, 3 males and 2 females ranging between 31–73 years of age. Only one of the five cases was from Bornholm island and the rest were infected in North Zealand.

Overview of TBE in Denmark

Table 1: Virus, vector, transmission of TBE in Denmark	
Viral subtypes, distribution	TBEV European (Western) subtype[7]
Reservoir animals	Roe deer[8]
Infected tick species (%)	2%[4]
Dairy product transmission	Not documented

Figure 1: Burden of TBE in Denmark over time[3,5,6]



Source Data: Appendix—Figure 1

One of the TBE cases in 2008 and one in 2009 were infected in Tokkekøb microfocus;[6]
all others were infected on Bornholm Island, Denmark.

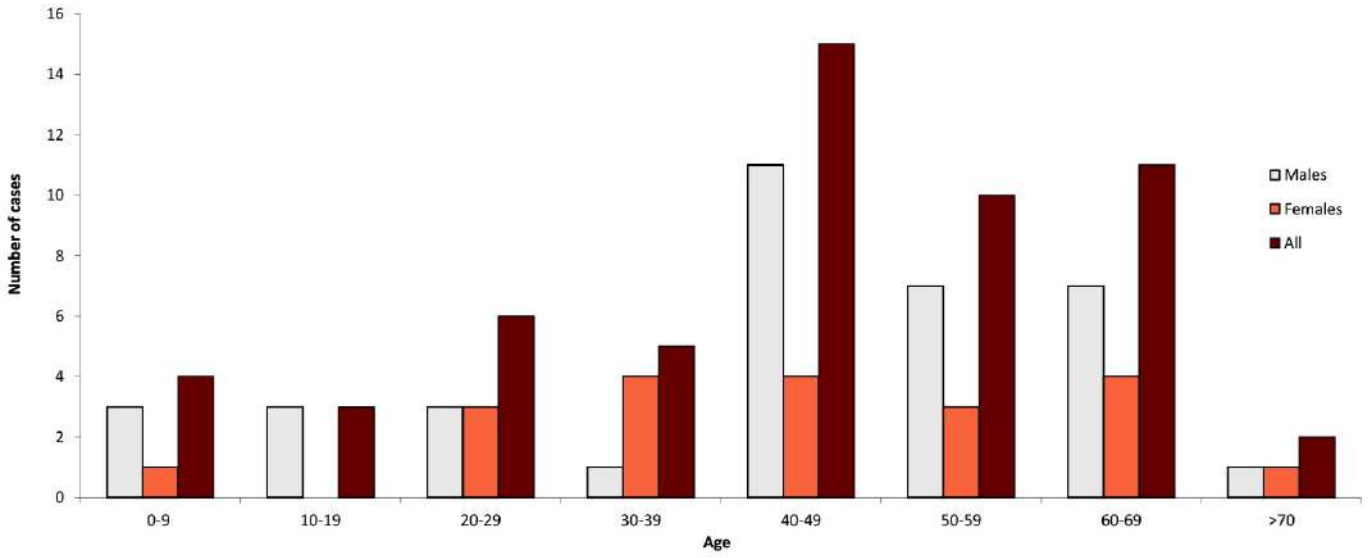
According to the Danish legislation, TBE is not a notifiable disease. However, since the SSI in Copenhagen performs centralized diagnostic testing, a line-item list is compiled for laboratory confirmed cases (since 2001).

Table 2: TBE reporting and vaccine prevention in Denmark	
Mandatory TBE reporting	TBE is not a notifiable disease in Denmark (DK) and there is no mandatory reporting
Other TBE surveillance	Statens Serum Institut (SSI) does the centralized TBE diagnostic in DK and compiles line-lists of confirmed cases
Special clinical features	Biphasic disease. Risk groups are people that regularly spend time in woods outside paths in areas where TBE is endemic (Bornholm)
	37.7% with permanent complications. No TBE deaths are registered in Denmark[3]
Available vaccines	Ticovac (Pfizer)
Vaccination recommendations and reimbursement	In 2001, the Danish Health Authorities recommended TBE vaccination for a defined at-risk population in Bornholm In 2009, the recommendations allowed reimbursement to regular visitors in endemic areas in DK[3,5]
Vaccine uptake by age group/risk group/general population	Unknown*
Name, address/website of TBE National Reference Center	Dept. Virology & Microbiology Diagnostic, Statens Serum Institut, 5 Artillerivej, DK-2300 Copenhagen S, Denmark (www.ssi.dk)

**In 2001, the Danish Health Authorities recommended TBE vaccination for a defined at-risk population in Bornholm. The vaccine coverage is not known, but starting in 2015 a prospective registration of all vaccines is mandatory in Denmark, which will clarify these issues.*

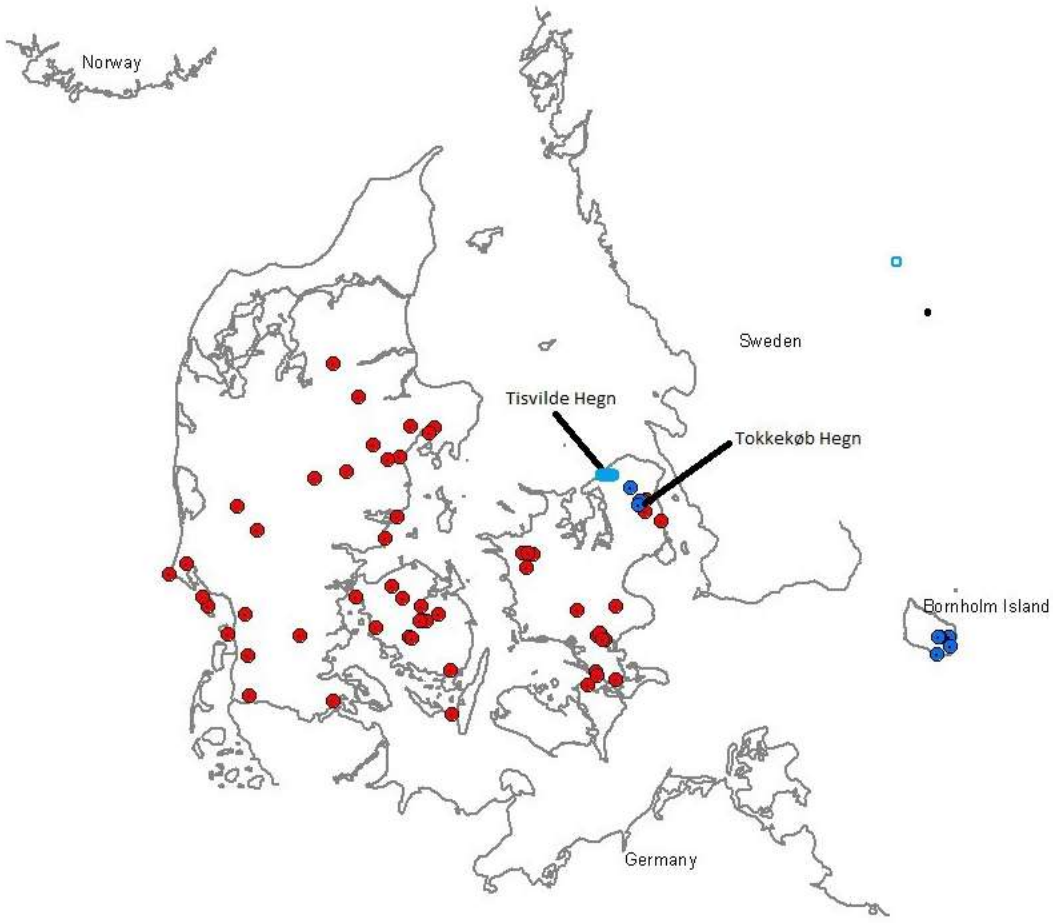
Figure 2: Age and gender distribution of TBE in Denmark[3,5,6]

Denmark 1994–2000 (14 cases) + 2001–2015 (38 cases)



Source Data: Appendix—Figure 2

Figure 3: TBEV-isolation and TBE cases in Denmark[7]



Isolate TBEV European (Western) subtype, T2; Tokkekøb Hegn, North Zealand[7]

Figure of Denmark showing endemic Bornholm and the TBEV microfocus Tokkekøb Hegn, North Zealand (TBEV isolate T2, 2011) and Tisvilde Hegn (2019); red dots indicate tick sampling from animals, blue dots indicate flagging.[7]

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	

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	3	1	4
10-19	3	0	3
20-29	3	3	6
30-39	1	4	5
40-49	11	4	15
50-59	7	3	10
60-69	7	4	11
>70	1	1	2

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Citation:
Fomsgaard A. TBE in Denmark. Chapter 12b. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 6th ed. Singapore: Global Health Press; 2023. doi:10.33442/26613980_12b9-6

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Chapter 12b

TBE in Estonia

Kuulo Kutsar

E-CDC risk status: endemic (data as of end 2022)

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.

Overview of TBE in Estonia

Table 1: Virus, vector, transmission of 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 (%)	*I. persulcatus* 3.8%, *I. ricinus* on mainland 0.6%–0.8% and on Saaremaa island 3.0% (data from 2011)
Dairy product transmission	Documented but rare

Table 2: TBE reporting and vaccine prevention in Estonia

<table>
<tr><td>Mandatory TBE reporting</td><td>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</td></tr>
<tr><td>Other TBE surveillance</td><td>Laboratory and epidemiological surveillance</td></tr>
<tr><td>Special clinical features</td><td>Biphasic disease: meningitis, meningoencephalitis, or meningoencephalomyelitis.
Risk groups: people who often spend time outdoors (in nature)</td></tr>
<tr><td>Available vaccines</td><td>TBE vaccination by age groups in Estonia, 2018–2021

<table>
<tr><th></th><th>1–14 years</th><th>15–17 years</th><th>Adults</th><th>Population (Estonia)</th></tr>
<tr><td>2018</td><td></td><td></td><td></td><td>1,319,133</td></tr>
<tr><td>Vaccination</td><td>5,717</td><td>1,123</td><td>10,567</td><td></td></tr>
<tr><td>Revaccination</td><td>4,374</td><td>1,618</td><td>17,997</td><td></td></tr>
<tr><td>2019</td><td></td><td></td><td></td><td>1,324,820</td></tr>
<tr><td>Vaccination</td><td>8,253</td><td>897</td><td>16,817</td><td></td></tr>
<tr><td>Revaccination</td><td>4,181</td><td>1,324</td><td>17,856</td><td></td></tr>
<tr><td>2020</td><td></td><td></td><td></td><td>1,328,889</td></tr>
<tr><td>Vaccination</td><td>8,344</td><td>845</td><td>16,033</td><td></td></tr>
<tr><td>Revaccination</td><td>3,716</td><td>1,295</td><td>14,767</td><td></td></tr>
<tr><td>2021</td><td></td><td></td><td></td><td>1,330,068</td></tr>
<tr><td>Vaccination</td><td>5,409</td><td>630</td><td>11,024</td><td></td></tr>
<tr><td>Revaccination</td><td>2,962</td><td>875</td><td>13,308</td><td></td></tr>
</table>
Vaccines available: ENCEPUR CHILDREN and ENCEPUR ADULTS, FSME-IMMUN and FSME-IMMUN Junior</td></tr>
<tr><td>Vaccination recommendations and reimbursement</td><td>Vaccination recommendations 1998. No reimbursement; self-paid</td></tr>
<tr><td>Vaccine uptake by age group/risk group/general population</td><td>Vaccine uptake by general population (vaccinated and revaccinated): 2018 – 3.1%; 2019 – 3.7%; 2020 – 3.4%; 2021 – 2.6%</td></tr>
<tr><td>Name, address/website of TBE NRC</td><td>Institute for Health Development, Lab for Virology
http://www.tai.ee/en/about-us/national-institute-for-health-development</td></tr>
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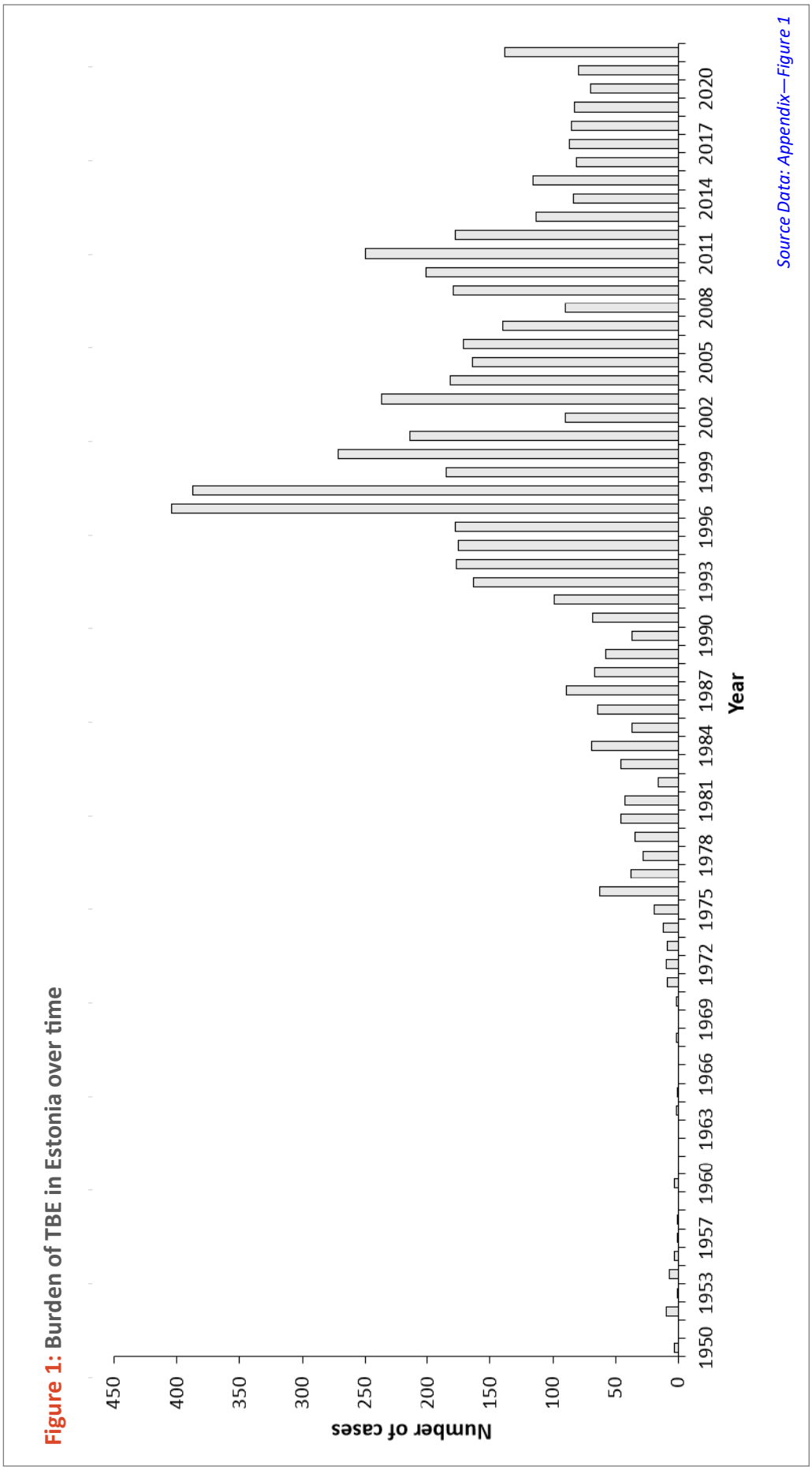
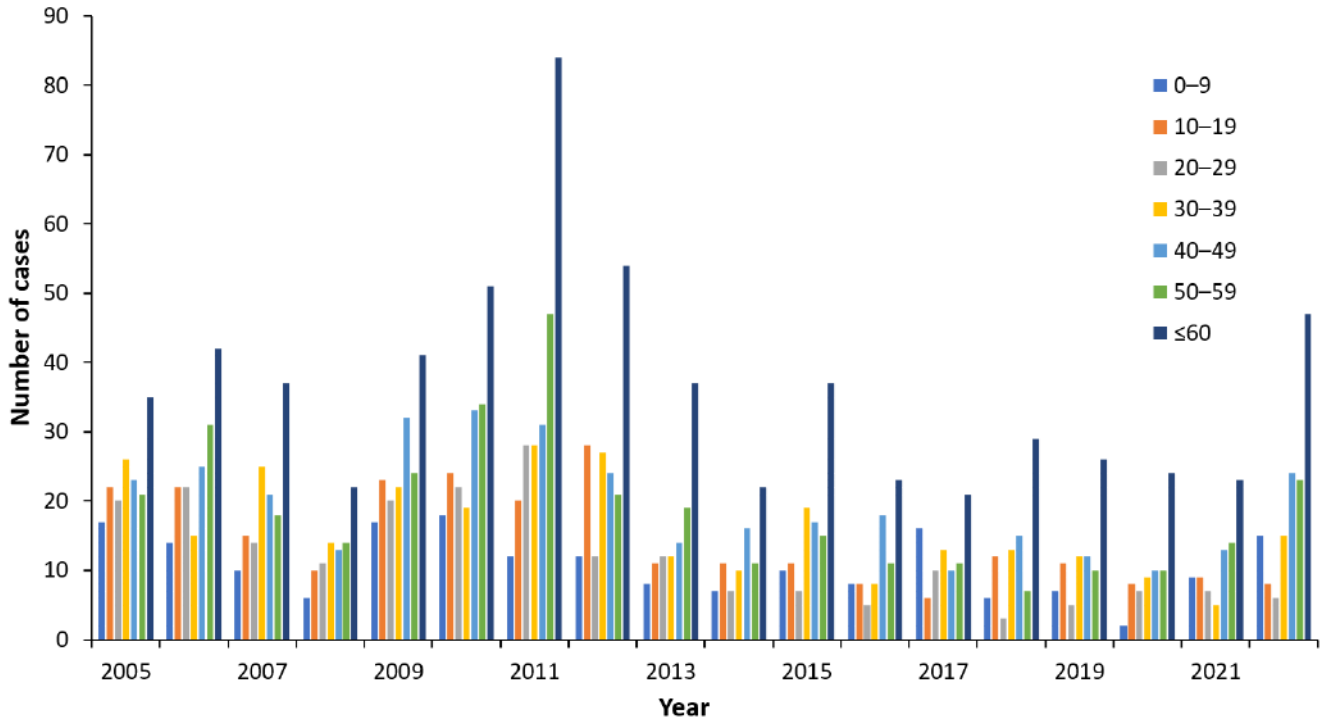


Figure 1: Burden of TBE in Estonia over time

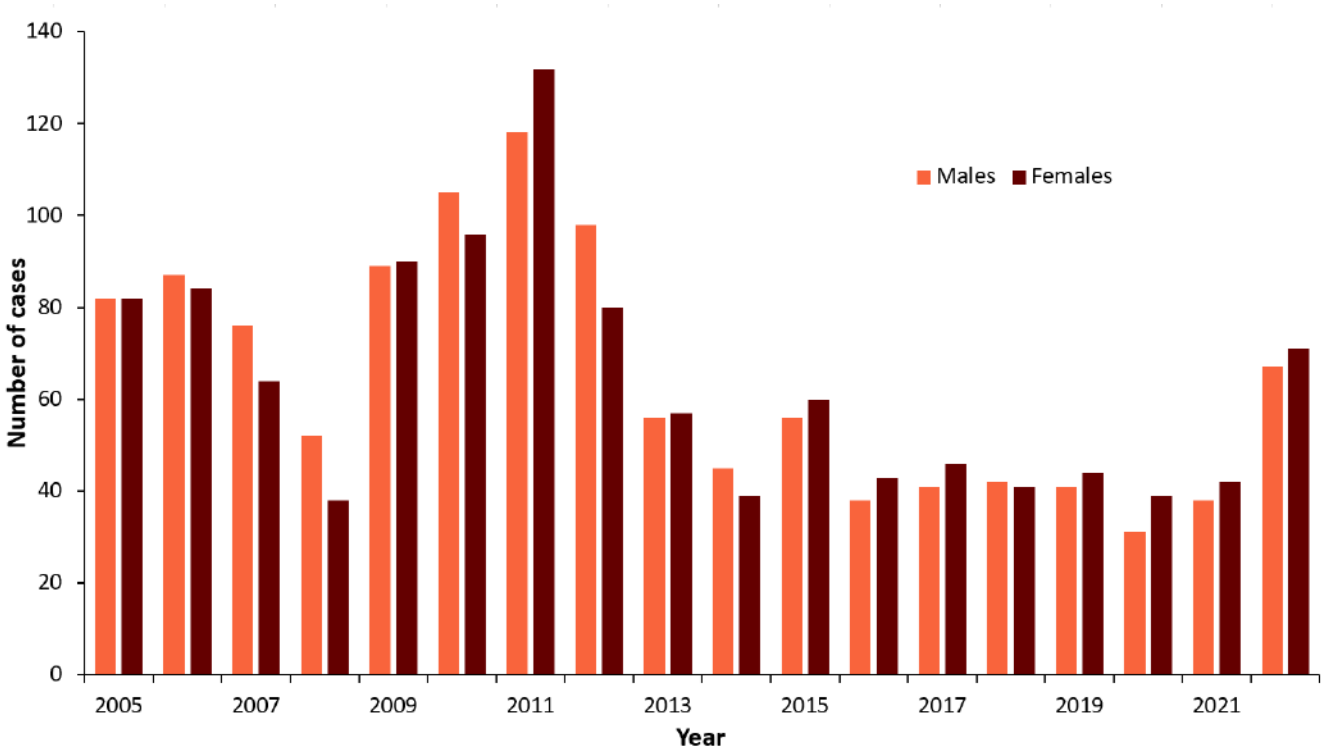
Source Data: Appendix—Figure 1

Figure 2: Age distribution of TBE in Estonia, 2005–2022



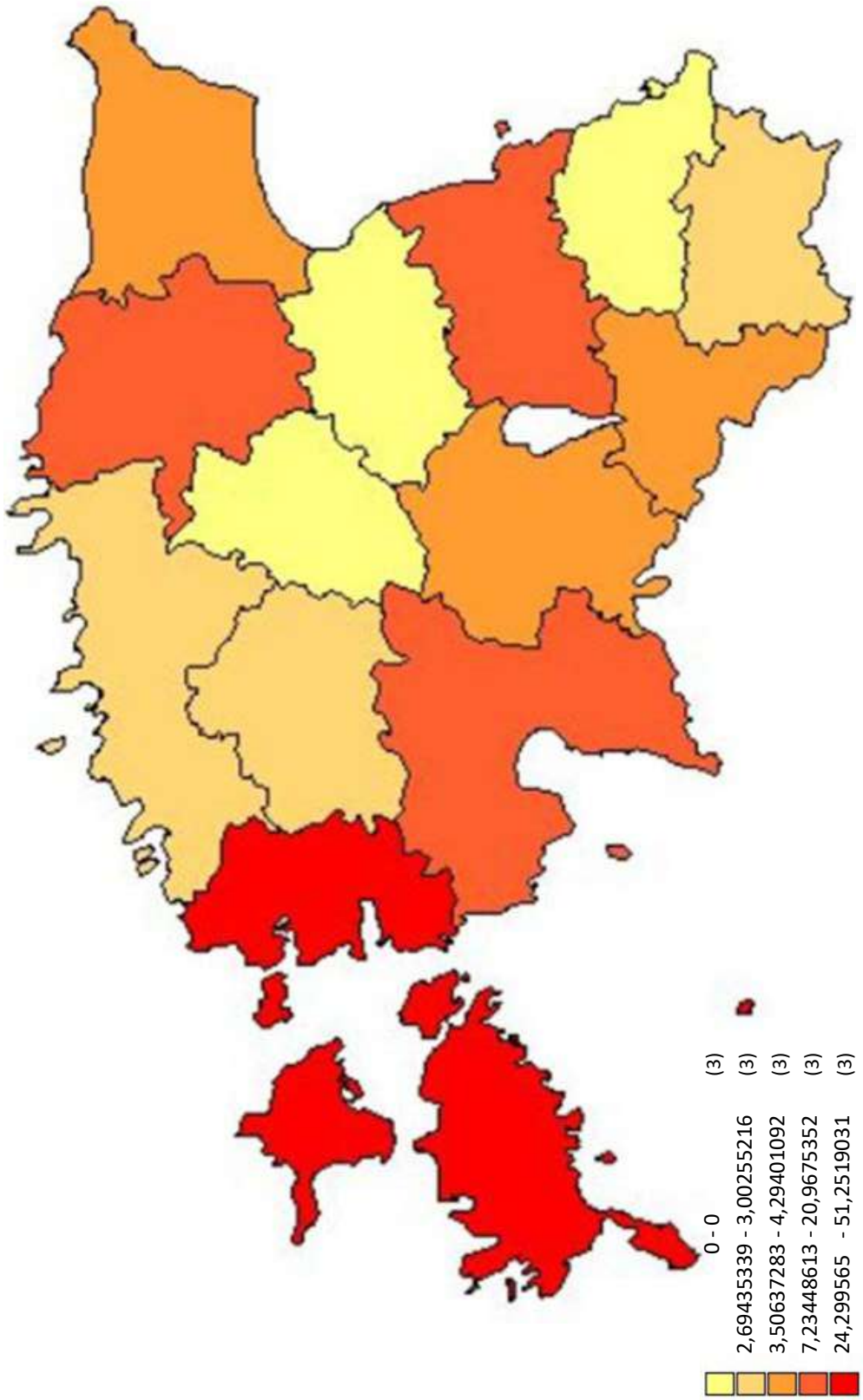
Source Data: Appendix—Figure 2

Figure 3: Gender distribution of TBE in Estonia, 2005–2022



Source Data: Appendix—Figure 3

Figure 4: TBEV-isolation and TBE cases, 2018



Source Data: Appendix—Figure 4

Contact: kkutsar@hotmail.com

Citation:
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Appendix

Source data: Figure 1

Year	Number of TBE cases	TBE incidence /10^5
1950	3	0.3
1951	0	0
1952	10	0.9
1953	1	0.1
1954	7	0.6
1955	3	0.3
1957	1	0.1
1958	1	0.1
1959	0	0
1960	3	0.2
1961	0	0
1962	0	0
1963	0	0
1964	2	0.2
1965	1	0.1
1966	0	0
1967	0	0
1968	2	0.2
1969	0	0
1970	2	0.2
1971	9	0.7
1972	10	0.7
1973	9	0.7
1974	12	0.8
1975	19	1.3
1976	63	4.4
1977	38	2.6
1978	28	1.9
1979	35	2.4
1980	46	3.1
1981	43	2.9
1982	16	1.1
1983	46	3.0
1984	69	4.5
1985	37	2.4
1986	64	4.1

Year	Number of TBE cases	TBE incidence /10^5
1987	89	5.7
1988	67	4.3
1989	58	3.7
1990	37	2.3
1991	68	4.4
1992	99	6.5
1993	163	10.8
1994	177	11.8
1995	175	11.8
1996	178	12.1
1997	404	27.8
1998	387	27.0
1999	185	12.8
2000	272	19.8
2001	215	15.8
2002	90	6.6
2003	237	17.5
2004	182	13.5
2005	164	12.2
2006	171	12.7
2007	140	10.4
2008	90	6.7
2009	179	13.3
2010	201	15.0
2011	250	18.7
2012	178	13.3
2013	113	8.4
2014	84	6.5
2015	116	8.8
2016	81	6.2
2017	87	6.6
2018	85	6.5
2019	83	6.3
2020	70	5.3
2021	80	6.0
2022	138	10.4

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

Source data: Figure 3

Year	Males	Females
2005	82	82
2006	87	84
2007	76	64
2008	52	38
2009	89	90
2010	105	96
2011	118	132
2012	98	80
2013	56	57

Year	Males	Females
2014	45	39
2015	56	60
2016	38	43
2017	41	46
2018	42	41
2019	41	44
2020	31	39
2021	38	42
2022	67	71

Source data: Figure 4

District	2005		2006		2007		2008		2009	
	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5
Tallinn (capital)	56	14.1	21	5.3	24	6.1	14	3.5	23	5.8
Lääne-Virumaa	2	3.0	9	13.5	2	2.9			4	5.9
Harjumaa	27	21.6	4	3.2	10	8.0	6	4.8	9	7.1
Hiiumaa	2	19.4	1	9.3	2	19.6	5	47.6	2	19.8
Ida-Virumaa	11	10.5	23	22.1	9	8.7	1	1.0	7	6.9
Järvamaa			2	5.2	1	2.7	2	5.5		
Jõgevamaa			7	18.7	2	5.4			6	16.3
Läänemaa	3	10.7	2	7.2	2	6.9	5	18.0	1	3.6
Narva	9	12.8	10	14.3	5	7.2	3	4.3	19	27.5
Pärnumaa	15	16.7	23	25.7	21	23.5	19	21.3	30	33.9
Põlvamaa	6	18.8	1	3.2	1	3.2	5	16.0	2	6.4
Raplamaa			2	5.4			1	2.7	1	2.7
Saaremaa	14	39.6	10	28.4	29	82.7	14	40.0	22	63.1
Tartumaa	17	11.4	31	20.8	20	13.4	10	6.7	35	23.4
Valgamaa	1	2.9	7	20.1			3	8.7	5	14.6
Viljandimaa	1	1.8	1	1.8	7	12.4	1	1.8	9	16.1
Võrumaa			17	44.0	5	13.0	1	2.6	4	10.5
Total	**164**	**12.2**	**171**	**12.7**	**140**	**10.4**	**90**	**6.7**	**179**	**13.3**

District	2010		2011		2012		2013		2014	
	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5
Tallinn (capital)	23	5.8	25	6.3	26	6.5	20	4.9	16	3.9
Harjumaa	13	10.3	24	18.9	12	9.3	16	9.9	10	6.2
Hiiumaa	4	39.6	8	79.7	2	20.0	3	34.7	4	46.6
Ida-Virumaa	8	7.9	6	6.0	6	6.1	0	0.0	3	3.4
Järvamaa	2	5.5	2	5.5	2	5.6	0	0.0		
Jõgevamaa	9	24.5	2	5.5	5	13.7	3	9.5	1	3.2
Läänemaa	2	7.3	15	54.8	3	11.0	2	8.1	2	8.2
Lääne-	4	6.0	4	6.0	3	4.5	4	6.6	3	5.0
Narva	16	23.3	11	16.1	11	16.2	2	3.2	1	1.6
Pärnumaa	39	44.1	45	50.9	35	39.7	27	30.6	10	12.3
Põlvamaa	9	29.0	9	29.1	5	16.3	2	7.2	1	3.6
Raplamaa	2	5.5	6	16.4	5	13.7	0	0.0	7	20.2
Saaremaa	15	43.2	51	147.2	21	60.8	9	28.3	14	44.1
Tartumaa	27	18.0	17	11.3	24	15.9	16	10.5	7	4.6
Valgamaa	7	20.5	8	23.5	2	5.9	3	9.8	1	3.3
Viljandimaa	10	18.0	9	16.2	5	9.1	1	2.1	4	8.4
Võrumaa	11	29.0	8	21.2	11	29.4	5	14.8		
Total	**201**	**15.0**	**250**	**18.6**	**178**	**13.3**	**113**	**8.4**	**83**	**6.3**

District	2015		2016		2017		2018	
	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5
Tallinn (capital)	23	5.6	20	4.7	27	6.4	12	2.8
Harjumaa	19	11.8	10	6.5	10	6.5	4	2.6
Hiiumaa	6	69.9	1	10.7	2	21.4	3	32.1
Ida-Virumaa	1	1.1	3	3.5	2	2.3	3	3.8
Järvamaa	1	3.3			1	3.3		
Jõgevamaa	3	9.6			1	3.2		
Läänemaa	4	16.4	1	4.1	2	8.1	5	24.1
Lääne-Virumaa	7	11.7			1	1.7	5	8.3
Narva	5	8.1	2	3.3	3	4.9	2	3.2
Pärnumaa	15	18.1	14	16.9	11	13.3	18	20.9
Põlvamaa	1	3.6	2	7.1	1	3.5		
Raplamaa			1	2.9			1	3.0
Saaremaa	7	22.0	10	29.9	19	56.7	17	51.0
Tartumaa	16	10.5	12	8.3	6	4.1	11	7.4
Valgamaa					1	3.3	1	3.4
Viljandimaa	3	6.3	2	4.2			2	4.2
Võrumaa	5	15.0	3	8.8			1	2.7
Total	**116**	**8.8**	**81**	**6.2**	**87**	**6.6**	**85**	**6.5**

District	2019		2020		2021		2022	
	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5	No. of cases	Incidence /10^5
Tallinn (capital)	16	3.7	6	1.4	10	2.3	33	7.5
Harjumaa	11	6.9	5	3.1	8	4.8	15	2.4
Hiiumaa	9	95.9	0	0.0	0	0.0	3	35.3
Ida-Virumaa			0	0.0	1	1.3		
Järvamaa	1	3.3	0	0.0	1	3.3		
Jõgevamaa	1	3.4	0	0.0	0	0.0	3	10.8
Läänemaa	1	4.8	2	9.7	4	19.6	1	4.9
Lääne-Virumaa	3	5.0	4	6.7	3	5.1	6	10.2
Narva	2	3.3						
Pärnumaa	12	14.0	12	14.0	12	13.9	27	31.5
Põlvamaa	3	11.9	6	23.7	3	12.2	7	29.2
Raplamaa	2	6.0	2	6.0	3	9.0	3	8.9
Saaremaa	8	24.1	12	36.1	9	27.2	12	38.3
Tartumaa	4	2.6	11	7.3	19	12.4	12	7.6
Valgamaa	1	3.5	3	10.5	1	3.5	1	3.6
Viljandimaa	5	10.7	5	10.7	2	4.3	7	15.4
Võrumaa	4	11.1	1	2.8	4	11.3	5	14.6
Total	**83**	**6.3**	**70**	**5.3**	**80**	**6.0**	**138**	**10.4**

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Chapter 12b

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]

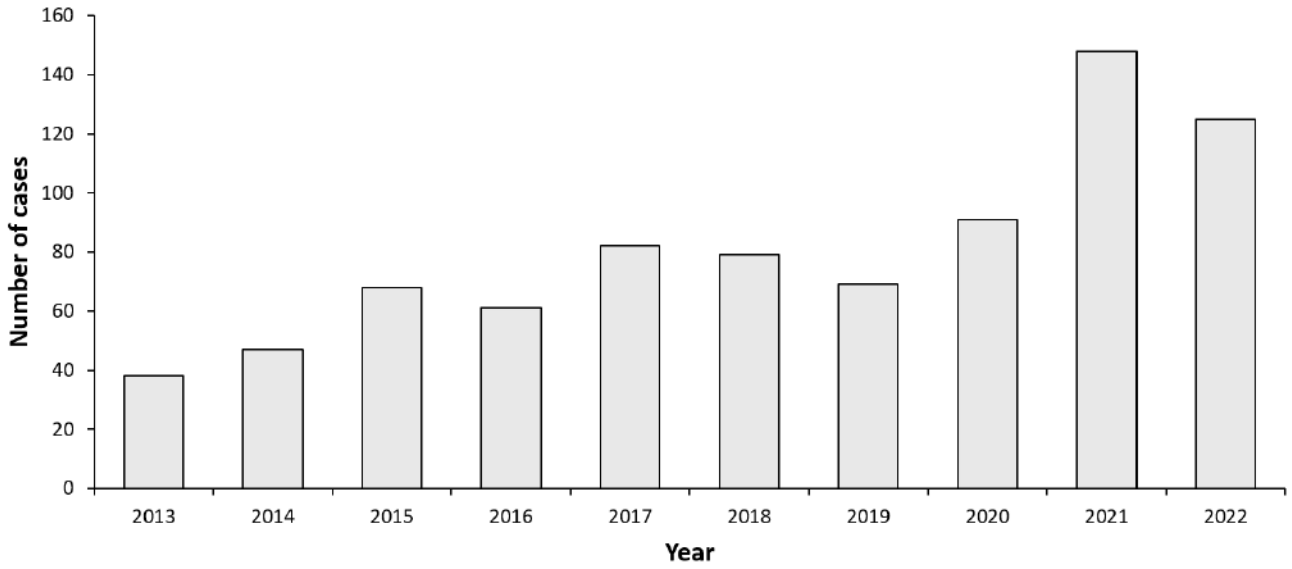
The overall prevalence of TBEV in ticks in Finland is reported to be 1.6%.[9] TBEV prevalence was higher in *I. persulcatus* (3.0%) than in *I. ricinus* (0.2%) in 2015 based on ticks sampled by crowdsourcing[9] but varies greatly within Finland.

Overview of TBE in Finland

Table 1: Virus, vector, transmission of 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]

Table 2: TBE reporting and vaccine prevention in Finland	
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 incidence rates vary from 0 to >15/100,000.

Source Data: Appendix—Figure 1

Figure 2: Number of TBE cases during 2017–2021[16]

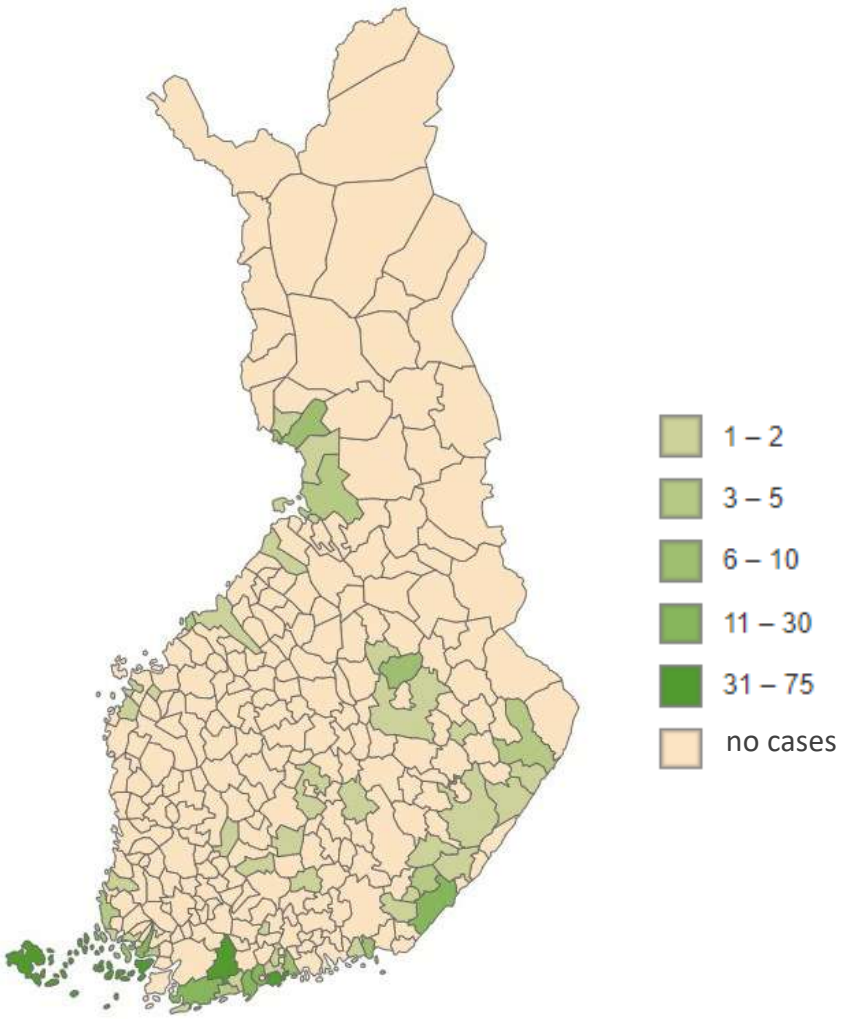
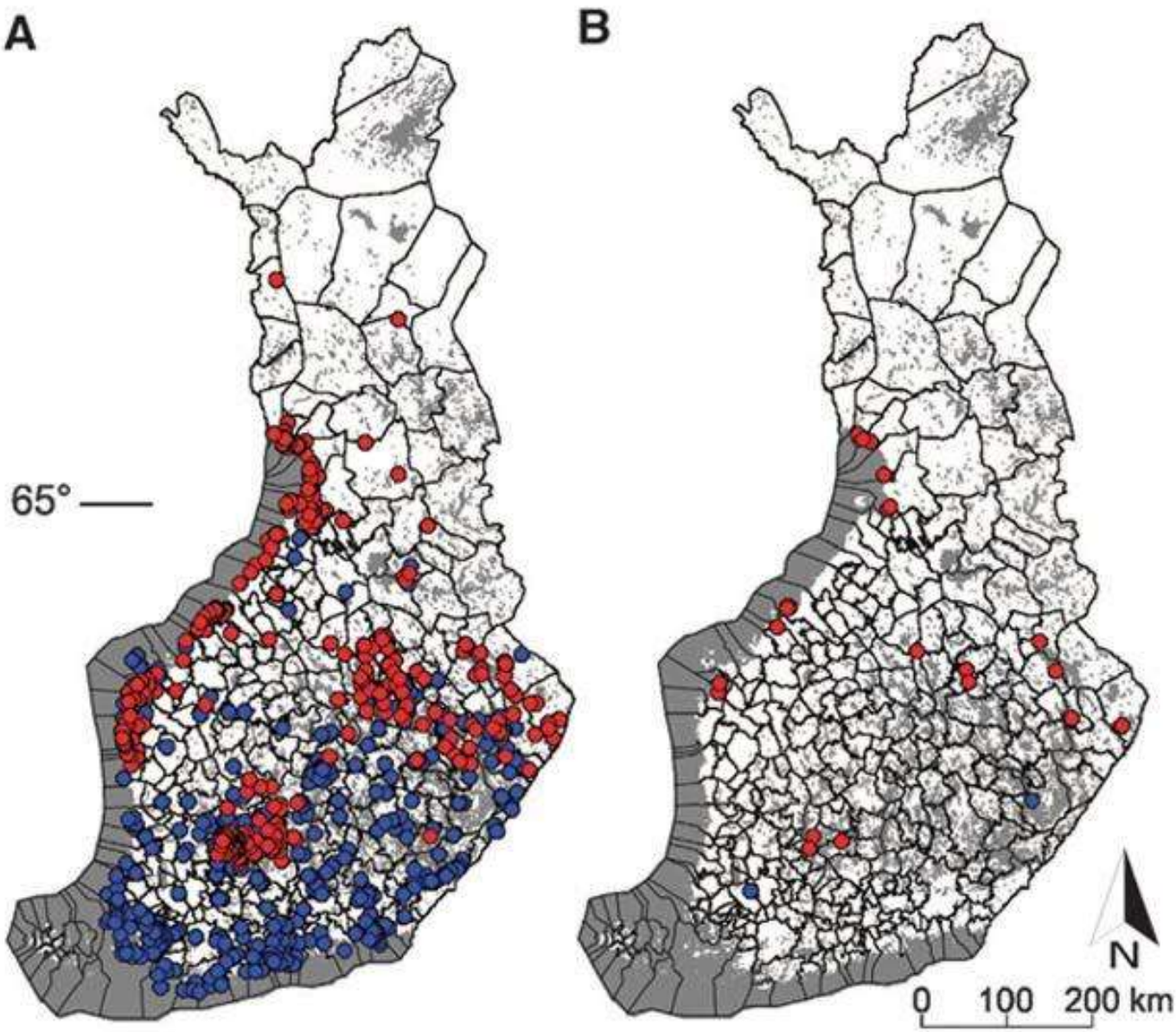


Figure 3:



(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]

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doi:10.33442/26613980_12b11-6

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

Year	Number of cases	Incidence / 10^5
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

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Chapter 12b

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.

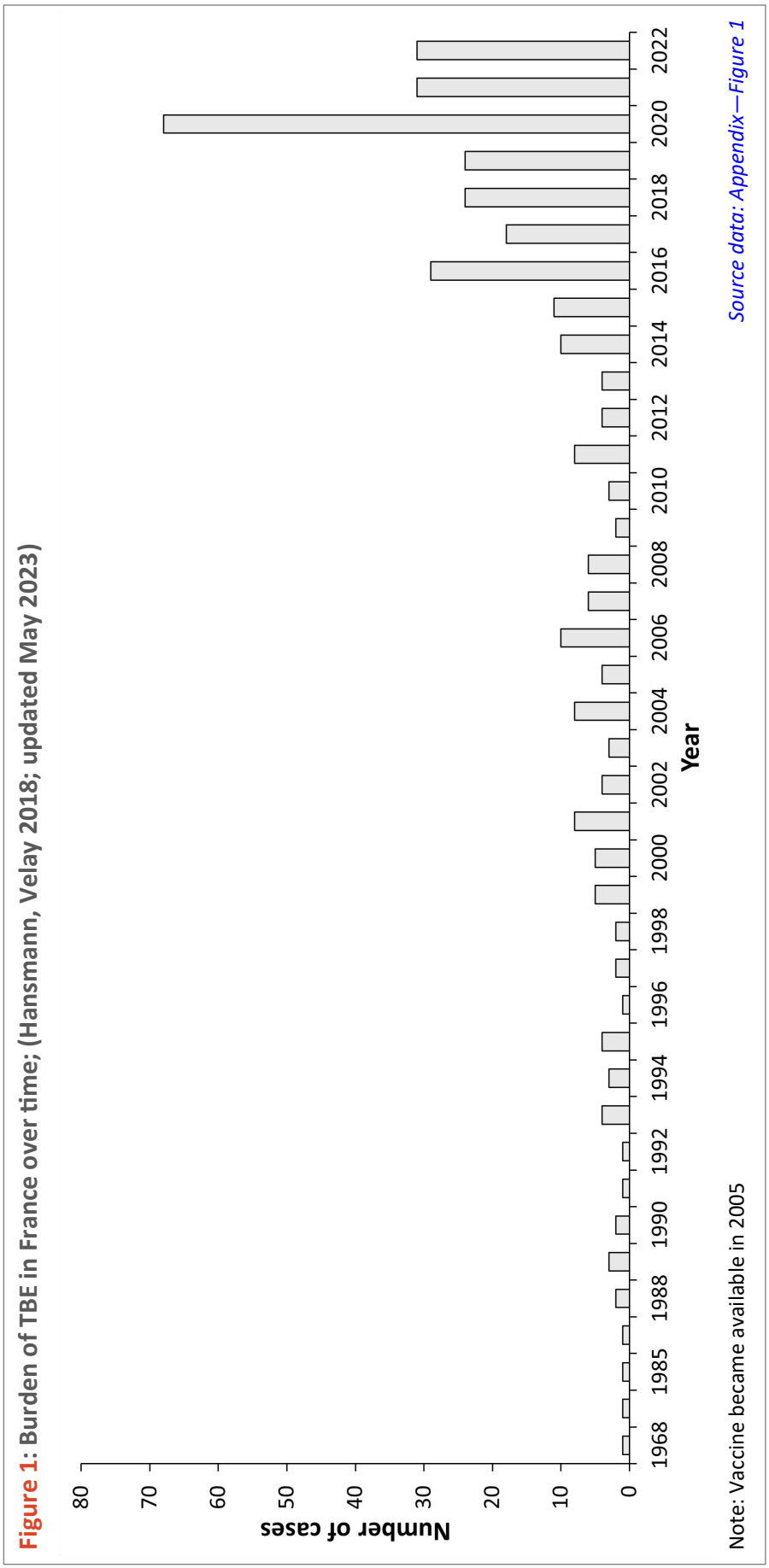
Two clusters were highlighted in the ARA region in an area not previously known to be at risk.

Table 1: Virus, vector, transmission of 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	Possible but unproven[5]

Table 2: TBE reporting and vaccine prevention in France	
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

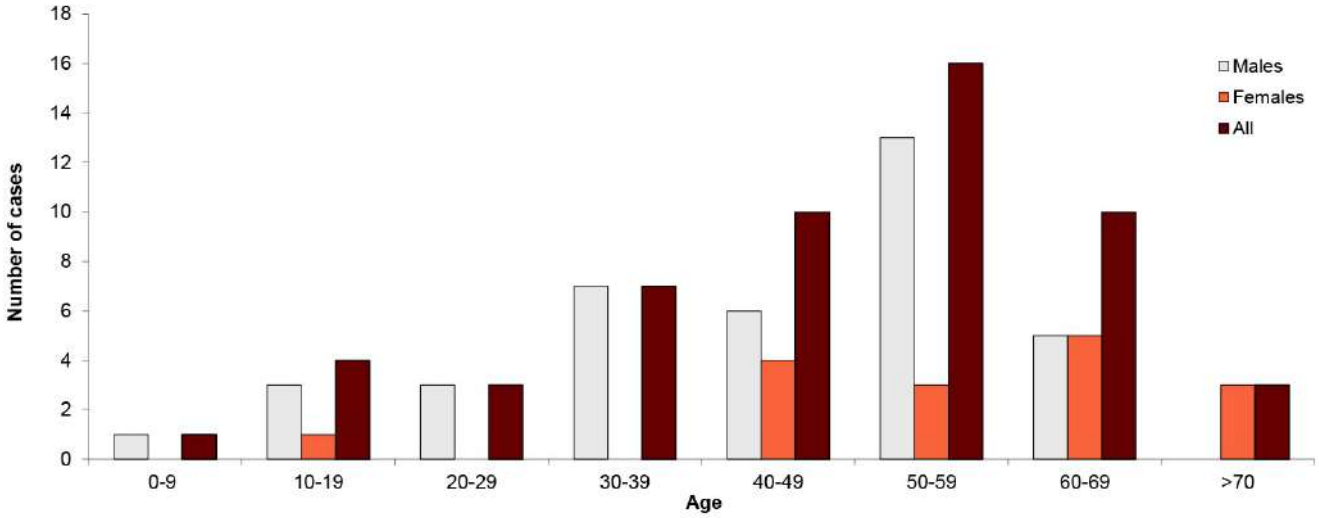
Figure 1: Burden of TBE in France over time; (Hansmann, Velay 2018; updated May 2023)



Note: Vaccine became available in 2005

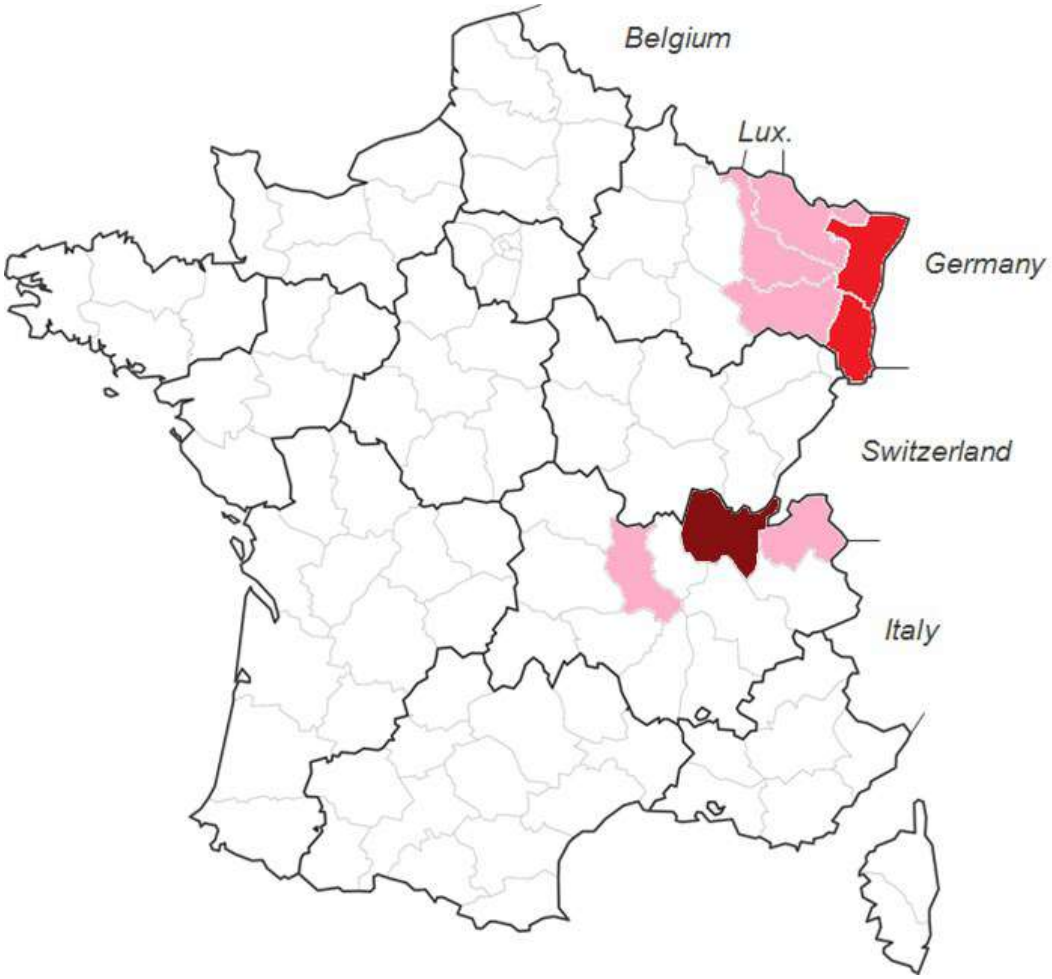
Source data: Appendix—Figure 1

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

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Citation:
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Chapter 12b

TBE in Germany

Gerhard Dobler and Ute Mackenstedt

E-CDC risk status: endemic (data as of end 2022)

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 formed 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 western (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 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).

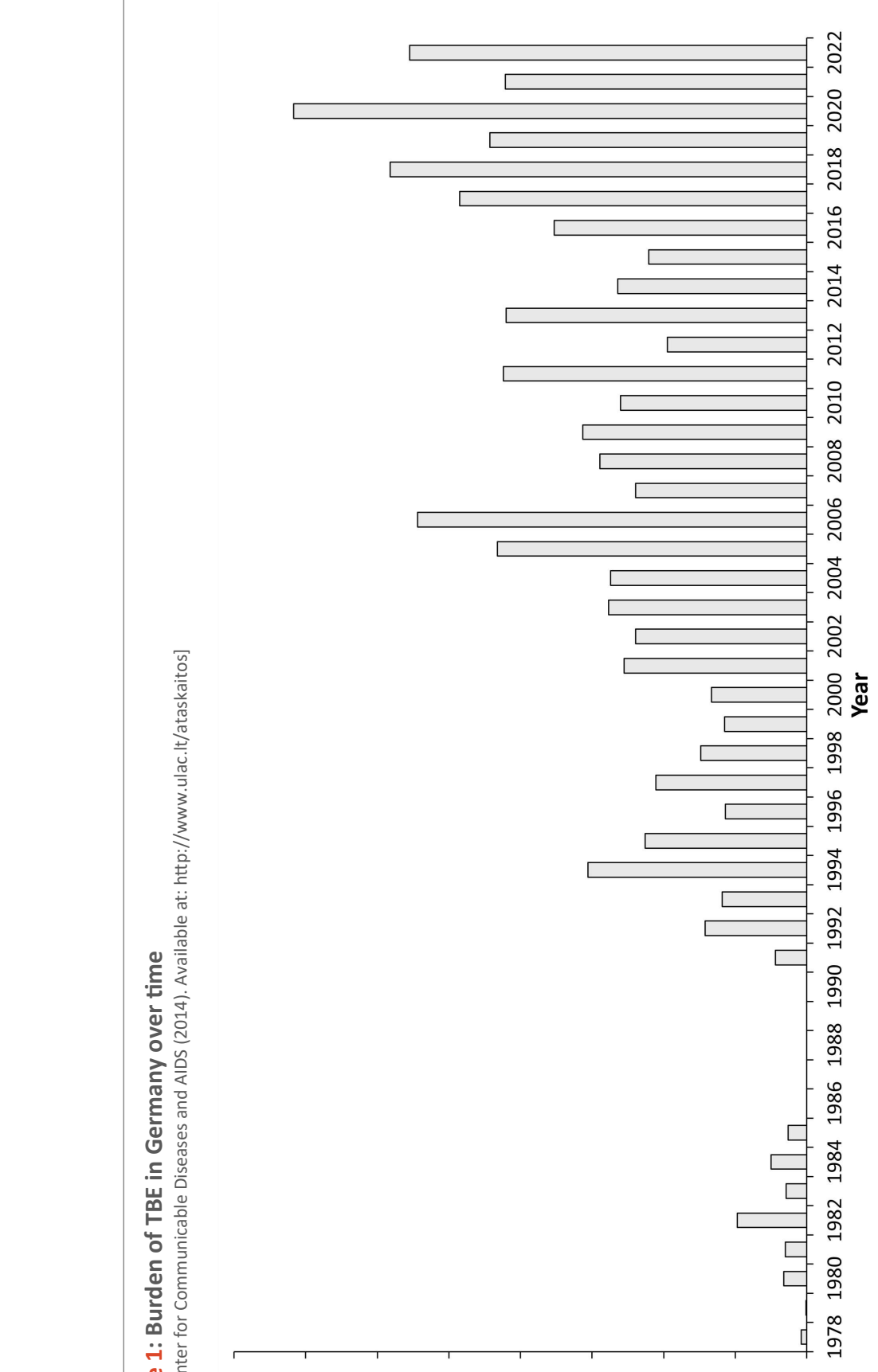
Overview of TBE in Germany

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 (Dobler, personal communication)

Figure 1: Burden of TBE in Germany over time

[The Center for Communicable Diseases and AIDS (2014). Available at: http://www.ulac.lt/ataskaitos]



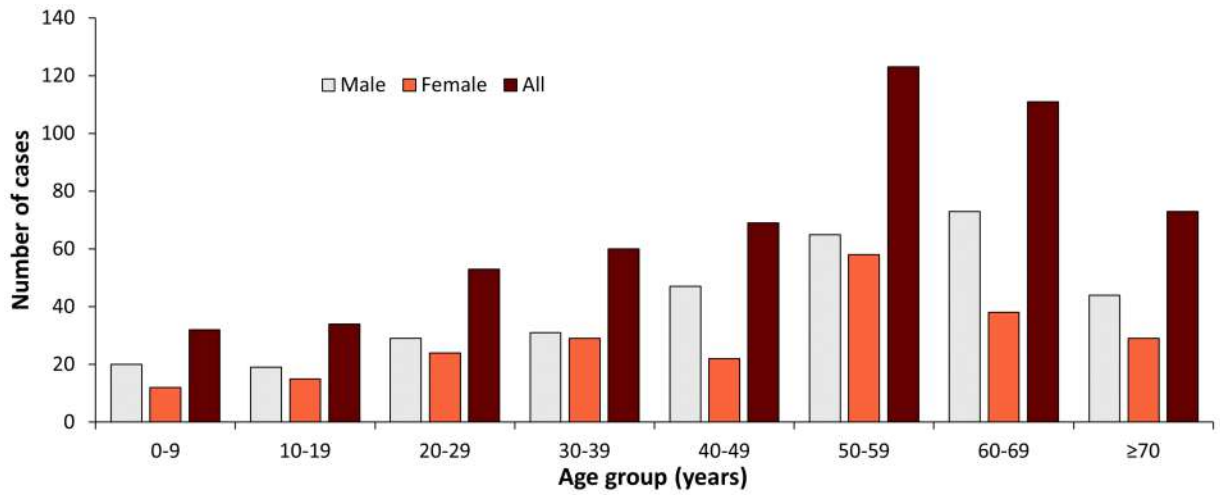
Source Data: Appendix—Figure 1

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 15% to 50%, depending on the district (Survey of the German Society of Consumption Research)
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

Source data: Figure 2

(2022, with data for 2010–2021 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**

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Citation:
Dobler G, Mackenstedt U. TBE in Germany. Chapter 12b. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 6th ed. Singapore: Global Health Press; 2023. doi:10.33442/26613980_12b13-6

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Chapter 12b

TBE in Hungary

Anna Nagy, Ferenc Schneider, Eszter Mezei, András Lakos

E-CDC risk status: endemic (data as of end 2022)

History and current situation

Hungarian scientists were among the pioneers in Europe as the tick-borne encephalitis virus (TBEV) was isolated in 1952.[1] However, most of their observations were published in the Hungarian language, and therefore cannot easily be accessed by the international medical community. Here the relevant Hungarian data are summarized.

Before 1997, the average annual number of tick-borne encephalitis (TBE) cases reported to authorities was around 300, and as of that year it has 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":

1. During the 5 years before 1997, a total of 1,800,000 TBE vaccine doses were sold by pharmacies (Figure 1—the population of Hungary was around 10 million people), 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.

2. The incidence data from the Hungarian military are similar to that of the civilian population: no case has been reported since 2003. "Underreporting" in this context would be practically impossible.[5] 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.

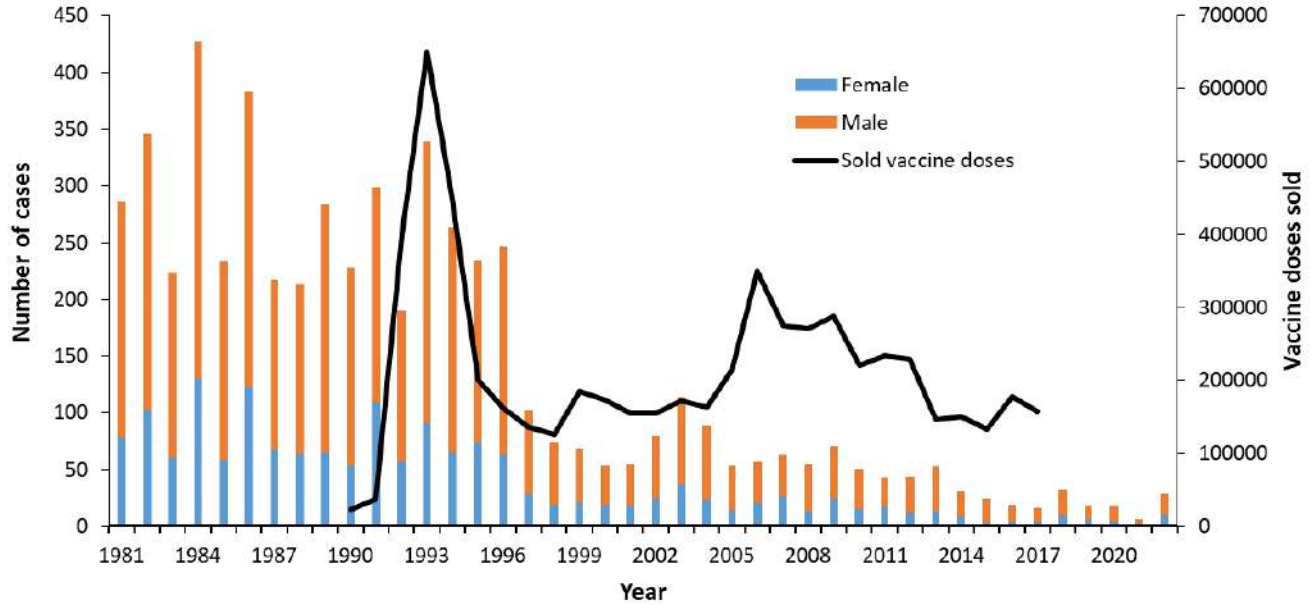
Overview of TBE in Hungary

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 (%)	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.

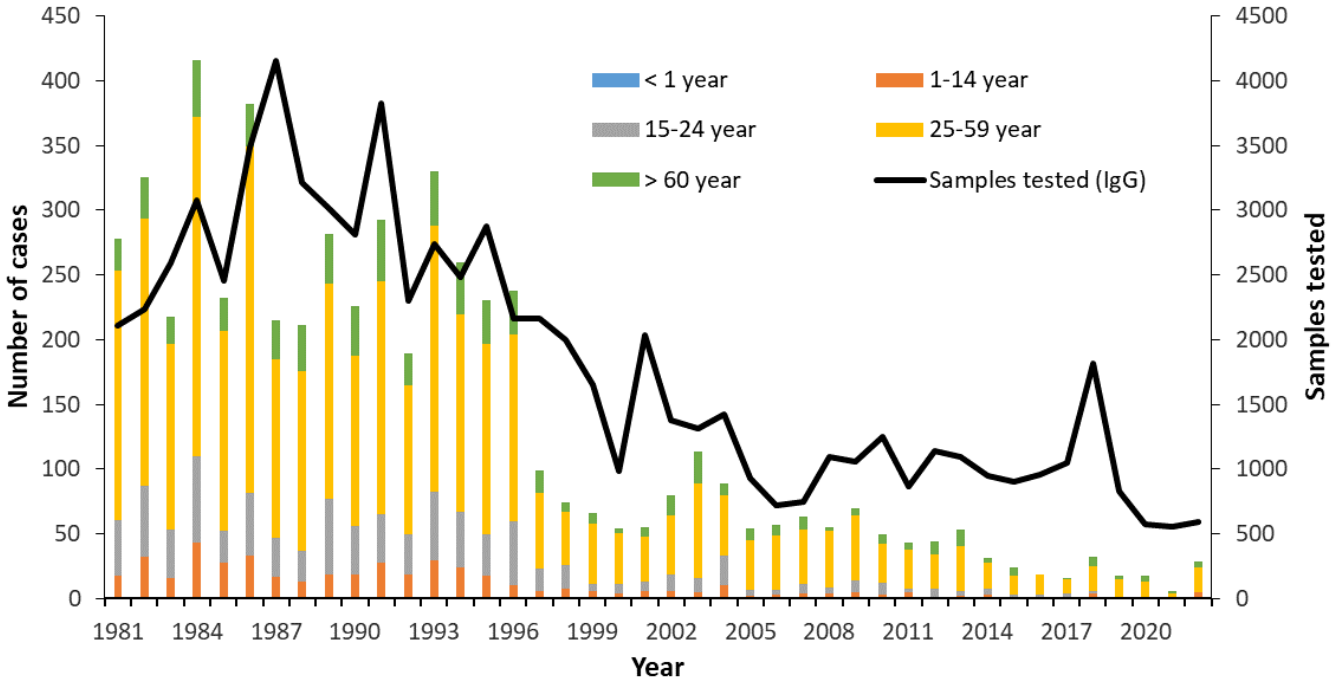
Table 2: TBE reporting and vaccine prevention in Hungary	
Mandatory TBE reporting	Every physician who establishes a diagnosis of TBE must report it. Practically, these are hospital-based specialists for infectious diseases, pediatricians, internists, and neurologists. Case definition: clinical symptoms 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 1670 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 are not available from 2018.)

Figure 2: Burden of TBE in Hungary from 1981 to 2022.[24-25] Age distribution and the requested number of diagnostic tests.

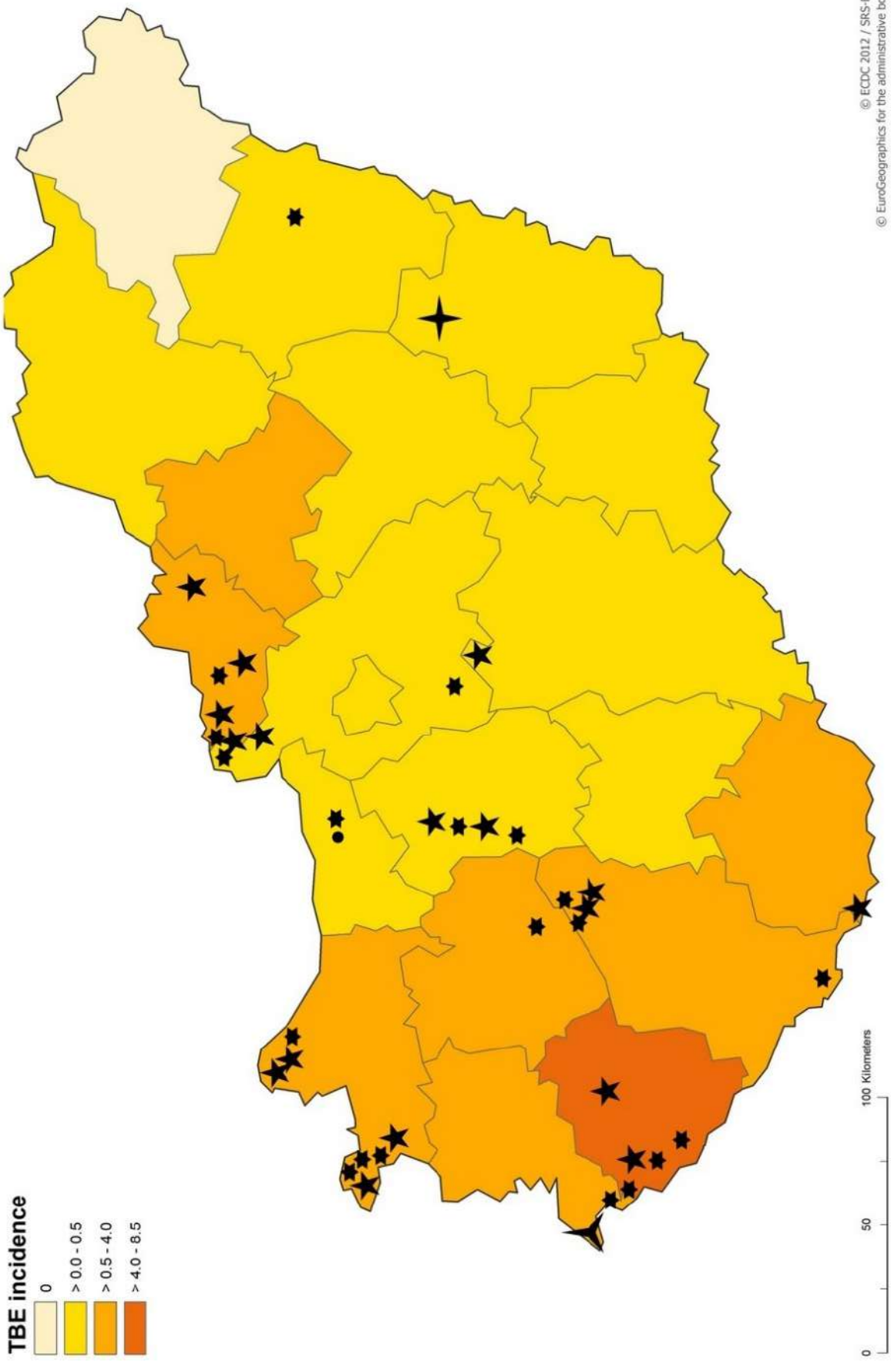


Source Data: Appendix-Figure 2

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-pronged stars (Molnár, 1979),[23] five-pronged stars (Gerzsenyi, 1980 and 1985),[9,10] four-pronged star (Pintér, 2013),[11] and three-pronged 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

N/A: data not available

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TBE in Italy

Valentina Tagliapietra, Flavia Riccardo, Martina Del Manso and Giovanni Rezza

E-CDC risk status: endemic (data as of October 31, 2022)

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 in Italy are geographically clustered in the forested and mountainous regions and provinces in the northeast part of the country, as suggested by TBE case series published over the last decade.[2-5] A national enhanced surveillance system for TBE has been established since 2017.[6] 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, ad hoc studies and international literature.[1]

TBE has been recorded in Italy since 1967, with foci of infection in the northeast (Trento, Belluno and Gorizia) and central (Florence and Latina) provinces.[7-10] TBE presence in central Italy has not been confirmed by further studies on ticks and serosurveys conducted afterwards,[11-12] nor by human cases, suggesting the disappearance of these small endemic foci.

Serological investigations of people at risk, such as forestry rangers, hunters, and mushrooms collectors, have been performed in order to get information on the TBE virus (TBEV). Circulation in the pre-alpine and alpine regions reporting seroprevalence values of 0.6%, 1.07% and 3.2% in Friuli-Venezia Giulia,[13] Trento province[14] and Turin province,[15] respectively. Interestingly, Turin province has never reported human cases of TBEV infection, so far.

A retrospective study conducted in 2015 in the northeast regions, 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 around 70% of them were between 30 and 70 years of age. 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 (incidence rate ratio [IRR]=1.05 per 1 calendar year increase, 95% confidence interval [CI]: 1.02–1.08, P>0.01). The majority of identified TBE cases occurred between April and October, consistent with the seasonality of tick activity. Areas with IR greater than 10 per 100,000 appear to be concentrated in 3 main foci: 1 in the Autonomous Province of Trento (IR=41.6), 1 in the Belluno Alps in Veneto (IR=35.9), and the third at the extreme northeast section of Friuli-Venezia Giulia (IR=42.6).[3] 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. Of note, the IR for municipalities with a mean altitude >800 m a.s.l. appears to be 5 times higher than for municipalities with a mean altitude <200 m a.s.l.[3]

A national TBE surveillance system recording neuro-invasive TBEV infections was established since 2017. In 2020, the number of notified cases reached a record, with 55 cases mainly from four northeastern Italian regions and provinces: Trento, Bolzano, Friuli-Venezia Giulia and Veneto (Fig. 3). In addition to these well-known positive areas, another two regions were added although they reported intermittent sporadic cases, namely Emilia Romagna with 2 cases in 2020 and Lazio with 1 case in 2019 (Fig. 3). Average annual incidence per 100.000 inhabitants doubled its value from 0.77 in 2017 to 1.42 in 2020. In particular, the province of Trento showed a sharp increase in the 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.[16] 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.

The observed increase of TBE cases was associated with the expansion of tick populations resulting from climatic factors, increasing abundance of ungulates, and changes in human behavior and land use, in addition to increased recognition and reporting of TBE cases.[22-23] Although the

distribution of human cases is consistent with that of the competent tick vector, the widely dispersed distribution of ticks in the environment and their very low TBEV prevalence (usually below 1%), make them an unsuitable indicator of TBEV infection risk. For these reasons, entomological studies, even if performed in endemic regions, cannot be translated into a direct human risk, and other factors should be considered in order to address public health efforts toward TBE hazard. For example, 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,[22] but this relationship is not always positive and at a threshold density level of ungulates TBEV prevalence decreases.[24] Transmission of TBEV from infected nymphs to co-feeding uninfected ticks on rodents is considered the most efficient route for 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 reflect in the maintenance of a TBE hotspot.[25]

Vaccination for TBE is currently recommended in Italy among residents and occupationally exposed groups, in particular in rural endemic areas.[17] In affected regions and provinces, TBE is offered free of charge to risk groups and the resident population since 2013 in Friuli-Venezia Giulia and since 2018 in the Autonomous Provinces of Trento and Bolzano. Affected regions and provinces have also made information on TBE vaccination available on websites.[18-21]

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. More studies are necessary to disentangle the complex factors that are involved in the circulation and maintenance of TBEV in an endemic focus and early-warning predictors should be better assessed. Human cases are currently reported from northeastern regions (Friuli-Venezia Giulia, Veneto and in the Provinces of Trento and Bolzano), with the highest incidence rates being reported in areas between 400 and 600 m a.s.l. TBE vaccine is offered to residents living in high-risk areas, but its impact on disease occurrence in the affected communities is not yet evaluated.

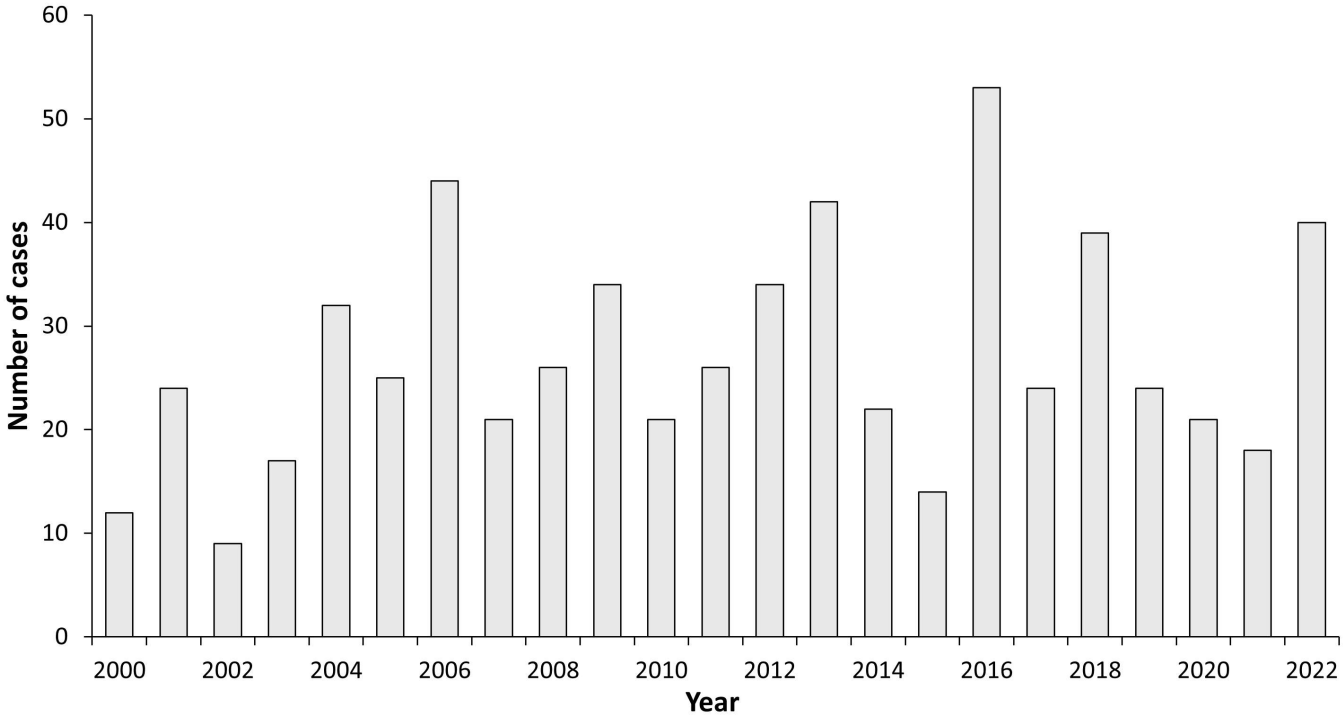
Overview of TBE in Italy

Table 1: Virus, vector, transmission of TBE in Italy (northeastern)

Viral subtypes, distribution	European TBEV subtype; northeast regions: Friuli-Venezia Giulia, Veneto, Trentino-Alto Adige (Fig. 3)
Reservoir animals	Ticks and small rodents. Consumption of milk and milk products from infected goats, sheep, or cows
Infected tick species (%)	*I. ricinus*
Dairy product transmission	Not documented

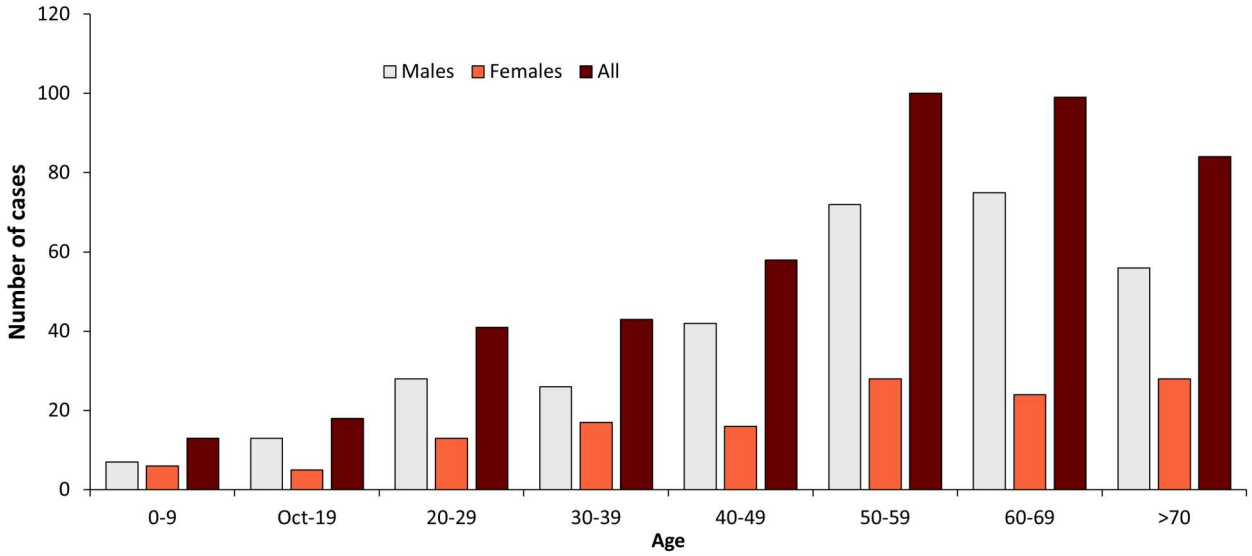
Table 2: TBE-reporting and vaccine prevention in Italy (northeastern)	
Mandatory TBE reporting[16,6]	Reported by Department of Infectious Diseases, National Institute of Health, Italy in collaboration with all the Infectious Diseases Units and Public Health Districts. Case definition: clinical criteria are any symptoms of inflammation of the CNS (for example, meningitis, meningoencephalitis, 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. Surveillance has been enhanced at the national level since 2017.
Special clinical features[13-15]	Biphasic disease is not reported. At-risk groups are defined by occupational risk (i.e. agricultural workers and forest or lumber workers) or risk hobbies (i.e. hiking/trekking, mushroom foraging). Presumed place of exposure and date of tick bite are recorded.
	Sequelae (information available on 193 cases): 18.1% with permanent sequelae, and 28.5% with temporary sequelae. Case-fatality rate: 0.7%
Available vaccines	TICOVAC 0.5 mL (Pfizer Srl)
Vaccine recommendations and reimbursement, and uptake by age group/risk group/ general population	Friuli-Venezia Giulia: vaccination is free of charge for residents. Veneto: vaccination is not free of charge; recommended for those who live in the woods or in rural areas at risk for TBE. Trentino-Alto Adige: vaccination is free of charge for residents.
Name, address/website of TBE National Reference Center	Prof. Giovanni Rezza Dipartimento Malattie Infettive Istituto Superiore di Sanità Viale Regina Elena, 299 00161 Roma, Italia Website: https://www.iss.it/?p=27

Figure 1: Reported human cases of TBE, Italy, 2000–2022[26-28]



**Data on vaccination rate : Appendix—Figure 1*

Figure 2: Age and gender distribution of reported human cases of TBE, Italy, 2000–2016



Source Data: Appendix—Figure 2

Figure 3: Regions in northeastern Italy reporting TBE cases
(BZ=Autonomous Province of Bolzano; TN=Autonomous Province of Trento;
[BZ+TN=Trentino-Alto Adige] VEN= Veneto; FVG= Friuli-Venezia Giulia
ER=Emilia Romagna; L=Lazio)

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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	0.04	
2020*	21	0.035	
2021**	18	0.03	
2022***	40	0.068	

* Neuroinvasive laboratory confirmed TBEV infections
** 18 total with 4 imported cases
*** Total cases from January 1, 2022 to October 31, 2022

Note: Data on vaccine coverage are not available for 2015–2022

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	7	6	13
10-19	13	5	18
20-29	28	13	41
30-39	26	17	43
40-49	42	16	58
50-59	72	28	100
60-69	75	24	99
>70	56	28	84

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Chapter 12b

TBE in Japan

Kentaro Yoshii

E-CDC risk status: affected, possibly endemic (data as of end 2022)

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.[5] 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 in 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 central to the southern parts of Hokkaido.[2-4,7-12] TBEV was isolated from dogs, wild rodents and from *Ixodes ovatus* ticks, which are the predominant ticks in the area. Sequence and phylo-genetic analysis classified the TBEV isolates as Far-Eastern subtype. Besides, antibodies against TBEV were detected in deer and wild rodents in the Tochigi and the Shimane 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.[3,6,12]

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.

Table 1: Virus, vector, transmission of TBE in Japan	
Viral subtypes, distribution	Far-Eastern subtype Central and southern parts of Hokkaido[3-5, 7-12] There is evidence for nationwide - distribution of the TBEV (see text above)
Reservoir animals	Wild rodents[4,10,12]
Infected tick species (%)	*I. ovatus* (0.05%–0.33%)[8,9]
Dairy product transmission	Not reported

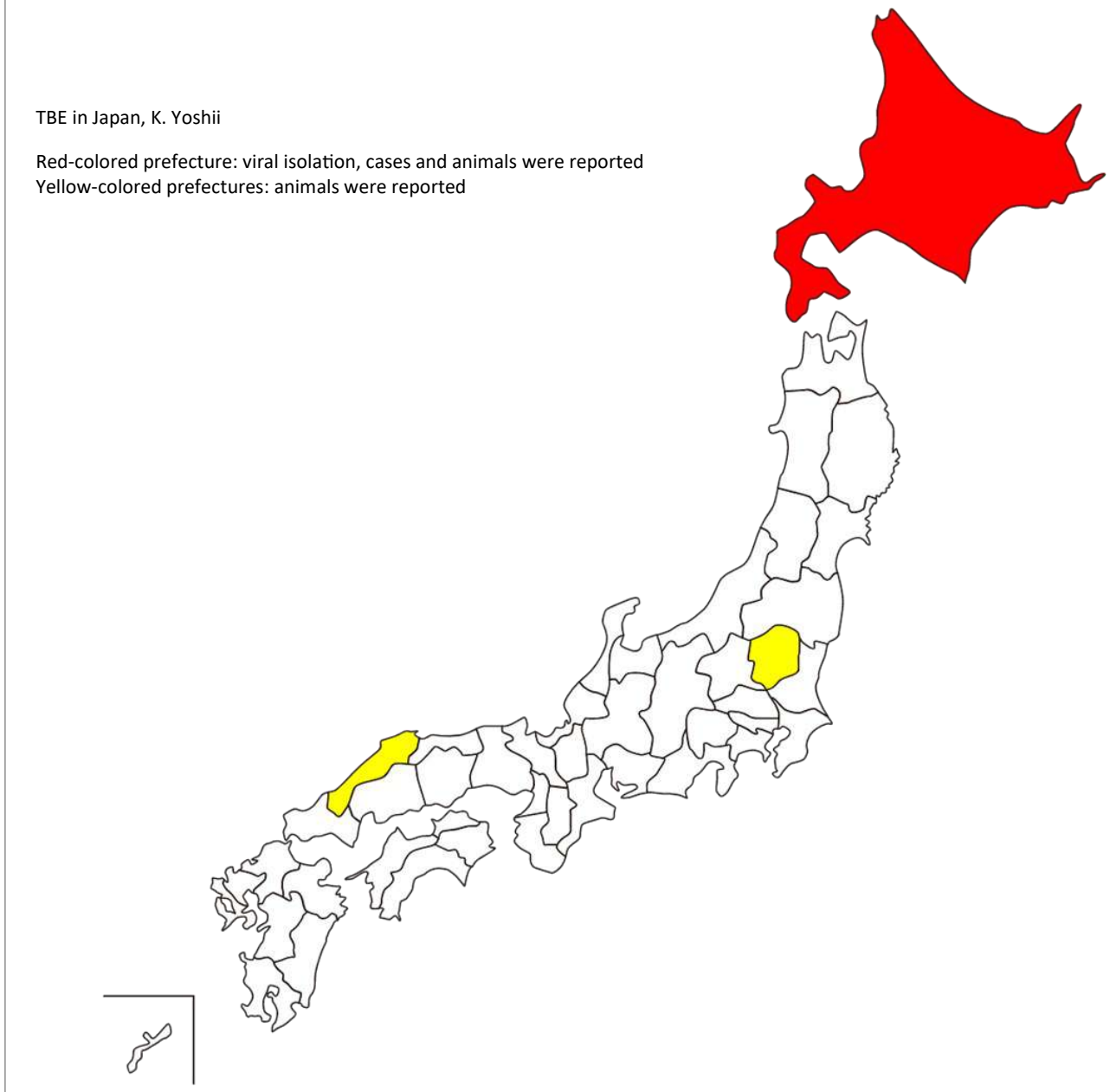
Table 2: TBE reporting and vaccine prevention in Japan	
Mandatory TBE reporting	Laboratory confirmed cases must be reported by physicians. Case definition: isolation of TBEV or detection of TBEV genomic ribonucleic acid by RT-PCR from blood or cerebrospinal fluid; detection of IgM antibodies against TBEV from blood or cerebrospinal fluid; detection of significant increase in neutralizing antibodies against TBEV in paired serum.
Other TBE surveillance	No
Special clinical features	Encephalitis and meningitis with typical neurological symptoms.
Available vaccines	No
Vaccination recommendations and reimbursement	No
Vaccine uptake by age group/risk group/general population	No
Name, address/ website of TBE NRC	No

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 the 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.

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,[4] and the second patient was a male person in his 40s (2016). The third and fourth patients were male in their 70s (2017). The fifth patient was a female in her 40s (2018). Retrospective survey revealed infection with TBEV in one Lyme disease-suspected patient with meningoencephalitis[9] and two asymptomatic cases in Japan Self-Defense Forces members in Hokkaido.[10]

Figure 1: TBEV-isolation, TBE cases and animals with TBEV-antibodies in Japan



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Chapter 12b

TBE in Kazakhstan

Andrey Dmitrovskiy

E-CDC risk status: endemic (data as of end 2022)

History and current situation

The first isolation of the TBEV in Kazakhstan was achieved in the Almaty region by M.P. Chumakov in 1941 (only one strain from one patient) during the expedition organized by the Central Institute of Epidemiology and Microbiology (Moscow). 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 also isolated from additional patients by local scientists from the Institute of Epidemiology and Microbiology, Laboratory of Virology in Alma-Ata by Prof. E. I. Demikhovsky. Isolation had been accomplished from CSF samples up to 8 days of illness and also from brain tissue on day 12.[1]

In Kazakhstan, the clinical manifestations of TBE were first described by Steblov E.M., again in the Almaty region, and the disease had been named "Almaty encephalitis". Moreover, Steblow 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 pictus* ticks (in 11 pools; 45%) in Zailiysky Alatau and 12 additional strains in Jungarsky Alatau (720 ticks – 12 pools – 100%).[5] In the 1960s the Arbovirus Infections Laboratory of the Institute of Epidemiology, Microbiology and Hygiene (Alma-Ata) under the direction of Prof. 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 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%.[8]

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 *D. pictus* 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] With no typical TBE cases in steppe foci in recent years, only 15 districts in 2 regions (Almaty and Eastern Kazakhstan)[15] are still on the list of TBE-endemic areas. However, in 2016, new cases appeared in "old" endemic zones in the Akmola region.[17-21]

In 2020, 32 cases were registered, including 6 cases in Akmola and 4 cases in Northern Kazakhstan regions that are not officially endemic. Only one case was registered in the Almaty region and no cases were registered in such major cities as Almaty and Nur Sultan. We explain this by the development of the COVID-19 pandemic and the implementation of restrictive anti-epidemic measures during the tick activity season (April–May), when people could not move freely and travel to endemic zones.

In 2021, the incidence of tick-borne encephalitis continued to decrease (by more than 20% compared to 2020), including a decrease in the number of cases in children (4

and 3 cases, respectively). At the same time, 2021 was characterized by an increase in the number of cases in the "new" endemic territory – the North Kazakhstan region (9 cases compared to 4 in 2020) and the appearance of cases in "non-endemic" territories – Zhambyl region (1 case).

In 2022, the number of confirmed TBE cases has increased to 32 (25% more compared to 2021). The highest number of cases was noted in the Almaty region (together with Almaty city) (13 cases), followed by the East Kazakhstan region (9 cases). The incidence was still registered in the "new" endemic regions – Akmola region (together with Astana, former Nur Sultan, city) with 6 cases, North Kazakhstan region with 3 cases and Zhambyl region with 1 case. Thus, the data of the former Kazakh Institute of Epidemiology on the wider endemicity of TBE, in addition to the Almaty and East Kazakhstan regions, are confirmed. We can also talk about the spread of endemicity in the Zhambyl region.

Overview of TBE in Kazakhstan

Table 1: Virus, vector, transmission of TBE in Kazakhstan

Viral subtypes, distribution	Siberian subtype, Almaty region[12] Siberian subtype, Eastern Kazakhstan region[13]
Reservoir animals	*No information available*
Infected tick species (%)	By virology studies (1970):[14] • 74% of natural foci are located in the mountains • 26% are in the steppe, forest-steppe foci • In the mountain foci, 51% of collections are *I. persulcatus* and 30.8% are *D. pictus* • In the steppe, 97%–99% of collections are *D. marginatus* and 1%–3% are *D. pictus* • In the forest-steppe zone, *D. marginatus* and *D. reticulatus* occur equally often • 90% of TBE patients are in the mountain foci The tick infection rate of long-term data: In the mountain foci of Zailiyskiy and Dzhungarskiy Alatau (Almaty region)[14] • *I. persulcatus* – 83/26 of positive pools (each pool - 10 to 30 ticks) – 31.3%; • *D. pictus* – 65/19 – 29.2% The steppe foci of Central Kazakhstan – • *D. marginatus* – 134/44 – 32.7% The steppe foci of Northern Kazakhstan – • *D. marginatus* – 15/5 – 33.3% Forest-steppe – • *D. marginatus, D. pictus* – 23/5 – 16.6% By ELISA on TBEV Ag (2014–2015):[15] *I. persulcatus* 18.6%–21.8% *D. marginatus* 32.1%–74.2% *D. reticulatus* 33.3%–33.3% *D. niveus* 34.8%–45.4% *H. punctata* 33.3%–47.0% *R. turanicus* 14.8%–15.7% By PCR in Almaty region (2014–2016)[16] • Talgar *I. persulcatus* 504 ticks/103 pools pos. 22 (21.3%) • Esyk *I. persulcatus* 79/17 pos. 5 (29.4%) *Haemophysalis punctata* 444/96 pos. 1 (1.0%) • Tekeli *I. persulcatus* 610/123 pos. 19 (15.4%) *D. marginatus* 50/12 pos. 1 (8.3%)
Dairy product transmission	Not documented—rare—frequent

Table 2: TBE reporting and vaccine prevention in Kazakhstan	
Mandatory TBE reporting	Any healthcare worker who has any reason to suspect that the patient has TBE.[11] A case of tick-borne encephalitis is reported if one of the following is present: 1. isolation of TBEV from blood or cerebrospinal fluid; 2. detection of TBEV RNA in PCR; 3. detection of IgM to TBEV by ELISA in serum or cerebrospinal fluid; 4. increasing titer of IgG antibodies to TBEV in ELISA. A probable case of TBE is reported with acute severe disease, accompanied by high fever, severe intoxication, and a syndrome of meningitis or meningoencephalitis, characterized by at least four of the following: 1. hyperemia and puffiness of face; 2. lethargy or agitation; 3. headache; 4. nausea and vomiting; 5. meningeal symptoms (stiff neck, Kernig's signs, Brudzinsky's signs in children) and one of the following: • tick bite; • contact with a tick; • epidemiologic link with a confirmed case. Possible cases – the definition of a suspected (possible) case in the TBE classification is not being used.
Other TBE surveillance	Unclear
Special clinical features	Biphasic disease? Usually no-risk groups? Local population in endemic zones
Available vaccines	Vaccine tick-borne encephalitis cultural concentrated purified inactivated sorbate "EnceVir" (ЭнцеВир ®) Russia Suspension for intramuscular injection; 1 dose (0.5 mL) in a vial One dose (0.5 mL) contains inactivated antigen of tick-borne encephalitis virus (TBE) in ELISA titer of at least 1:128 (active component) The course of vaccination consists of two injections with an interval of 1–7 months. Course of vaccination (two vaccinations) can be carried out throughout the year, including during the summer season but not later than two weeks prior to a visit to a TBE endemic zone. The optimal interval between the first and second vaccinations – 5–7 months (autumn-spring). If necessary, emergency prevention, including, at the beginning of vaccinations in the summer, the interval between vaccinations may be reduced to 14 days. Manufacturer scientific practical association: "Microgen", Russia, Tomsk.
Vaccination recommendations and reimbursement	Give year when recommendations / reimbursement started, year of changes, etc.
Vaccine uptake by age group/ risk group/general population	Medical organizations hold preventive, routine immunization against tick-borne encephalitis professionally threatened contingents (risk group).
Name, address/website of TBE NRC	Scientific practical center for sanitary and epidemiological expertise and monitoring (SPC SEEM), Parasitology Department #84, Auezov street, Almaty, 050008

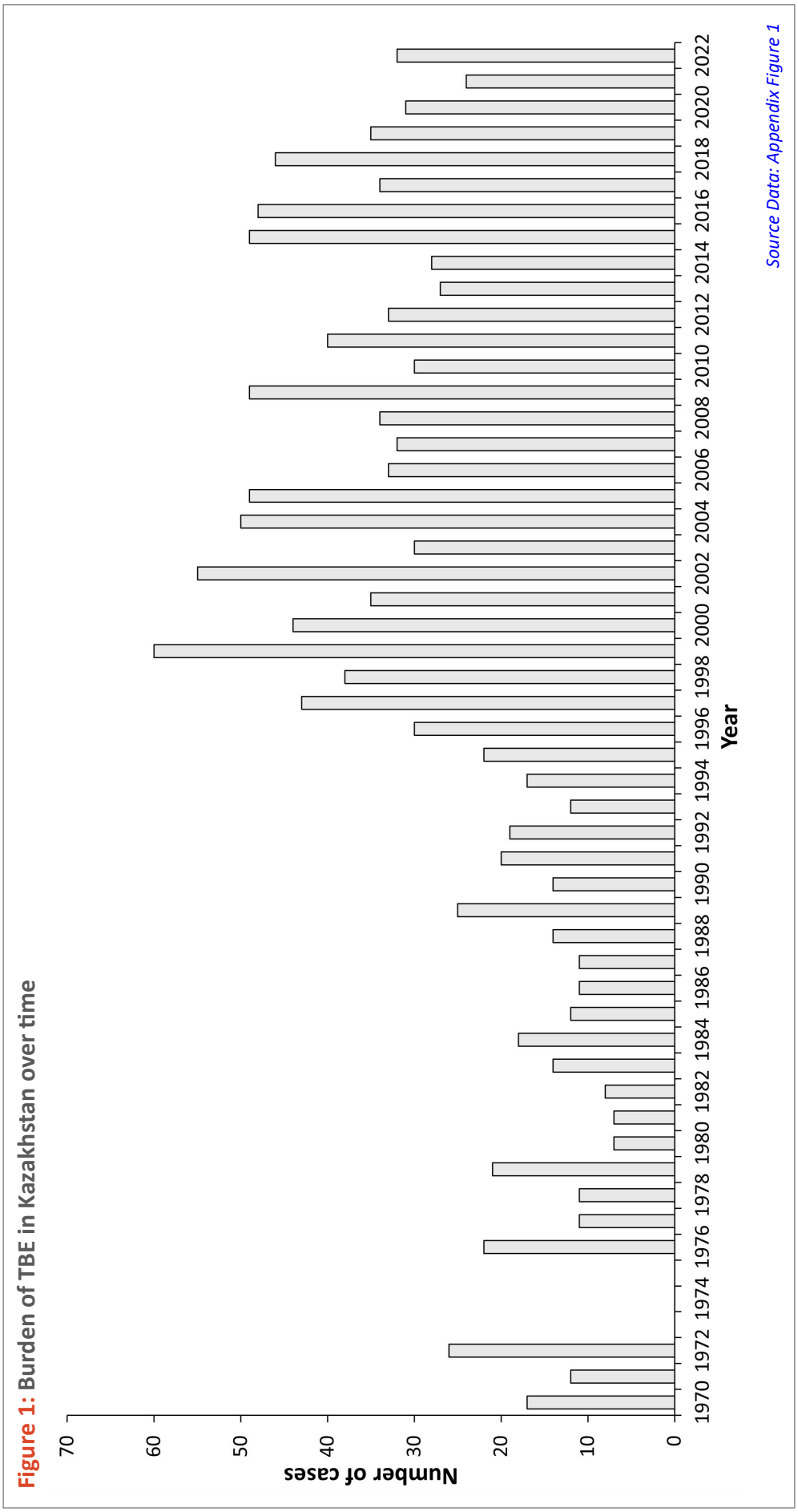
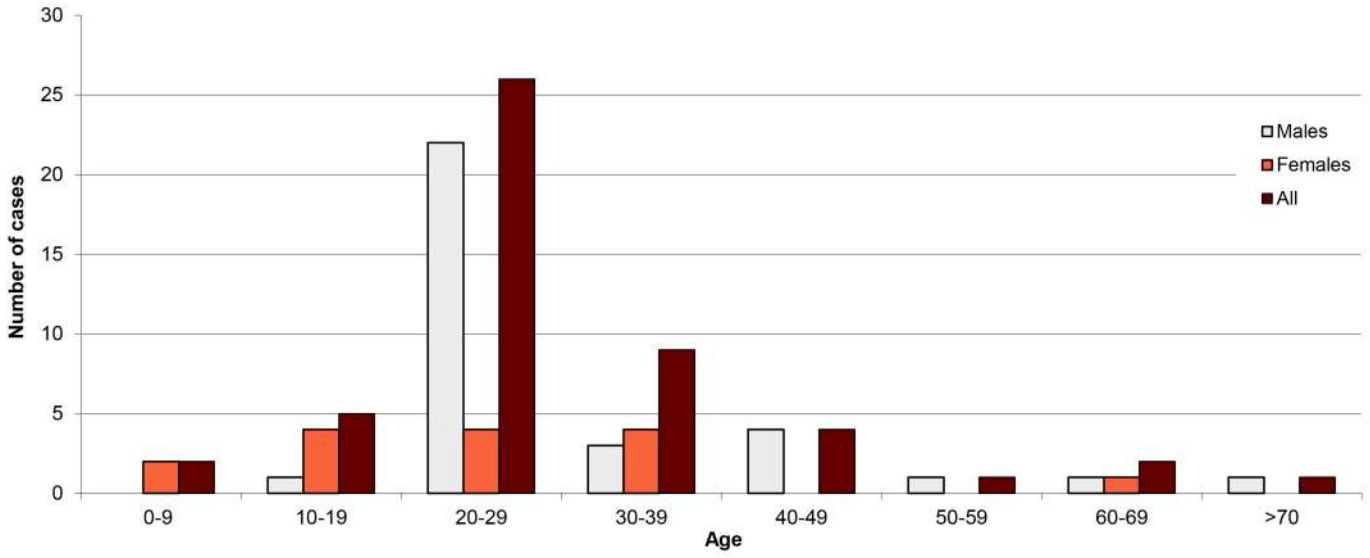


Figure 1: Burden of TBE in Kazakhstan over time

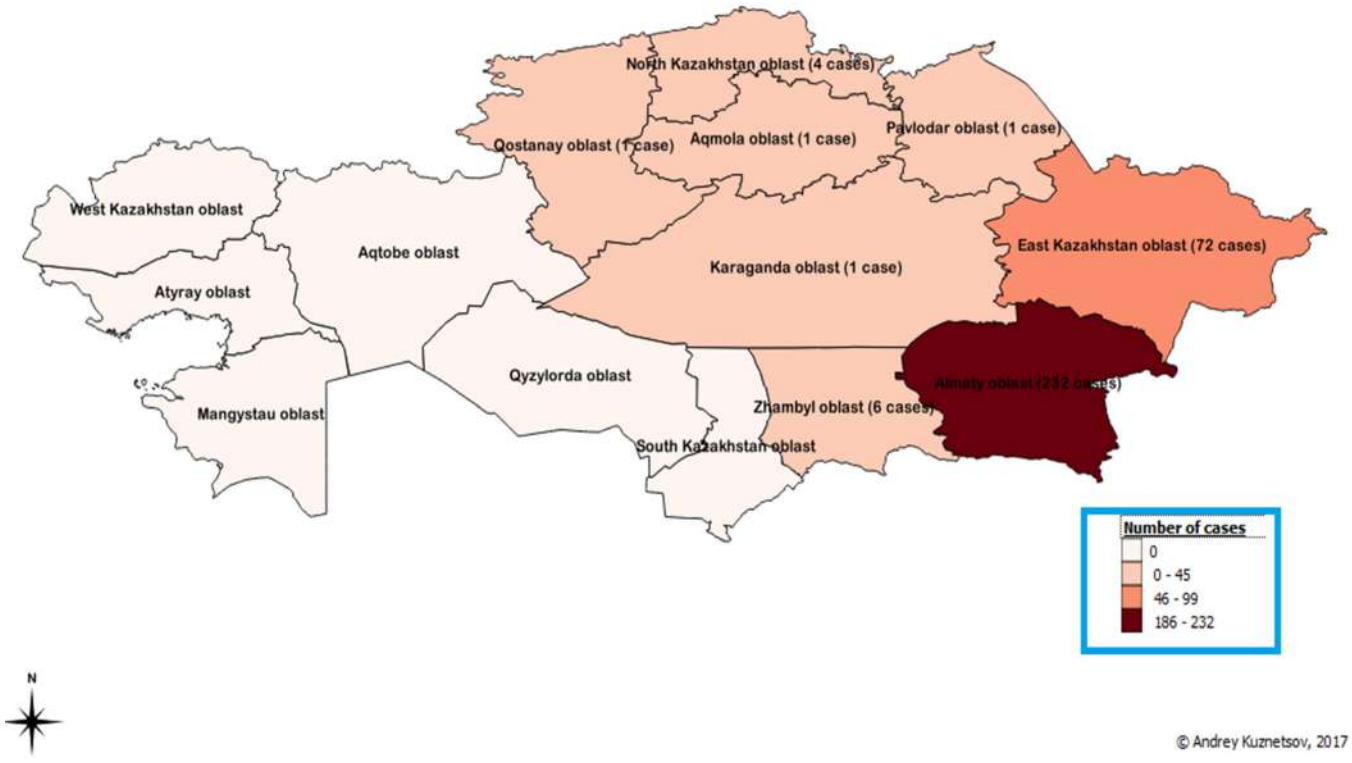
Source Data: Appendix Figure 1

Figure 2: Age and gender distribution of TBE in Kazakhstan



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).

The number of TBE cases in the Regions of Kazakhstan during 1970–1992
(Unfortunately, there is no current information for when and where isolated TBEV was verified, with the exception of the above historical information on the TBEV isolation in Almaty and Eastern Kazakhstan Regions.)

A feature of the epidemiology of tick-borne encephalitis in 2018-2019 was the registration of cases of CE in "unusual" areas-Akmola (9), North Kazakhstan (2), Kostanay (1), Zhambyl (1), and even the appearance of the case in the region where the incidence of TBE has never been recorded before (Kyzylorda-1)

Appendix

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

Source data: Figure 2

Age group	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

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TBE in Kyrgyzstan

Wilhelm Erber

E-CDC risk status: endemic (limited data available)

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

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Chapter 12b

TBE in Latvia

Dace Zavadska and Zane Freimane

E-CDC risk status: endemic (data as of end 2022)

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 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 suggests 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 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.

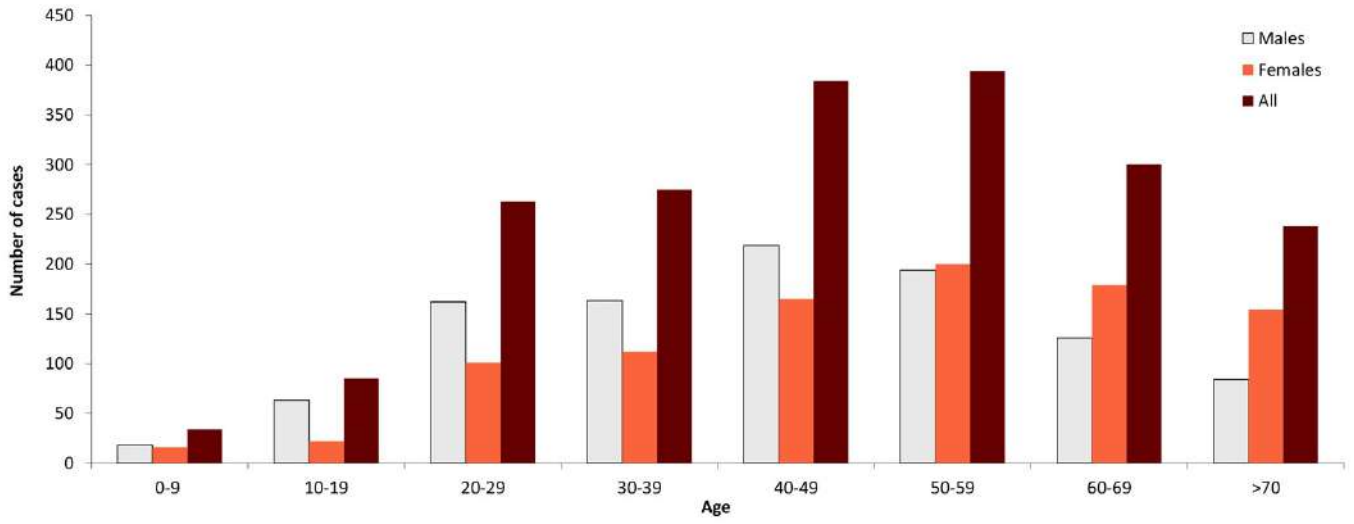
Vaccination remains the most effective protective measure against TBE.[10,11,12] In Latvia, there is only a partial National Immunization Program, which has provided vaccine free of charge for children living in highly endemic areas since 2006 and orphans/children without parental care in the whole country 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 an up to 86% market share 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 part 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

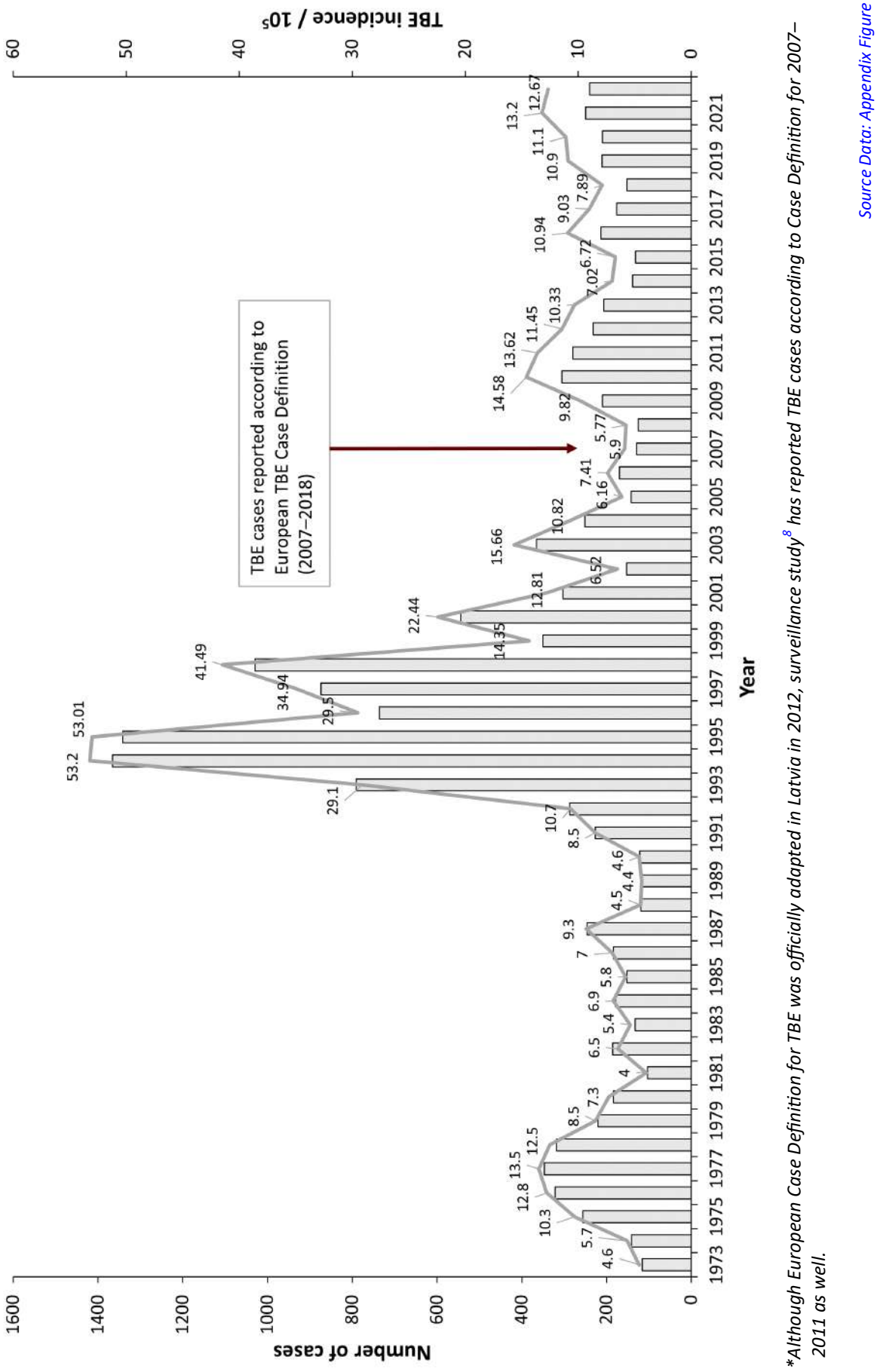
Figure 2: Age and gender distribution of TBE in Latvia (2007–2016, n=1973)[8]



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 covers 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]

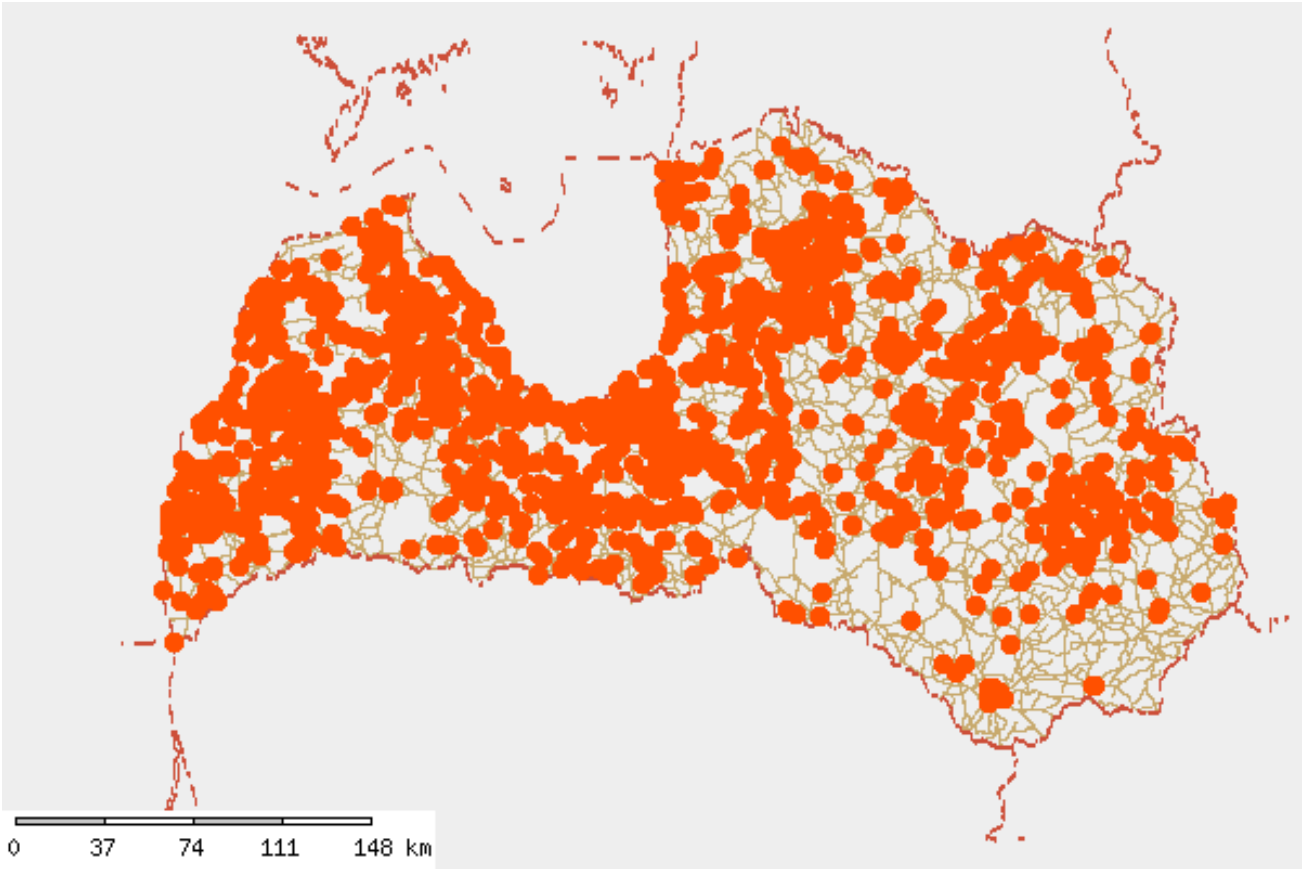
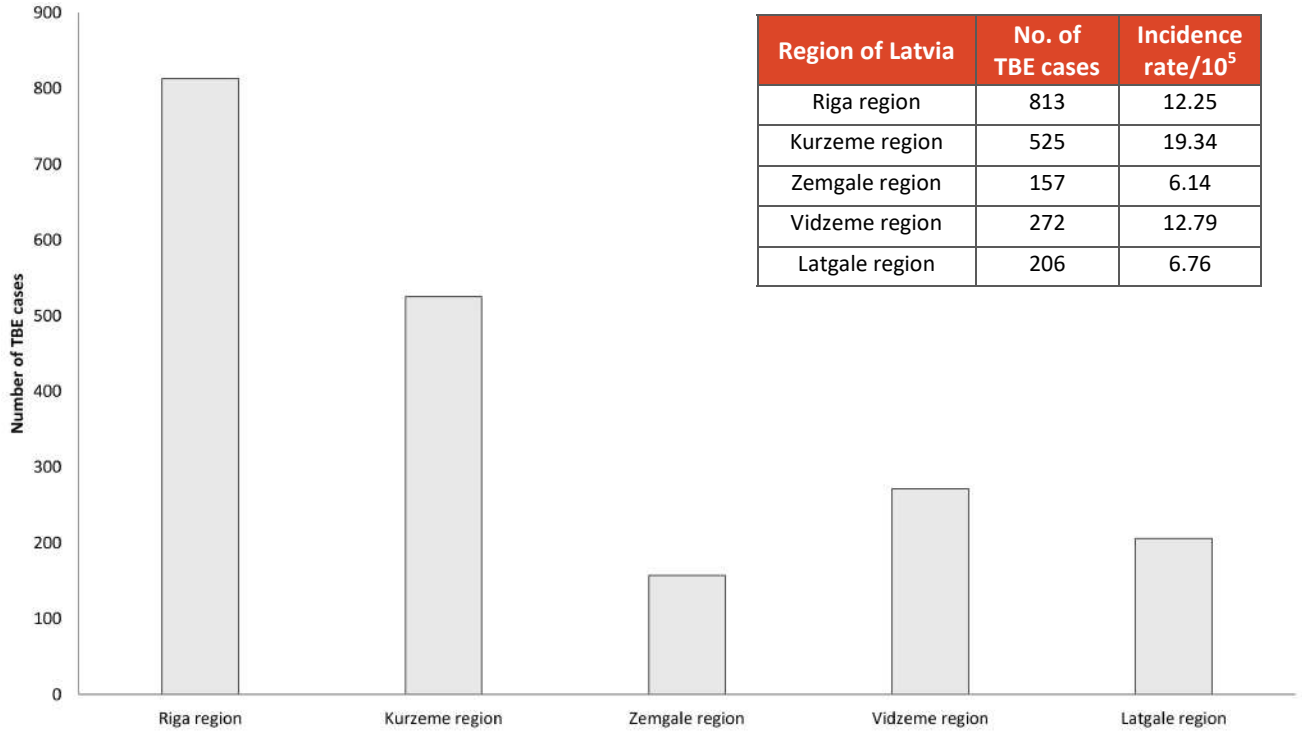


Figure 4: Burden of TBE ("CNS disease") by 5 regions of Latvia (2007–2016, n=1973)[8]



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

*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.

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Chapter 12b

TBE in Lithuania

Auksė Mickienė

E-CDC risk status: endemic (data as of end 2022)

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 most recent entomological studies have also detected *I. persulcatus* in the Rokiškis district.[7] Sequence analysis of Lithuanian TBEV strains isolated from humans and field-collected ticks has shown that the virus belongs to the European TBEV subtype.[4,8] The minimum infection rate of *I. ricinus* ticks in Lithuania varies from 0.1% to 1.84%.[4,9]

TBEV is found from ticks collected in all administrative districts of Lithuania and in 3 urban parks in the country.[3] The density of *I. ricinus* ticks during the spring peak of activity increased three-fold from 1995 (19 ticks per 1 km) to 2008 (57 ticks per 1 km),[3] and this increase has been correlated to increased numbers of TBE cases in humans.

TBEV seroprevalence in non-vaccinated healthy permanent residents in Lithuania is 3%. TBEV antibodies have been more frequently found in people who regularly visit the countryside or who consume unpasteurized goat milk, and the risk for seropositivity increases with age.[10] Also, a general correlation has been noted between seropositivity among domestic animals, TBEV prevalence in ticks, and cases of TBE in humans in some regions of Lithuania.[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). In 2012, 4.1% of the Lithuanian population lived in these three districts (123,255 of 3,003,641 permanent inhabitants of Lithuania); however, the total number of TBE cases in these districts comprised 17% (1,230 of 7,409) of all TBE cases registered in Lithuania between 1993 and 2011.[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 up to 2018. During the last 3 years, the highest TBE incidence rate in Lithuania was observed in Utena county, in the northeastern part of Lithuania and on the border to Latvia (2016 – 42.8/100 000, 2017 – 40.3/100 000, 2018 – 27.3/100 000).[13]

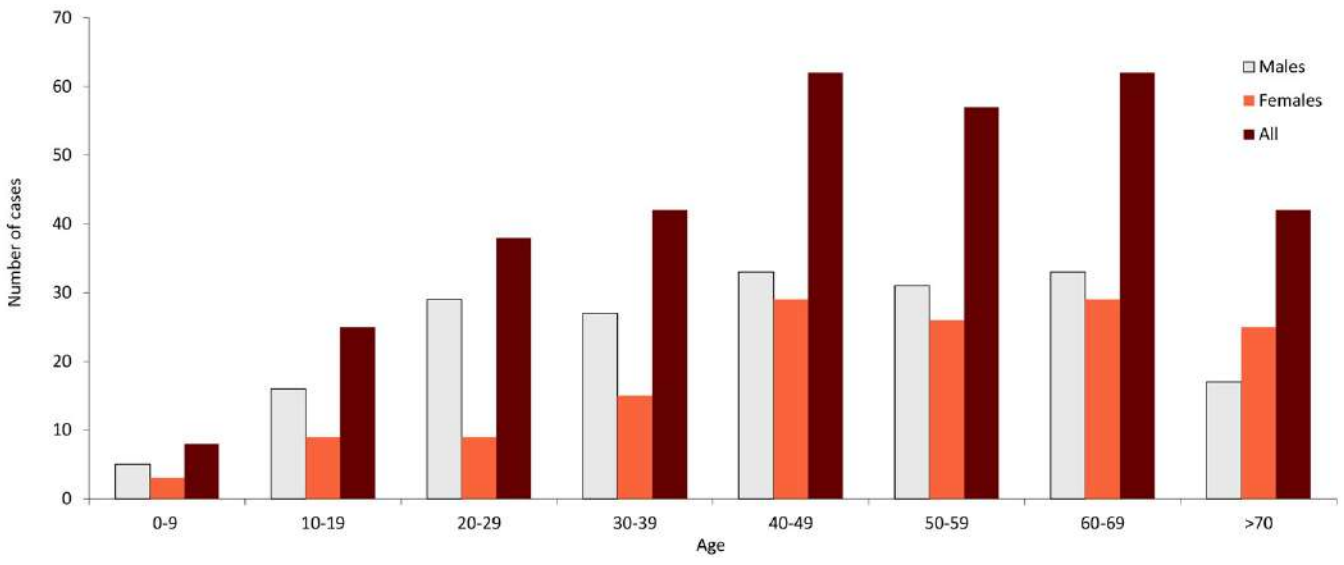
Presently, TBE is the most common viral infection of the CNS in Lithuania, with an average number of 395 cases per year; a total of 10,611 TBE cases was reported between 1990 and 2018.[13] Children (mainly school children and adolescents) comprise 8.7% of all TBE cases in the country. In the period from 1999 to 2018, children 0–3 years of age comprised 5.4% of all TBE cases in children (n=38), 4–6-year -old children comprised 11.2% (n=79), and 7–16 year-old children comprised 83.4% (n=589).[13] Retired and unemployed people are the major risk group for infection with TBEV in Lithuania; 56.4% of TBE patients are infected in the immediate areas surrounding their homes.[14]

Overview of TBE in Lithuania

Table 1: Virus, vector, transmission of TBE in Lithuania	
Viral subtypes, distribution	European TBEV subtype[4,8]
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%)[4,9]
Dairy product transmission	Rare[13]

Table 2: TBE reporting and vaccine prevention in Lithuania	
Mandatory TBE reporting	All hospitalized patients with CNS form of TBEV infection confirmed by serological methods (TBEV IgM ± IgG) are reported to the Lithuanian Center for Communicable Diseases and AIDS[13]
Other TBE surveillance	N/A
Special clinical features	Biphasic disease in 72.2% Risk groups: retired people, unemployed people, and permanent inhabitants of highly endemic areas[14] Moderate and severe sequelae in 30.8%. Mortality 0.75%[14]
Available vaccines	Encepur, Ticovac.[13] Total number of doses sold 2010–2015: 308,969
Vaccination recommendations and reimbursement	Vaccination of adults: the joint recommendations by Lithuanian Societies for Infectious Diseases, Internal and Family Medicine (2013; no reimbursement). Reimbursed for military recruits and forestry workers*
Vaccine uptake by age group/risk group/general population	Total number of doses sold 2010–2015:[13] Children (0–17 years) – 101,651 Adults (>18 years) – 207,318
Name, address/website of TBE NRC	The Lithuanian Center for Communicable Diseases and AIDS[13]

Figure 2: Age and gender distribution of TBE in Lithuania, 2010–2015



Source Data: Appendix—Figure 2

TBEV-isolation and TBE cases in Lithuania:

no information available

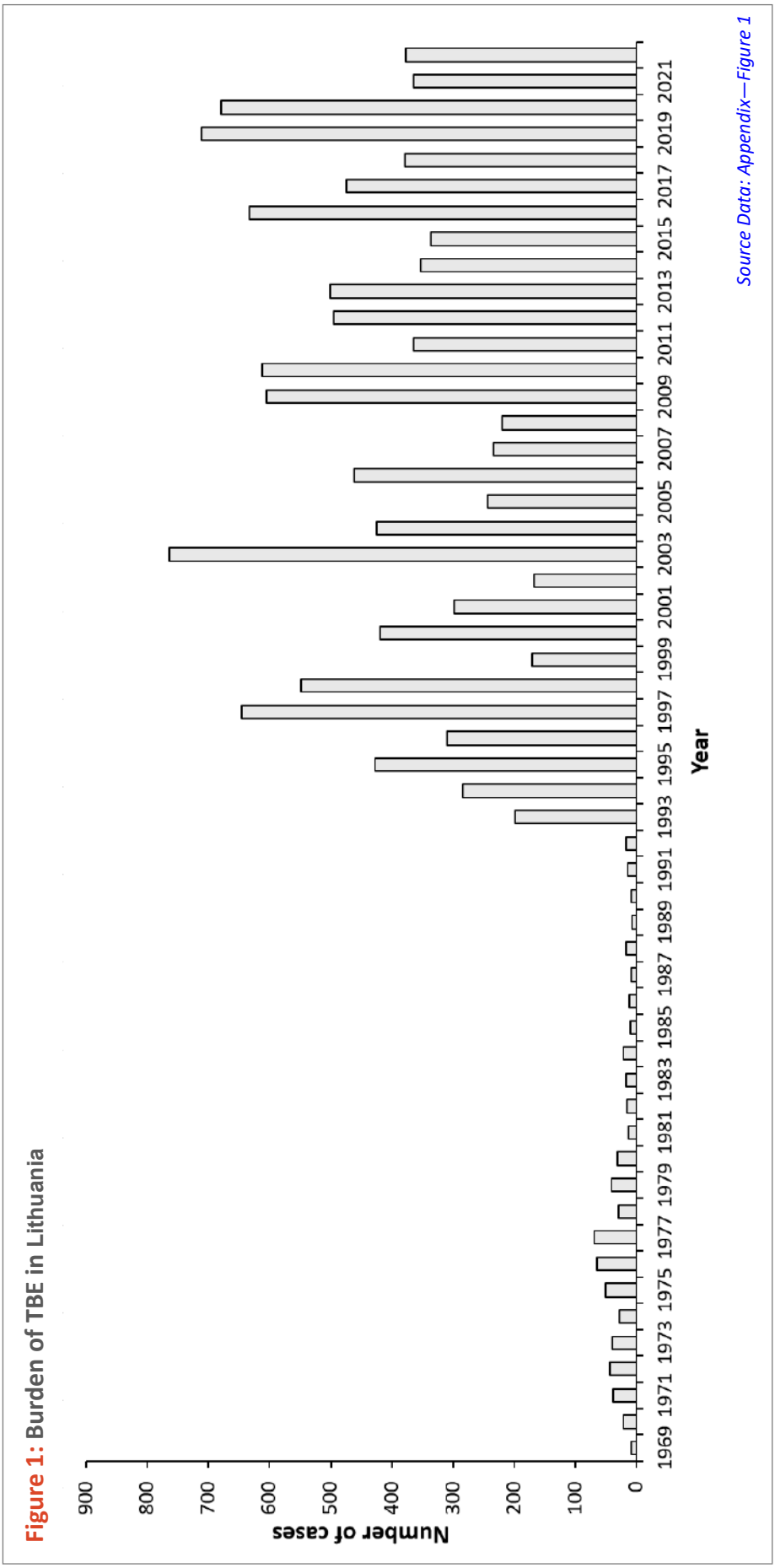


Figure 1: Burden of TBE in Lithuania

Source Data: Appendix—Figure 1

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

Source data: Figure 2

2010			
Age group (years)	Males	Females	All
0-9	17	7	24
10-19	30	20	50
20-29	43	19	62
30-39	34	31	65
40-49	59	59	118
50-59	71	56	127
60-69	41	57	98
>70	38	30	68

2012			
Age group (years)	Males	Females	All
0-9	9	5	14
10-19	21	13	34
20-29	37	21	58
30-39	34	17	51
40-49	52	33	85
50-59	59	43	102
60-69	42	37	79
>70	30	42	72

2014			
Age group (years)	Males	Females	All
0-9	4	2	6
10-19	17	12	29
20-29	25	14	39
30-39	19	13	32
40-49	27	22	49
50-59	53	39	92
60-69	26	30	56
>70	16	34	50

2011			
Age group (years)	Males	Females	All
0-9	7	1	8
10-19	20	12	32
20-29	20	17	37
30-39	29	24	53
40-49	35	33	68
50-59	34	31	65
60-69	30	39	69
>70	14	19	33

2013			
Age group (years)	Males	Females	All
0-9	4	8	12
10-19	16	10	26
20-29	36	16	52
30-39	39	21	60
40-49	53	35	88
50-59	67	53	120
60-69	36	43	79
>70	24	40	64

2015			
Age group (years)	Males	Females	All
0-9	5	3	8
10-19	16	9	25
20-29	29	9	38
30-39	27	15	42
40-49	33	29	62
50-59	31	26	57
60-69	33	29	62
>70	17	25	42

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TBE in Moldova

Wilhelm Erber and Tamara Vuković-Janković

E-CDC risk status: affected (limited data available)

History and current situation

Although there are no reliable data on the number of tick-borne encephalitis (TBE) cases or the percentage of infected ticks, based on the geography and the presence of TBE virus (TBEV) in all neighboring countries, it must be assumed that TBEV is present somewhere in Moldova.

The Far Eastern subtype (TBEV-FE) was detected in 3 different species of ticks collected from domestic animals and agricultural lands in the Republic of Moldova between 2010 and 2011 (*Ixodes ricinus, Dermacentor* spp. and *Haemaphysalis* spp.).[1] The World Health Organization (WHO) Centralized Information System for Infectious Diseases (CISID) collects data on the incidence of TBE; however, in 2005 and 2006, Moldova officially reported zero TBE cases.[2]

Overview of TBE in Moldova

Table 1: Virus, vector, transmission of TBE in Moldova	
Viral subtypes, distribution	Far Eastern subtype (TBEV-FE)[1]
Reservoir animals	Information not available
Infected tick species (%)	3.8% *I. ricinus* ticks (3/78), 3.9% *D. reticulatus* ticks (3/77) and 8.8% *Haemaphysalis punctate* ticks (3/34) were positive for TBEV RNA[1]
Dairy product transmission	Not documented

Burden of TBE in Moldova over time: no data available

Age and gender distribution of TBE in Moldova: no data available

TBEV-isolation and TBE cases in Moldova: no reported cases of TBE in the country

Table 2: TBE reporting and vaccine prevention in Moldova	
Mandatory TBE reporting	Not mandatory
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	National Centre of Public Health of Moldova (Ministry of Health) http://cnsp.md/ (available only in local language)

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Chapter 12b

TBE in Mongolia

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E-CDC risk status: endemic (data as of end 2022)

History and current situation

In Mongolia, TBEV 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] *I. 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.

Since 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]

A family 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 infections from 1993–2000. Five of them had died and had been recorded as “viral infections“. This is the first 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.

Between 2005–2022, 371 confirmed cases have been registered in Arkhangai, Bayankhongor, Bulgan, Darkhan-Uul, Dundgobi, Dornod, Orkhon, Uvurkhangai, Selenge, Tuv, Uvs, Khunsgul, Khentii provinces and Ulaanbaatar city. Most patients remembered a tick bite had occurred in the area of the Selenge (78%) and Bulgan (12%) provinces. During this period (2005–2022), there were 18 fatal cases (CFR 4.85%) attributed to severe meningoencephalitis (Fig. 1).

Since 2005, prevention measures such as vaccination, training and advocation among the population have been administered but human cases continue to be registered. Between 2014 and 2017, TBE cases and deaths increased annually, but declined in the last five years (2018–2022). TBE cases have also been recorded from areas without the main vector *I. persulcatus*. Moreover, an expansion of natural TBEV-foci has been 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 (Fig. 2). The majority of subjects were bitten by ticks when they had been collecting plants and picnicking during May and June.[7]

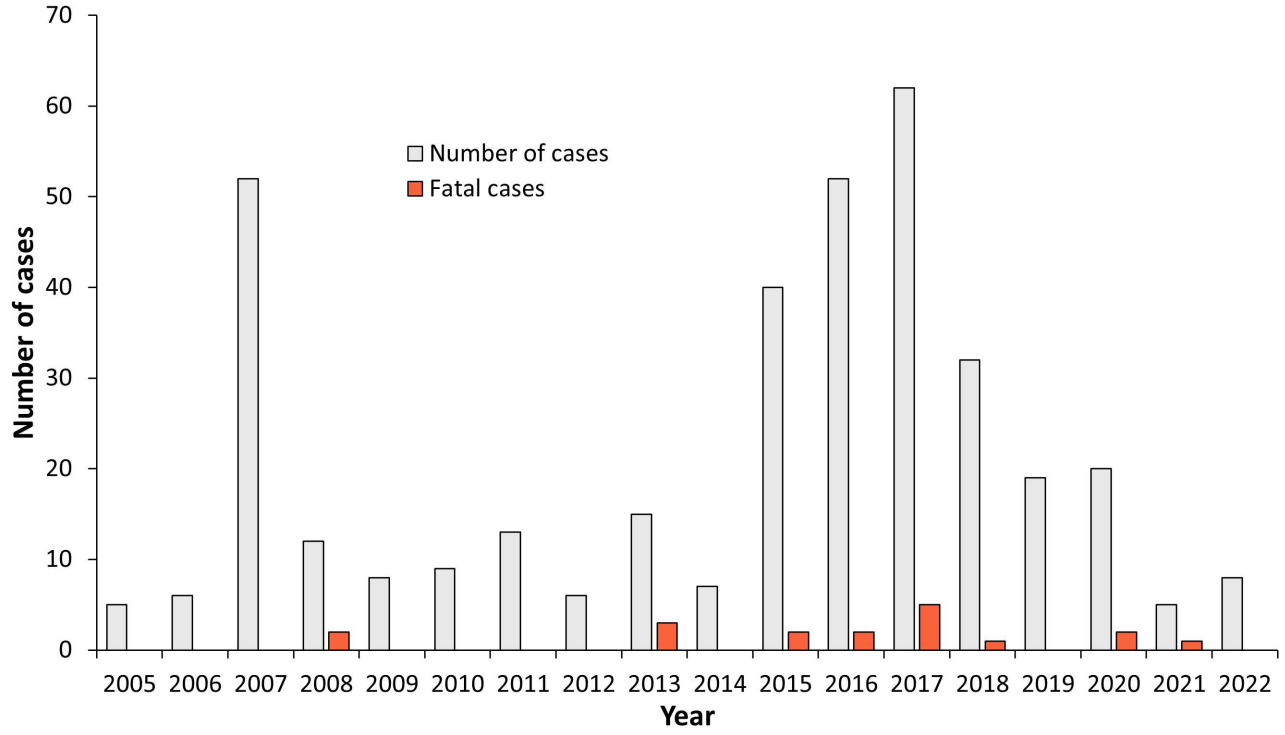
According to a survey of long-term neurological symptoms in 37 TBE-recovered individuals in Selenge province, 5 (16.1%) of them manifested with fever, 6 (19.4%) with paralysis, 8 (25.8%) with meningoencephalitis and 12 (38.7%) with meningitis when they were ill. After recovery between one to twelve years, 78.4% of them had headache, 30%–40% of them had fatigue, forgetfulness, decreased ability to concentrate and stiff neck, 10%–20% had hearing loss, paralysis, and a small percentage (3.2%) of them still had 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]

Table 1: Virus, vector, transmission of 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

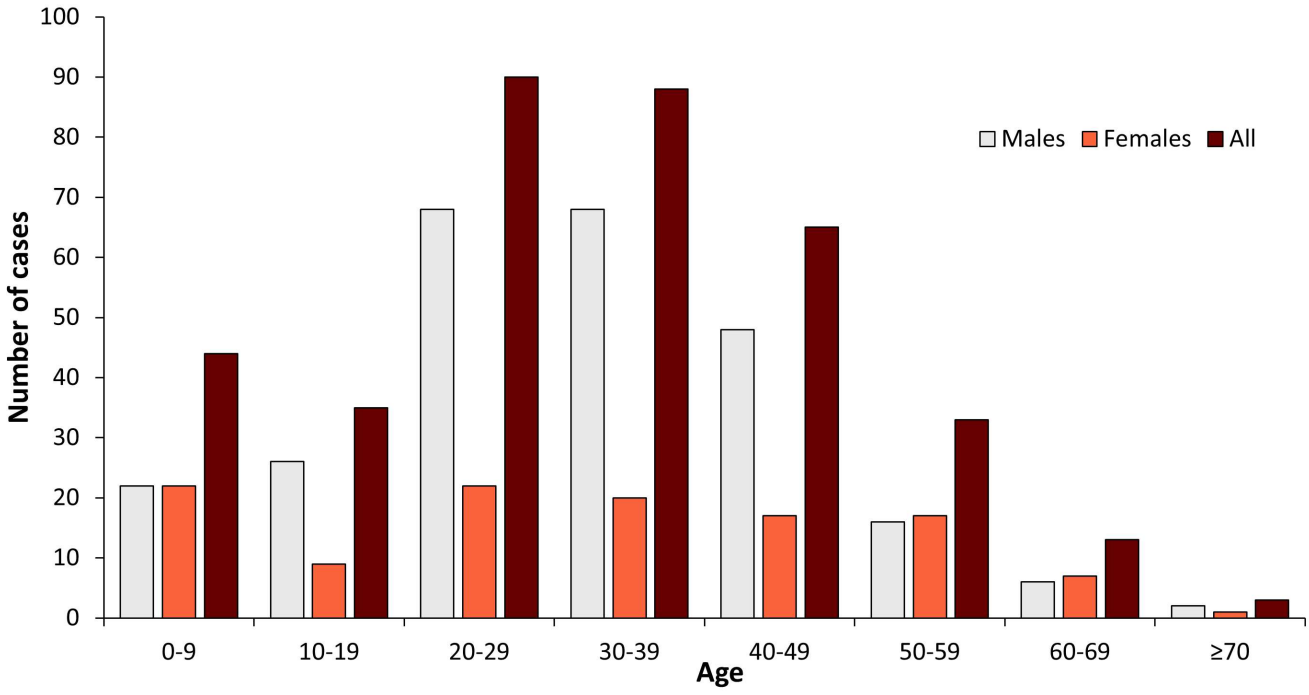
Table 2: TBE reporting and vaccine prevention in Mongolia	
Mandatory TBE reporting	Patients with clinical suspected TBE are reported to the National Center for Zoonotic Diseases (NCZD) where the diagnosis can be microbiologically confirmed (anti-TBEV-IgG and IgM by ELISA). Any patient with serologically 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: TBE cases and mortality, 2005–2022



Source data: Appendix - Figure 1

Figure 2: Age and gender distribution of TBE in Mongolia (2005–2022, n=371)



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 (Fig. 3).

According to surveillance efforts since 2006, 10,464 ticks have been collected. Following species identification, 14.7% (1,540) were classified as *Ixodes persulcatus*, 79.3% (8,300) were *Dermacentor nutalli*, 3.2% (341) were *Dermacentor 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 the 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% (Fig. 4).

Figure 3: Geographical distribution of TBE cases

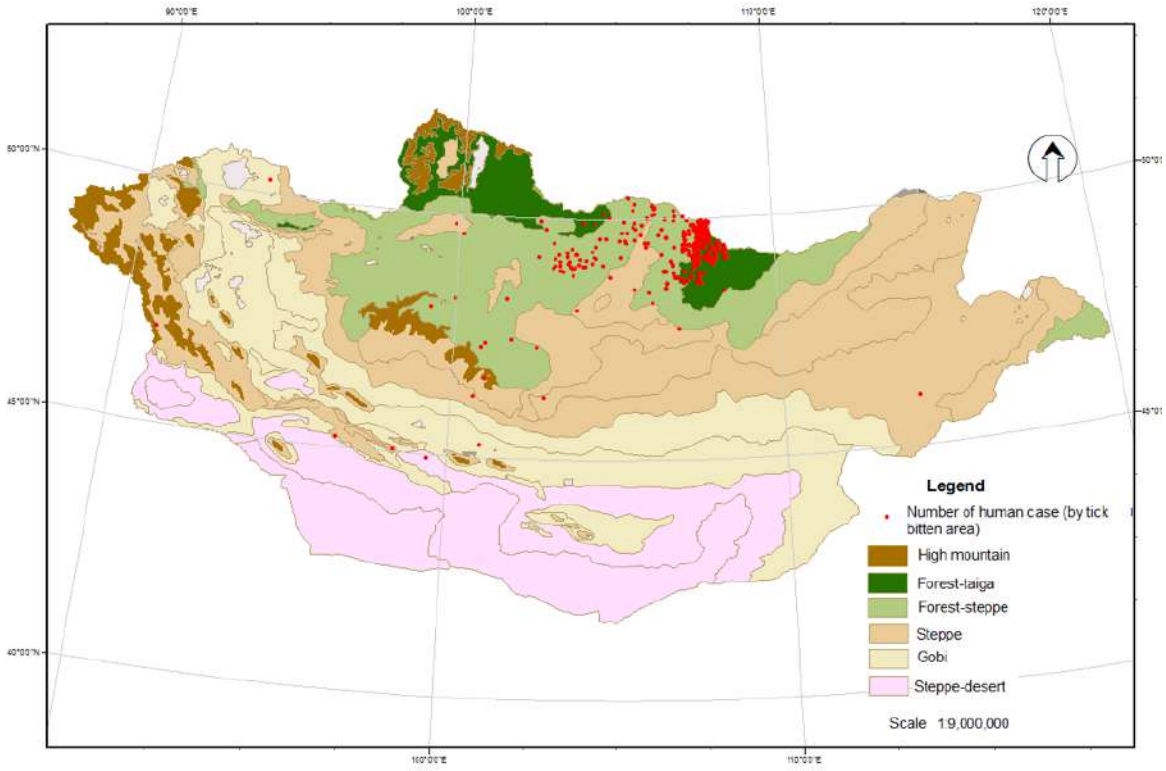
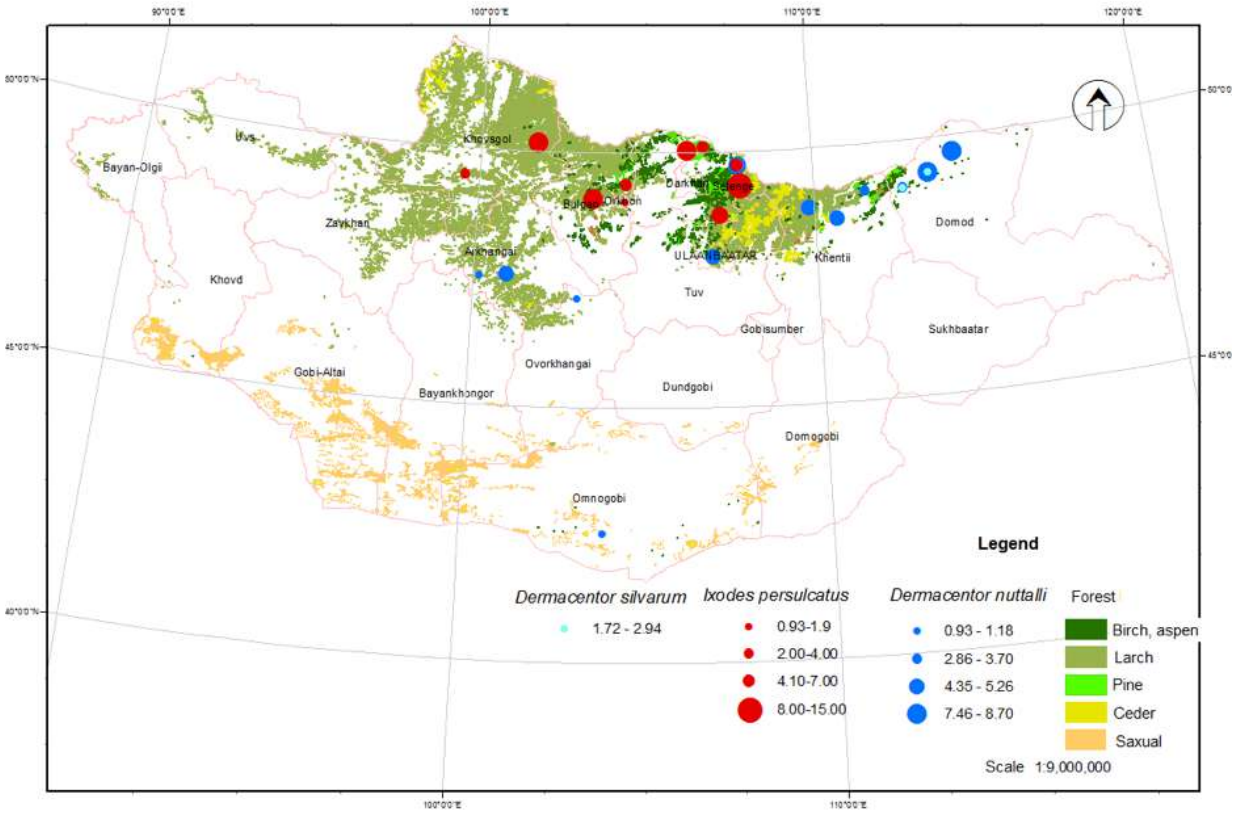


Figure 4: TBEV infection rate of three tick species



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

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	22	22	44
10-19	26	9	35
20-29	68	22	90
30-39	68	20	88
40-49	48	17	65
50-59	16	17	33
60-69	6	7	13
≥70	2	1	3
Total	256	115	371

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Chapter 12b

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 2022)

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 of these 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 out of 297 (2%) roe deer sera, collected in 2010, were found serologically positive for TBEV -infection.[2,3] 5 out 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 nucleotides and 2% on amino acid levels, respectively.

In 2016, soon after the CIb raised general awareness about the presence of TBEV in the Netherlands, the first 2 autochthonous TBE cases were reported.[5,6] Both patients were positive for TBEV by ELISA and virus neutralization test. The first patient most likely acquired TBEV when hiking at national park "Utrechtse Heuvelrug",[2,5] located in the center of the Netherlands (Fig. 3). A tick collected from this patient was RT-PCR-positive for TBEV. 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 national park "Sallandse heuvelrug" and frequently visited this park.[2]

Moreover, fourteen additional autochthonous human cases have been reported since. From the two autochthonous cases reported in 2022, one patient lived in a known endemic region in the east of the Netherlands, in the province of Gelderland and the other patient was reported outside the known TBEV loci on the island of Terschelling in the north of the country (Fig. 3). The reported case in Gelderland most likely acquired infection near his residence and the case in Terschelling lives in Leiden but contracted the infection during a holiday on the island. Additionally, three travel-associated TBEV infections were diagnosed in 2022 and most probably infected in Denmark, Sweden and Germany.

The number of laboratories implementing TBEV diagnostics stagnates at five with virus neutralization tests implemented at two. Despite the general availability of routine diagnostics in the Netherlands the number of diagnosed cases is still low. In 2017, a seroprevalence study conducted in roe deer identified additional potential TBEV foci, mainly located near the borders with Germany and Belgium (Fig. 4). In a study conducted in 2018 and 2019, ticks and/or rodents were sampled and tested for TBEV RNA on locations near to the places where seropositive roe deer were detected.[11] TBEV RNA-positive ticks were found in the known foci as indicated in Figure 1 but additionally TBEV RNA-positive rodents were found outside the known foci (Fig. 1). Since 2020, cases were identified near newly identified potential TBEV foci and we hope that the awareness among clinicians for this recently emerged disease will grow outside the known endemic regions.

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 for 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

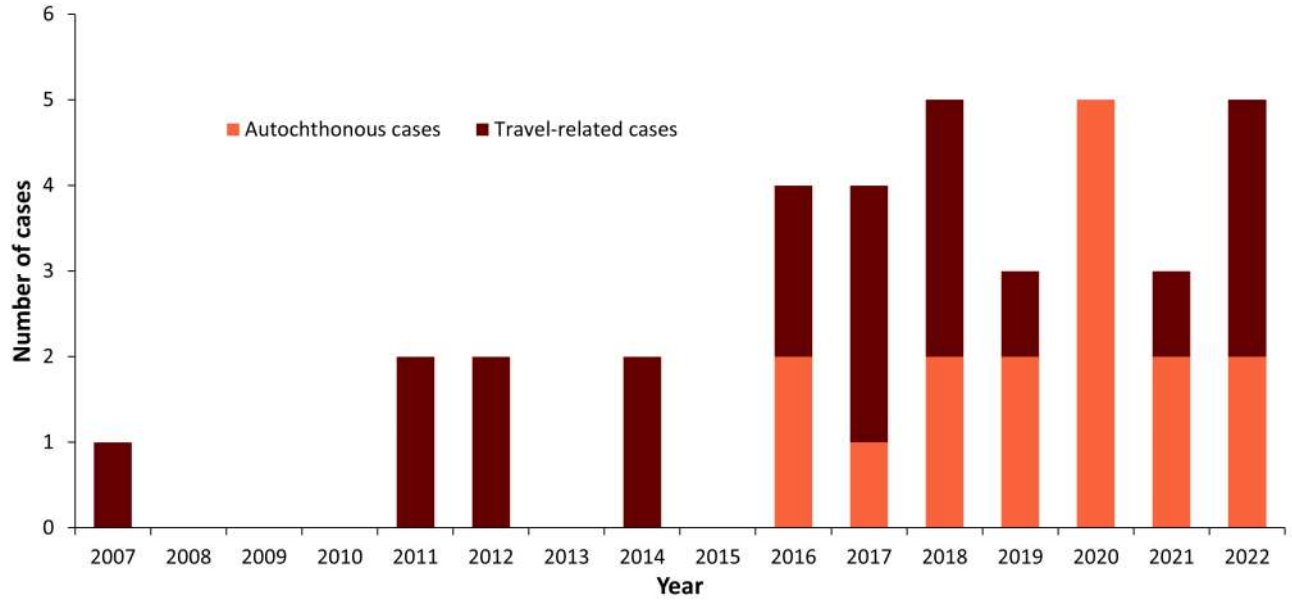
European TBEV strains have been detected in the Netherlands. Based on the fact that two autochthonous cases got infected near national park "Sallandse heuvelrug", it is highly likely that the divergent "Salland" strain found in this area can cause disease in humans, but this remains to be confirmed.

Overview of TBE in the Netherlands

Table 1: Virus, vector, transmission of 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

Table 2: TBE reporting and vaccine prevention in Netherlands	
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 by age group /risk group / general population	No information available
Name, address/website of TBE NRC	-

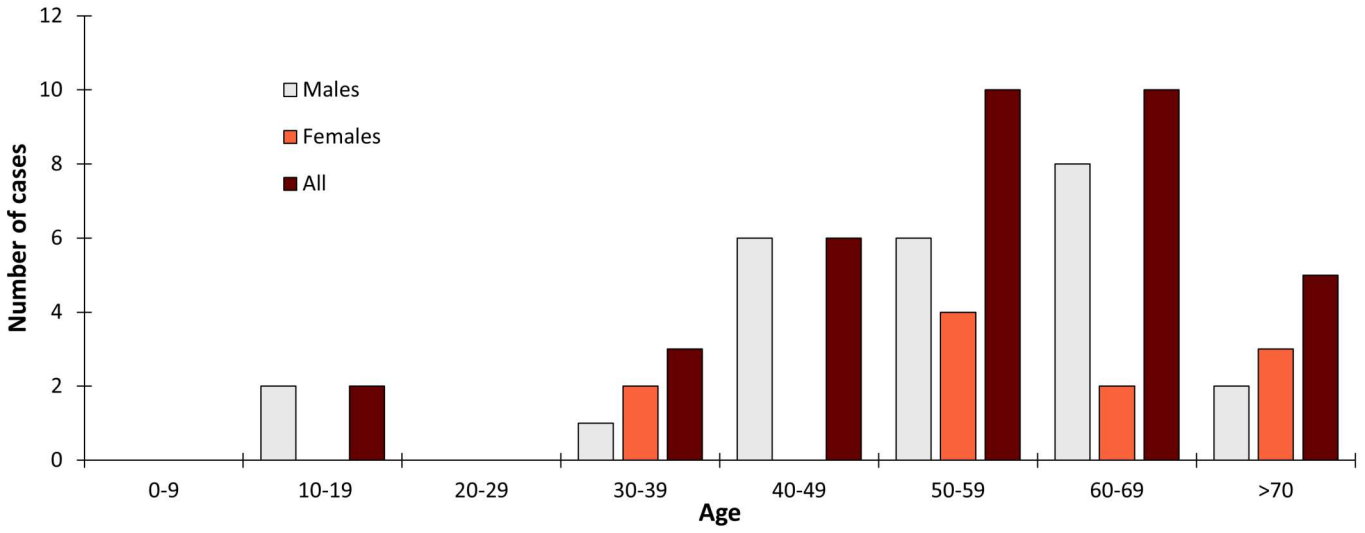
Figure 1: Burden of TBE in the Netherlands over time



Due to the low numbers of diagnostic requests and diagnosed infections, a reliable number for the incidence is difficult to provide.

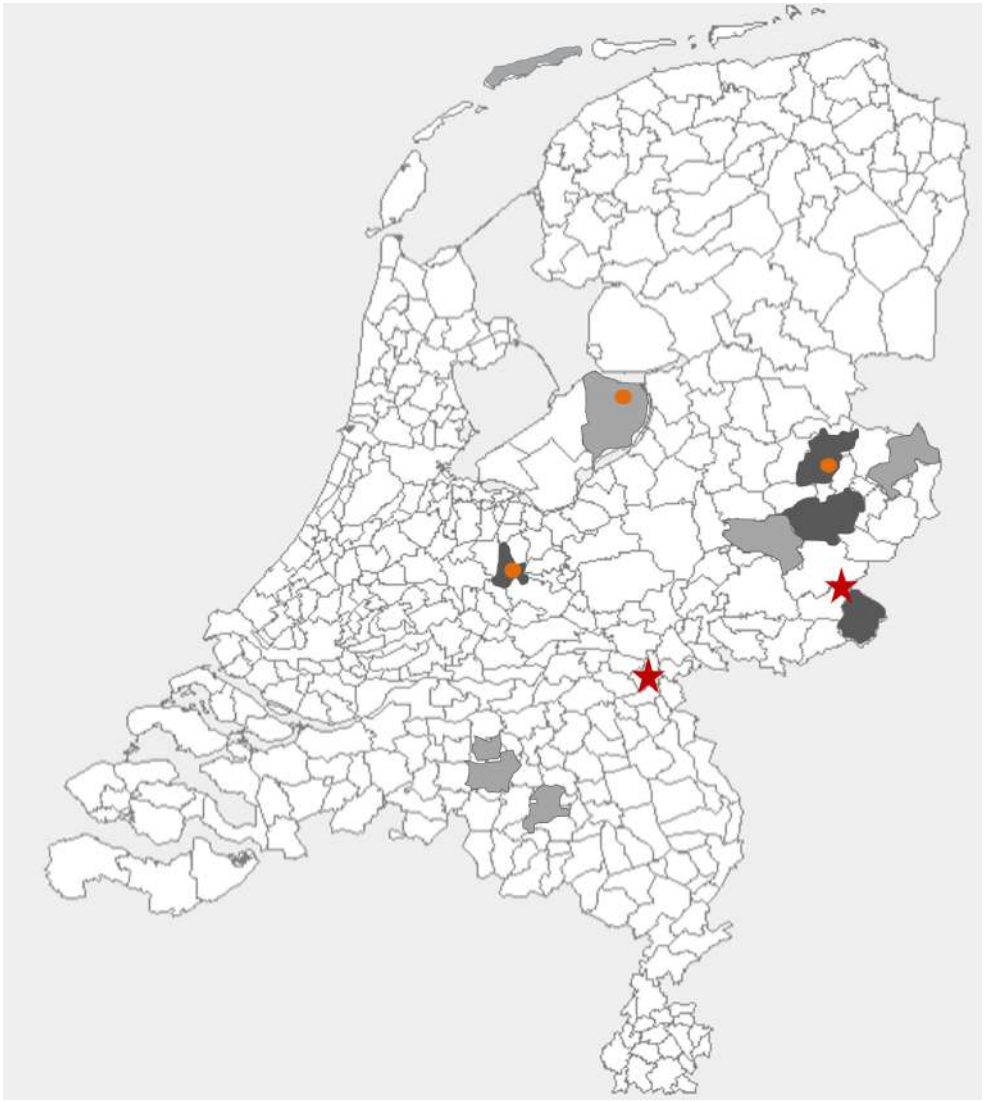
Source Data: Appendix—Figure 1

Figure 2: Age and gender distribution of TBE in the Netherlands



Source Data: Appendix—Figure 2

Figure 3: TBEV-isolation and TBE cases in the Netherlands



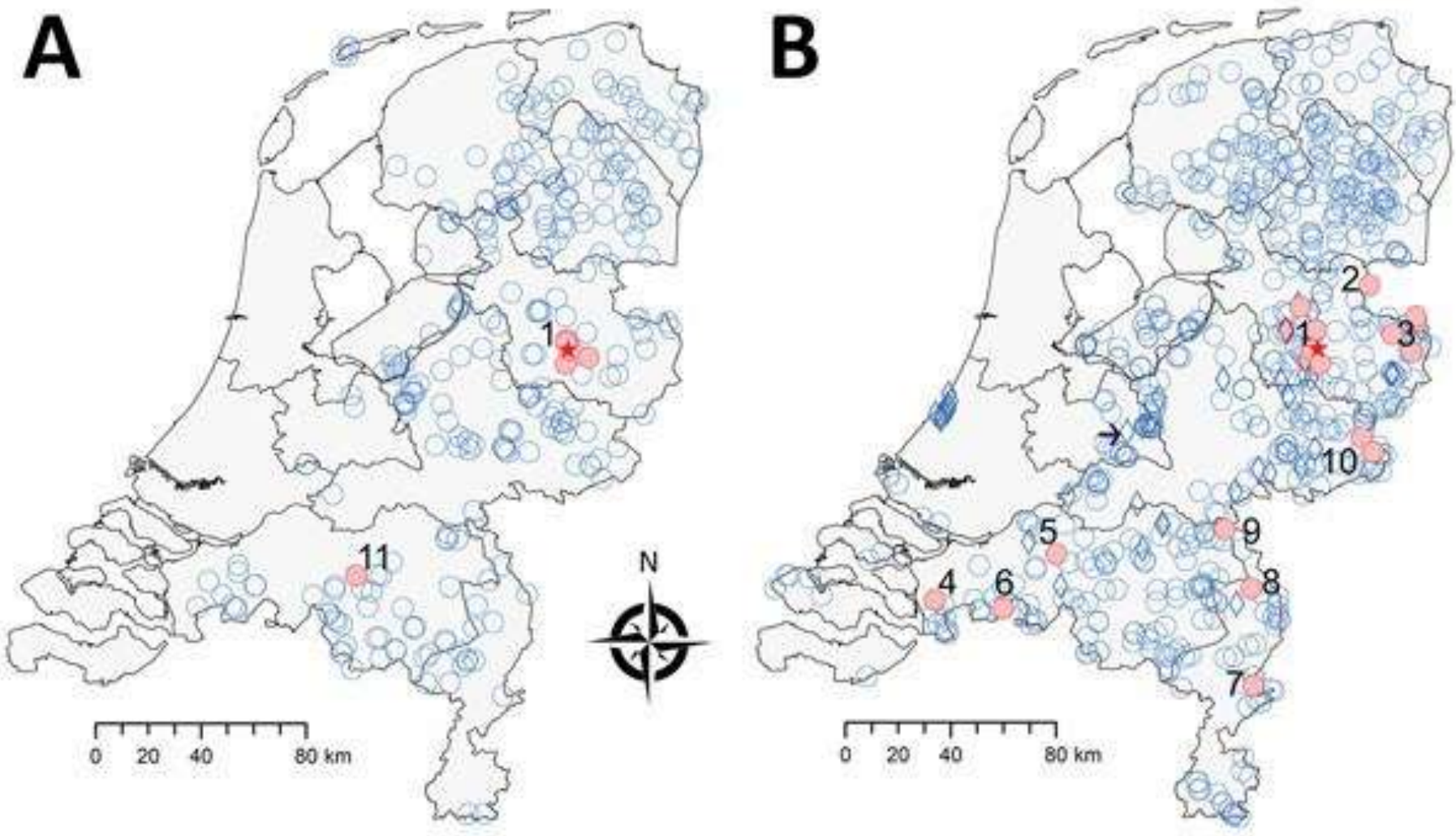
Municipalities where the autochthonous TBEV cases likely acquired infection are depicted in grey (light grey: 1 case; dark grey: 2 cases).

Locations where TBEV RNA-positive ticks were caught are indicated by an **orange** dot.

Locations of TBEV RNA-positive rodent samples are indicated with a **red** star.[11]

Figure 4: Geographic distribution of tick-borne encephalitis virus (TBEV) based on serosurveillance of roe deer during A) 2010 and B) 2017

Red indicates roe deer serum samples that showed positive results in the TBEV neutralization test, and **blue** indicates roe deer serum samples that showed negative results in this test or an ELISA. Numbers indicate confirmed or potential foci, and red stars indicate location of 2016 TBEV-RNA positive ticks in Sallandse Heuvelrug National Park. (Figure and accompanying legend are reprinted from reference[7]).



Appendix

Source data: Figure 1

Year	Number of cases
…	…
2006	0
2007	1 (1 travel-related)
2008	0
2009	0
2010	0
2011	2 (2 travel-related)
2012	2 (2 travel-related)
2013	0
2014	2 (2 travel-related)
2015	0
2016	4 (2 travel-related)
2017	4 (3 travel-related)
2018	5 (3 travel-related)
2019	3 (1 travel-related)
2020	5 (0 travel-related)
2021	3 (1 travel-related)
2022	5 (3 travel-related)

Source data: Figure 2

Age group (years)	Males	Females	All
0–9	0	0	0
10–19	2	0	2
20–29	0	0	0
30–39	1	2	3
40–49	6	0	6
50–59	6	4	10
60–69	8	2	10
>70	2	3	5

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Chapter 12b

TBE in Norway

Katrine M. Paulsen, Rose Vikse, Arnulf Soleng, Kristin S. Edgar, Heidi H. Lindstedt, Dagny H. Dorenberg, Berit S. Wiklund and Åshild K. Andreassen

E-CDC risk status: endemic (data as of end 2022)

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, and Vestfold and Telemark) are endemic for TBE. Further, Viken County (former Østfold, Akershus and Buskerud), and western and northern Norway to Brønnøy municipality is 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 245 TBE cases have been reported to MSIS per February 2021 (Fig. 1). Of these, 201 cases are autochthonous infections, while 44 cases were infected abroad or have an unknown infection history. The number of cases varies annually between 1 and 41 (Table 1 and Fig. 1). Data for 2018, 2019 and 2020 shows an increase in the number of cases, especially in the county of Vestfold and Telemark (MSIS, February 2021). 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 western or northern coastal areas, nor from 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 as the major vector of the European TBE-virus.[17-18] The geographical distribution of *I. ricinus* in Norway has been examined 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 ~66°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 in *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 Viken 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 and 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 coworkers. Five virus strains with 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, similar to the highly virulent "Hypr" strain.[29]

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 is in contrast to 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 on 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

Recently, a seroprevalence study in a TBE endemic area in southern Norway a TBEV seroprevalence of 3.1% (45/1,453) was found in the general adult population in Søgne municipality. Among individuals not vaccinated against TBEV and/or yellow fever, the seroprevalence of IgG antibodies to TBEV was 1.4% (6/419).[33] Furthermore, 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]

Conclusion

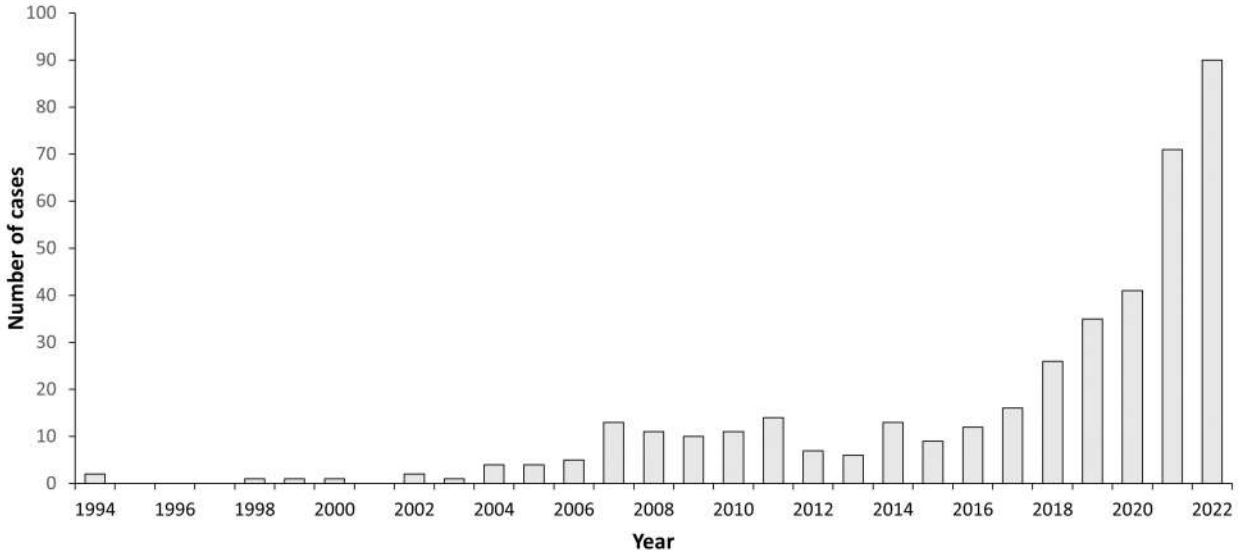
In summary, TBE is endemic in Norway and the number of human TBE cases has been increasing in recent years. Clinical TBE cases are only found in southern parts of Norway; however, the results from both prevalence studies in ticks and seroprevalence studies in humans and animals indicate that TBEV might 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: Viken, Vestfold and Telemark, Agder, Rogaland, Vestland, Møre and Romsdal, Trøndelag, and Nordland. Human TBE cases have been reported in the following counties: Agder, Vestfold and Telemark, and Viken (three municipalities: Drammen, Vestby and Fredrikstad). *Source: www.fhi.no Norwegian Surveillance System for Communicable Diseases (MSIS)*
Reservoir animals	Small rodents in the genera *Apodemus* and *Myodes.*[37]
Infected tick species (%)	*Ixodes ricinus* (0%–1.1% in nymphs and 0%–20.6% in adults).[6]
Dairy product transmission	Not documented. TBEV RNA has been detected in unpasteurized cow milk.[5]

Table 2: TBE-reporting and vaccine prevention in Norway	
Mandatory TBE-reporting	Hospitals and General Practitioners Only cases with meningitis/encephalitis are notifiable. Criteria: - Detection of specific antibody response in serum and/or cerebrospinal fluid and/or - Detection of TBEV in cerebrospinal fluid and/or serum by isolation and nucleic acid analysis *Source: www.fhi.no*
Other TBE-Surveillance	Ongoing studies: The Barents region project (ID B1710). Emerging infections. Capacity building on vector-borne infections in the Barents Region, Norway and Russia. EMERGING VIRUS: Vector-borne infections in Norway – Understanding the emergence of viral vector-borne diseases in a One Health perspective by studies of dynamics, distribution, climate, genetic diversity, biogeographic distribution, risk of infection, surveillance, and diagnosis. Observation and prognosis of TBE-patients (Telemark Sykehus HF). North-Tick (Interreg VB, North Sea program): Vector-borne pathogens/infections in the North Sea area. Experimental infection study of sheep with TBEV: Transmission to lambs via milk[38] TBFV net (EEA-project): surveillance and research on tick-borne flaviviruses Development of pipeline of whole genome sequencing of TBEV[39]
Special clinical features	No. 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 into the national immunization register, SYSVAK. According to SYSVAK, about 77,677 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 into SYSVAK was consensual up to 1/1/2020. The number of TBE vaccine doses actually 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*

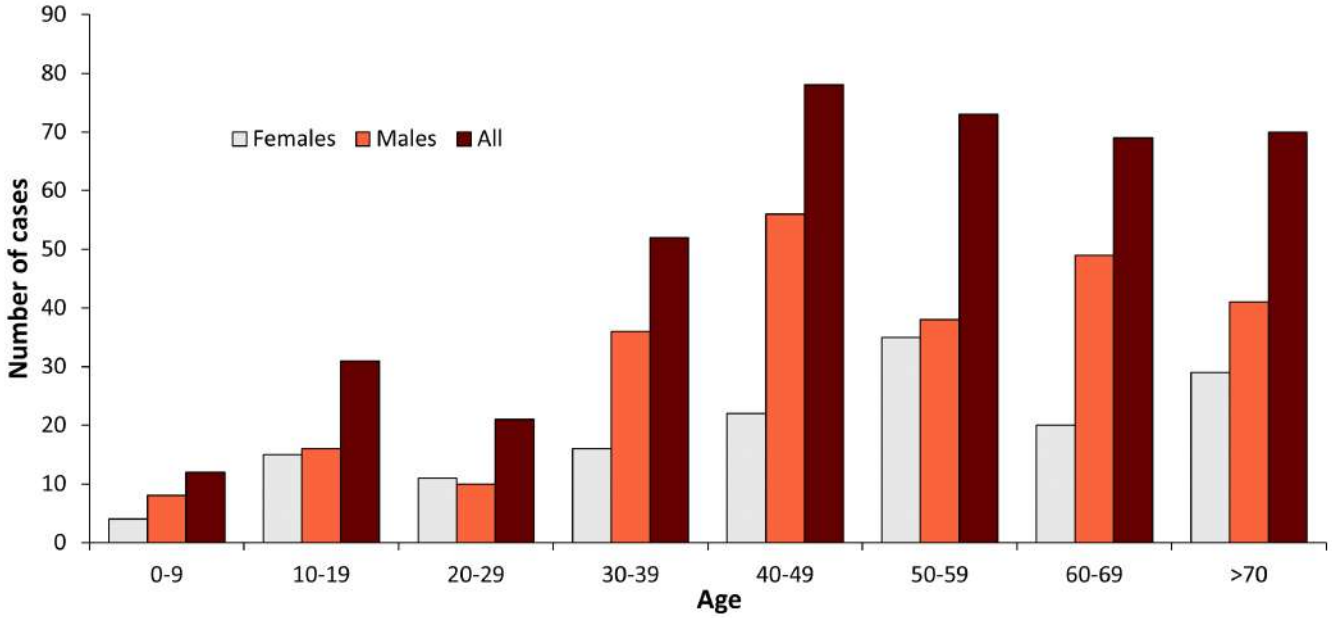
Figure 1: Burden of TBE in Norway over time*



*data per April 2023 (MSIS).
These data include 47 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 1994–2022*



*data per April 2023 (MSIS).
These data include 46 cases that have been infected abroad or have an unknown infection history.

Source Data: Appendix—Figure 2

Figure 3: TBE cases in Norway 1994–2019 (MSIS)

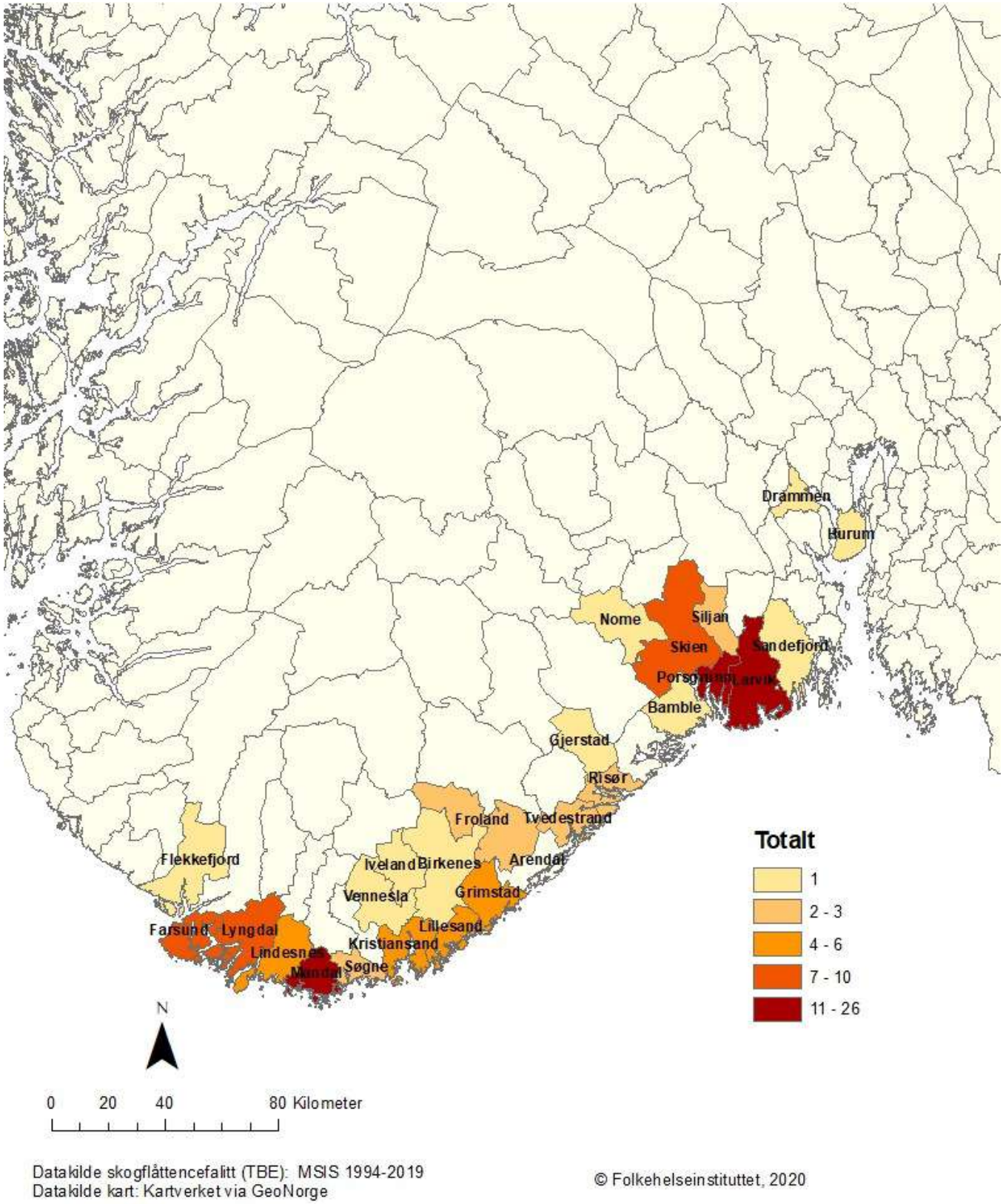
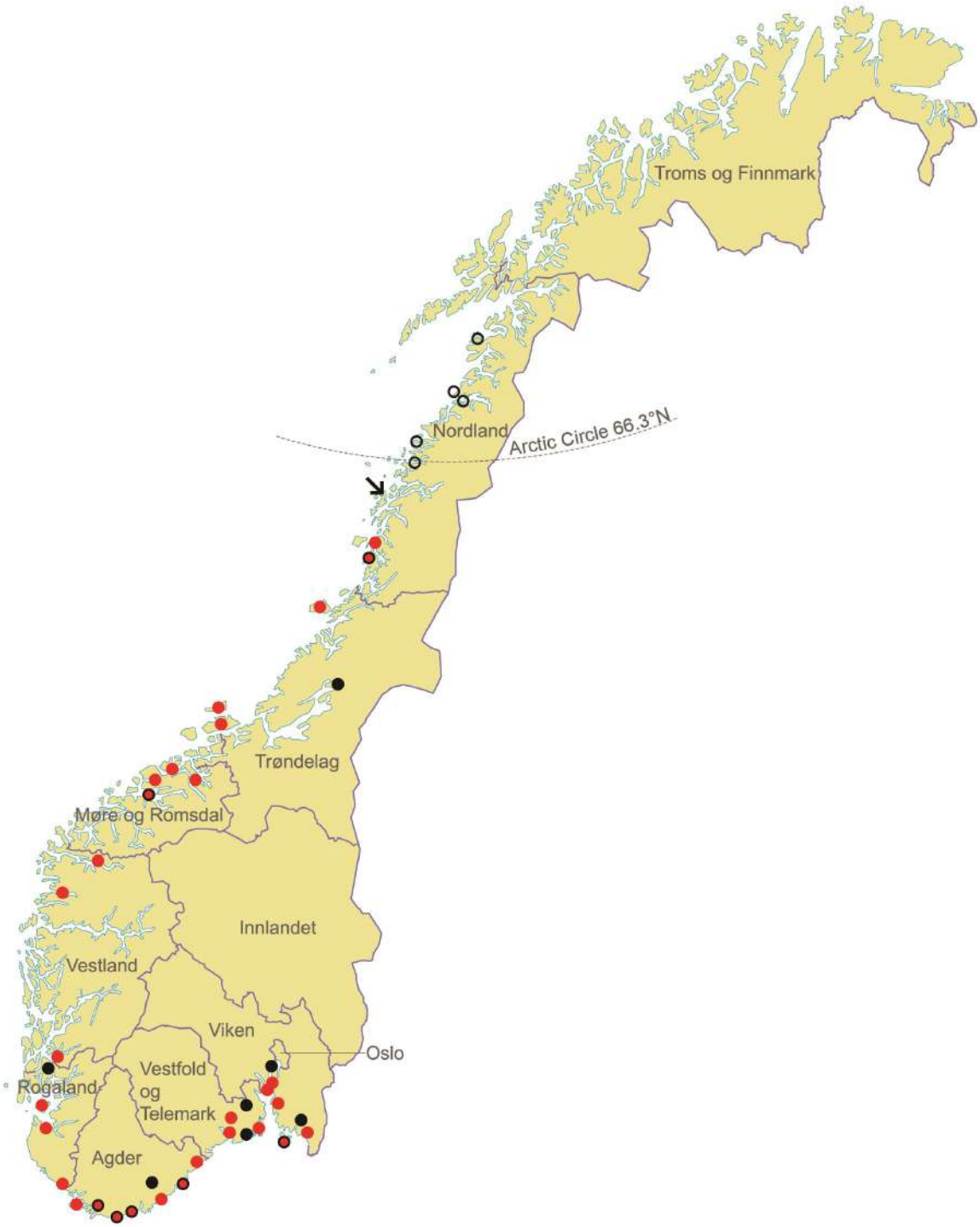


Figure 4: Geographical locations where tick-borne encephalitis virus has been detected in Norway from 2004 to 2020:
○ No ticks found, ● Ticks with TBEV, ● TBEV antibodies in animals, ● TBEV in ticks, cow milk, and TBEV antibodies in animals

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]



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

Source data: Figure 2

Age group (years)	Females	Males	All
0-9	4	8	12
10-19	15	16	31
20-29	11	10	21
30-39	16	36	52
40-49	22	56	78
50-59	35	38	73
60-69	20	49	69
>70	29	41	70

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Chapter 12b

TBE in Poland

Katarzyna Pancer and Włodzimierz Gut

E-CDC risk status: endemic (data as of end 2022)

History and current situation

Clinical symptoms of tick-borne encephalitis (TBE) were first described in Poland in 1948 by Demiaszkiewicz. All patients had been living in the Białowieża region (in northeastern Poland). Similar infections were described to those that had been diagnosed in the same region before World War II as complicated cases of typhoid fever or influenza.[1]

Twenty-eight cases of TBE were identified in 1952 among patients hospitalized in Nysa Kłodzka (in southwestern Poland). In 1954, 35 cases were identified in the Olsztyn region in northern Poland. More cases were recognized in the following years across different regions of Poland: northern (Gdańsk, Szczecin), central (Łódź), and southern (Kraków). This was the catalyst for scientific research studies by Przesmycki's team in selected regions of the country in the years 1953 through 1957.[2] In these studies, tick-borne encephalitis viruses (TBEV) were isolated from human specimens as well as from animal samples (small mammals) and vectors (ticks). Isolated viruses were determined to be TBEV, European sub-type.[2–4]

Seroprevalence studies were conducted in the late 1960s and early 1970s. Serum samples collected from ~17,000 blood donors and 20,000 forest workers living in different parts of Poland were examined. Distribution of positive serological results varied depending on the place of residence, ranging from 0.5% to 6.5% among blood donors and 7% to 27% among forest workers. These seroprevalence data also indicated high numbers of asymptomatic or non-severe infections among the tested populations.[2,5–9]

A distribution map of TBE cases and confirmed presence of TBEV in Poland was developed, based on results from seroprevalence studies, virus isolation and clinical data. Some regions were determined to be endemic for TBEV. These included provinces in the north-eastern part of Poland (Białystok, Olsztyn, Suwalki) and southwestern Poland (Opole).[3,4,6–12]

In total, 576 TBE cases were reported during the 23 years of surveillance (1970–1992); the annual number of reported TBE infections varied from 4 (1991) to 60 (1970), and the incidence ranged from 0.01/100,000 inhabitants to 0.2/100,000 inhabitants, respectively. In the 1980s, the number of reported TBE cases decreased to 14–19 cases annually because of abandonment of diagnostics tests.[2,13]

In 1993, when new commercial tests became available in Poland, the number of reported TBE cases increased more than 30-fold in comparison to 1992 (249 vs. 8 cases). In 1993, the incidence of TBE (0.65/100,000) was the highest observed since surveillance began in 1970. This trend continued into the 21st century and more than 300 TBE cases were reported in the years 2003 (339 cases), 2006 (317 cases), and 2009 (351 cases). The highest incidence (0.92/100,000) was reported in 2009. The annual number of reported TBE cases decreased to 149 in 2015.[13]

In total, 3,662 cases of TBE were reported in Poland between 2000 and 2015. The incidence varied from 0.33 to 0.92/100,000. A 3–4-year cycle was identified based on the reported numbers of TBE cases, with peaks observed in 2003, 2006, and 2009. TBE cases were identified in all regions of Poland except one: there was no diagnosed or reported TBE case in the Lubuskie Province, which is located at the Western border region along the banks of the Odra River. In contrast, more than 70% of the reported cases each year were diagnosed in two provinces in the northeastern part of Poland: Podlaskie (Białystok) with >45% reported TBE cases and an incidence >6/100,000, and Warmińsko-Mazurskie (Olsztyn) with 25% cases and an incidence >1.5/100,000. Also, outbreaks of TBE were observed in those same regions during spring-summer time.[13]

In contrast to Central European countries (Germany, Czech Republic, Austria, Switzerland) the reported Polish TBE case numbers in 2018 did not significantly increase in summer time. Also the total number of 197 TBE cases is ~30% lower than in previous years (279 cases in 2017, 283 cases in 2016) (Fig. 3). However, a similar phenomenon with an increased number of reported TBE cases during the summer time was observed in 2016; but, the total number of TBE cases in that year was comparable to the numbers reported in 2017 although higher than the number of TBE cases reported in 2015 (149 cases).[20] The total number of reported TBE cases in 2019 was 265; however, during the first 6 weeks of the year, the number of reported cases was higher in 2019 (14 cases) than in 2018 and 2020 (10 cases each year).

The age of TBE patients ranged from 3 to 80 years, but the majority of patients were >20 years old.[13] Almost 20% of all reported TBE cases were associated with work or visits in the area where TBEV-infected ticks were found. Moreover, food-borne transmission was documented in 1975 and

1995. The source of infection was fresh, non-pasteurized milk of cows (1975) or goats (1995) contaminated with TBEV.[14,15]

The mortality rate observed for the reported TBE cases in Poland ranged from 0.5% to 2.8% and was similar to that observed in other European countries where European subtype of TBEV (TBEV-EU) variants have been confirmed.[5,13]

Prevention of TBE is based on decreasing the probability of infection by limiting exposure to infected ticks (wearing appropriate clothing, use of insect repellents, etc.), by vaccination, and by appropriate preparation of milk (pasteurization, boiling). Since 1952 the commercial sale of milk in Poland is only allowed after thermal preparation. However, fresh milk is still available in local markets.[14,15]

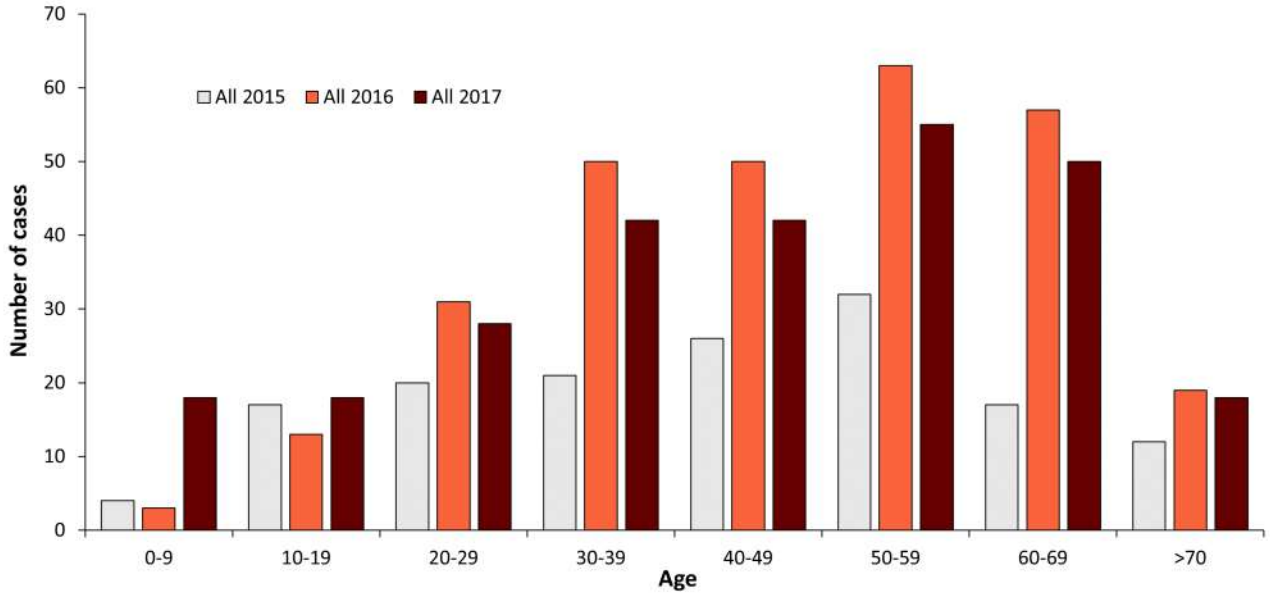
In Poland vaccination against TBEV started in the 1970s. At the beginning of this campaign, vaccination was done using the Russian (local brand name: "Vaccinum Encephalitis Ixodice"), which consisted of a formalin-inactivated TBEV-Siberian type. Since 1993 this vaccine was replaced by the two EMA-licensed vaccines with a TBEV-EU subtype as the seed virus for production (FSME-Immun (Pfizer) and Encepur (Bavarian Nordic)).[2,16–17] Both vaccines are available for use in children and adults. Vaccination against TBEV is recommended in Poland, especially for forest workers, foragers of forest undergrowth, and tourists. The costs of vaccination are not reimbursed, except through campaigns paid for by employers or local communities (medical service, forest workers etc.). In Poland, 27,849 persons were vaccinated in 2015, among them 11,516 below the age of 19 years.[18] The rather low rate of vaccination against TBE among people in Poland has no effect on the number of reported TBE cases and epidemiological characteristics of TBEV infection.

Overview of TBE in Poland

Table 1: Virus, vector, transmission of TBE in Poland

Viral subtypes, distribution	European subtype (also called Western European or Central European subtype)
Reservoir animals	Rodents, Tick[2,7]
Infected tick species (%)	*I. ricinus*, depending on region and used technique, range of "Minimum Infection Rate" from 0.00 to 1.96[3,4,7,10-12] *Dermacentor reticulatus*, depending on region and used technique, range of "Minimum Infection Rate" similar to *I. ricinus*[21-22]
Dairy product transmission	Rare (1975; 1995)[14,15]

Figure 2: Age distribution of TBE cases in Poland, 2015–2017

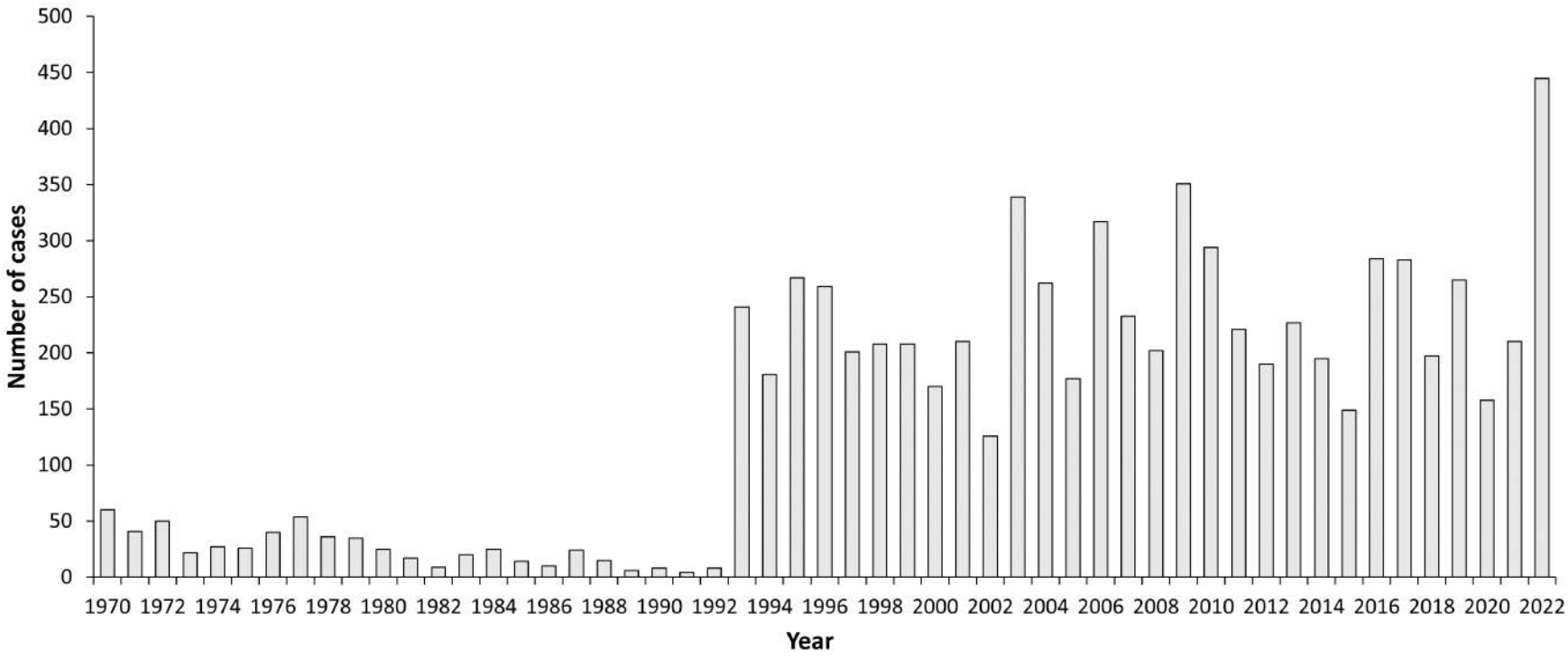


Source Data: Appendix Figure 2

Table 2: TBE reporting and vaccine prevention in Poland	
Mandatory TBE reporting	ONLY cases of neuroinfection Case definition—per ECDC (2.44)[19] **Clinical criteria**: any person with symptoms of inflammation of the central nervous system (CNS): e.g., meningitis, meningoencephalitis, encephalomyelitis, encephaloradiculitis **Laboratory criteria**: laboratory criteria for case confirmation At least 1 of the following 5 criteria: • TBE-specific IgM AND IgG antibodies in blood • TBE-specific IgM antibodies in cerebrospinal fluid (CSF) • Seroconversion or 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 specimen Laboratory criteria for a probable case: • Detection of TBE-specific IgM-antibodies in a unique serum sample • 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 **Epidemiological criteria**: exposure to a common source (unpasteurized dairy products) **Case classification** • **Probable case**: any person meeting the clinical and laboratory criteria for a probable case; any person meeting the clinical criteria and with an epidemiological link • **Confirmed case**: any person meeting the clinical and laboratory criteria for case confirmation
Other TBE surveillance	Obligatory reporting by diagnostic laboratory of any positive results from serological (IgM) examination to local health service in the patient's place of residence
Special clinical features	Contact with ticks; consumption of non-pasteurized dairy products[14,15] Mortality 0.5%–2.8%[13]
Available vaccines	Since 1993: • **FSME-Immun** (manufacturer: Pfizer) in 2 formulations (adults and children <16 years of age) • **Encepur** (manufacturer: Bavarian Nordic) in 2 formulations (for adults and children 1–11 years of age).
Vaccination recommendations and reimbursement	Recommendation for additional supplementary immunization – 1970s. No reimbursement*
Vaccine uptake by age group/ risk group/general population	In 2015, 27,849 persons; among them 11,516 who are <20 years of age[18]
Name, address/website of TBE National Reference Center/	Lack of reference laboratory or center – since 2004 (due to more stable/constant disease situation)

**In Poland, vaccination against TBE is recommended (but not financed from the budget of the Ministry of Health) for persons in areas with severe occurrence of the disease, in particular:*
forest workers, foragers (e.g., persons who harvest mushrooms, berries, etc – commercially or recreationally), stationed military, guards brigade and border, farmers, young people in practice (outdoor play and recreation), tourists and visitors to camps and colonies.

Figure 1: Burden of TBE in Poland [2,13,16,18]



Source Data: Appendix Figure 1

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

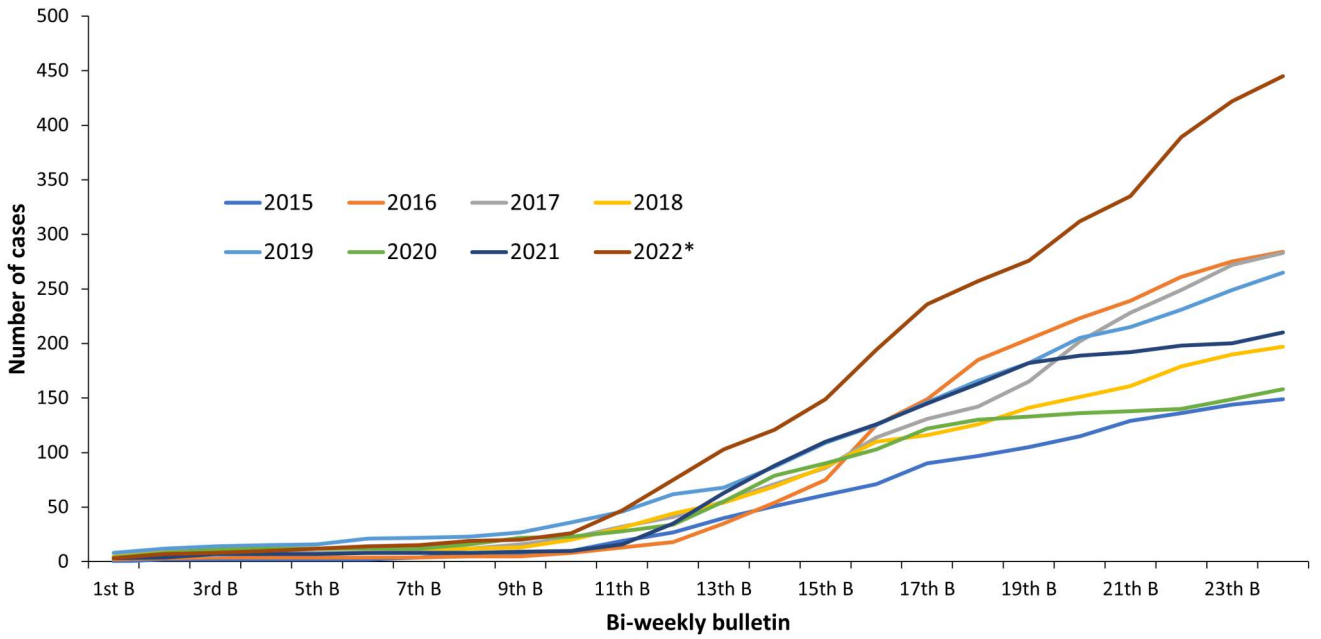
[c]Diagnostics based on ELISA method in hospital and Sanitary Service laboratories with confirmation in Reference Laboratory NIH; 1993–2003 recommended vaccination against TBEV-EU (not reimbursed)

[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

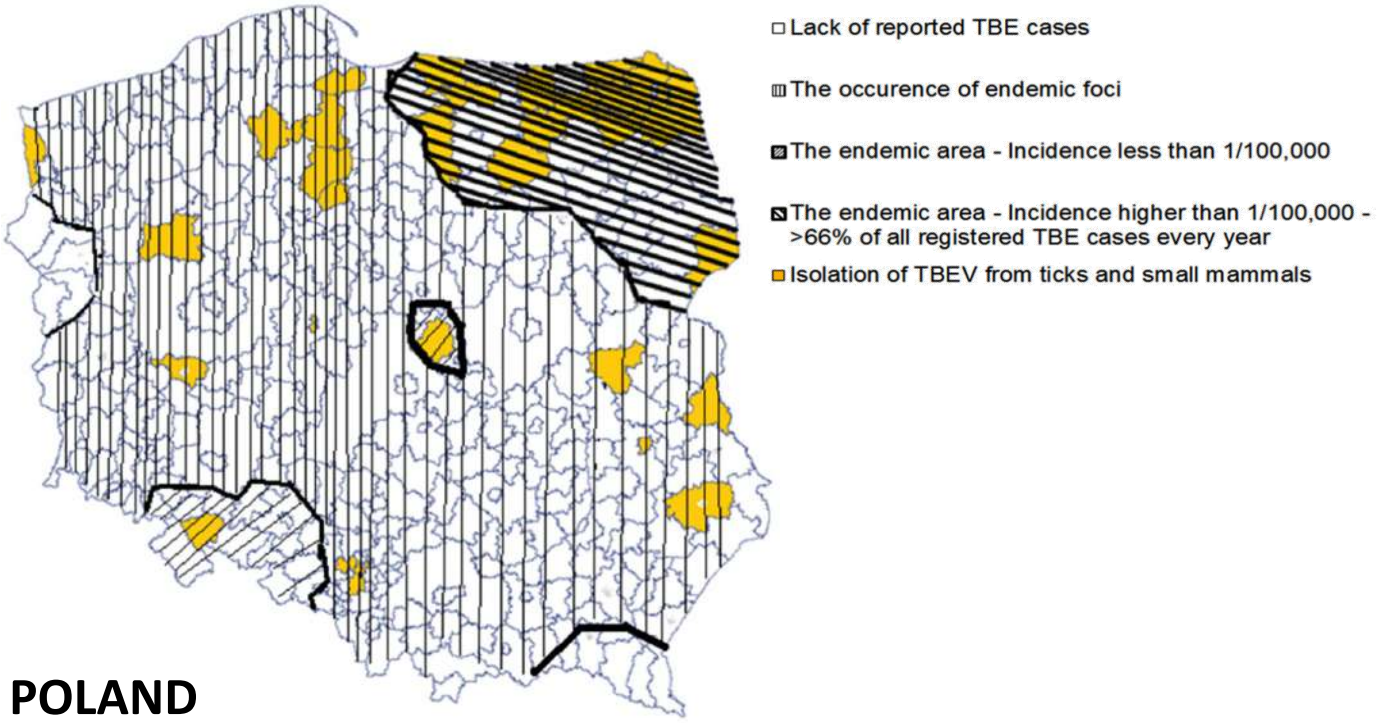
[#]From 1970 to 1985 confirmation based on HI test; since 1993, IgM ELISA for confirmation (and local synthesis of TBEV-specific IgG in CSF)

Figure 3: The cumulative number of reported TBE cases in Poland by bi-weekly period (B)



*Data is not verified

Figure 4: TBEV-isolation and TBE cases in Poland[3–4,6–7,9–12]



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

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

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TBE in Romania

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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]

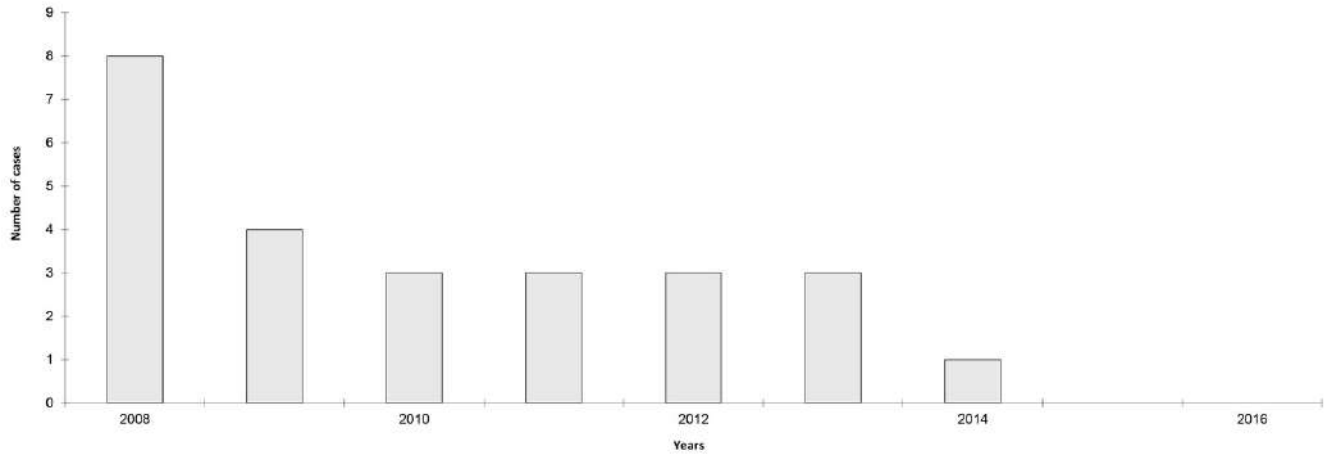
Overview of TBE in Romania

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/

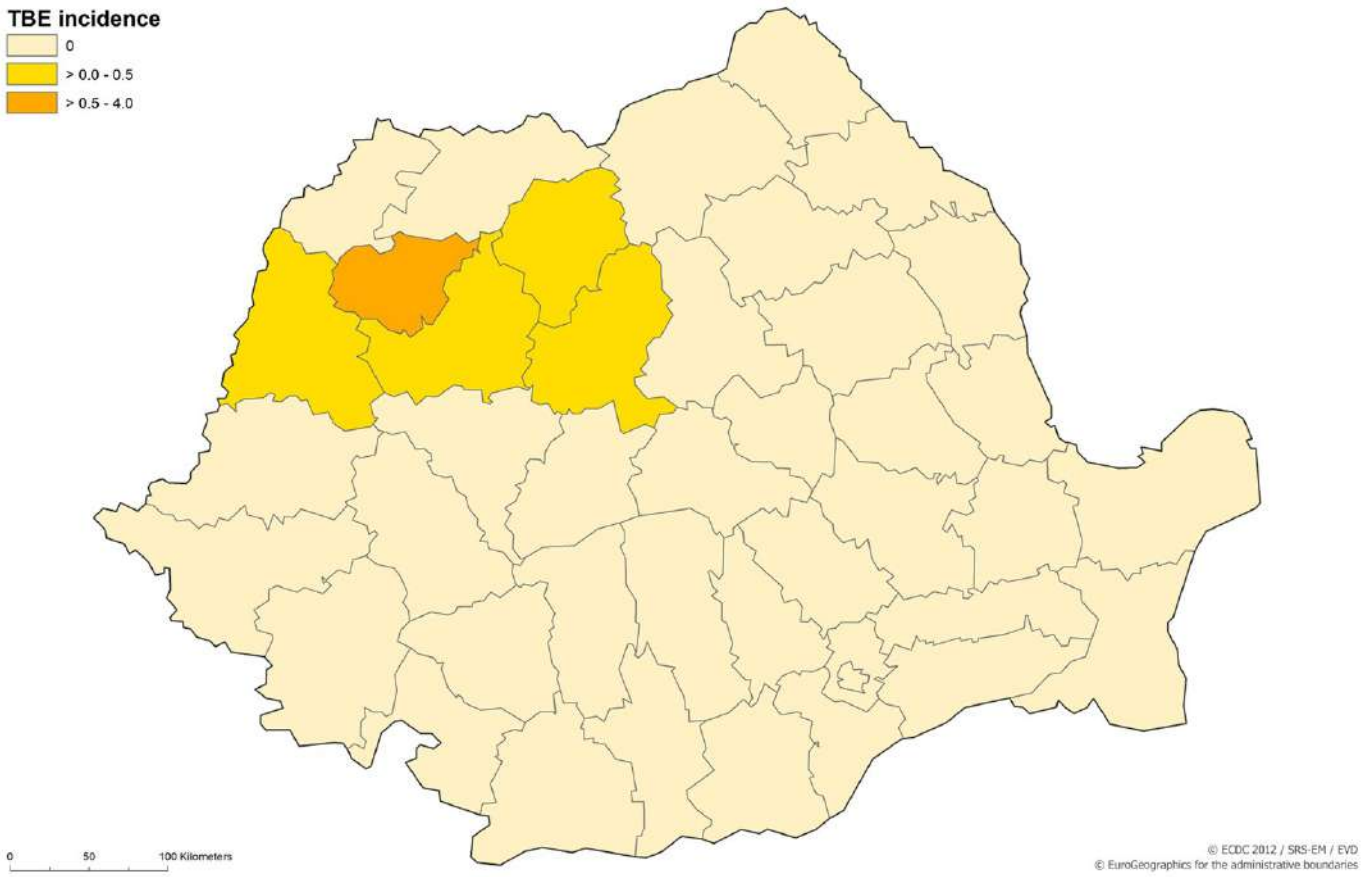
Table 3: Seroprevalence rates against TBEV in humans and animals in different counties of Romania			
County	**No. of sera**	**Study Ionescu et al. 2008[6]**	**Study Salat et al. 2017[9]**
Alba	49 human	4.0%	
	190 animal	0%	
Bihor	119 sheep		27.7%
Bistrita-Nasaud	626 human	4.6%	
	100 sheep		12.0%
Caras 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	

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Chapter 12b

TBE in Russia

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E-CDC risk status: endemic (data as of end 2022)

History and current situation

TBE was first revealed in the Far-East Taiga Forest in the Soviet Union in springs and summers between 1933–1935[1] and it was further investigated as of 1937 at 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 the first strain of TBE virus (TBEV) was isolated. The clinical disease spectrum in humans and the respective pathology were described and the effectiveness of immunoglobulin-therapy was shown.[5] Based on morphological studies since 1937, 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" formalin-inactivated vaccine from the Far East TBEV subtype "Sof'in" (1st generation of vaccines).[17,18] Vaccine impact was established at the level of 98%, but it frequently induced serious adverse events. A live attenuated vaccine based on the Elantsev strain had not been licensed due to severe complications (encephalitis) in the vaccinated group.[19] In 1950–1960 a 2nd generation of TBE vaccine was introduced which used chicken embryonic cell culture for virus reproduction.[20] The vaccine was upscaled in 1961–1966 and tested in Western Siberia with high effectiveness. In the 1980s, another new type of TBE vaccine was licensed and is currently in use, a concentrated purified lyophilized 3rd generation vaccine.[21,22]

The Siberian subtype dominance of the TBEV (over 60% of endemic areas) in the Russian Federation was demonstrated by numerous virological studies.[8,9]

Only two species of ticks are epidemiologically significant in Russia: *I. persulcatus* in the Asian part and some additional areas in the European part (Yaroslavl, Sverdlovsk, Omsk, Irkutsk, Primorsky regions) and *I. ricinus* in the European part. There is a less epidemiologically significant species – *D. pictus* – confirmed as a carrier of the TBEV in Udmurtia.[11-15]

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 foci, 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.[14] Over time, two disease peaks were observed in Russia (Fig. 1). In the mid-1950s, over 5,000 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, because the vector population 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 indices of TBE morbidity reached the highest values ever recorded.[15] 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.

In two periods (1997–2006 and 2007–2016) all Russian Federation (RF) endemic regions were divided into three groups with an either low (≤2.9 per 100,000 population), moderate (3.0–8.4 per 100,000) or high (≥8.5 per 100,000) TBE incidence (Fig. 2A and B).

Between 1997 and 2006, the average TBE incidence in Russia was 4.0 ± 0.05 per 100,000 totaling 58,585 cases in 55 regions of Russia. At that time the number of regions known to be endemic for the disease grew progressively from 41 in 1997 to 47 in 2001 and 2003. The group of regions with a high incidence per 100,000 population included 15 regions, where a total of 48,166 (82.2%) of TBE cases were registered: Tomsk (40.2), Krasnoyarsk (32.6), Udmurt Republic (28.8), Altai (27.0), Khakassia (26.4), Tuva (23.0), Irkutsk (20.9), Kurgan (16.8), Tyumen (15.9), Buryatia (15.3), Perm (13.8), Kemerovo (11.6), Sverdlovsk region (11.0), Novosibirsk (10.8) and Chelyabinsk (8.6). Nine regions were included into the moderate incidence group with 6 482 registered cases (11.1%), i.e., six regions in the European part of Russia [Republic of Karelia (7.6), Kirov

(6.6), Vologda (4.5), Kostroma (4.0), Arkhangelsk (3.6) and Novgorod regions (3.2)]; and 3 regions in the Asian part (Altai (5.9), Zabaikalsky (5.6) and Primorsky (5.3) regions). In 28 regions, low incidence rates were registered (25 regions in the European part and 3 in the Asian part of Russia).

In 2007–2016, the incidence has been 1.9 ± 0.04 per 100,000 with 27,351 TBE cases registered. During this decade, the most intensive epidemic process occurred in the Asian endemic areas. High incidence rates in the European part of the RF were registered in the Kirov region (8.8%). In the Asian part, a cluster was formed between the bordering regions of Altai (16.7), Krasnoyarsk (16.2), Tomsk (16.2), Khakassia (10.6) and Tuva (10.6). Moderate incidence rates were established in 6 regions in the European part and 8 regions in the Asian part, low incidence in 23 regions (Figure 2, B). In summary, the incidence of TBE in the RF has significantly decreased over the past decade in all regions except in Kirov, where an incidence increases from 6.6 ± 0.7 per 100,000 in 1997–2006 to 8.8 ± 0.8 per 100,000 in 2007–2016 was observed. The registered frequency of tick bites remained constant over time (1944–2016) and is at the level of 400,000–550,000 per year.[16]

The number of endemic regions of Russia increased from 37 (1956) to 48 (2019). The distribution of TBE in Russia has territorial unevenness, with the largest number of cases recorded in the Siberian Federal District (45%–48% of the total incidence of TBE in the Russian Federation), while in the Volga Region – 17.4%–21.1%, in the Urals – 14%–17%, in the North-West – 12.8%–14.3%, in the Central – 2.4%–3.8%, in the Far East – 1.5%–2.2%.

Middle Ural area is an active natural focus of TBE; TBE cases have been recorded since the 1930s. At present, all 94 administrative territories of the Sverdlovsk Region are endemic for the TBE. Sverdlovsk region is a good example of a typical Russian TBE endemic area. 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:

1. 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;

2. 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;

3. 1986–1989: the period of acaricidal (DDT) air spraying of the forests, TBE incidence decrease, ≤200 TBE cases per year;

4. 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;

5. 2000 to present: TBE incidence decrease associated with routine TBE vaccination of the adult population and universal routine immunization of children.[26]

Given the high incidence of TBE, vaccination has become a leading preventative measure in the Sverdlovsk region. Four tactics of vaccination were realized in Regional Immunization Program (Fig 3):

1. 1990–1996 – Selective specific TBE vaccination – immunization of the occupational risk groups;

2. 1997–2001 – Adult population routine TBE vaccination;

3. 2001–2008 – Routine children ≥ 7 years of age vaccination and mass immunization of adults;

4. 2008 to present – Universal routine vaccination of children from 15 months of age and mass immunization of adults.[26]

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. 4) and led to the TBE incidence decrease. 98% TBE vaccination field effectiveness in 2016 (Fig. 6).[26,27,28]

To summarize current TBE data from Russia, in 2020 there were 471,630 visits[30] (in 2019 – 580,069 visits; in 2018 – 518,510 visits; in 2017 – 509,323 visits) to medical centers due to a tick-bite with ~25% of the cases occurring in children; were registered 967 cases of TBE (0.66 per 100,000), in 2019 – 1,781 cases of TBE (1.2 per 100,000), in 2018 – 1,727 cases of TBE (1.18 per 100,000), in 2017 – 1934 cases (1.3 per 100,000). There is a current tendency of TBE incidence reduction in the Russian Federation. In the period 2007–2019, 265 people died from TBE, in 2020 – 18 deaths.[24,25,29,30]

In 2019, primary and booster series of TBE vaccines were administered to 3.2 million people. In the last 6 years, the planned annual immunization rates have not exceeded 3.3

million people per year, which is about 4 times lower than required due to insufficient awareness and absence of a national immunization program – regions are purchasing vaccines themselves according to the local budget available (Fig. 6).[16,23-25]

The means of 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. Compared with 2011, these measures were more than doubled, when in 2016 in the RF over 17,600,000 m^2 of the most populated and actively used by people areas (i.e., parks, camps and recreation zones, hospital, hotels, school and kindergarten territories) were deployed in endemic regions.[23]

Table 1: Virus, vector, transmission of TBE in Russia

Viral subtypes, distribution	European, Siberian, and Far Eastern TBEV subtypes
Reservoir animals	Vertebrate reservoir animals assumed
Infected tick species (%)	6.3% infected tick from people after tick bite 5.7% infected tick from natural foci
Dairy product transmission	Rare (goat, cow milk)

Table 2: TBE reporting and vaccine prevention in Russia	
Mandatory TBE reporting	**TBE case definition:** Any person who has had a tick bite and who has been in the endemic area of TBE during a tick activity period or who has consumed goat milk and has symptoms of CNS inflammation (e.g., meningitis, meningoencephalitis, encephalomyelitis) or fever. **Laboratory criteria for case confirmation:** TBE specific IgM or/and IgG antibodies in blood Seroconversion or four-fold increase of TBE-specific antibodies in paired serum samples. or/and TBE specific IgM antibodies in CSF or/and Detection of TBE viral nucleic acid in a clinical specimen. PCR of SF (not obligatory). All TBE cases with laboratory confirmation are referred to the *Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (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" 3.1.3.2352-08)*
Other TBE surveillance	**Endemicity definition:** The territory is considered endemic for TBE with the combined presence of the following components: • carriers of infection in the territory (in natural and anthropogenic foci), • confirmed by laboratory methods, presence of the pathogen in ticks, selected in a planned manner and removed from people, • presence of immunity to tick-borne encephalitis virus among the unvaccinated population, • presence of immunity to tick-borne viral encephalitis among animals – tick hosts, provided that ixodid ticks have been spread throughout the territory for a 5-year period; or: • with laboratory confirmation of cases of tick-borne viral encephalitis with an active examination of febrile patients with an unknown diagnosis, patients with meningeal conditions and with symptoms of focal lesions of the brain and spinal cord of an unknown etiology, • presence of carriers of infection in the territory (in natural and anthropurgic foci), • confirmed by laboratory methods, presence of the pathogen in ticks, selected in a planned manner and removed from people, • immunity to tick-borne viral encephalitis virus among the unvaccinated population; or: • when registering confirmed cases of tick-borne viral encephalitis diseases, • presence of carriers of infection in the territory (in natural and anthropurgic foci), confirmed by laboratory methods, presence of the pathogen in ticks, selected in a planned manner and removed from people, • presence of immunity to the tick-borne encephalitis virus among the unvaccinated population. *(Source: Sanitary regulations "Prevention of tick-borne encephalitis" 3.1.3.2352-08)*

Special clinical features	1%–10% – TBEV meningoencephalitis or meningoencephalomyelitis, 35%–40% – TBEV meningitis 35%–40% – fever + anti-TBEV IgM or IgG increase 1%–3% – chronic TBEV with no reliable data available on neurological sequelae Mortality rate 1%–2% Risk groups: permanent inhabitants and visitors of endemic areas; mainly acquired during leisure activities, occupational risk groups *(Source: Sanitary regulations "Prevention of tick-borne encephalitis" 3.1.3.2352-08)*
Available vaccines	**Russian TBE vaccines:** • **Klesch-E-Vac** for children 0.25 mL and for adults 0.5 mL (FEDERAL STATE BUDGETARY SCIENTIFIC INSTITUTION Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of Russian Academy of Sciences, Moscow) (*Source: http://chumakovs.ru/en/products)* • **Tick-borne encephalitis vaccine concentrated purified inactivated adsorbed culture dry** 0.5mL (FEDERAL STATE BUDGETARY SCIENTIFIC INSTITUTION Chumakov Federal Scientific Center for Research and Development of Immune-and- Biological Products of Russian Academy of Sciences, Moscow) • **EnceVir®Neo** for children 0.25 mL (NPO Microgen, Tomsk) • **EnceVir®** for adults 0.5 mL (NPO Microgen, Tomsk) (Russian vaccines have boosters every 3 years) **European vaccines**: • **Encepur adult** 0.5 mL (Bavarian Nordic, Germany) • **Encepur baby** 0.25 mL (Bavarian Nordic, Germany) • **FSME-IMMUN** 0.5 mL (Pfizer, Austria) • **FSME-IMMUN junior** 0.25 mL (Pfizer, Austria) *(Source: http://www.microgen.ru/en/)*
Vaccination recommendations and reimbursement	National Immunization Calendar for epidemic indications (Order of the Ministry of Health of the Russian Federation №125n, part 2): endemic regions have the right to implement local immunization program (RIP) 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")*
Name, address/ website of TBE NRC	Irkutsk Anti-Plague Research Institute of Rospotrebnadzor, Irkutsk, Russian Federation *(Source: http://irknipchi.ru)*

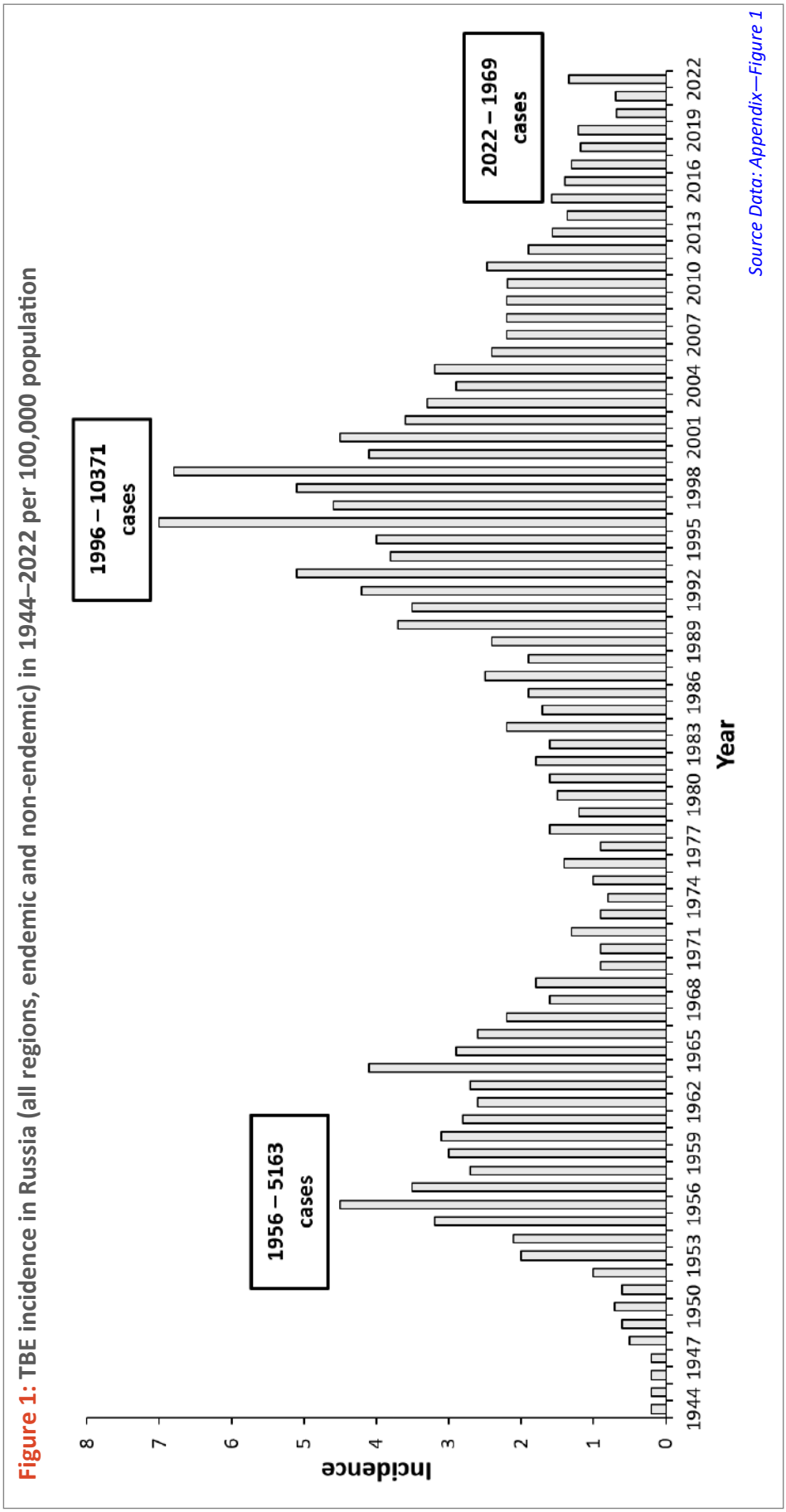


Figure 1: TBE incidence in Russia (all regions, endemic and non-endemic) in 1944–2022 per 100,000 population

Source Data: Appendix—Figure 1

Figure 2: TBE incidence (per 100,000 population) in the Russian Federation 1997–2006 (A) and 2007–2016 (B)

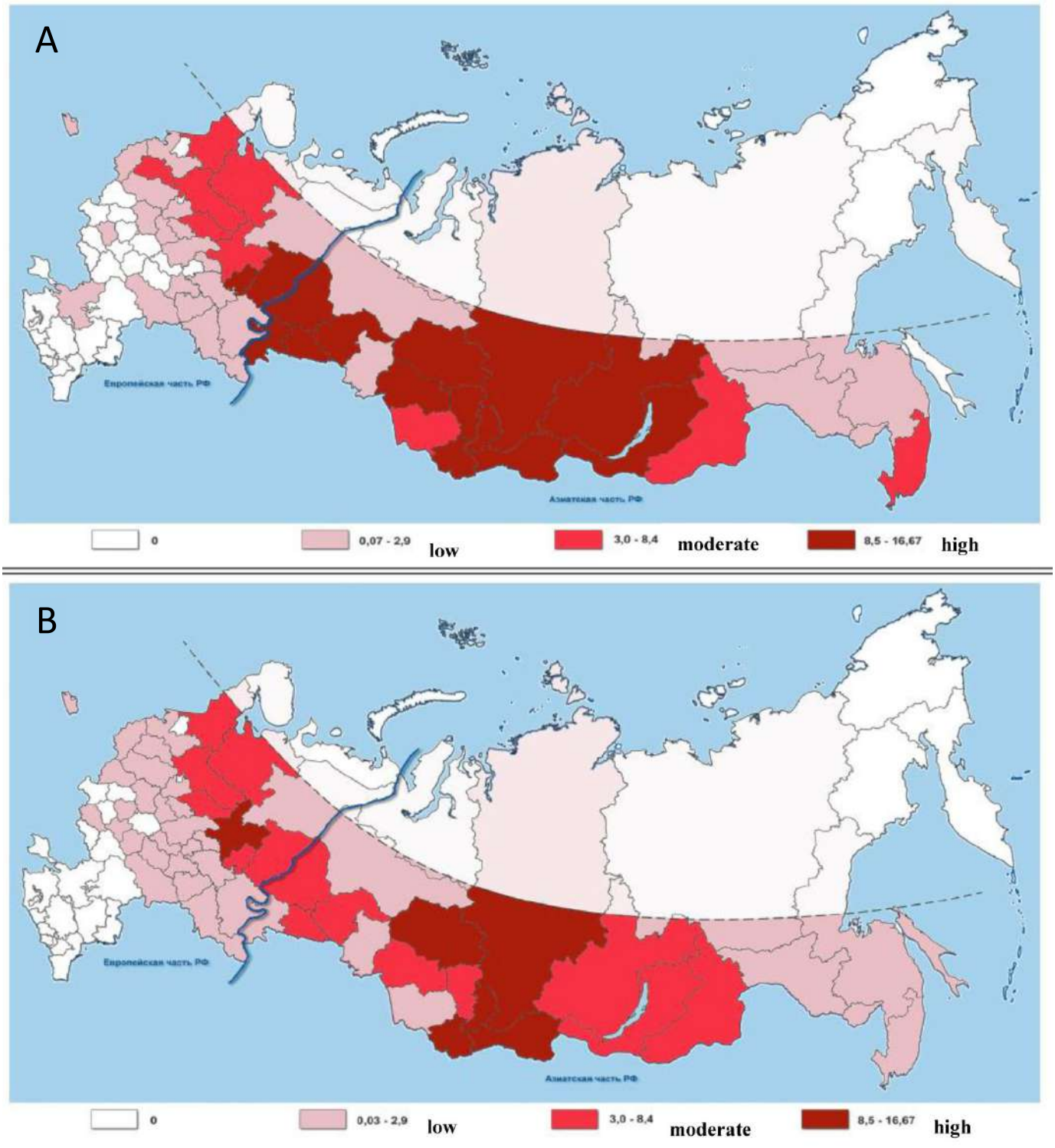
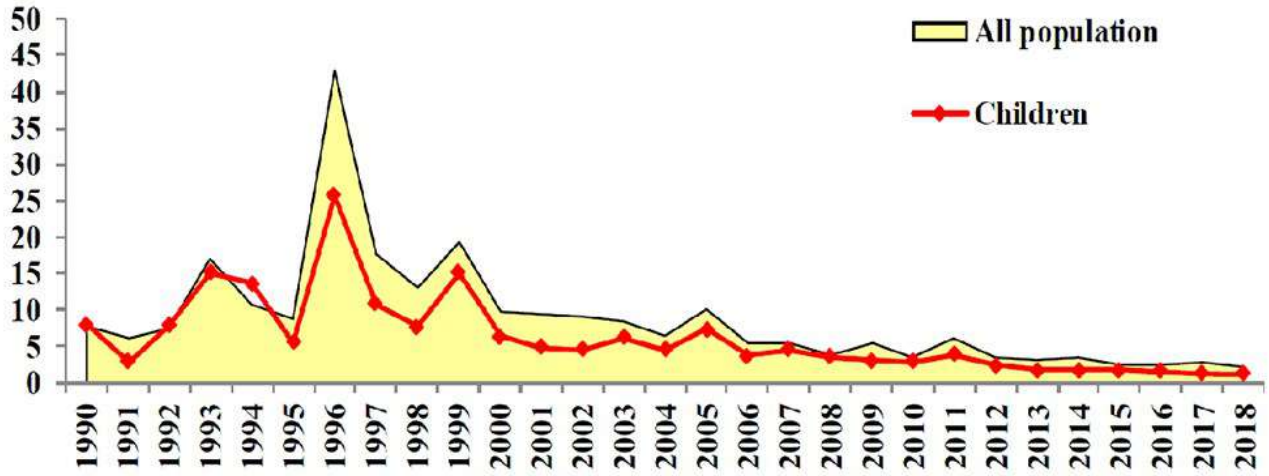


Figure 3: TBE Incidence in Sverdlovsk region by preventive tactics period in 1990–2018 (per 100,000 population, children under 14 years old)



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 4: Annual TBE vaccine uptake by the number of doses in Sverdlovsk region, Russia (%)

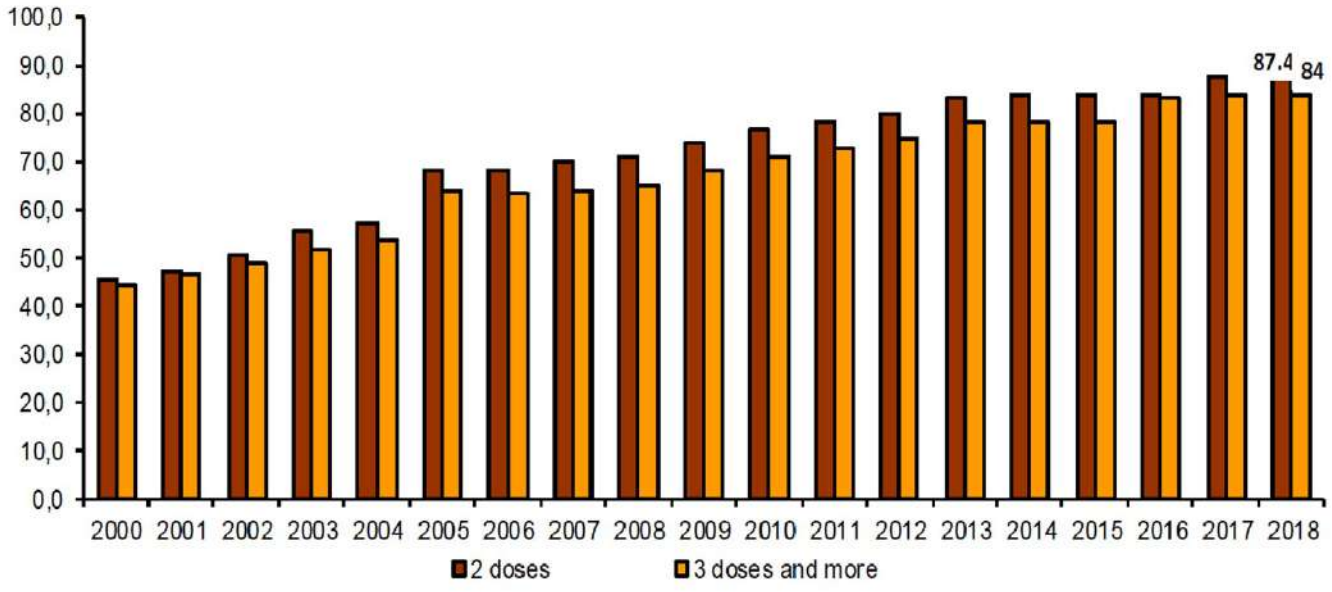
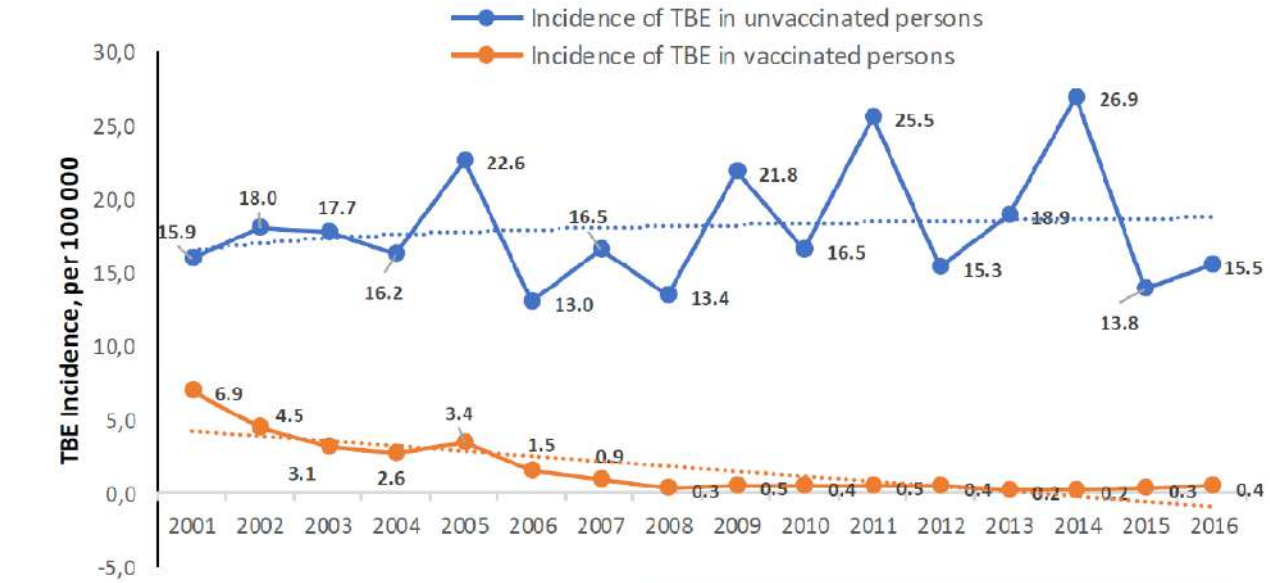


Figure 5: TBE incidence in vaccinated and unvaccinated persons in 2000–2016 in Sverdlovsk region (per 100,000 vaccinated/unvaccinated populations)



Field effectiveness. %	63,1	76,7	83,8	84	85	88,5	94,5	97,8	97,7	97,6	98	97,3	99,2	99,3	98	98

Figure 6: Incidence of tick bite cases per 100,000 population, population seroprotection by vaccination (%), and rate of tick infection (ELISA, %) in the Russian Federation between 2009–2016

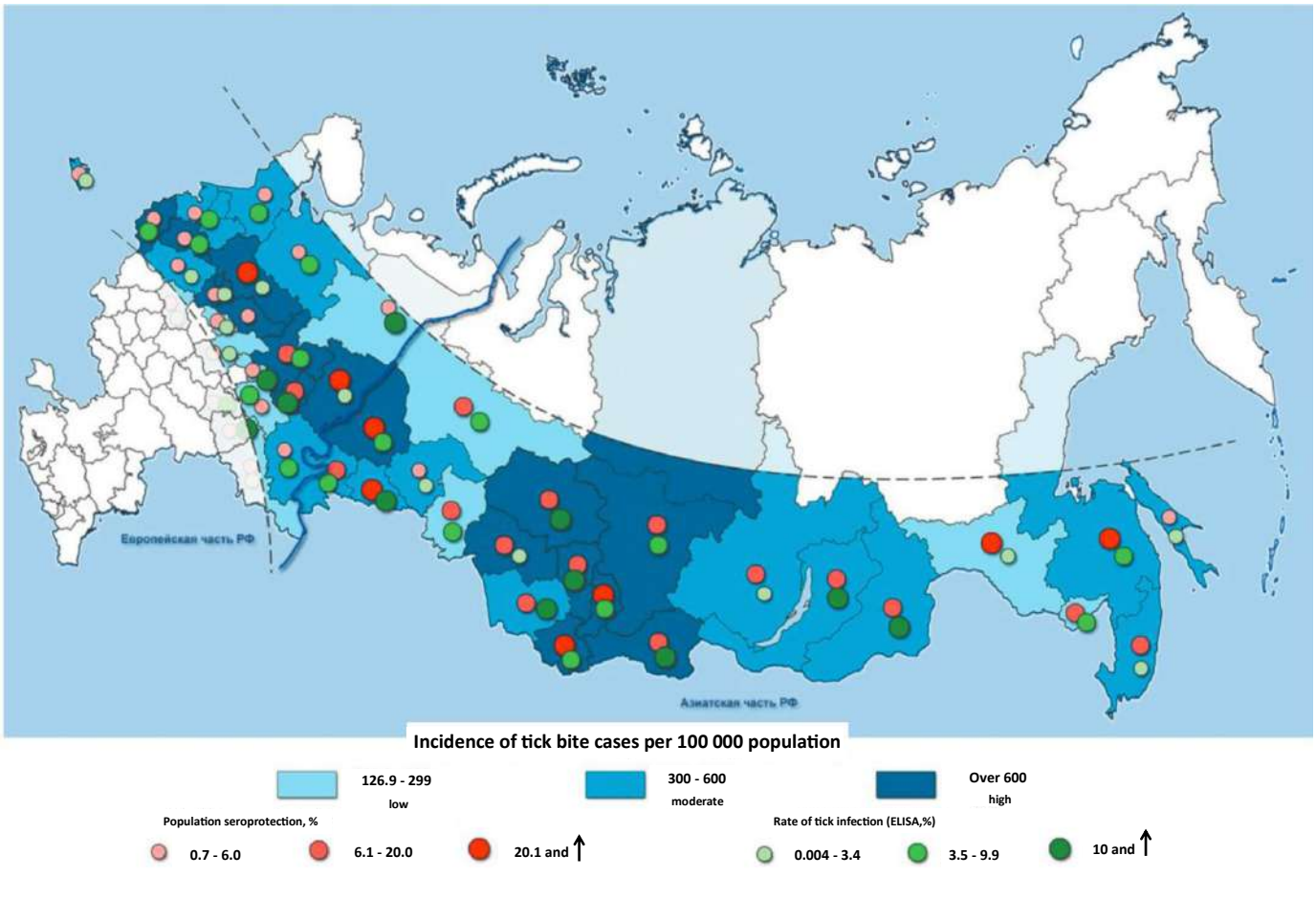


Table 3: Age distribution of TBE in Russia (2016–2019)*

Year	Age													
	Up to 1 year		1–2 years		3–6 years		7–17 years		≥18 years		All	
	Number of TBE cases	Incidence (10^5)	Number of TBE cases	Incidence (10^5)	Number of TBE cases	Incidence (10^5)	Number of TBE cases	Incidence (10^5)	Number of TBE cases	Incidence (10^5)	Number of TBE cases	Incidence (10^5)
2016	3	0.16	19	0.50	85	1.21	165	1.04	1763	1.5	2035	1.39
2017	2	0.1	10	0.26	76	1.05	148	0.9	1698	1.45	1934	1.3
2018	2	0.1	14	0.36	48	0.64	139	0.83	1524	1.3	1727	1.18
2019	1	0.06	26	0.7	79	1.03	149	0.87	1520	1.3	1775	1.21

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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	1969	1.34

*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

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30. Report 2 "Information about infectious and parasitic diseases" 2020 Data of the Federal Center for Hygiene and Epidemiology

Chapter 12b

TBE in Serbia

Vladimir Petrović, Elizabeta Ristanović, Aleksandar Potkonjak

E-CDC risk status: endemic (no new data available as of May 2023)

History and current situation

Tick-borne encephalitis virus (TBEV) was first isolated in the former Yugoslavia in 1953 from the blood of infected human patients in Slovenia.[1] The virus was isolated from ticks in 1954, also in Slovenia.[2] Afterward, in the western part of the country a number of tick-borne encephalitis (TBE) foci were registered, while in the Republic of Serbia such foci were not registered. In the period to following 1969, no new infections with TBEV could be confirmed in the Republic of Serbia through the routine serological testing of samples from more than 1,000 patients with clinical signs of meningitis and encephalitis, as conducted in laboratories of the Institute of Immunobiology and Virology "Torlak" in Belgrade.[3]

In the period from 1962 to 1969, a total of 1,726 serum samples collected from healthy individuals in the Republic of Serbia were tested by hemagglutination inhibition for the presence of antibodies to TBEV and 1.1%–52.6% were positive. The highest percentage of seropositive persons was registered in the region of Sandžak-Raška (Novi Pazar and its surroundings: 52.6%) in the province of Kosovo (37.8%) and in Western Serbia (19.4%). In the area of Banat in the Province Vojvodina, antibodies were found in 8.4% of tested sera, while in the territory of Belgrade city the seropositivity rate was 7.3%. In Eastern Serbia (Zaječar and surroundings) – 3.6% samples were seroreactive, 2.0% in Central Serbia and 1.1% in the Srem area in Vojvodina. These results clearly document - along with the isolation of the TBEV (Western subtype) from ixodid ticks from the area of Pešter, Sandžak-Raška in 1972 – the existence of TBEV-hot spots, i.e., active foci of tick-borne encephalitis in the Republic of Serbia.[4]

Overview of TBE in Serbia

In the Republic of Serbia, TBE is subject to mandatory reporting rules under the 2004 law protecting the population from infectious diseases. That year, a single case of TBE disease was recorded by the Institute of Public Health of Serbia, followed by 6 cases in 2005 and 1 case in 2006.[5] In 2012, the Institute of Public Health of Serbia reported a total of 4 cases of TBE, while in 2013 and 2014, no cases of the disease were registered. In 2015, in central Serbia, 4 cases of disease were registered; all cases were males over 45 years old.[6–10]

From 2016–2018, 19 new cases of TBE disease were officially reported to the Institute of Public Health of Serbia. In 2016, in central Serbia (Belgrade region) 1 case of TBE disease was registered (male, age distribution group 40–49 years) and 5 cases of TBE were registered in 2017 (all cases in central Serbia (Belgrade region-3 cases and Podunavlje District-2 cases). All cases were males and more than 20 years old. The largest number of TBE cases (13) was registered in 2018 in central Serbia (Belgrade region: 4 cases; Podunavlje District: 4 cases). Six of these cases were males and 7 cases were females, 1 in the 20–29 years age group, 3 cases in the 40–49 years group, 1 case in the 50–59 year group and 8 cases were older than 60 years.[11–13]

From January 1, 2014 until October 31, 2015, serum samples from 200 animals (40 dogs, 20 horses, 40 wild boars, 40 cattle, 40 roe deer, and 20 goats) were collected. All serum samples were tested using commercial all-species ELISA kits (Progen Biotechnik, Germany). Anti-TBEV IgG antibodies were found in 7 dogs (17.5%), 1 horse (5%), 5 wild boars (12.5%), 1 cow (2.5%), and 1 roe deer (2.5%). None of the goats tested were positive for anti-TBEV IgG antibodies.[11]

At the same time, PCR and sequencing confirmed the presence of the Western subtype of TBEV (TBEV-EU) in *Ixodes ricinus* ticks, detected in 1 of the 50 (2.0%) *I. ricinus* ticks in Vojvodina province (Fruška Gora mountain) and in 30 of the 450 (6.6%) *I. ricinus* ticks in the vicinity of Belgrade (Manastirska šuma, Rakovica).[14] In 2017, 1 case of TBE disease in horses with acute neurological symptoms was PCR confirmed in Branicevo District.[15]

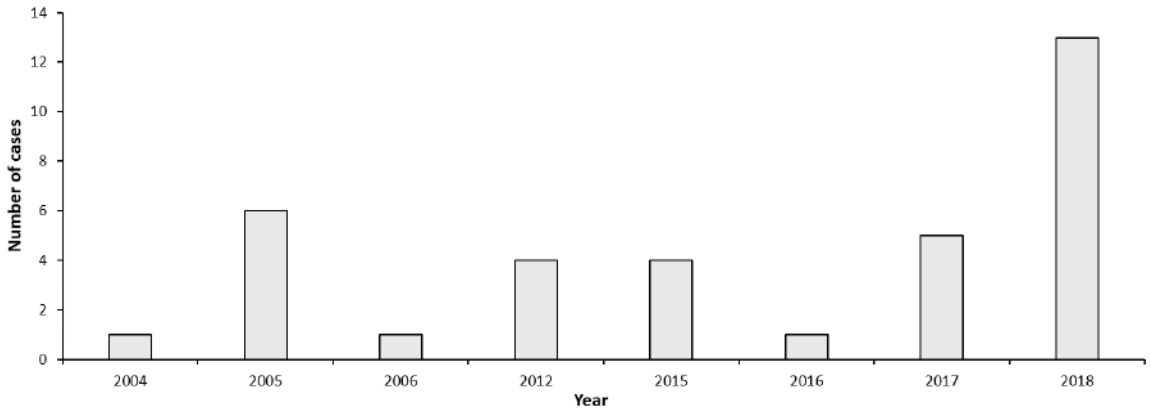
Serbia is "endemic" as several human cases have been reported with seasonal recurrences and the presence of TBEV in *I. ricinus* ticks and/or animals.

Table 1: Virus, vector, transmission of TBE in Serbia

Viral subtypes, distribution	Presence of Western subtype of TBEV was detected in the regions of Vojvodina province, Belgrade, and Pešter[4,14]
Reservoir animals	Seropositive species of animals: dogs, horses, wild boars, cattle, and roe deer[14]
Infected tick species (%)	*I. ricinus*; 2% and 6.6% at the two different localities[14]
Dairy product transmission	Not documented

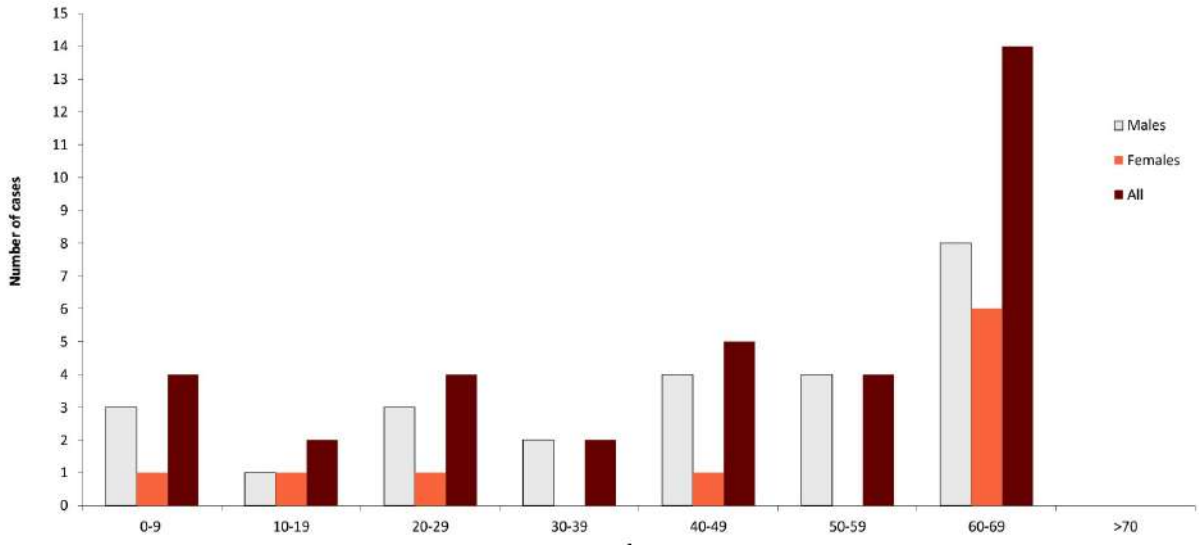
Table 2: TBE reporting and vaccine prevention in Serbia	
Mandatory TBE reporting	Since 2004, under the Law on Protection of Population from Infectious Diseases
Other TBE surveillance	Since January 2020, surveillance according to the EU Clinical Case Definition is introduced in all hospitals in Autonomous 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	No information available
Available vaccines	No information available
Vaccination recommendations and reimbursement	Recommended immunization schedule of persons of a certain age against TBE is introduced from April 1, 2019 as a part of National Immunization Program. Primary series of two or three doses followed by booster after one to three years is recommended. There is no reimbursement. Immunization is to be paid out of pocket.
Vaccine uptake by age group/risk group/general population	Active immunization is recommended as measure of protection of population in endemic areas and for professionally exposed as well as those exposed during recreational activities in endemic areas. There is no information available on vaccine uptake.
Name, address/website of TBE NRC	No information available

Figure 1: Burden of TBE in Serbia over time[6–10]



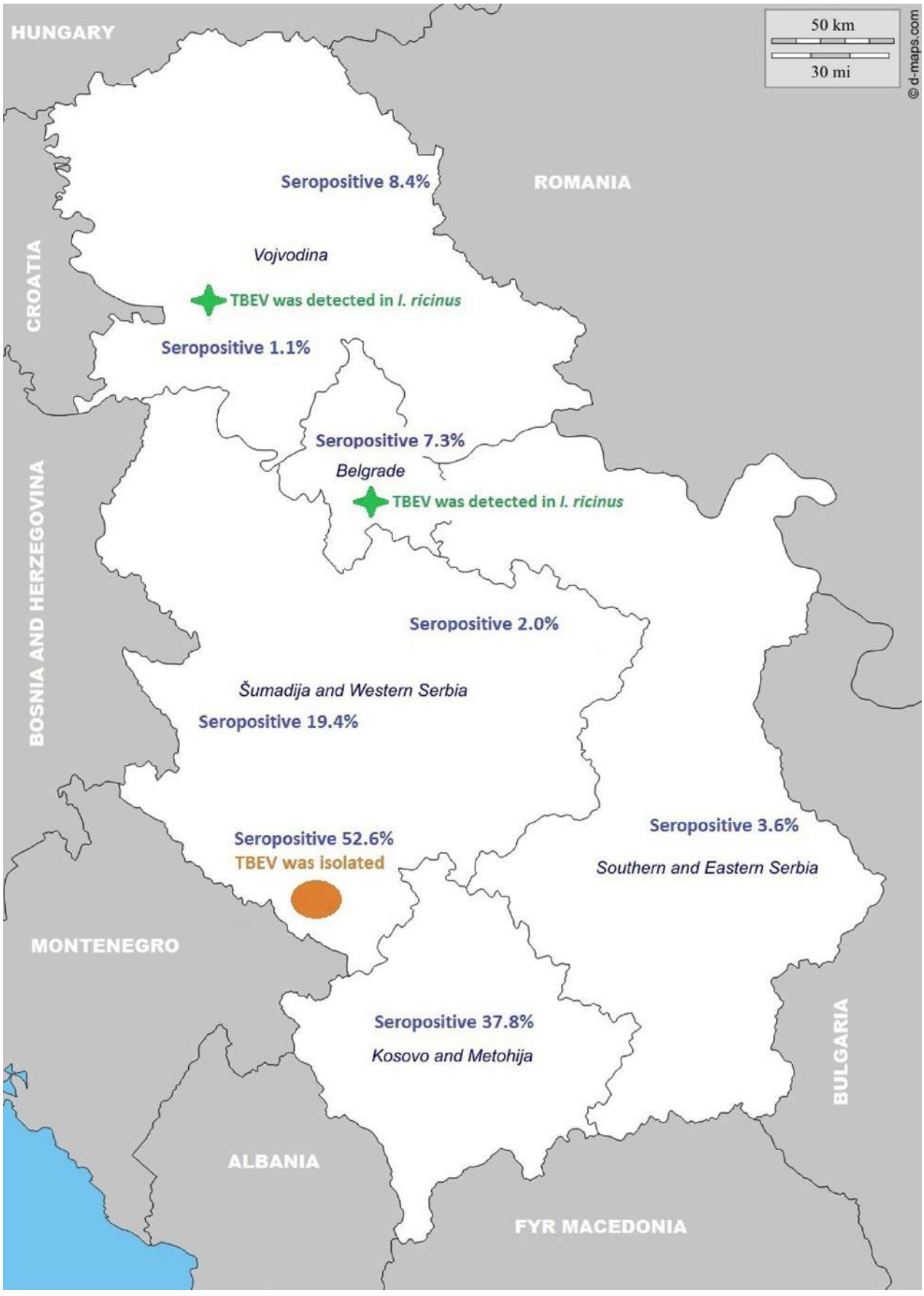
Source Data: Appendix-Figure 1

Figure 2: Age and gender distribution of TBE in Serbia[6–10]



Source Data: Appendix-Figure 2

Figure 3: TBEV-isolation and TBE cases in Serbia[4,11]



Appendix

Source data: Figure 1

Year	Number of TBE cases	TBE incidence /10^5
2004	1	0.01
2005	6	0.08
2006	1	0.01
2012	4	0.06
2015	4	0.06
2016	1	0.01
2017	5	0.07
2018	13	0.19

Source data: Figure 2

Age group (years)	Males	Females	All
0-9	3	1	4
10-19	1	1	2
20-29	3	1	4
30-39	2	0	2
40-49	4	1	5
50-59	4	0	4
60-69	8	6	14
>70	0	0	0

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Citation:

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Chapter 12b

TBE in Slovakia*

Jana Kerlik

E-CDC risk status: endemic (data as of end 2022)

History and current situation

The former Czechoslovak Republic was one of the first countries in Europe where the tick-borne encephalitis virus (TBEV) was identified. This discovery was made in 1947, when Rampas and Gallia observed a high incidence of disease identified as "Czechoslovakia encephalitis", and TBEV was isolated from *Ixodes ricinus*.[1]

In 1951, for the first time ever, and again in Czechoslovakia, the alimentary transmission of TBEV from infected animals to humans was confirmed during a large outbreak in Rožňava. There were 271 hospitalized and serologically confirmed tick-borne encephalitis (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 tick-borne encephalitis TBE focus in Rožňava, the goats were found with high anti-TBEV titers.[3]

In the last few years, we have observed an increasing trend of TBE cases in Slovakia, for which there are several potential contributing factors. First, tick populations thrive because of extreme temperatures, high humidity, and the presence of snow cover during the winter, which acts as insulation. Early springs, as well as summers that are not too hot or dry, also may also be important positive factors for tick survival and reproduction success. Other factors are ecological, demographic, and socioeconomic conditions, for instance, changes in land usage such as increased forestation or newly created gardens, and the growing popularity of outdoor pursuits such as hiking and fishing.[4] Slovakia is well known in Europe for TBE alimentary outbreaks. Over the last few years, there has been a growing trend in the number of food-borne TBE outbreaks. Slovaks like to consume products made from raw goat and sheep milk. Moreover, raw goat milk has been recently promoted as a product to improve health and immunity in humans. In one case, a patient under immune-suppressive therapy in Slovakia drank raw goat milk in order to improve his health status, and he died from TBE.[5]

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.). Some insurance companies partially reimburse TBE vaccine in Slovakia.[6,7]

**Note: Readers may also wish to review the accompanying chapter for the Czech Republic, given the geographic proximity and national history of these countries.*

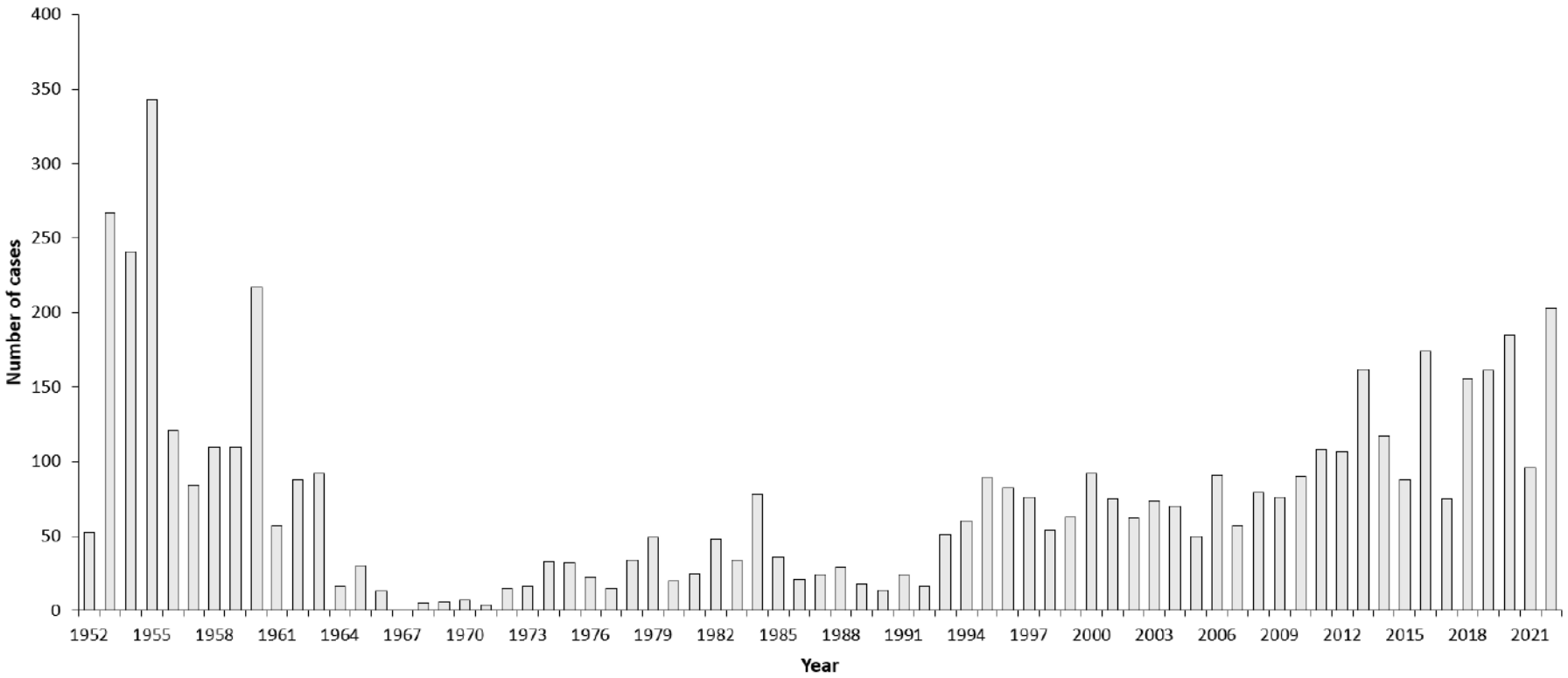
Overview of TBE in Slovakia

Table 1: Virus, vector, transmission of TBE in Slovakia

Viral subtypes, distribution	European subtype[1] At present, there are around 50 known endemic TBE foci in Slovakia. 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, by inference about 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 areas of the country.[9] In the last few years, we have observed an increasing trend of TBE incidence.[10]
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 TBEV were obtained. Therefore, moles can represent not only an important host animal, but may also be considered a reservoir of TBEV 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 TBEV strains were isolated from *I. ricinus* collected on 2 *Turdus merula*[13] Lúky pod Makytou, 1981: 5 strains of TBEV isolated from ticks and organs of *Apodemus flavicollis* (in 15% infected)[14] Western Slovakia (6 localities), 1981–1986: 6 TBEV strains isolated from organs of *C. glareolus* (4), *Apodemus flavicollis* (1), *Sorex araneus* (1)[15] Záhorská Ves, 1990–1992: 8 TBEV isolates from organs of *C. glareolus* (6), *Apodemus flavicollis* (1), *Apodemus sylvaticus* (1)[16] Košická Belá, 2013: TBEV from the brain sample of *Buteo buteo*[17]
Infected tick species (%)	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% ticks were infected by TBEV 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% female ticks infected by TBEV[19] Devín, 1973: 0.1% nymphs and 1.1% female ticks infected by TBEV[20] Slovakia, 1981: In Slovakia there are 2 types of TBEV natural foci – Carpathian and Pannonian. In Carpathian natural TBEV foci, there were 2.6% ticks infected by TBEV. In the Pannonian natural TBEV foci, there were 0.1% ticks infected by TBEV[21] Kurínec, 1982: 0.8% nymphs and 6% 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 2.5% infected ticks). In Pannonian elementary foci (ranging from 0.07% to 6.0%; average 0.9% infected ticks)[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 TBEV in ticks, carried out during 1985–1990 by the Virology Institute of the Slovak Academy of Sciences in Bratislava, showed that the TBEV 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 TBEV[25]
Dairy product transmission	During 2007–2016 we observed a growing trend in the number of TBE outbreaks. A total of 26 outbreaks (those with 2 or more people) were responsible for 142 TBE cases (13.9%) in the studied period. There were 13 family outbreaks with at least 2 linked TBE cases in a single outbreak. Larger outbreaks with 3 or more cases were recorded 13 times. The highest number of outbreaks (6) was recorded in 2013 (4 family outbreaks, 2 large outbreaks). In 2016, there was the largest number of TBE cases (44) reported in a single outbreak over the past 30 years.[10] The most probable and also confirmed common factor in the transmission of TBEV during outbreaks was goat milk and its products (61.5%, 16 outbreaks). Sheep's milk and products 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 TBEV was identified epidemiologically. Sheep cheese was considered as the TBEV transmission factor in the TBE outbreak with the highest number of TBE cases (44) over the past 30 years by retrospective case control study.[26] In 4 outbreaks TBEV was serologically confirmed in goats. In 3 outbreaks TBEV was tested directly in sheep milk. In 1 outbreak the sheep milk was TBEV positive, however it was not the milk from which the incriminated cheese was made. In another 2 outbreaks, TBEV was not found in the sheep milk. 2018 again was a peak year for alimentary transmission: in 5 outbreaks altogether 21 TBE cases were recorded, 3x from consumption of sheep cheese, 1x from consumption of goat cheese, 1x from consumption of goat milk, in all cases the TBEV was detected in milk.

Table 2: TBE reporting and vaccine prevention in Slovakia	
Mandatory TBE reporting	According to Slovak legislation, physicians, hospitals, national reference centers, and laboratories are obliged to report TBE cases comprehensively via paper-based forms, e-mails, or by phone (more urgent) to the individual Regional Public Health Authorities, which passes the information to the Epidemiological Information System (EPIS), or they can report TBE cases directly to EPIS.[6 (§ 4)] The TBE case definition set by Commission Implementing Decision (August 8, 2012) was adopted in Slovakia at the end of 2012.[26] • *Possible case*: not applicable • *Probable case*: any person meeting the clinical criteria (fever, aseptic meningitis/encephalitis) and the laboratory criteria for probable case - OR- any person meeting the clinical criteria with an epidemiological link • *Confirmed case*: any person meeting the clinical criteria (fever, aseptic meningitis/encephalitis) and laboratory criteria for case confirmation
Other TBE surveillance	No data available
Special clinical features	*Note: In the TBE search interface, EPIS offers the following categories: meningeal form (includes biphasic form, but also encephalitis), febrile form, and neurological form (paresis). If greater specification is desired, each case must be reviewed individually for details such as course of disease, which is quite complicated given the large number of cases.* • Meningeal form (including encephalitis): 68.5%[10] • Febrile form: 21.8%[10] • Other neurological form: 8.8%[10] • Asymptomatic form (during outbreak): 0.2%[10] • Unspecified report: 0.7%[10] The meningeal form of the disease was observed in almost the same ratio after alimentary transmission and tick-borne transmission (66.3% vs 69.1%). The febrile form affected 24.9% patients after consumption of TBE infected milk products and 20.9% tick-bitten patients. Most TBE cases were recorded among retired persons (19.7%). High-risk occupations were identified in 15.3%, including general workers (111), foresters (17), farmers (12), field workers (9), and railway workers (7).[10] Other groups affected by TBE: students (9.1%), unemployed persons (8.0%), and children (6.6%). Other professions (e.g., women on maternity leave, food producers, health professionals, educators, social workers and others) were affected by the disease in 33.1% of cases.[10] In most TBE cases (85.9%) a recovery was observed. In 6 cases, permanent consequences (palsy, neurological complications) were recorded. Three cases resulted in death (0.3% mortality). In 2 cases, an infectious cause was involved; in 1 case death was from another cause. In 13.2% of cases the impact of the disease was not specified.[10] In a study, we have diagnosed post-encephalitic syndrome in 27.2% of patients who most often reported headache, tremor of upper limbs, fatigue, and lack of concentration.[28]
Available vaccines	FSME-IMMUN; FSME-IMMUN junior
Vaccination recommendations and reimbursement	According to the law, employees of the virology laboratories in Slovakia that work with TBEV must be vaccinated[6 (§ 8, section 5)] Vaccination is recommended in persons who are professionally exposed to increased risk of selected diseases such TBE. Physicians usually vaccinate subjects who are forestry and water management (including students of forestry schools) employees, agricultural workers, surveyors, geologists, tourist hiking guides, employees of the mountain hunts and lifts, employees of recreation facilities, members of police forces and customs officers, professional soldiers and reserve soldiers called for extraordinary service, and employees performing work associated with the operation and maintenance of the tracks.[6 (§ 10, section 2)] Some insurance companies partially reimburse TBE vaccine in Slovakia.[7]
Vaccine uptake by age group/risk group/general population	TBE immunization data on the Slovak population are not available, but according to numbers of sold vaccine doses and child immunization control, the estimated vaccination coverage in Slovakia is 1% (1.3/100 000).[10] None of the TBE patients seen in the country during the period 2007–2016 had all 3 vaccine doses. Most cases were not vaccinated (98.8%). In 9 (0.9%) cases, a vaccination record was not found or was not available. Partial vaccination was recorded in 3 cases.[10]
Name, address/ website of TBE NRC	NRC for arboviruses and hemorrhagic fevers Public Health Authority of Slovakia Trnavská cesta 52 826 45 Bratislava, Slovakia elena.ticha@uvzsr.sk

Figure 1: Burden of TBE in Slovakia over time[1]



Source Data: Appendix—Figure 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

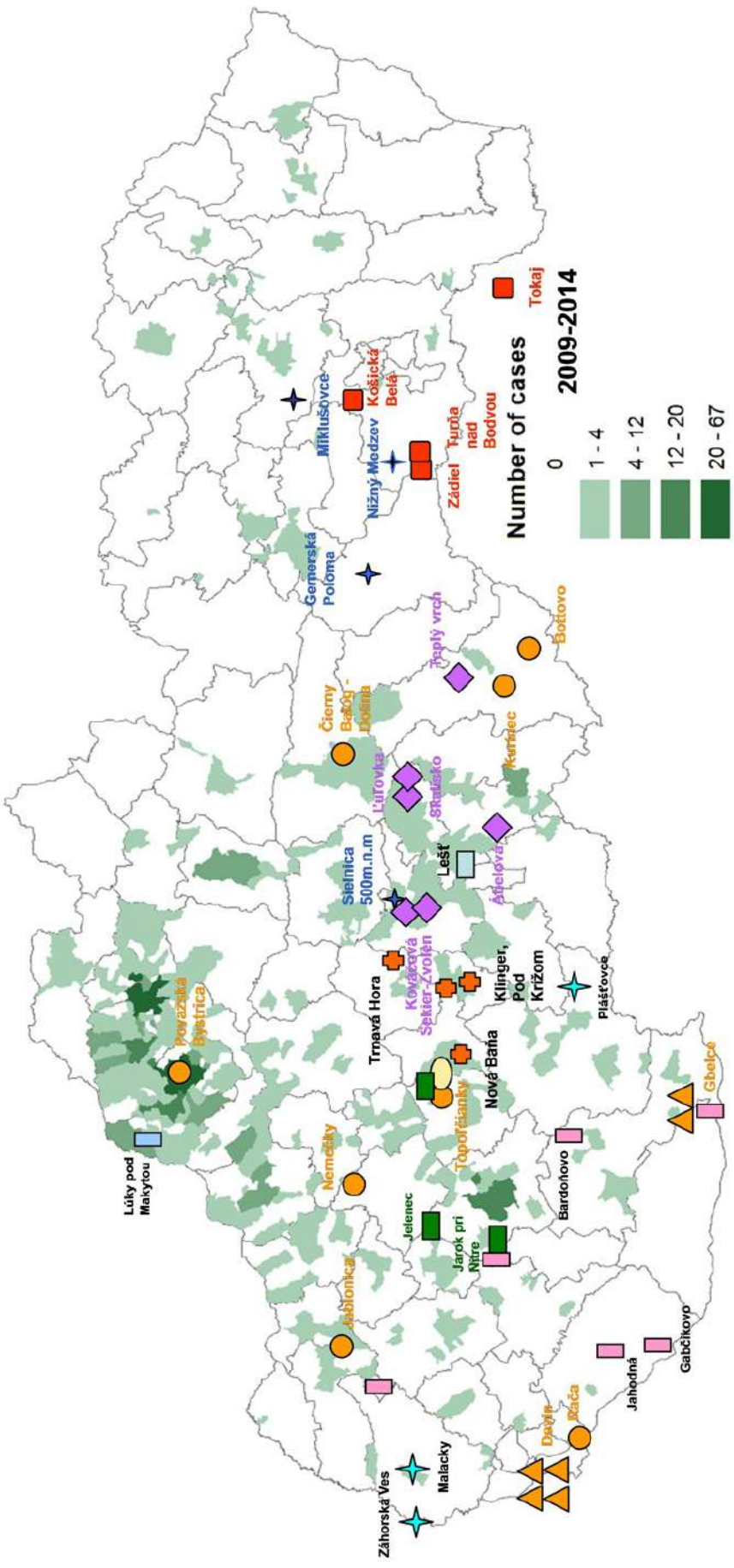


Figure 2: TBEV isolation (1972–2015) and TBE cases (2009–2014) in Slovakia

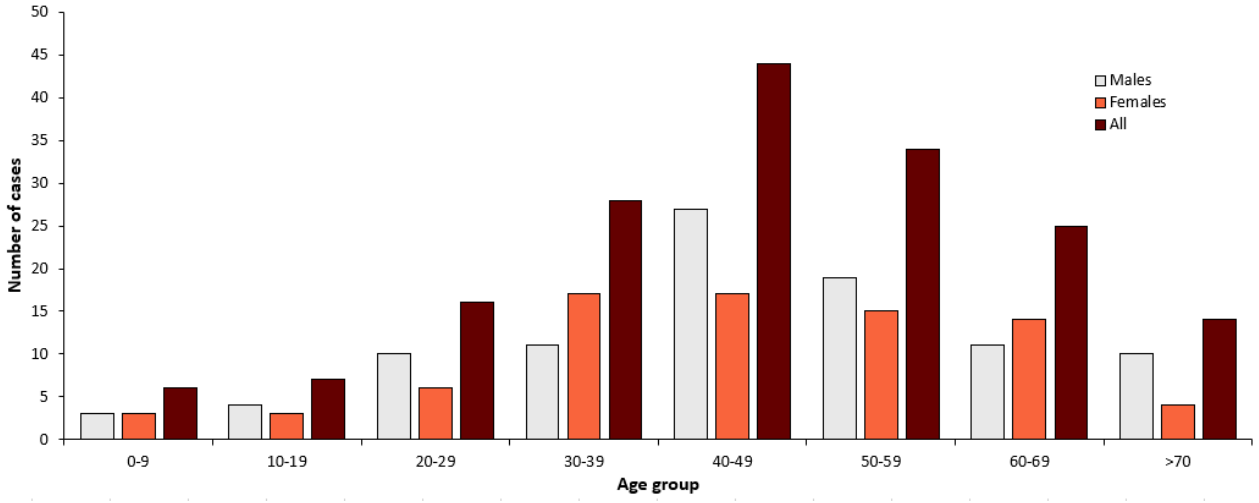
More TBEV isolations carried out in Slovakia exist than are shown on the map. However, data from some isolations were not published and some data from isolations are difficult to find. The most isolations were carried out by the Virology Institute, Slovak Academy of Sciences during the second half of the last century. Since 2000, there has been a decrease in updating TBEV foci in Slovakia.

References for Figure 2

	1964 Tribeč region (Topoľčianky, Jelenec)	Ticks *I. ricinus*	Grešíková M, Nosek J.	Isolation of Tick-borne Encephalitis Virus from *I. ricinus* Ticks in the Tribec region. *Bull Wld Hlth Org.* 1967;36 Suppl. 1:67-71.
	1965 Tribeč region (Jarok pri Nitre, Topoľčianky, Jelenec)	Blood and brain samples from *Talpa europaea*	Kozuch O, Grulich I, Nosek J.	Isolation of Tick-borne Encephalitis Virus from *I. ricinus* Ticks in the Tribec region. *Bull Wld Hlth Org.* 1967;36 Suppl. 1:67-71.
	1967 Tribeč region (Topoľčianky – 2 TBEV strains isolated from *Ixodes ricinus* collected on 2 *Turdus merula*)	Ticks *I. ricinus* collected on the birds	Grešíková M, Nosek J.	Isolation of Tick-borne Encephalitis Virus from *I. ricinus* Ticks in the Tribec region. *Bull Wld Hlth Org.* 1967;36 Suppl. 1:67-71.
	1972–1983 Panonian TBE focus type (most of the southern part of Slovakia – Devín, Gbelce)	Ticks *I. ricinus*	Grešíková M. Gresíková M, Kozuch O, Sekeyová M, Nosek J.	[Tick-borne encephalitis – permanent public health problem]. VEDA Bratislava 1999. 100 p. [Monograph in Slovak]. Studies on the ecology of tick-borne encephalitis virus in the Carpathian and Pannonian types of natural foci. *Acta Virol*. 1986;30 (4):325-31.
	1982 (Kurínec – 4 TBEV strains)	Ticks *I. ricinus*	Grešíková M, Palanová A, Pötheová A, Teplan J, Sekezová M, Kohutová V.	[Contribution to harnessing natural foci of tick-borne encephalitis in south central Slovakia]. *Bratisl Lek Listy*. 1983;80, No 4:385 -512. [Monography in Slovak].
	1973–1984 Carpathian TBE focus type (Carpathian mountains – Rača, Jablonica, Bottovo, Topoľčianky, Ćierna Dolina, Nemečky, Kurínec)	Ticks *I. ricinus*	Grešíková M. Gresíková M, Kozuch O, Sekeyová M, Nosek J.	[Tick-borne encephalitis – permanent public health problem]. VEDA Bratislava 1999. 100 p. [Monography in Slovak]. Studies on the ecology of tick-borne encephalitis virus in the Carpathian and Pannonian types of natural foci. *Acta Virol*. 1986;30 (4):325-31.
	1973 (Devín – 2 strains *Bratislava 188, Bratislava 204*)	Ticks *I. ricinus*	Grešíková M.	[Isolation of tick-borne encephalitis strains Bratislava from ticks *I. ricinus* collected by Devin pathway]. *Bratisl Lek Listy*. 1975;64, No 1: 1-128. [Article in Slovak].
	1981 (Lazy pod Makytou – 5 TBEV strains from *Apodemus flavicollis*)	Ticks *I. ricinus*	Nosek J, Kožuch O, Grešíková M, Lysý J, Labuda M, Teplan J, 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, No 3:257-263. [Article in Slovak].
	1981–1986 (Western Slovakia – 6 TBEV strains: Plavecký Mikuláš –2x *C. glareolus*, 1x *A. flavicollis*, Gabčíkovo – 1x *C. glareolus*, Gbelce – 1x C. glareolus, Jahodná – 1x S. araneus.	Tissues of animals	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(6):537-44.
	1987 (Bardoňovo, Jarok)	Ticks *I. ricinus*	Cannot find publication	

	1980 (Plášťovce – *strain 114*) 1990 (Záhorská Ves from *Myodes glareolus* – *strain CGI223*, Malacky – *strain 285*)	2 strains (285, 114) from *ticks I. ricinus* and 1 strain (CGI223) from the brain *of Myodes glareolus*	Frey S, Essbauer S, Zöler G, Klempa B, Dobler G, Pfeffer M.	Full genome sequences and preliminary molecular characterization of three tick-borne encephalitis virus strains isolated from ticks and a bank vole in the Slovak republic. *Virus Genes.* 2014;48:184-8.
	1990–1992 (Záhorská Ves - 8 TBEV isolates: 6 from tissues of *Clethrionomys glareolus*, 1 from *Apodemus flavicollis*, 1 from *Apodemus sylvaticus*)	Tissues of animals	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.
	1996–1997	Ticks *I. ricinus*	Labuda M, Elecková E, Licková M, Sabó A.	Tick-borne encephalitis virus foci in Slovakia. *Int J Med Microbiol.* 2002;291 Suppl 33:43-7.
	2002–2007	Ticks *I. ricinus*	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. [online] http://www.sevs.sls.sk/images/public/Reviewed_Abstracts_Zoonoses_2016.pdf
	2006	Ticks *I. ricinus*	Koči J, Tarageľová V, Derdáková M, Selyemová D, Cíglerová I, Lenčáková D, et al.	[Tick seasonal dynamics and prevalence of tickborne pathogens in Slovakia.] *Labudove dni 2009: Proceedings of extended abstracts*. Bratislava: Virological Institute SAS, 2009. [Abstract in Slovak].
	2012–2013	Ticks *I. ricinus*	Csank T.	Not published
	2013 Košická Belá	From one brain sample of *Buteo buteo*	Csank T, Bhide K, Bencúrová E, Dolinská S, Drzewnioková P, Major P, et al.	Detection of West Nile virus and tick-borne encephalitis virus in birds in Slovakia, using a universal primer set. *Arch Virol*. 2016;161:1679-83.
	1972–2002	Ticks *I. ricinus*	Not published in print, present on the website of Public Health Authority of Slovak Republic	[Natural foci of tick-borne encephalitis in Slovakia] [Internet] Public Health Authority of Slovak republic; 2017 [Cited 2017 Jan 9]. Available at: http://www.uvzsr.sk/index.php?option=com_content&view=article&id=395:prirodne-ohniska-klieovej-encefalitidy-na-slovensku&catid=68:epidemiologia&Itemid=76. [In Slovak].
	2015	Ticks *I. ricinus*	Kerlik J, Avdičová M., Musilová M., Dobler G., Molčányi T., Csank T, et al.	Presence of tick-borne encephalitis virus in ticks from selected locations in Slovakia. Zoonoses – Protection of public and animal health reviewed abstracts from the 5th scientific congress. Slovak Medical University, Bratislava, 2016. 322 p. [online]. http://www.sevs.sls.sk/images/public/Reviewed_Abstracts_Zoonoses_2016.pdf.

Figure 3: Age and gender distribution of TBE in Slovakia in 2016



Source Data: Appendix—Figure 3

Reference

[Epidemiologický informačný systém] [Internet] Epidemiological Information System; 2017 [Cited 2017 Jan 5]. Data export 2015. Available at: www.epis.sk [In Slovak].

Figure 4: TBE trend in Slovakia, 2003–2022

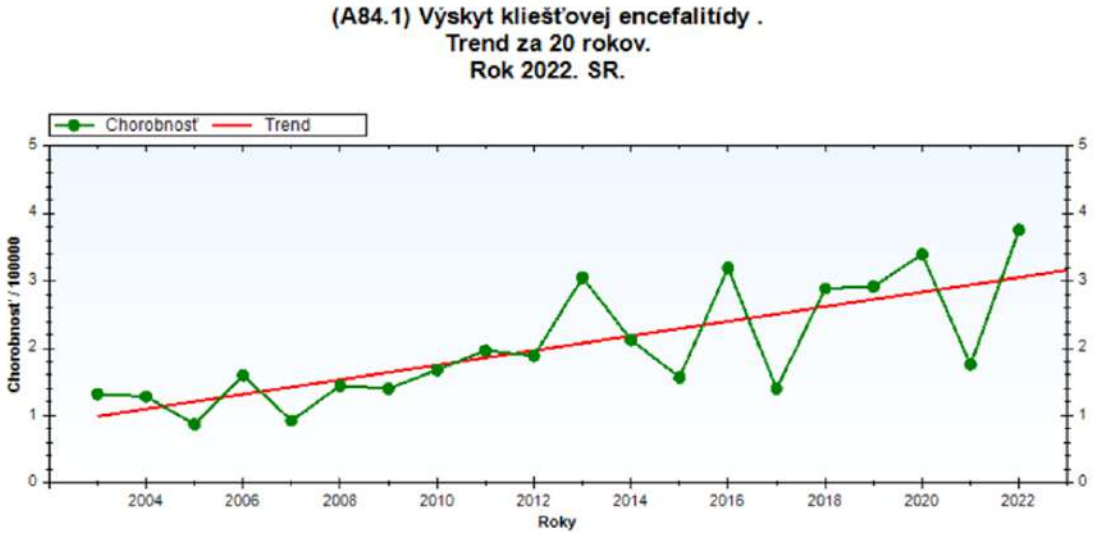


Figure 5: TBE age-specific morbidity in Slovakia, 2022

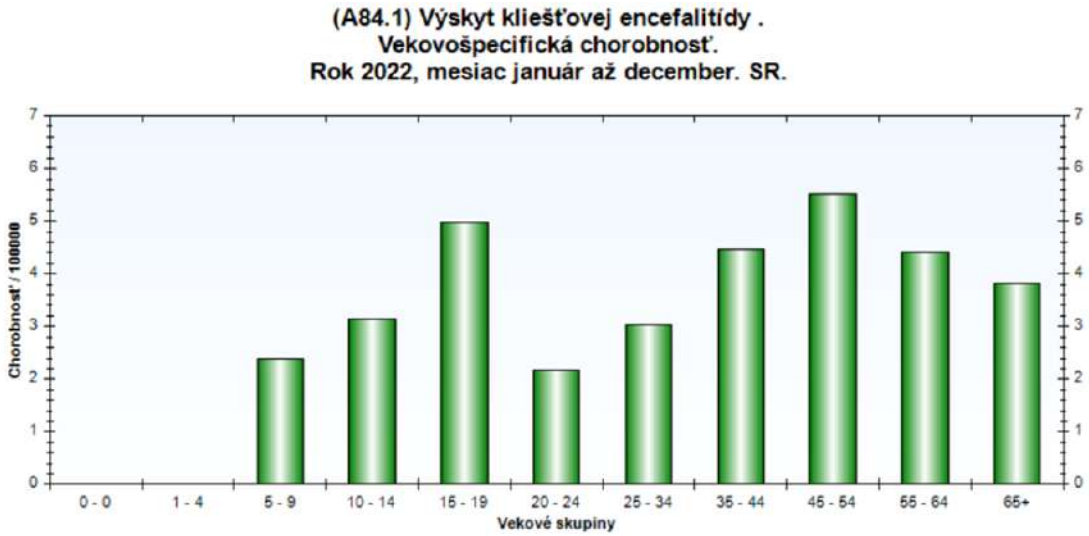


Figure 6: Number and trend of TBE alimentary outbreaks in Slovakia, 2005–2022

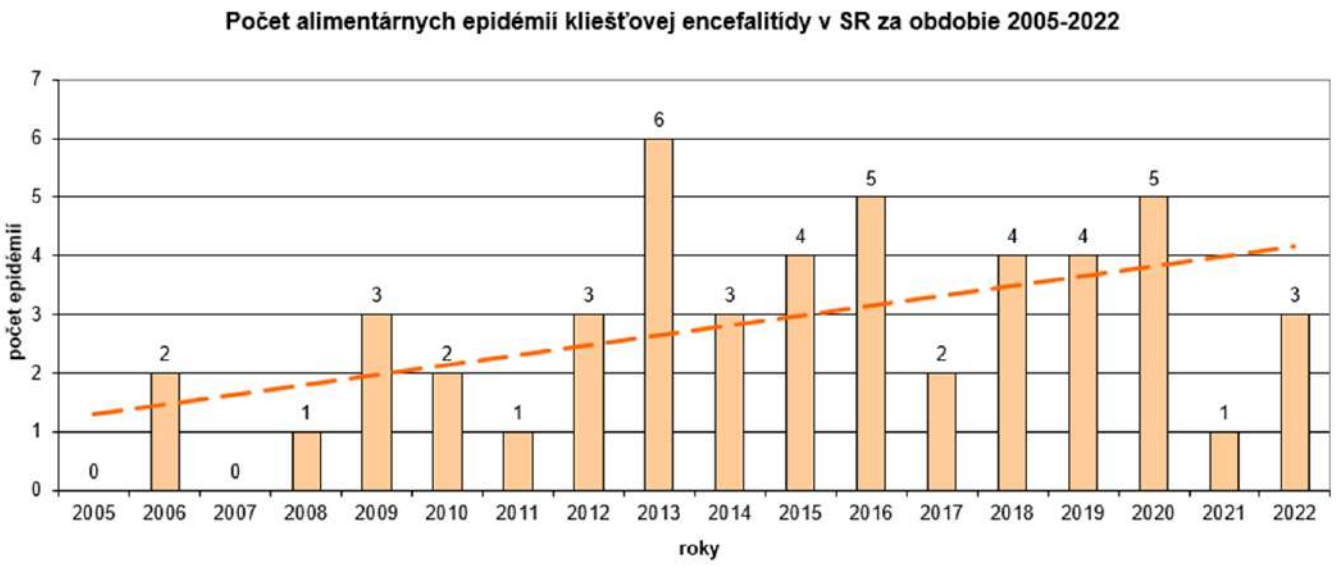
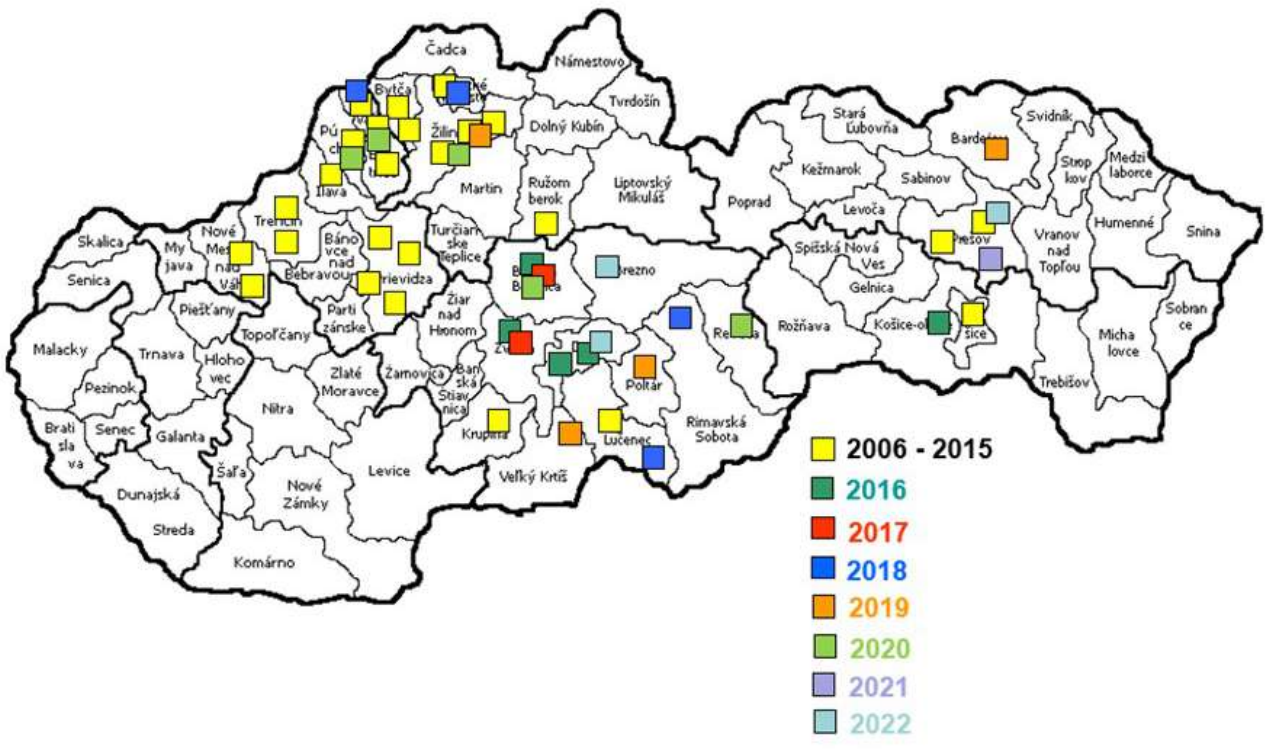


Figure 7: Geographical distribution of TBE alimentary outbreaks in Slovakia, 2006–2022



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
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
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

**According to ECDC classification, Slovakia is in 2/3 areas endemic. There were 161 TBE cases last year.*
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: Figure 3

Age group	Males	Females	All
0-9	3	3	6
10-19	4	3	7
20-29	10	6	16
30-39	11	17	28
40-49	27	17	44
50-59	19	15	34
60-69	11	14	25
>70	10	4	14

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TBE in Slovenia

Zoran Simonović and Tamara Vuković-Janković

ECDC risk status: endemic (data as of end 2022)

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 *I. ricinus.*[2]

Notification of TBE cases as well as deaths due to TBE has been mandatory in Slovenia since 1977. Only cases with central nervous system involvement and laboratory confirmation are notified. Surveillance data has been collected within the communicable diseases surveillance system by the National Institute of Public Health of Slovenia (NIPH).

The number of TBE reported cases in Slovenia varies every year. In the period from 1983 to 2016, the number of annually reported TBE cases was between 62 and 531 (incidence rates between 3.0 and 26.6/100,000), which amounts to a mean of 206 cases/year, and a mean annual incidence rate of 10.3/100,000. In contrast to reports on increasingly higher incidence rates of TBE during the last two decades from many endemic countries, in Slovenia the reported incidence rates during the last 35 years have shown no apparent increasing or decreasing trend. Occurrence of the disease presumably fluctuates due to climatic factors influencing tick activity and population number of small forest mammals, different weather conditions during summer months in different years and other possible factors (e.g., changes in leisure activities) that have not been investigated yet.

TBE virus is present in all Slovenian regions. Although some regions in Slovenia have higher incidence of TBE than others, TBE occurs throughout the country, with the most affected areas in the north and central regions (Fig. 1). In some administrative units average annual TBE incidence rates exceed 45/100,000 (Fig. 2).

TBE infections occur seasonally, in Slovenia mostly between April and November, with a peak in June and July.[5] In recent years, an increase of the cases in the elderly has been observed. Since 1994, TBE incidence rates have been the highest in the 55–64 age group in most years, with males being more frequently affected than females.[6] In men, the 65–74 age group and in women the 45–54 age group followed, with the second highest rates in most years. In contrast to the TBE incidence, the disease burden expressed in DALYs was higher in children aged 5–14 years than in adults aged 50–74 years.[7]

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-

Figure 1: Reported TBE cases in Slovenia between 2008 and 2014 by region[3]

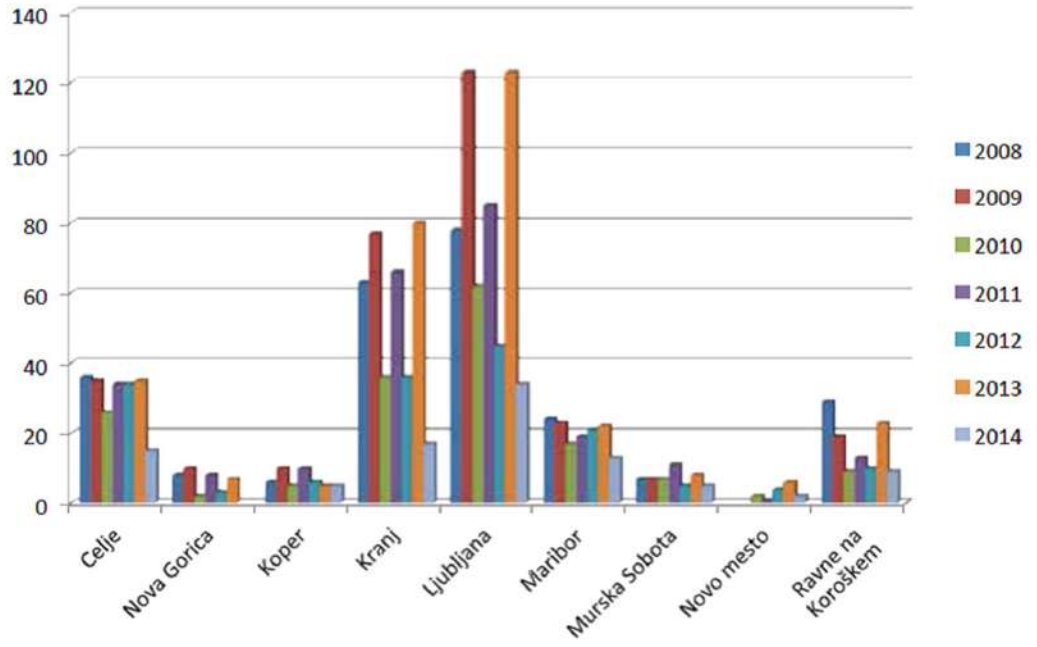
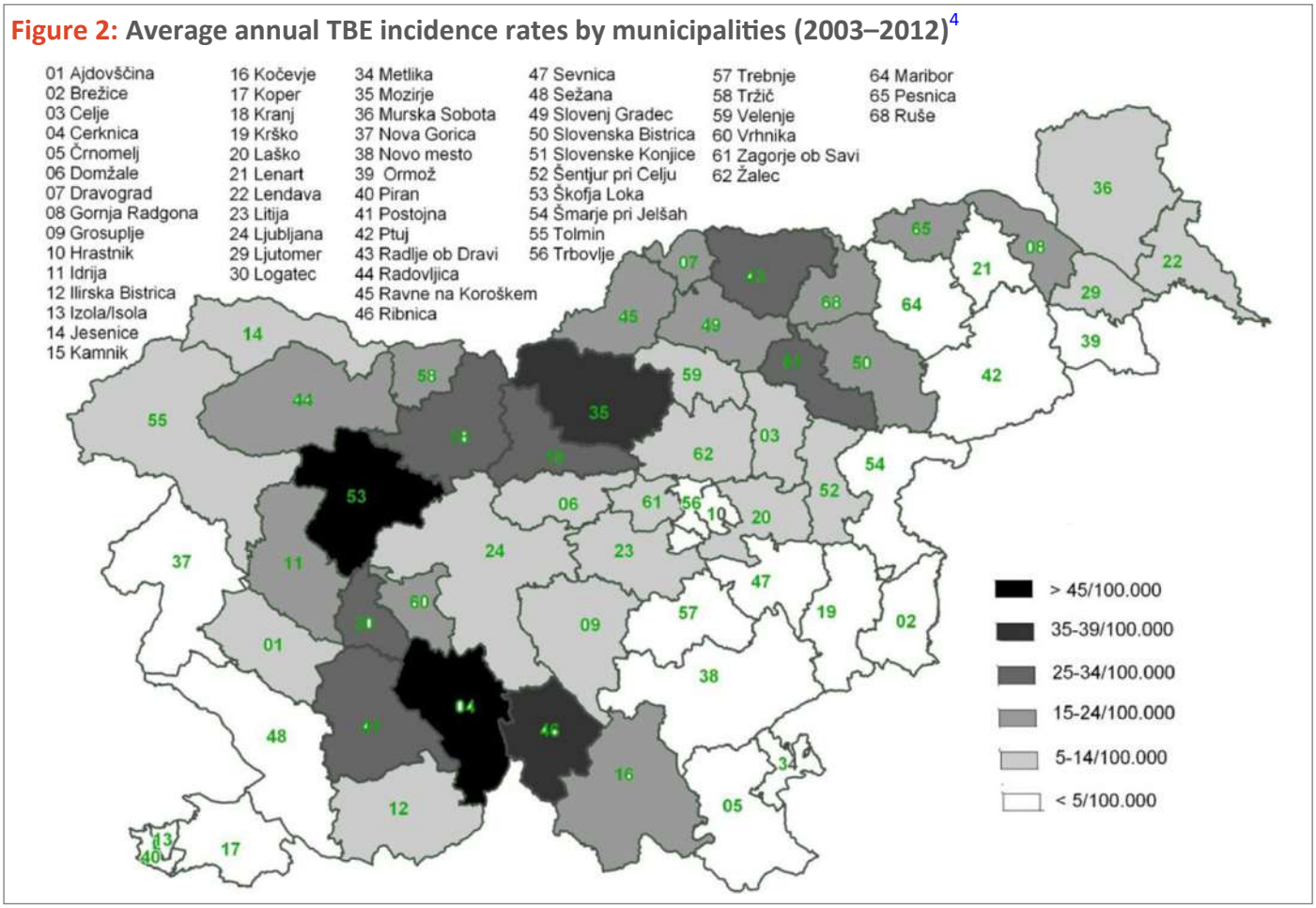


Figure 2: Average annual TBE incidence rates by municipalities (2003–2012)[4]



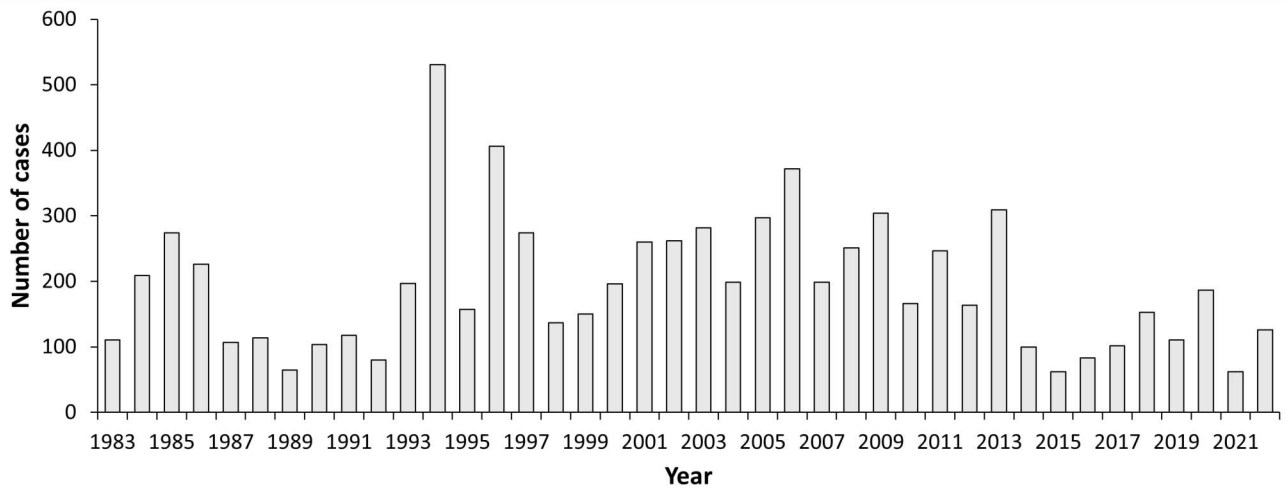
processing industries and construction. The risk is also higher among farmers, if their farmlands are located near forested areas, which present a natural habitat for ticks. There have also been observations of increased TBE incidence among people who visit forests for recreational purpose or forest 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 hours in 23.5% of TBE cases. Long attachments (more than 24 hours) 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.[8]

Overview of TBE in Slovenia

Table 1: Virus, vector, transmission of TBE in Slovenia	
Viral subtypes, distribution	European subtype (TBEV-EU); great heterogeneity of the viruses with geographical clustering seen for viruses with the same genetic characteristics.[9]
Reservoir animals	Rodents; TBE virus antibodies were detected in 5.9% of rodent sera. Bank voles had higher rate of infection than mice.[10]
Infected tick species (%)	In Slovenia, the main vector is *Ixodes ricinus*, and the prevalence of TBE ticks infection is 0.47%.[11]
Dairy product transmission	Cases of alimentary TBE 2012–2014: 6 (4 in small outbreak in 2012).[12]

Table 2: TBE reporting and vaccine prevention in Slovenia	
Mandatory TBE reporting[13,14]	Reporting is mandatory. Only confirmed cases are reported formally to clinicians. Clinically diagnosed CNS infection of TBE must be confirmed by at least one of the following: Case definition: laboratory-confirmed patient • The presence of specific serum IgM and IgG antibodies • The presence of specific IgM antibodies in cerebrospinal fluid (CSF) • IgG seroconversion to TBEV • The presence of the TBEV genome in the clinical specimen • Isolation of TBEV from the clinical specimen.
Other TBE surveillance	Information not available
Special clinical features	Information not available
Available vaccines	FSME-Immun and Encepur[15]
Vaccination recommendations and reimbursement[16-20]	TBE vaccination was introduced in 1986. A national TBE vaccination policy and recommendation has been implemented only for high-risk groups: • Since 1986, mandatory for high-risk workers (e.g., foresters, hunters, farmers, gardeners, soldiers, laboratory workers) – reimbursed by employers • Since 1990, mandatory for students at high risk (e.g., forestry, wood processing) – reimbursed within compulsory national health insurance • Since 1991, recommended for all individuals living in or travelling to endemic areas including children from 1 year of age – paid by vaccinated individuals themselves. In 2019, the vaccination against TBE, funded by the Health Insurance Institute of Slovenia, is available to children born in 2016 and adults born in 1970. Vaccination is performed by selected personal physicians or pediatricians. Persons from these age groups who have already been vaccinated against tick-borne meningoencephalitis are eligible for the next three doses of the TBE vaccine (primary vaccination or booster). Previously unvaccinated adults 49 years old and children 3 years old, will be included in the vaccination program every year; thus, gradually increasing the protection of the Slovenian population against TBE.
Vaccine uptake by age group/ risk group/ general population	In 2007, the estimated proportion of the general population age 15 years and older who reported to have ever been vaccinated against TBE was 12.4%. In 2014, according to official data from National Institute of Public Health of Slovenia the number have increased to 16%. No further estimates of vaccine coverage have been performed.[18]
Name, address/website of TBE National Reference Center	National Institute of Public Health of Slovenia http://www.nijz.si/en

Figure 3: Burden of TBE in Slovenia over time[3,4]



Source Data: Appendix—Figure 3

Figure 4: Age and gender specific incidence rates of TBE in Slovenia in year 2018[3]

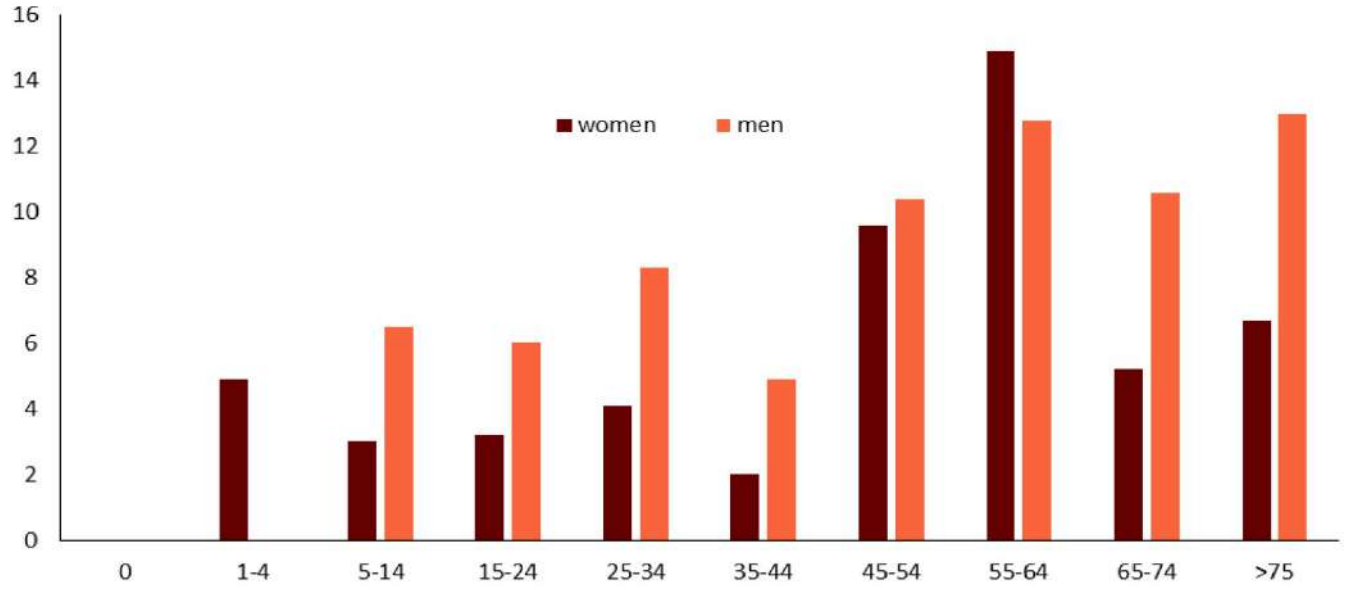


Figure 5: Mean annual incidence per 100,000 of tick-borne encephalitis, by age and gender, Slovenia, 2009–2013[8]

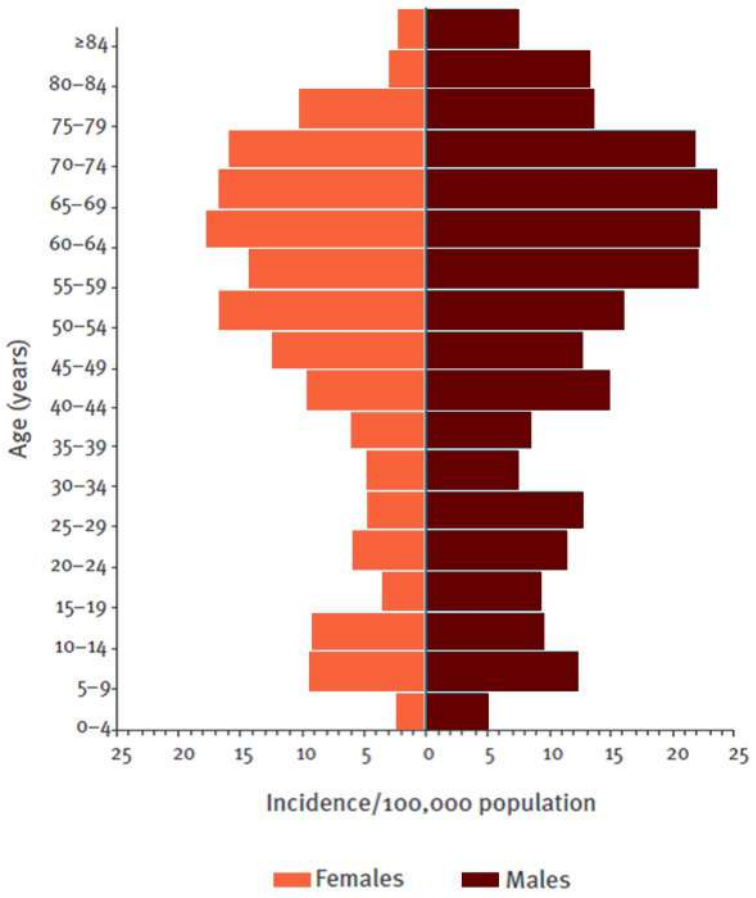


Figure 6: Geographical distribution of TBE virus isolation from rodents only, 2005–2008
TBEV-isolation in Slovenia[10]

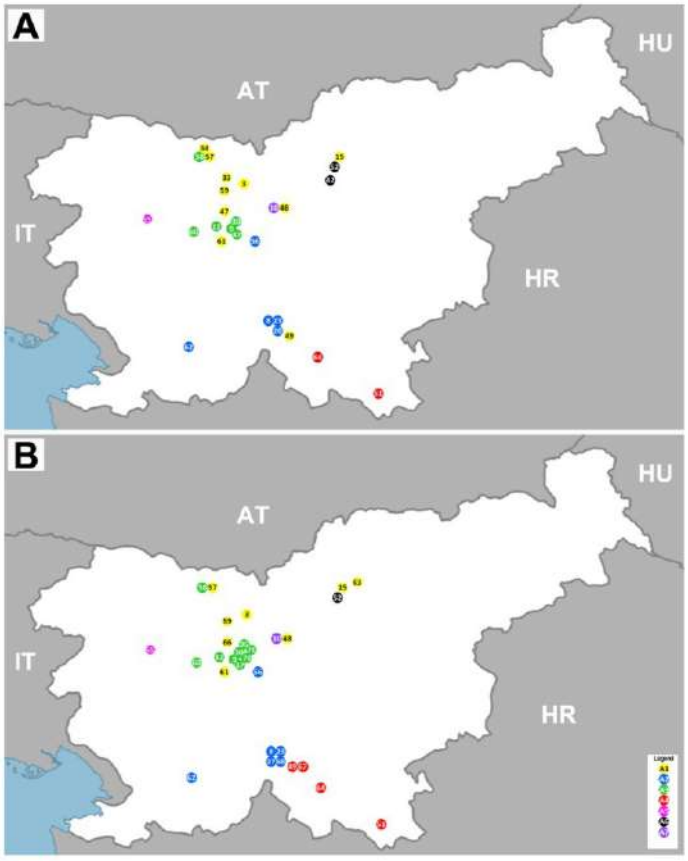
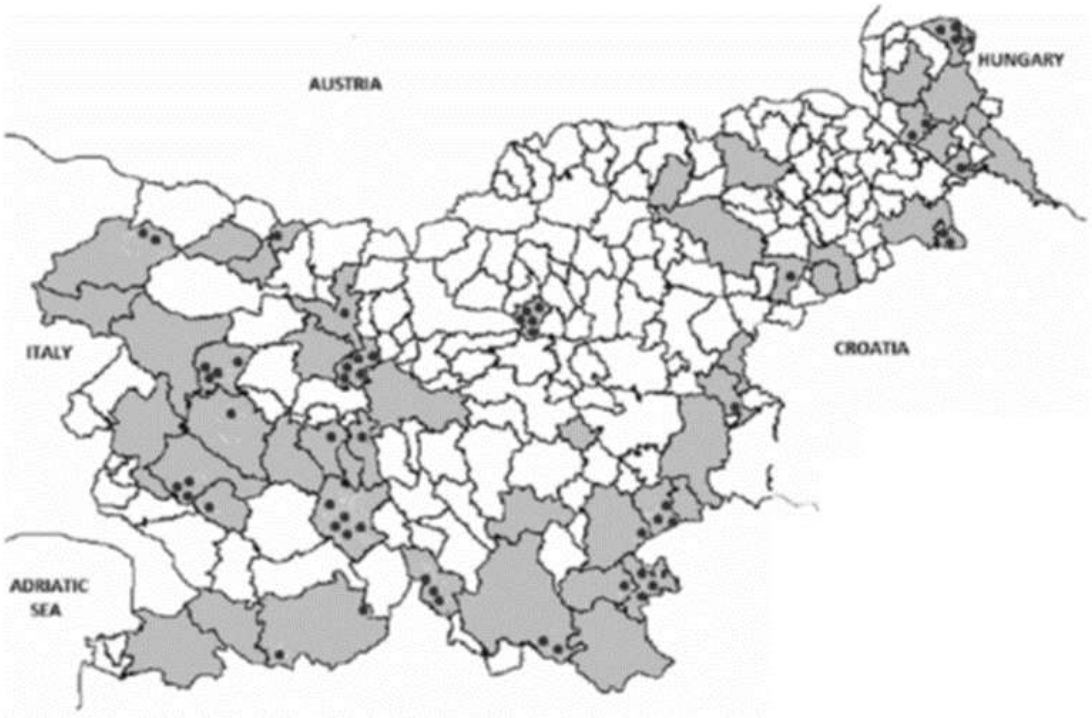


Figure 7: Map of municipalities in Slovenia, showing sites where tick-borne encephalitis virus (TBEV) was detected in rodents (represented in dots) and municipalities (gray colored) where rodents were captured (2000–2008).



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Appendix

Source data: Figure 3

Year	Number of cases	Incidence / 10^5
1983	111	5.56
1984	209	10.47
1985	274	13.72
1986	226	11.32
1987	107	5.36
1988	114	5.71
1989	65	3.26
1990	104	5.21
1991	118	5.91
1992	80	4.01
1993	197	9.87
1994	531	26.59
1995	157	7.86
1996	406	20.33
1997	274	13.72
1998	137	6.86
1999	150	7.51
2000	196	20.33
2001	260	13.02
2002	262	13.12
2003	282	14.12
2004	199	9.97
2005	297	14.90
2006	372	18.63
2007	199	9.90
2008	251	12.40
2009	304	14.90
2010	166	8.10
2011	247	12.00
2012	164	8.00
2013	309	15.00
2014	100	4.85
2015	62	3.00
2016	83	4.00
2017	102	4.94
2018	153	7.60
2019	111	4.21
2020	187	9.35
2021	62	2.94
2022	126	5.97

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Chapter 12b

TBE in South Korea

Song Joon Young

E-CDC status: imperiled – unknown if affected or endemic
(no new data available as of May 2023)

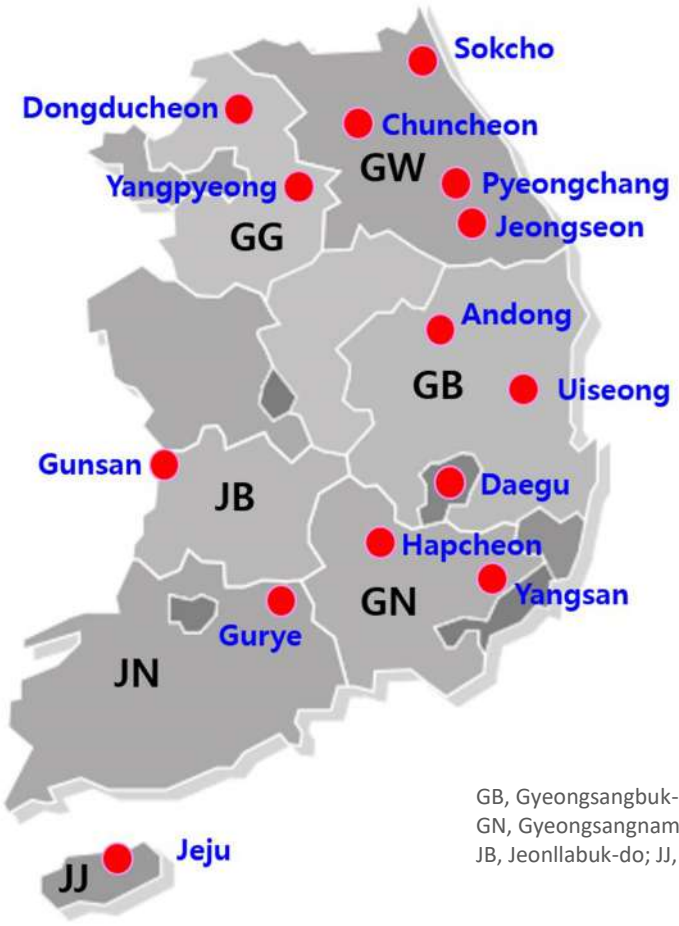
History and current situation

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 one 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 *I. nipponensis* became predominant when harvested from small mammals.[6]

According to the results, TBEV was detected in numerous regions (Figure 1):

- 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).[1-5]

Figure 1: Geographical locations where 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.

The first study was conducted in 12 regions of 5 provinces of South Korea in 2005–2006.[1] TBEV was detected from *H. 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.

Table 1: Virus, vector, transmission of 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

Table 2: TBE reporting and vaccine prevention in South Korea

Mandatory TBE reporting	Yes: TBE is a group 4 Nationally Notifiable Infectious Disease in South Korea[7] Case definition: laboratory-confirmed patient 1. Clinical criteria: person with symptoms of inflammation of the central nervous system, including meningitis, meningo-encephalitis, encephalomyelitis, 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) - Seroconversion 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	Not available

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 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. nipponensis* (0.3%, 42/13,053), and *I. 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.[5] 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. In phylogenetic analysis, the TBEV strains identified in this study belonged to the Western subtype also.

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.

Overview of TBE in South Korea

In South Korea, TBE is designated as a group 4 Nationally Notifiable Infectious Disease, requiring immediate reporting for laboratory-confirmed cases.[8]

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 Centers for Disease Control and Prevention (KCDC) 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 KCDC 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 May 2023, no human cases of TBE have been reported.[9]

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Chapter 12b

TBE in Sweden

Åke Lundkvist

E-CDC risk status: endemic (data as of end 2022)

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. 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 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.

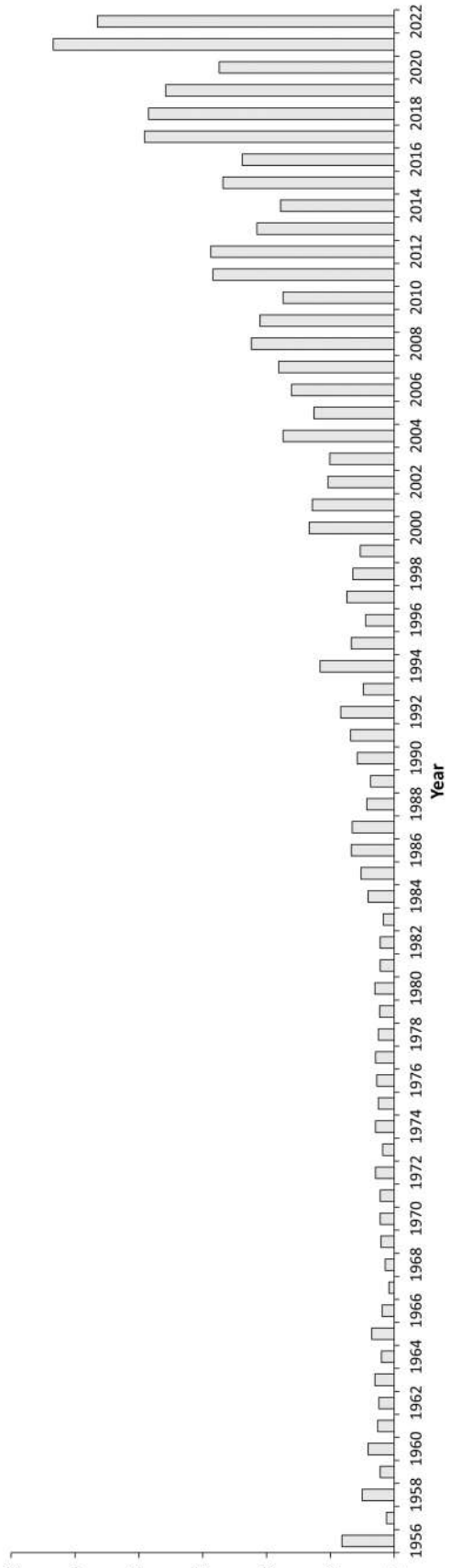
iv. Climate change and weather events associated with higher temperatures further influenced the infection prevalence in the tick population and therefore also the annual incidence in humans.

Overview of TBE in Sweden

Table 1: Virus, vector, transmission of 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

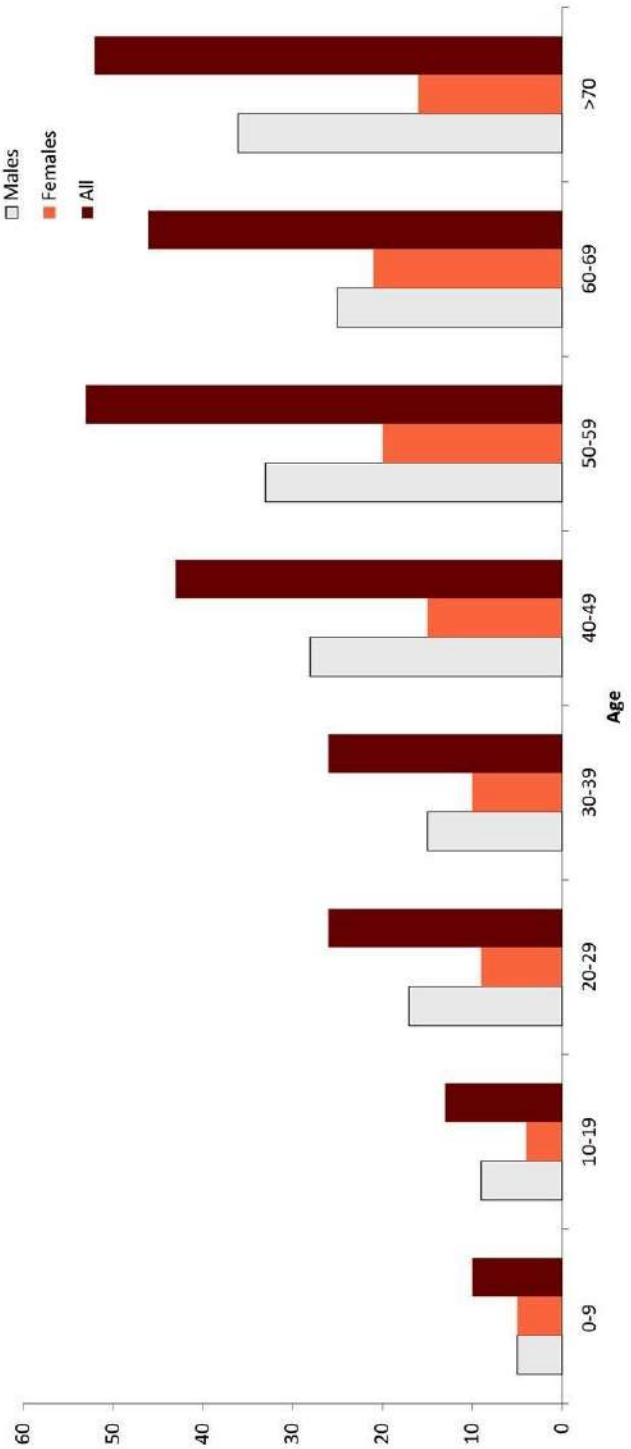
Table 2: TBE reporting and vaccine prevention in Sweden	
Mandatory TBE reporting	Each diagnostic laboratory plus the responsible physician report to the Public Health Agency of Sweden **Case definition**: **TBEV-infection (viral TBE)** **Suspected case:** - Epidemiological link - Clinical symptoms consistent 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



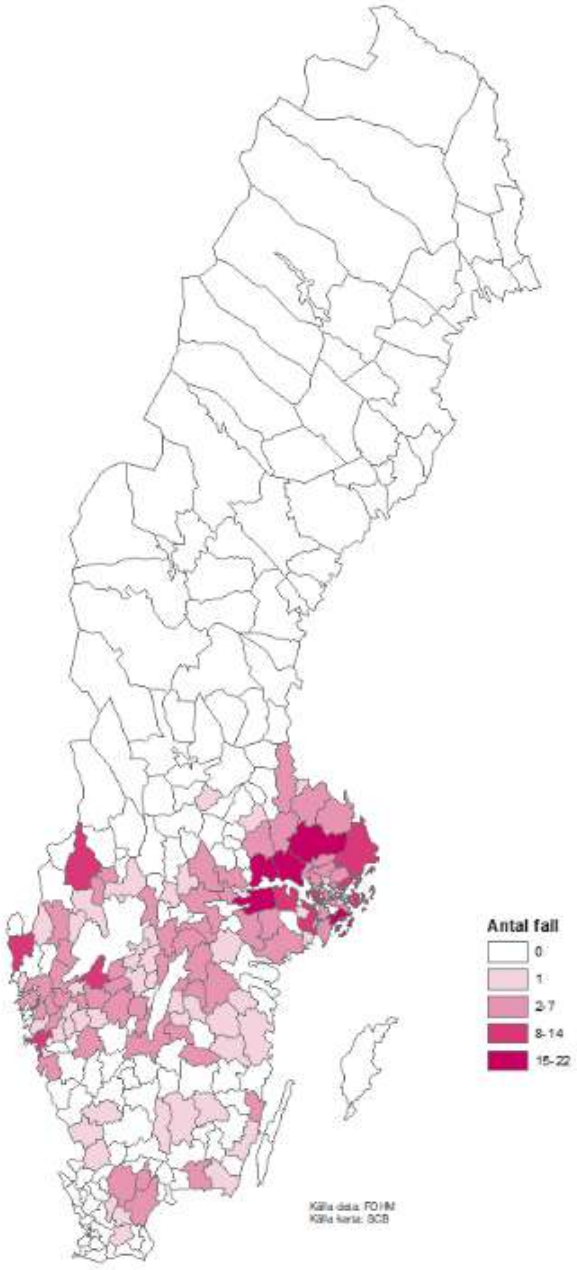
Source Data: Appendix—Figure 1

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

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	36	16	52

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Chapter 12b

TBE in Switzerland and Liechtenstein

Daniel Desgrandchamps and Klara M. Posfay-Barbe

E-CDC risk status: endemic (data as of end 2022)

History and current situation

The first serological reports of tick-borne encephalitis (TBE) in Switzerland date back to the early 1970s [T. Krech. Dissertation, University of Berne, 1980]. Surveillance started in 1984, and TBE became a notifiable disease in 1988. Most cases are reported between April and October following tick bite exposures below an altitude of 1500–2000 meters.[1,2]

Tick-borne encephalitis virus (TBEV) has been identified in ticks from almost all regions of Switzerland and in Liechtenstein. Accordingly, human cases are found in almost all regions. Most cases occur in the north-eastern, central, and midwestern regions of the country, but in recent years, new endemic regions have been detected in western, and southern Switzerland. TBE has thus become endemic in almost the entire country.

In 2013, a procedure allowing the identification of regions which qualify for a local TBE vaccination recommendation was adopted for Switzerland and Liechtenstein.[3] Data from cases notified over the previous 10 years ("high risk areas", Fig. 3a) were combined with data from the historical map of Swiss endemic regions and "natural clusters". The resulting Swiss map was used until 2018 for the definition of regions where TBE vaccination is recommended for exposed people (Fig. 3b).

However, in view of the increasing numbers of reported TBE cases in recent years, Swiss and Liechtenstein health authorities decided in 2019 to consider their entire countries – except for the cantons of Geneva and Ticino – as an at-risk area in which TBE vaccination is recommended for all individuals with possible exposure (both as residents or as visitors),[2] see Fig. 3c.

Currently, vaccination is recommended and reimbursed by health insurance for individuals older than 6 years of age living in or visiting endemic regions. In children aged 1–5 years, the indication shall be based on individual considerations. Unlike in other countries and in contrast to the label, a booster dose is recommended only every 10 years.[3]

As elsewhere in Europe, the proportion of "mild cases" is lower and the number of more serious cases increases with age. However, more serious disease patterns like meningoencephalitis have also been reported in children below the age of 6 years over the later years (E. Altpeter, FOPH, personal communication). Less than half (45%) of symptomatic patients reported a tick bite within 4 weeks of disease onset.[5] Less than 2% of cases experienced relevant tick bites outside of Switzerland.

Approximately 80% of all symptomatic patients are hospitalized.[1] The mean duration for hospitalization was 9 days (interquartile range 5–11 days), and duration increased linearly with age (5 days in children less than 14 years old to 14.6 days for patients older than 70 years).[5]

Overview of TBE in Switzerland

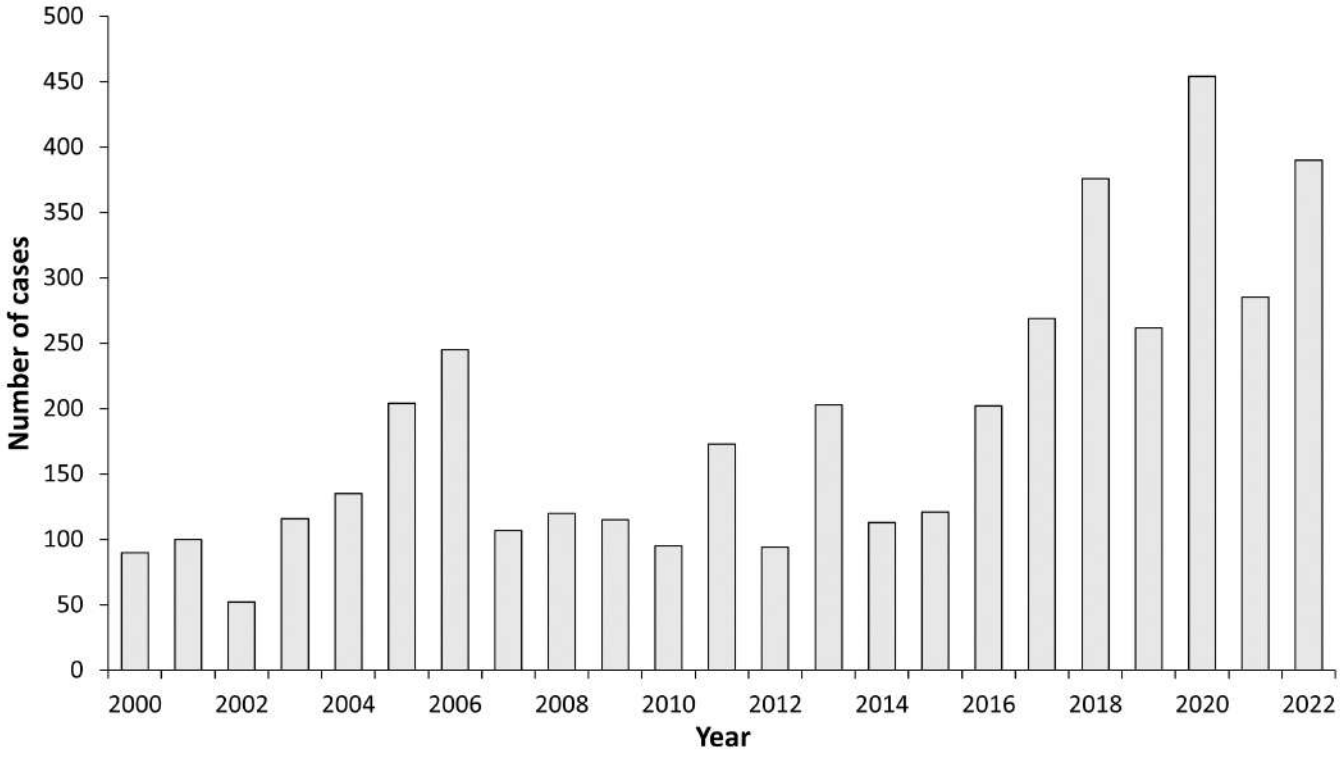
Table 1: Virus, vector, transmission of TBE in Switzerland

Viral subtypes, distribution	European subtype; 97%–98.4% similar to the reference Neudoerfl strain, strain Genbank = U27495; mostly: strain NETBE7, HQ883372 & NETBE8 (HM450136, HM450137, HM450138, HM450140, HM450141)[6,7]
Reservoir animals	Small mammals such as rodents, birds[6,7]
Infected tick species (%)	*I. ricinus*. 1.6%–9.9% in areas <2000 meters altitude[6,8]
Dairy product transmission	Not documented

Table 2: TBE reporting and vaccine prevention in Switzerland

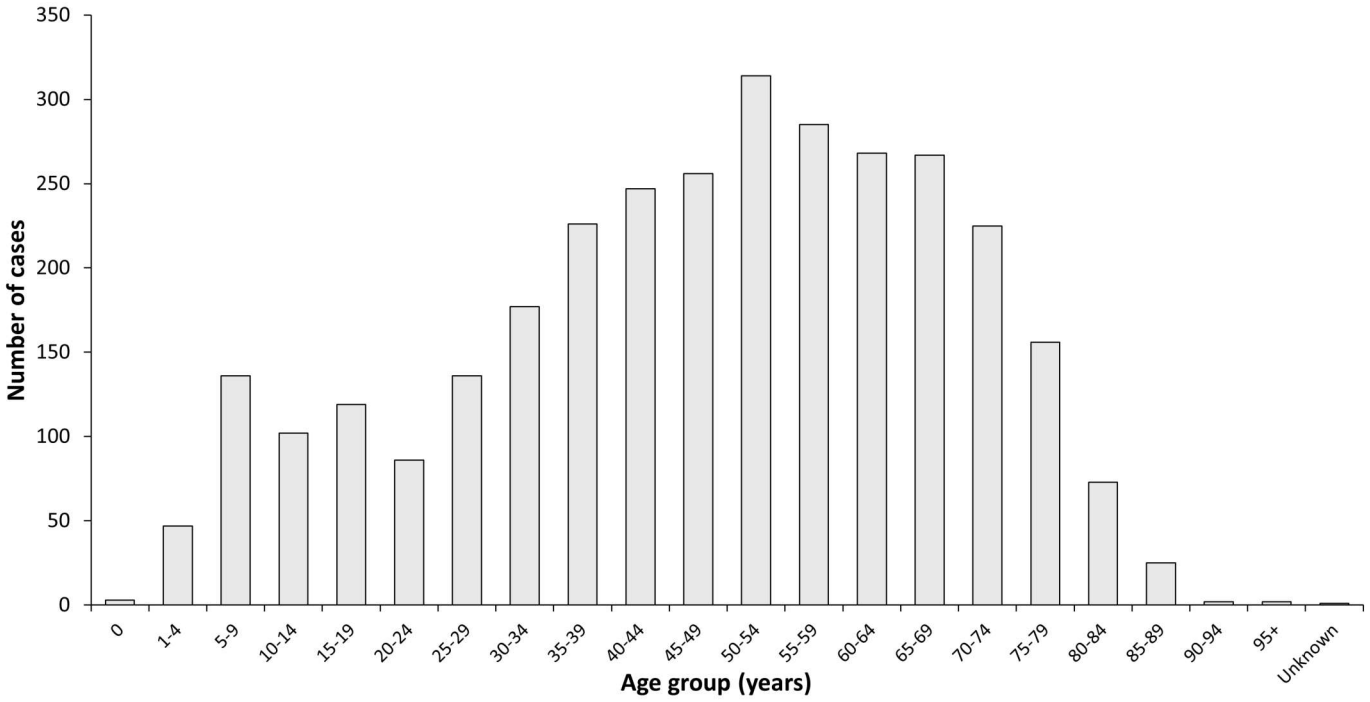
Mandatory TBE reporting	Notifiable disease since 1988 Tick bites and Lyme borreliosis have been reported via a sentinel group (general practitioners and pediatricians in the entire country) since 2008[5,9]			
Categorization[5]	Case classification	Laboratory criteria		Clinical criteria
	Not a case	Positive IgM serology		No ILI & no neurological symptoms
	Possible case	a)	Positive IgM serology	ILI or non-specific neurological signs & symptoms
		b)	Positive IgM + positive IgG serology*	Any
	Probable case	a)	Positive IgM serology	Meningitis, meningoencephalitis, encephalomyelitis or pareses
		b)	Positive IgM + positive IgG serology*	ILI or non-specific neurological signs or symptoms
	Confirmed case	a)	Positive IgM + positive IgG serology*	Meningitis, meningoencephalitis, encephalomyelitis, or pareses
		b)	TBE-RNA detection by PCR	Meningitis, meningoencephalitis, encephalomyelitis, or pareses
IgG, immunoglobulin; IgG, immunoglobulin; ILI, influenza-like illness; PCR, polymerase chain reaction *Or anti-TBE IgG serum antibody seroconversion or ≥4-fold rise in anti-TBE IgG serum antibodies				
Special clinical features	No Swiss data			
	% with sequelae: 25%; mortality: 1%			
Available vaccines[10]	Encepur N® (Bavarian Nordic); FSME-Immun® (Baxter/Pfizer). Number of doses sold: not available			
Vaccination recommendations and reimbursement[10]	Recommendations and reimbursement for vaccination in 2006			
Vaccine uptake by age group/risk group/general population[11]	**Average national vaccination uptake (3 doses), 2014–2016:** 8 years old: 22%–31% 16 years old: 33%–45% **High-risk area** (canton of Thurgau): 8 years old: 40%–53% 16 years old: 64%–75%			
Name, address/ website of TBE National Reference Center	National Reference Center for Tick-borne Diseases, SPIEZ LABORATORY is a division of the Federal Office for Civil Protection LABOR SPIEZ Austrasse 3700 SPIEZ - Switzerland https://www.labor-spiez.ch/de/die/bio/dediebionrz.htm nrzk@babs.admin.ch			

Figure 1: Burden of TBE in Switzerland 2000–2022[2,4]



Source Data: Appendix—Figure 1

Figure 2: Age distribution of TBE in Switzerland 2009–2022[4]



Source Data: Appendix—Figure 2

Figure 3a: High risk areas[3]
(local clusters of TBE notifications over the last 10 years, as per March 2022)

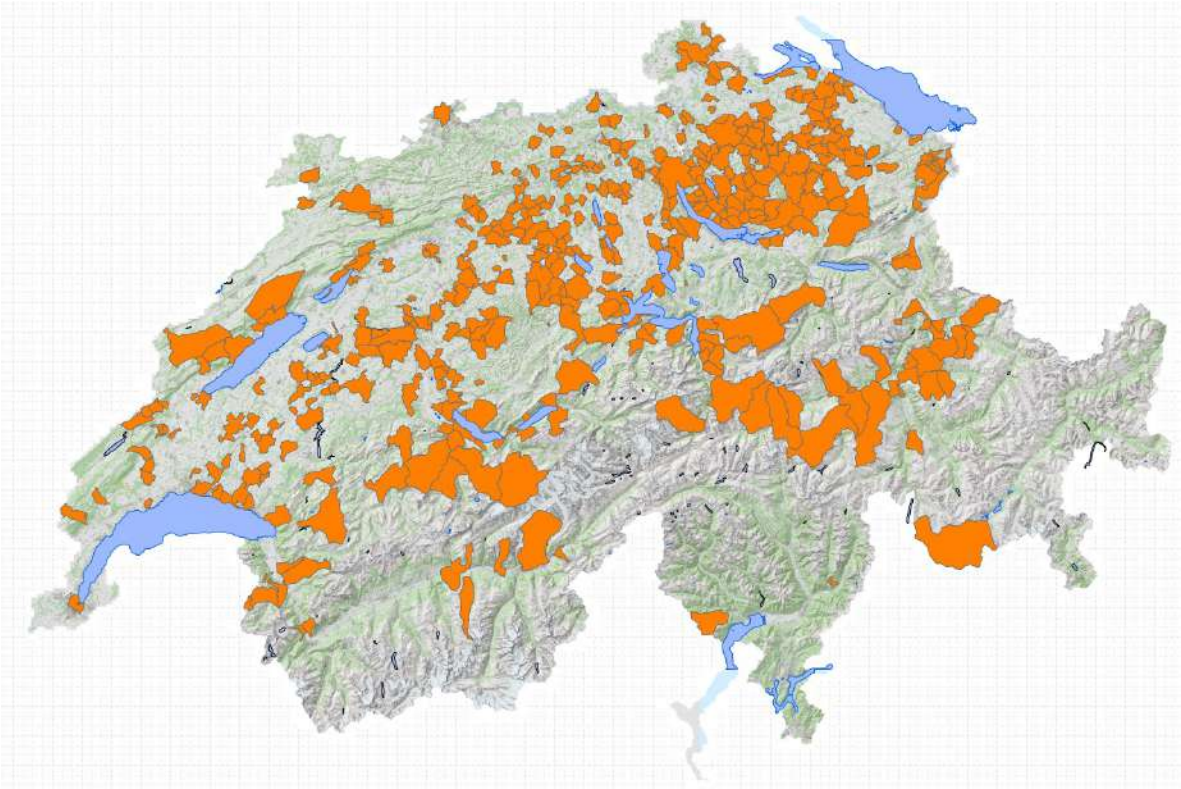


Figure 3b: Defined risk areas in Switzerland,[3] where vaccination was recommended for exposed people until end of 2018.

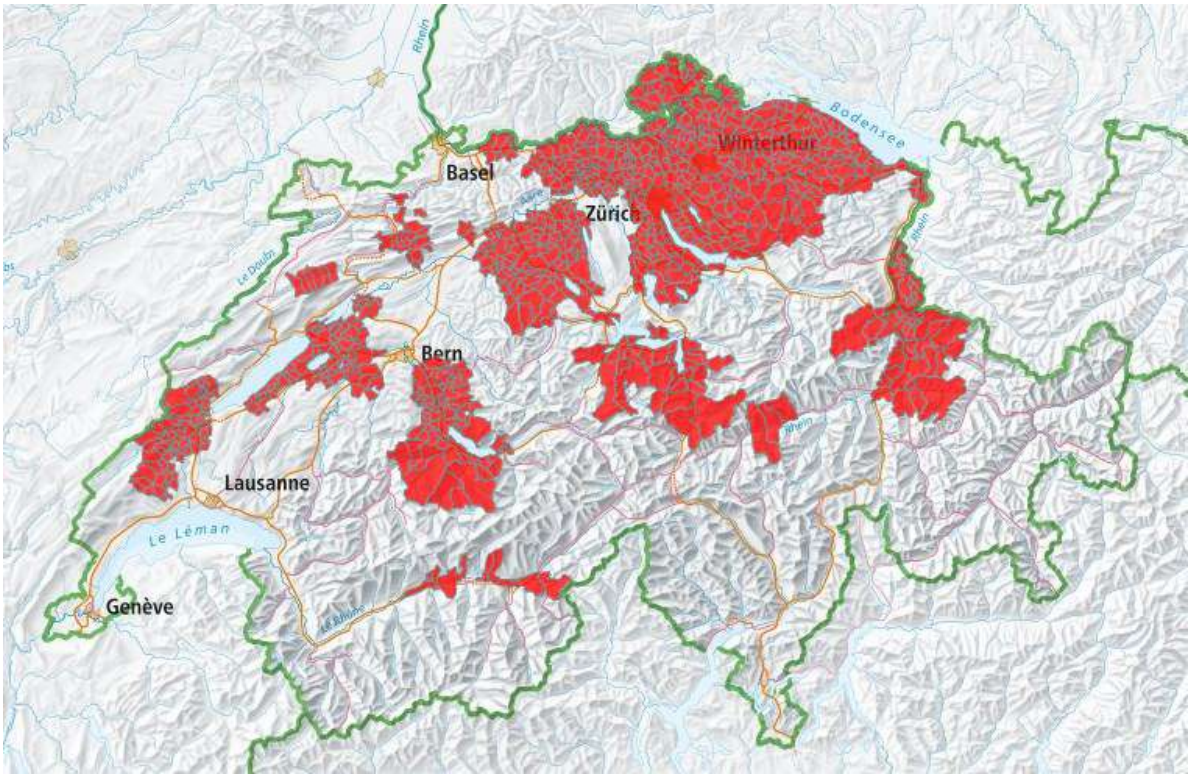


Figure 3c: Extended risk areas with recommended TBE vaccination for all exposed individuals (residents and visitors) as per March 2022[2]

Latest update: https://map.geo.admin.ch/index.html?lang=en&topic=ech&bgLayer=voidLayer&layers=ch.swisstopo.swisstlm3d-karte-farbe,ch.bag.zecken-fsme-faelle,ch.bag.zecken-fsme-impfung,ch.bafu.vec25-seen&layers_opacity=1,0.75,0.75,1&E=2614954.88&N=1168709.15&zoom=1&catalogNodes=457,532,687,1743,720,727,653,614,458

Acknowledgments

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Updates for 2022 cases provided by Dr Kyra Zens, University of Zurich, Institute for Experimental Immunology, Switzerland.

Contact: daniel@desgrandchamps.ch

Citation:

Desgrandchamps D, Posfay-Barbe MK. TBE in Switzerland and Liechtenstein. Chapter 12b. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 6th ed. Singapore: Global Health Press; 2023. doi:10.33442/26613980_12b33-6

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Appendix

Source data: Figure 1[2,4]

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	391	4.45

Source data: Figure 2[4]

Age group (years)	Number of cases	Incidence/10^5
0	3	0.25
1–4	47	0.96
5–9	136	2.28
10–14	102	1.73
15–19	119	1.95
20–24	86	1.26
25–29	136	1.74
30–34	177	2.09
35–39	226	2.68
40–44	247	2.90
45–49	256	2.88
50–54	314	3.45
55–59	285	3.38
60–64	268	3.77
65–69	267	4.39
70–74	225	4.19
75–79	156	3.60
80–84	73	2.38
85–89	25	1.31
90–94	2	0.21
95+	2	0.83
Unknown	1	N/A

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10. FOPH. Recommendations for vaccination against TBE [Empfehlung zur Impfung gegen Zeckenenzephalitis]. *Bulletin de l'OFSP*. 2006;13:225-31.

11. FOPH. https://www.bag.admin.ch/bag/de/home/gesund-leben/gesundheitsfoerderung-und-praevention/impfungen-prophylaxe/informationen-fachleute-gesundheitspersonal/durchimpfung.html

TBE in Tunisia

Elyes Zhioua

E-CDC risk status: imperiled country (data as of end 2022)

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 12b. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 6th ed. Singapore: Global Health Press; 2023. doi:10.33442/26613980_12b21-6

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Chapter 12b

TBE in Ukraine

Igor Nebogatkin, Olga Onishchuk, Oleksandr Hnatiuk,
Wilhelm Erber and Tamara Vuković-Janković

E-CDC risk status: endemic (no new data available as of May 2023)

History and current situation

The available data indicate that infection with tick-borne encephalitis virus (TBEV) is the most common arbovirus infection in Ukraine. Natural TBE foci are mainly located in the Polissya territories (Volyn, Rivne, Zhytomyr, Kyiv, and the Chernigiv region), as well as the Pre-Carpathian and Trans–Carpathian regions; a highly intensive distribution of TBEV was also observed in the entire mountain forest zone of Crimea and in Volinskij.

During 2003–2010, 223 cases of TBEV-seropositive patients from 14 areas of Ukraine were diagnosed. As diagnostic systems are not affordable for most medical institutions, these reported figures might grossly underestimate the true extent of the disease in Ukraine.[1]

TBE in the Ukraine has been studied since 1955, i.e., for 65 years. Official data are shown in Table 1, broken down into 5 periods ($p<0.051$).

For the years between 2004 and 2020 the following official analysis is available:

The annual numbers of occurrences of the disease are shown in Fig. 1. However, 10 cases were imported from Russia, Belarus, the Czech Rep. and elsewhere. Cases were noted between the beginning of May and October with a peak incidence in July–August, 1 case was determined as late as December (Fig. 2). Most of the patients were infected when visiting the forest for different purposes: picking berries and mushrooms, haymaking, harvesting firewood, grazing pets, and recreation. There was also 1 laboratory infection.

The age distribution is presented in Fig. 3, the average age is 37.59±1.88. Gender analysis showed that women (50.59%) and men (49.41%) were infected in approximately equal proportions 1:1. 93.18% of these cases were not vaccinated, 6.82% of cases[6] were vaccinated.

People were infected in 10 regions and 19 districts of Ukraine during the analyzed period. Majority of the inflicted people lived in the Volyn region and Crimea. 57 local TBE cases were detected in Volyn region from 2004 to 2020 according to official statistics, which amounted to 67.86% of all cases in Ukraine. Natural foci as of 01.01.2020 are shown in Figure 4.

However unofficial reports indicate about 50 cases annually.[2]

Quite recently 8 TBEV isolates were identified from ticks among 6 study sites in the southern Ukraine. This study confirmed that the TBEV-EU (European subtype) is present in the southern region of Ukraine, which overlaps with the TBEV-FE (Far Eastern subtype) and TBEV-Sib (Siberian subtype), showing the heterogeneity of TBEV circulating in Ukraine.[3] Sites where the TBE virus has been identified are shown in Figure 5.

A study exploring the potential relationship between the ecosystems, vectors, and the presence of tick-borne infections in the Western Ukraine identified TBEV by PCR in 6.3% and 14.5% of *Ixodes ricinus* and *Dermatocentor reticulatus* as convectors.[7]

Table 1

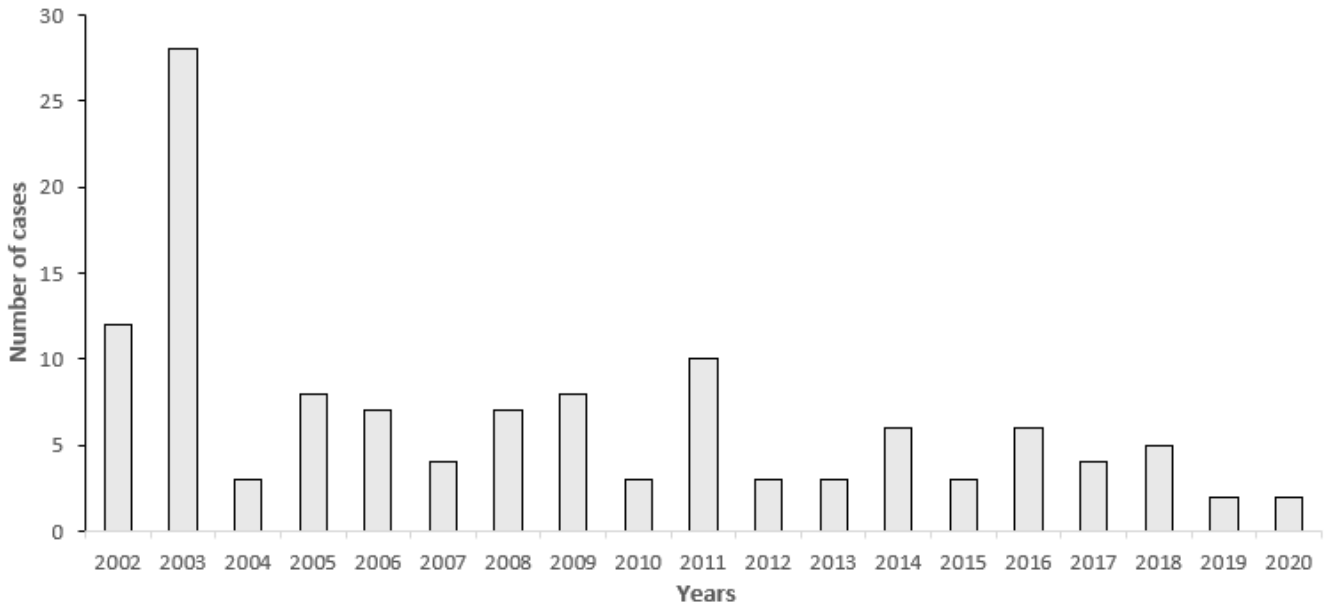
Years	Mean annual TBE cases	Number of years
2004–2019	5.19±0.58	16
1995–2003	33.89±5.24	9
1974–1994	7.43±1.88	21
1966–1973	0	8
1955–1965	7.43±1.88	11
all	9.72±1.68	65

Overview of TBE in Ukraine

Table 2: Virus, vector, transmission of TBE in Ukraine	
Viral subtypes, distribution	Heterogeneity of circulating TBEV: European (TBEV-EU), Far Eastern (TBEV-FE), and Siberian (TBEV-Sib) subtypes.[3]
Reservoir animals	Information not available
Infected tick species (%)	The main vector is *Ixodes ricinus*. *Dermacentor reticulatus*, *D. marginatus*, and *Hyalomma marginatum* were also found to take part in the circulation of virus; infection rate ranges from 0.11% to 0.81%.[3,5]
Dairy product transmission	Data not available

Table 3: TBE reporting and vaccine prevention in Ukraine	
Mandatory TBE reporting	Public Health Center
Other TBE surveillance	Not applicable to Public Health Center
Special clinical features	Information not available
Available vaccines	FSME-Immun, FSME-Immun Junior, EnceVir, TBE vaccine Moscow[6]
Vaccination recommendations and reimbursement	Recommendation for high-risk population living in endemic areas
Vaccine uptake by age group/risk group/general population	Data not available
Name, address/website of TBE NRC	Public Health Center of Ministry of Health https://www.phc.org.ua/

Figure 1: Annual disease numbers for the years 2004–2020



Source Data: Appendix—Figure 1

Figure 2: The number of TBE cases 2004–2019 in Ukraine by month

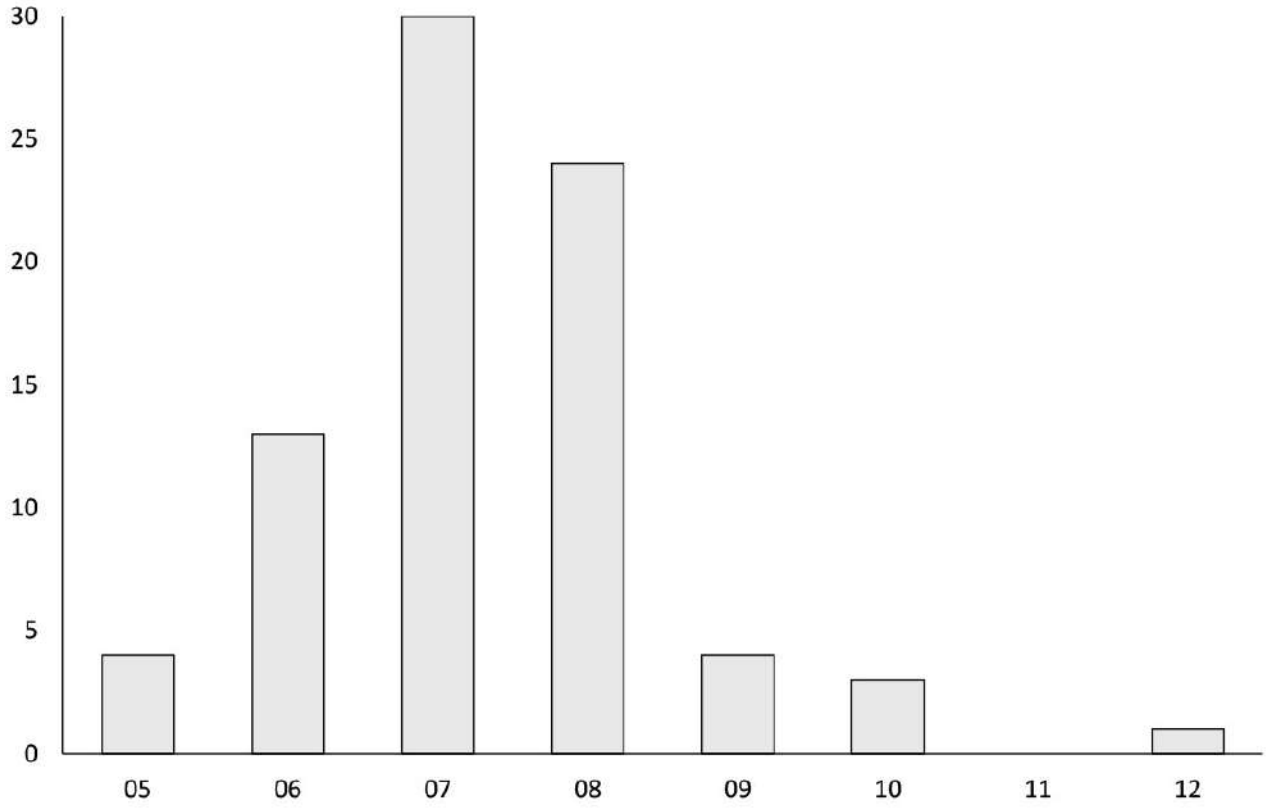


Figure 3: Distribution of TBE patients by age in Ukraine

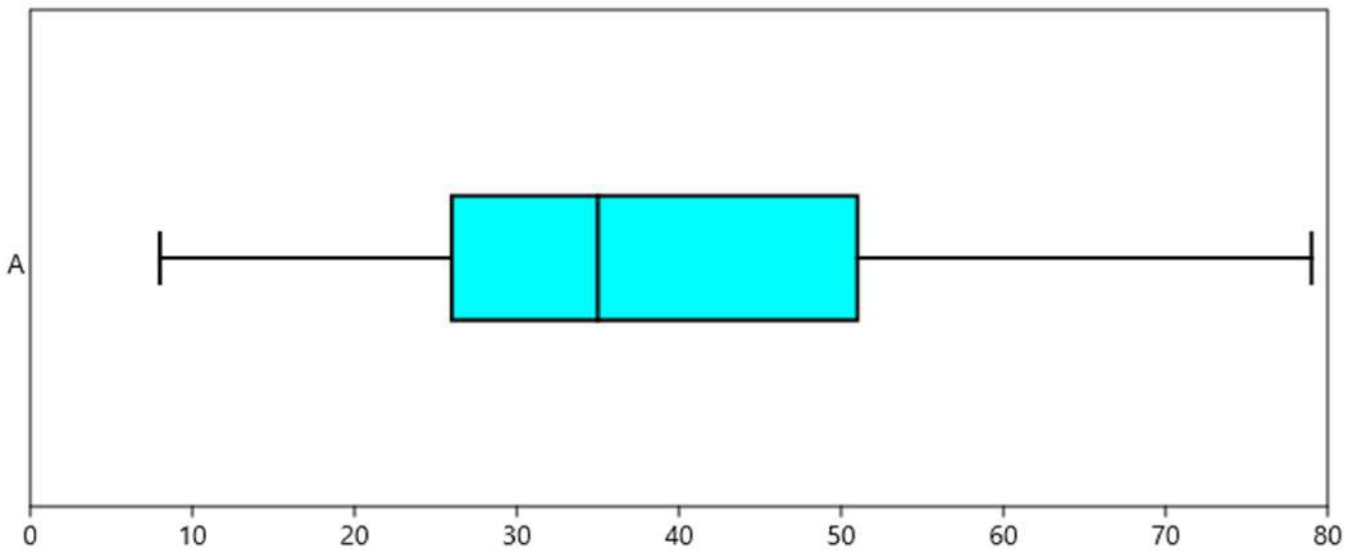


Figure 4: Natural TBE foci as of 01.01.2020

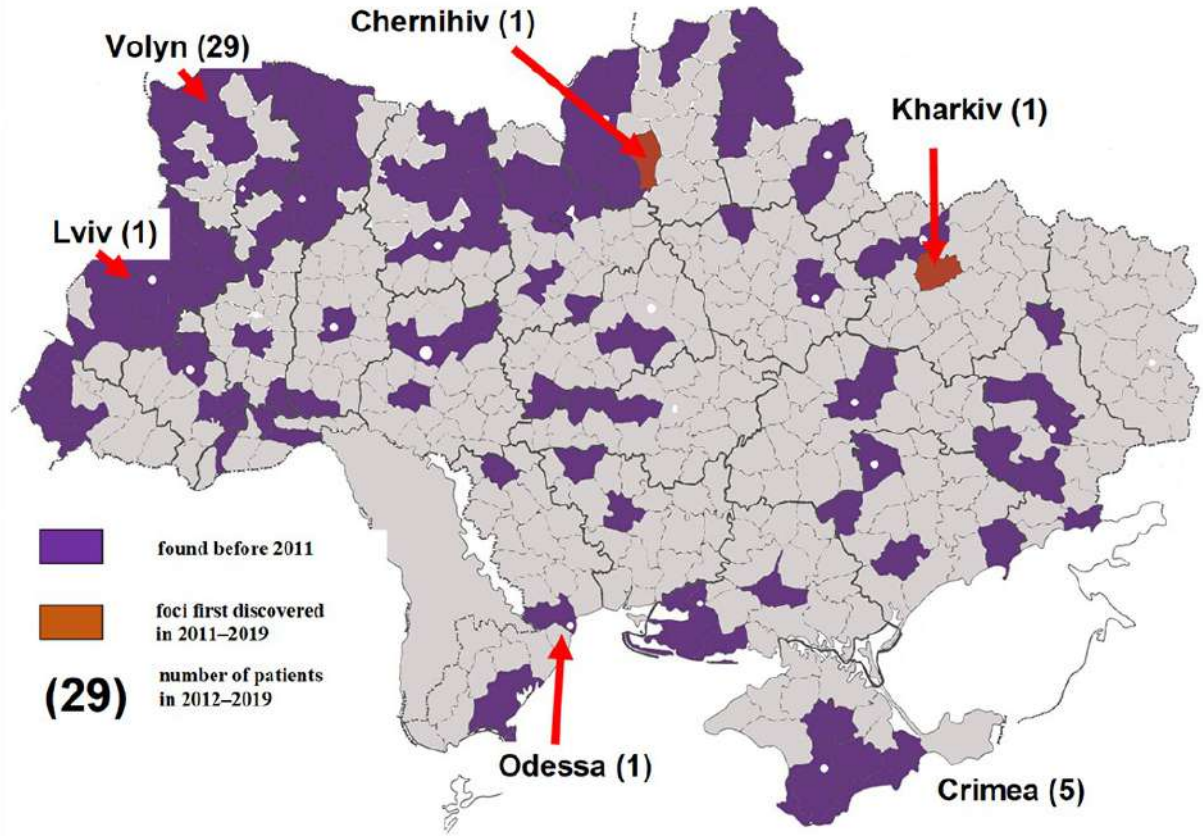


Figure 5: Identified sites where TBEV has been isolated in Ukraine[3]

Appendix

Source data: Figure 1

Year	Number of cases	Incidence / 10^5
2002	12	0.03
2003	28	0.07
2004	3	0.01
2005	8	0.02
2006	7	0.02
2007	4	0.01
2008	7	0.02
2009	8	0.02
2010	3	0.01
2011	10	0.02
2012	3	0.01
2013	3	0.01
2014	6	0.01
2015	3	0.01
2016	6	0.01
2017	4	0.01
2018	5	0.01
2019	2	0.0
2020	2	0.01
2021	No data	
2022	No data	

Age and gender distribution of TBE in Ukraine:
no available data

TBEV-isolation and TBE cases in Ukraine:
no available data

Contact: niv_zoo@ua.fm

Citation:
Nebogatkin I, Onishchuk O, Hnatiuk O, Erber W, Vuković-Janković T. TBE in Ukraine. Chapter 12b. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 6th ed. Singapore: Global Health Press;2023. doi:10.33442/26613980_12b34-6

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Chapter 12b

TBE in United Kingdom

Maya Holding, Heinz-J. Schmitt and Gillian Ellsbury

E-CDC risk status: affected, unknown if endemic (data as of end 2022)

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 caused by LIV in livestock are mainly from Scotland, parts of North and South West England and Wales.[4]

A large-scale surveillance project searching for the presence and prevalence of the TBEV and Louping Ill Virus (LIV) in deer as sentinel animals was established in the UK 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, an unanticipated 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 animals in ELISA-positive regions tested positive by LIV/TBE PCR5 and 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]

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 there 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 on July 15th.[3] An un-engorged tick had been removed from the child´s neck during a picnic in the New Forest National Park, south-west of London, on 6th July, 2019. Two days after arrival back home in Germany, the child developed fever and focal seizures and meningo-encephalitis was diagnosed in a pediatric hospital. Tests for various infectious diseases were all negative, whereas TBEV-IgG and IgM were positive. The mother had never received any TBE vaccination nor had she ever suffered from TBE. A confirmatory diagnosis was not possible as this was based on serology, therefore cross-reactivity with LIV could not be excluded.[3] Hospitalization lasted 15 days and the child was well at a follow-up visit 6 weeks later. Based on the timing of the events and incubation period, it is not possible that the child was infected in Germany. A second "probable TBE case" was diagnosed in a patient from Hampshire in July 2020.[8]

To summarize, overall serological evidence supported by PCR detection and sequence analysis of TBEV-EU RNA indicates that the TBEV circulates within the Thetford Forest and the Hampshire/Dorset border areas. There has been two probable autochthonous TBE cases in one of these areas, although it is not known whether the TBEV-UK Hampshire strain was the cause of disease in this instance. Work is ongoing to understand the risk of TBEV to the UK human population.

In 2022, there were 2 possible cases, pending classification.

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

Table 2: TBE reporting and vaccine prevention in the United Kingdom	
Mandatory TBE 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
Available vaccines	TicoVac® and TicoVac Junior®[11]
Vaccination recommendtions and reimbursement	The UK Joint Committee on Vaccination and Immunisation agreed that this should be further reviewed, once more data were available, especially around whether certain occupational groups were at increased risk.[12]
Vaccine uptake by age group/ risk group/general population	Uptake of vaccine not known
Name, address/website of TBE NRC	Rare and Imported Pathogens Laboratory (RIPL) Public Health England 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]

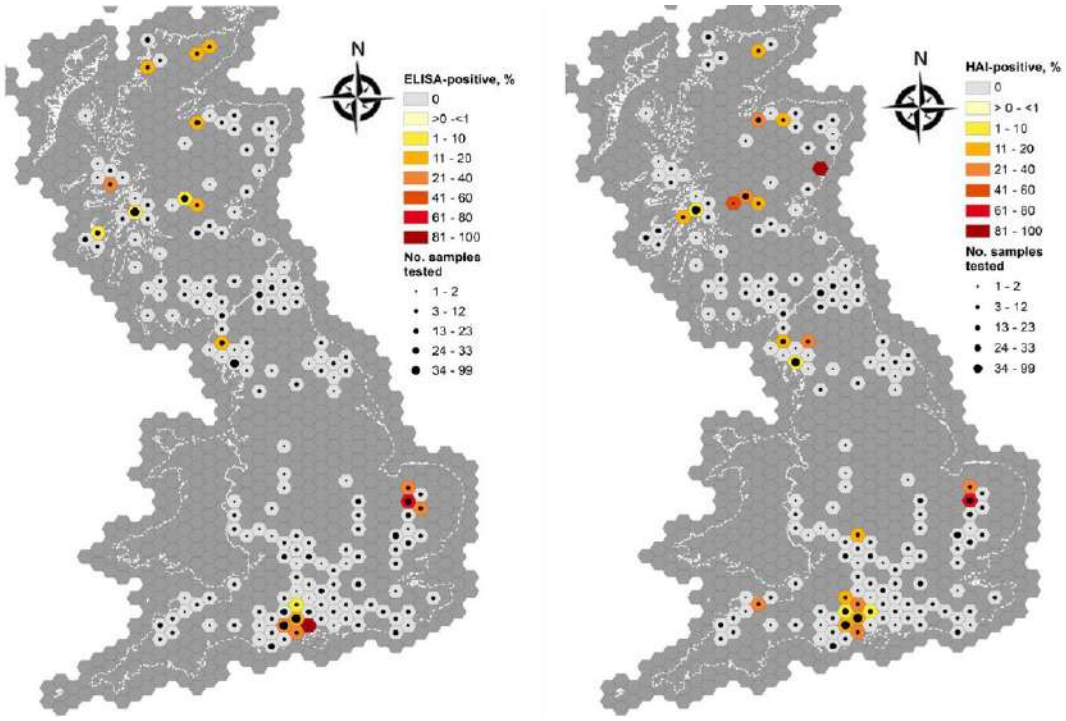
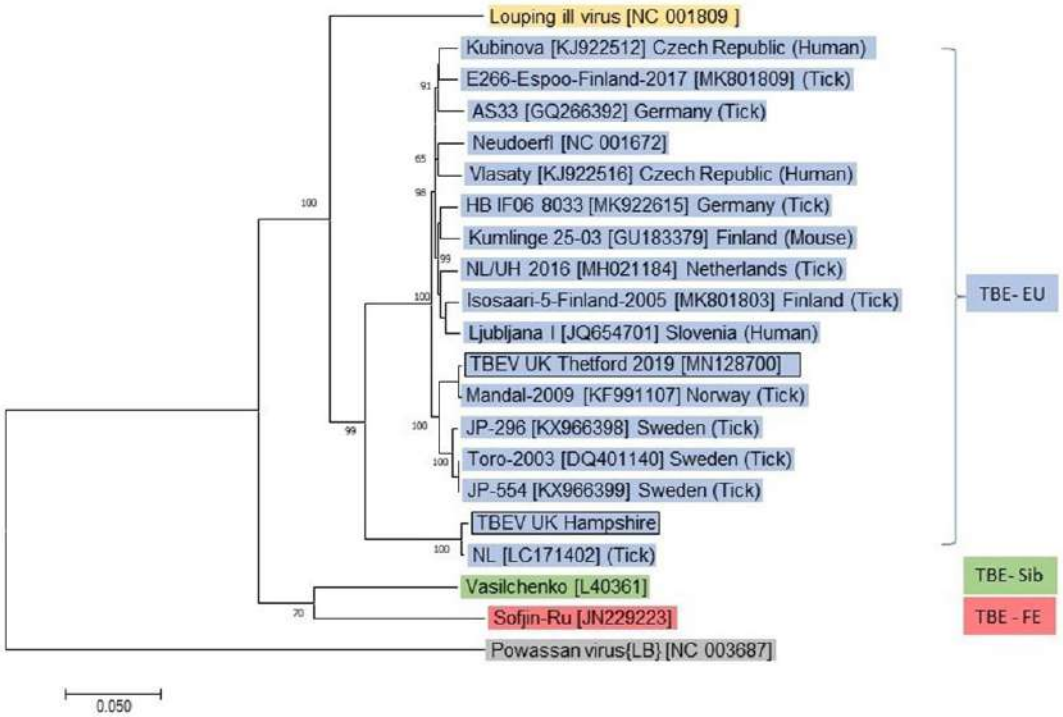
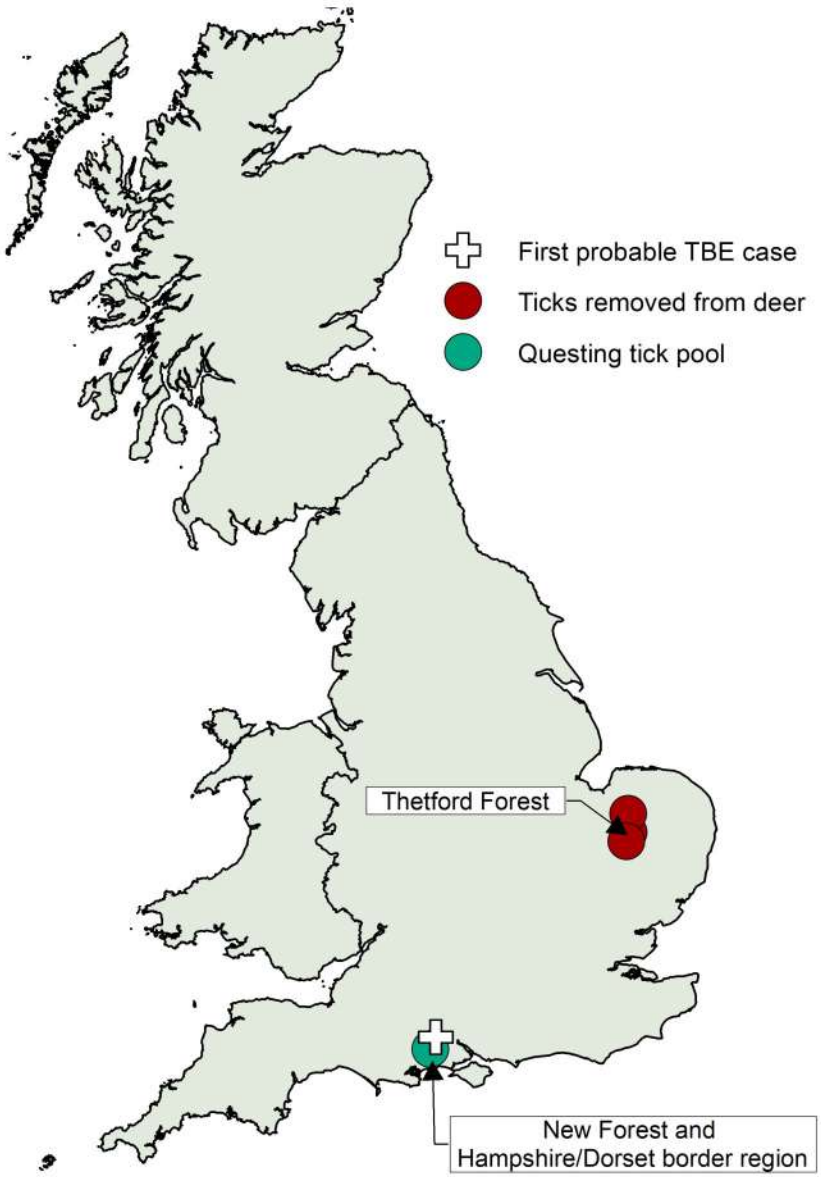


Figure 2: Phylogenetic tree highlighting the TBEV UK-Thetford and TBEV-UK Hampshire strains (figure and accompanying legend are adapted and reprinted from reference)[2]



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: Geographic locations of areas in which TBEV was detected in ticks and the first probable autochthonous TBE case



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Chapter 12c

Global distribution of the TBEV

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Lidia Chitimia-Dobler and Heinz-Josef Schmitt

In the map below, the areas highlighted in orange indicate TBEV endemic regions as documented either by 1) TBEV detection in ticks or other animals; or 2) detection of specific TBEV antibodies in reservoir animals or human sera; or 3) microbiologically confirmed locally acquired TBE cases in humans who contracted the disease in the respective region. This map does not reflect the incidence of the disease or the prevalence of the virus in a given area.

See: https://tbenews.com/tbe/chapter-12c-tbe-risk-map/

**This map may be different from "official" TBE risk maps from local authorities. Differences between the map above and country maps are explained by the fact that Public Health officials try to quantify the risk for TBE and thus indicate only those areas as "TBE risk areas" where a certain incidence threshold of TBE case numbers or TBE incidence is reached. This approach, however, does neither take into account the (in-) completeness of testing of all encephalitis cases for TBE as well as vaccine uptake nor the existence of TBEV in regions with low risk of exposition to humans (i.e. high TBE risk areas, however only rarely visited by humans). The goal of the map is to give a more real impression of the distribution of the TBEV, according to available virological/ serological data – since all other data are biased by incomplete surveillance.*

Moreover, the map presented here may not entirely be complete, and very likely TBEV infections, and thus TBE may occur in additional ("new") areas.

Chapter 13

TBE as a matter of public health

Michael Kunze , Wilhelm Erber and Martin Haditsch

Key Points

- The incidence of TBE ranges from 'only single sporadic cases' to >50/10^5 per year depending on the region and on the year of analysis; it is usually 1-10/10^5 in endemic regions in central Europe.
- his number may be considered as 'low' – not only as an individual risk but also from a public health perspective.
- If an individual does contract TBE, however, the disease may deeply change her/his life due to the need for acute hospital care and due to potentially severe and long-term sequelae. In 1–2% (-20%) of cases, TBE may even result in death.
- No specific treatments exist for TBE. The severity of the disease and high frequency of long-term sequelae result in high public awareness and concerns about tick bites in endemic areas. Public health officials in TBE-endemic areas need to address these concerns; moreover, they need to address the concerns of travelers at risk.
- The principal public health measures aim at reducing TBE cases by reduction of exposure and preventive vaccination.
- Recommendation/reimbursement of TBE vaccination still is under discussion from side of healthcare payer perspective as well as from the individuals perspective considering long term sequelae.

Introduction

Descriptive epidemiology is the cornerstone of information for public health considerations. In this regard, as outlined in various chapters of this book, tick-borne encephalitis (TBE) poses specific challenges:

1. TBE presents as a non-specific CNS disease to family physicians, general practitioners, pediatricians, internal medicine specialists, neurologists, and other medical specialists. Especially outside of endemic areas, TBE is often not diagnosed because physicians are not aware of the differential diagnosis, and they do not order the appropriate test to confirm TBE infection. This phenomenon is particularly important in countries or regions where the burden of disease of TBE and perhaps even the presence of the TBE virus (TBEV) have not been fully studied. In some countries, even the costs and limited availability of serological testing for TBE serve as barriers to reaching a correct diagnosis.

2. In many countries, incomplete reporting of TBE is likely. This fact starts a vicious cycle in which low TBE incidences or even periodic lack of human TBE cases result in low awareness and further underdiagnosis of the disease. As long as the risk is low (by regional / national definition) some 'official maps' published by governmental bodies do not indicate this risk for TBE in a specific region because a special 'incidence threshold' is used as a condition before TBE risk is communicated. Thus, lack of case finding and case reporting results in missed opportunities for prevention.

3. With vaccine uptake being unknown in many instances, reporting case incidences results in artificially low numbers and thus an underestimation of true TBE risk.

4. The risk of TBEV infection is influenced by seasonal patterns of tick bites and transmission, the environment, personal behaviour, personal protection measures and, of course, (vaccination-induced or natural) immunity. The details of the interactions among all parameters (reservoir animals, tick activity, migrating birds, climate, the environment/landscape and its changes over time, human behaviour, etc.) and the resulting risk for TBE is largely unknown to date and – due to the complexity – difficult to assess.

5. The risk of TBE disease in general and the severity of specific symptoms depend on age, immunological status, underlying diseases, routine medications, TBEV viral load, and the specific infecting TBE strain. Not only has the epidemiology of TBE posed issues for public health officials, but it also appears fair to state that perhaps with the exception of Austria, TBE is 1) largely underreported and 2) mostly neglected by public health authorities. Several reasons may explain this phenomenon:

6. TBE vaccination results in protection of the individual only. There is no herd protection because the viral reservoir exists outside the human population and – with the exception of an extremely low risk of transmission via breast-feeding or blood transfusion – TBE is not transmitted between humans.

7. Classical TBE infection (i.e., infection involving the CNS) is relatively rare, so cost-benefit analyses are likely negative, particularly if long-term sequelae and social costs are not accounted for. The prerequisite for a vaccination program to be effective is a high vaccine uptake and this requires appropriate funding not only for the vaccine but also for its administration. The results then of such a program are 'no disease' – and absence of diseases is not a success story in the popular media or in elections unless rigorous (and again expensive) surveillance is undertaken to assess field effectiveness and document the success. Valid surveillance again needs appropriate funding – and so another vicious cycle emerges, where a perceived 'rare disease' is not considered to justify or even be eligible for public health expenditures.

8. Public health officials often become more active largely when there are common threats, and symptomatic TBE is relatively rare (see Chapter 11b). Moreover, TBE vaccination results in individual protection only and not in any herd protection .(As indicated above apart from breast-feeding and blood transfusions in very rare instances TBEV has not been proven to be transmitted between humans). So, while TBE vaccination is highly effective, it does not result in any impact on the population in general; thus, TBE vaccination is often not paid for by public health resources.

9. In some instances local governments prioritize tourism / travel over public health concerns and may be not in favour of indicating their region being classified as a TBE-risk-area

We strongly believe – against all these arguments and despite the perceived low incidence of TBE – that this disease deserves a high level of public health attention because it poses a risk to any human living in or traveling to or through TBE endemic areas and because the disease may frequently result in long-term disability and, in some cases, even death. Rightfully so, the public should be concerned and, if correctly informed, would certainly opt for an adequate public health response.

As a response to all these public health challenges, and to encourage the control of TBE in Eurasia, an international effort was launched in 1999 with the aim to investigate and alleviate this situation. International experts created a new body, the International Scientific Working Group on Tick-Borne Encephalitis (ISW-TBE; www.iswtbe.com).

This Working Group gathers data from internationally recognized scientific experts from TBEV endemic and non-endemic regions with extensive personal experience in the field and a high level of commitment to improving the knowledge of and response to TBE.[1]

Epidemiology of TBE from the public health perspective

As outlined in more detail in Chapters 3, 11, and 12 of this book, TBEV is mainly transmitted through tick bites. Food-borne infections through unpasteurized milk and milk products have no major impact in terms of epidemiology but are of increasing importance due to the growing popularity of more 'natural' (unprocessed and raw/ unprepared) foods. In contrast to the otherwise sporadic cases, food-borne TBE-infections occur as outbreaks with sometimes high numbers of cases (see chapter 11). Consequently, these types of TBE infection occur even in western European countries. This has become a major public health debate pitting 'healthy food' activists and enthusiasts against health officials with obligations to enforce food regulations. Thus, governments are challenged to find solutions.

Most natural TBE foci are well described, but new TBE affected areas have recently emerged (e.g., Japan, The Netherlands and UK in 2019, respectively; see Chapter 12b). Roughly 3,200-12,000 tick -borne encephalitis (TBE) cases are reported annually from countries where the disease is endemic[2,3], but this figure is believed to be a significant underestimation of the actual number. TBE has also become an international public health problem because of the increasing mobility of people traveling to risk areas. Today, the risk of infection is especially high for all people living in, going through (and having a stop-over) or visiting endemic areas who pursue leisure activities outdoors, and TBEV infections may even be acquired in city parks. In most regions, the main risk has shifted from an occupational to a leisure time health risk. As a result, over the last 30 years, a continuous increase in TBE morbidity has been observed in Europe,[4] and both the importance and awareness of TBE have increased in endemic areas and in the recent past in travelers, too.

Circulation of TBEV also depends on the population density of ticks and their hosts (see Chapter 3). Virus prevalence in the tick population within TBEV foci is determined by the duration of viremia in hosts because the virus is mostly ingested by ticks while engorging on a viremic host. Virus circulation in nature is also influenced by the percentage of immune hosts in a particular region.

Climate is another determinant of tick-borne disease dynamics. Even if major discrepancies in annual TBE incidences cannot be explained by recorded temperature increases alone, the seasonal shifts in reported cases of TBE in central and northeastern Europe suggest that TBEV transmission dynamics have changed – perhaps as a result of warmer temperatures and changes in humidity.[5] In addition the density of rodents (esp. those feeding on beech nuts which again is related to climate [change]) seems to be positively correlated which TBE case counts. Of note, a much higher percentage of TBE-positive individuals (whether locals or travelers) has been observed among risk groups[6] such as:

- individuals working in agriculture and forestry
- hikers, ramblers, joggers, and other people engaged in outdoor sports
- foragers of mushrooms and berries
- anyone who spends time outdoors (e.g. having a picnic, walking, gardening, dog-walking, or sunbathing on the grass).

Today, most people (90%) in Europe who will ultimately develop TBE visit endemic areas in pursuit of recreational activities. In central Europe and the Baltic states, recent increases in TBE may have arisen largely from changes in human behaviour that have brought more people into contact with infected ticks[7] (e.g. mountain biking, playing golf or jogging instead of playing tennis). Infection with TBEV may also happen at home when infected ticks inadvertently are brought in with harvested items from the outdoors (e.g., wildflowers or Christmas trees) on clothing, or by domestic animals (e.g., dogs).[8] Moreover, TBEV infections are increasingly reported to occur in gardens – even in urban areas.

TBE affects all age groups. The severity of the disease increases with age. Older generations and retired people are more active today and especially at risk of acquiring TBE. This is especially true for elderly travelers (both domestic as well as from other regions) since Europe is generally considered a safe destination requiring no specific preparation, and that can meet the needs of elderly people or those with chronic or underlying illnesses – including those that depend on a “high-standard medical infra-structure".

In children, too, TBE can run a severe course and may lead to permanent sequelae (see chapter 6). Retrospective studies have shown TBE infection to occur in infants as young as 3 months.[9] A higher incidence of TBE has been reported in boys (boy: girl ratio 7:3), who more often show signs of focal encephalitis.[10]

General aspects of TBE prevention

No therapy, and specifically no antiviral agent, is available against TBEV. Control of reservoir animals and of ticks is not feasible and/or has limited to no impact on TBE incidence. Prevention thus relies on 1) avoidance of exposure and 2) vaccination. Success of vaccination is based on TBE awareness among those at risk and – perhaps more importantly – those counseling them. A key challenge for public health authorities is to encourage precaution without causing alarm.[11]

Primary prophylaxis

Behavior

Since ticks may transmit diseases other than TBE (borreliosis being most common in TBE endemic regions), the avoidance of exposure to ticks is crucial. Not entering TBEV-endemic areas would be the safest way to avoid any risk of TBE infection. This may be an option for travelers, but it does not solve the problem for the population living in TBEV- endemic areas. For anyone entering endemic areas, the TBE risk can be reduced by personal behaviour like not running or walking through high grasses or on narrow paths that present repeated and unavoidable contact with bushes during seasons and in areas with tick activity. Persons at risk should be aware of the fact that ticks transmitting TBE often are so-called “questing ticks” (in contrast to some tropical species which are hunting ticks) and that a contact time of 0.1 second is sufficient for the attachment of ticks to the skin.

Additional recommendations (below) also may reduce the risk for TBE.

Protective clothes and repellents

1. As ticks attach to any spot on the host and from there try to reach an uncovered part of the skin, adequate clothing may help to make access to the skin more difficult for ticks. Protective clothes must be completely closed to be really effective, but this may not be accepted by people spending their leisure time or holidays in endemic areas during the warm season.

2. If we apply terminology strictly, then discriminating between types of repellents is important. In the narrow sense (s.s.) repellents include formulations that repel (keep off arthropods like ticks), while insecticides act as neurotoxic agents that paralyze or even kill arthropods after contact. The expression ‘repellent’ in the broad sense (s.l.) combines both means of action and will be used henceforth for simplicity.

For the impregnation of clothes, permethrin or other pyrethroids are recommended. The impregnation of clothes usually provides long-lasting protection (weeks to months), even though the solutions typically used for soaking clothes are water-based. For skin impregnation, products with proven efficacy like N-diethyl-3-methylbenzamide (formerly N, N-diethyl-m-toluamide / DEET; in higher concentrations, i.e., preferably >20%), (p)icaridin or p-menthane-3, 8-diol (PMD) are recommended. The efficacy of cutaneous repellents decreases in a comparably short time (a few hours at maximum), which in addition to chemical characteristics depends on factors such as the concentration of the chemical compound, the user's degree of sweating, and environmental moisture. Whereas the water solubility of these products primarily might be judged as a disadvantage, this quality allows quick removal from skin or mucous membranes should they become contaminated unintentionally.

Vector control

As with other vector-borne diseases, strategies to reduce vector density have been implemented in the past. From the beginning of the 1950s to the end of the 1970s, this was the leading strategy of TBE prevention in Russia.[12] However, these large-scale control measures using tetrachlorvinphos, DDT, or Hexachlor did not produce the desired effect: no significant impact was observed on human infections.

Since the virus persists not only in ticks, but also in a large number of wild animals, particularly small mammals, such measures are unlikely to eradicate or even control the disease.

Secondary prophylaxis

(Early) tick detection and removal

Ticks do not immediately penetrate the skin of the host. Some time is always required until the tick finds the most appropriate location for its bite. After the tick bite, TBEV is immediately transmitted to the host by means of the tick's saliva. Even if the tick is already firmly attached to the skin, early removal is still advised to help to avoid other potential infections like those with Borrelia spp., where transmission of bacteria takes place between 1 and 3 days after the tick has attached itself to a human host. Thus, if a tick is detected and immediately removed after attachment, the risk of certain infections in humans is reduced substantially.[13]

Tick removal should follow a number of rules: screening the body after outdoor activities is always an important first measure. Adherent ticks should be removed as atraumatically as possible (https://amp.usatoday.com/amp/2189393002).

Table 1: General primary and secondary preventive measures

	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

This can be done using a fine-tipped tweezer, long fingernails, or especially notched cards. The key is to pick the tick at the part closest to the skin and to tear it off without rotation and without squeezing the body, which could result in an increased influx of pathogens. Not recommended are any attempts to drown a tick by bathing or using 'home remedies' like suffocating a tick with a drop of glue, nail polish, or oil, or burning it with a match or lit cigarette. According to most authors, any advantage offered by the seemingly easier removal of the tick is by far outweighed by the disadvantage of an increased burden of infectious particles being released while the tick is struggling to death.

To overcome another misconception: if a black dot should happen to remain in the wound after tick removal, this is not the head of the tick but some part of the biting apparatus only. Taking into account the anatomy of the tick as an arachnid, the head and (in the case of TBEV), the salivary glands are sources of infection Once these are safely removed by the recommended actions and even if these resulted in incomplete removal, the window of TBEV transmission certainly would be closed.

In summary, all preventive measures described above and directed against ticks offer limited protection, only. This reinforces the need for immunological, i.e. vaccine-induced protection. A summary of prevention recommendations is provided in Table 1.

Cost-benefit analysis of TBE vaccination

Economic evaluation of TBE vaccination has become an increasingly important step in the process of including TBE vaccination in the immunization programs and/or making recommendations. However, there are only a few cost-effectiveness evaluations of the TBE vaccine.

In 1981, an overall TBE vaccination campaign was introduced in Austria[14] which ultimately resulted in a substantial reduction of TBE cases.[15] The economic benefit (reducing costs for inpatients care, loss of productivity and premature retirement) of that campaign was evaluated to be EUR 24 million for the years 1981 to 1990 and EUR 60 million between 1991 and 2000.[16, 17]

A Slovenian study showed cost-effectiveness of TBE-vaccination from a healthcare payers perspective, when starting vaccination at the age of 18 years and continuing up to 80 years of age.[16]

In Estonia vaccination of persons ≥50 years of age is calculated to be cost-effective from the health care payer's perspective. However, the authors stated that vaccination of the older population only has a limited impact on incidence reduction in the total population.

In 1996, a crude estimation of cost effectiveness of TBE-vaccination in the Stockholm area was done, and it was calculated that based upon the TBE-incidence at that time as well as on the costs of vaccination, mass vaccination would be an unrealistic alternative[18]. However more than 20 years later much higher incidences in the unvaccinated population are reported. A health economic analysis in Sörmland County, a highly TBE-endemic area adjacent to Stockholm County, 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 therefore concluded that introducing a structured vaccination program will be cost effective at all ages, but it would be specifically more cost effective if it started in childhood.[19]

Such analyses are mainly based on a health care perspective, and the program would compete with other resources in the health-care sector. Therefore it is important to establish the long-term costs and health outcomes of a local TBE vaccination strategy in order to understand if funding of a TBE vaccination program yield better health outcomes at a reasonable cost.[20] Differences in the underlying assumptions and disease modelling approaches as well heavily influence the outcomes of such analyses too as shown for TBE vaccination (see Fafangel 2016[21] - versus Smit 2015[22]). Moreover, TBE can be associated with a high productivity loss beyond the health care sector. Increasing vaccination and age groups can be the most effective and efficient strategy to reduce the burden of TBE and protect the whole population health.[10] Considering those consequences one may thus be in favour of a vaccination program or at least a vaccination recommendation. Out-of-pocket costs may have a positive impact on individual´s private consumption that is not included in the analysis from a health care perspective.

Although cost-benefit analyses are often closely linked to official recommendations for vaccination,[20] this aspect is of limited value when it comes to a disease that often leads to chronic sequelae and even death but on the other hand can be easily prevented. Here, ethical considerations are the main issue. This is especially the case in affluent societies where economic resources and systems for prevention are readily available.

Recommendations for TBE vaccination

Recommendations for TBE vaccination vary considerably across the countries in which TBEV foci are found (see also Chapter 12a). In areas where TBEV is highly endemic and where the average pre-vaccination incidence of clinical disease is ≥5 cases/100,000 persons per year, the World Health Organization (WHO) and the European Centre for

Disease Prevention and Control (ECDC) both recommend that vaccination be offered to all age groups, including children.[24] However, this is always dependent on the evidence known so far, on the quality of the surveillance system, and does not necessarily reflect real changes in risk areas which have occurred in the past few decades. The changing epidemiology of TBE includes increasing TBE infection rates outside known endemic areas mostly north and south; case-based discoveries of new TBE foci (e.g. The Netherlands and the UK) and new areas with TBEV identification in ticks; TBEV transmissions in higher altitudes; and changed nutrition behavior resulting in an increase of risky eating habits (such as the consumption of raw milk and other raw dairy products). Furthermore, experience in several countries has shown that the recommendation to vaccinate risk groups only has no substantial impact on the annual TBE incidence. This is exemplified by the Austrian experience, where an Austria-wide vaccination campaign was started in 1981 targeting the general population in contrast to vaccinating so-called at-risk persons before, only. Subsequently the vaccination coverage of the Austrian population increased and the TBE disease numbers were drastically reduced. (see Fig. 1)

The documented increase in non-vaccinated persons may be due to an increase in outdoor leisure activities as well as the fact that an increasing proportion of the population is more mobile and therefore moves from non-risky to risky areas on a frequent basis.

TBE awareness and vaccination rates

Awareness promotion is the key element in TBE control, in combination with vaccination of the general public, starting with specific risk groups, e.g., forestry workers, hunters, and military personal. The results from a cross-sectional study involving 11 European countries showed:[25]

- Overall awareness of TBE (83%) was lower than awareness of influenza (98%) or measles (92%). Of all respondents, 68% were aware of the TBE vaccine, and 25% had received >=1 vaccination(s) against TBE.
- Vaccination rates for TBE were lowest in Finland and Slovakia (up to 10%). Much higher vaccination rates were seen in Latvia and Estonia with 53% and 31%, respectively, and highest in Austria (85%). In German endemic areas, vaccination rates varied widely (20-80%) with highest rates in a few regions like the Odenwald, where vaccine uptake even approaches 100%.
- Compliance among respondents who received >=1 TBE vaccination(s) was 61%. First and second booster injections were received by 27% and 15% of respondents, respectively.
- Strongest motivators for vaccination were fear of TBE (38%) and residence/spending time in high-risk areas (31–35%). Main reasons for not receiving vaccinations were the belief that vaccination was unnecessary (33%) and that there was no risk of contracting TBE (23%).

One of the main aspects in issuing recommendations and creating awareness is the definition of a 'risk area.' The Robert Koch-Institute in Germany, for example, defines and recommends vaccination for a 'high-risk area' as follows: wherever the TBE incidence over a floating 5-year period is significantly higher than 1/100,000 population.[15] Austria, on the contrary, does not restrict vaccination recommendation to a numeric incidence. Any person living in or traveling to an endemic area is 'at risk' and should be vaccinated. For details on vaccination recommendations in European countries, see Chapter 12a.

The Austrian example: A success story

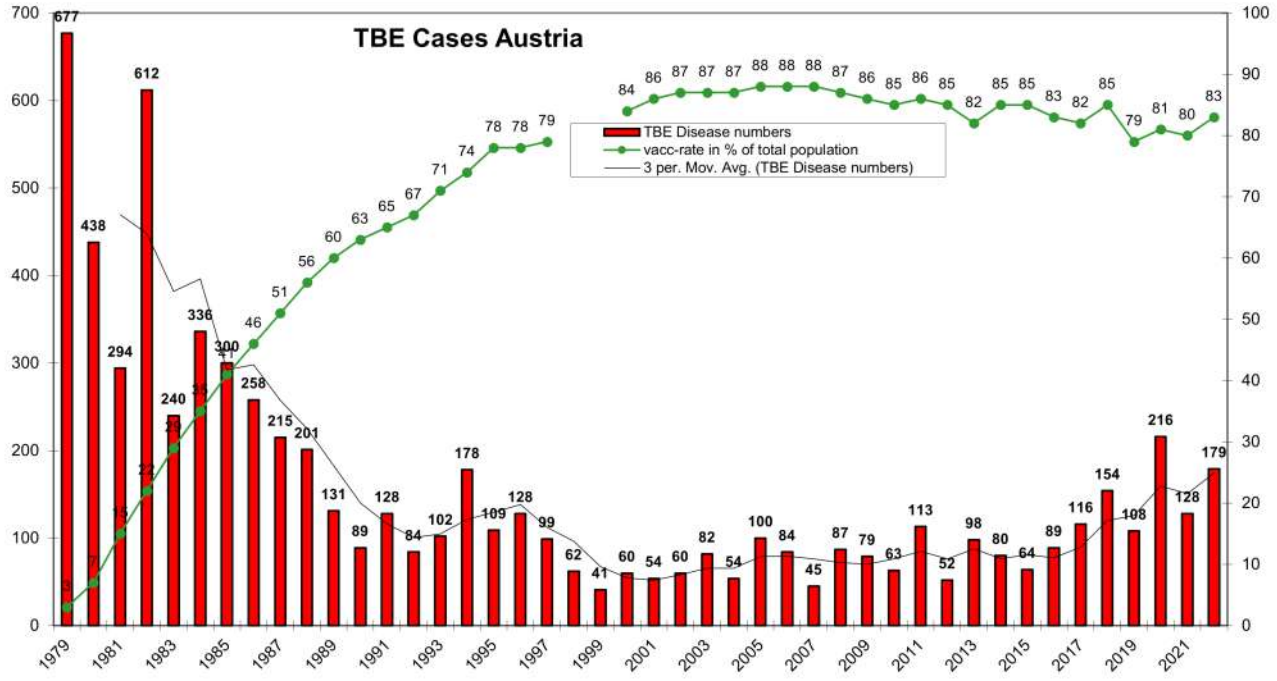
Austria is the only European country that implemented as early as 1981 an annual, more or less nationwide TBE awareness and vaccination campaign that targets the whole population; this has led to a substantial decline in the number of TBE cases in Austria. The Austrian example shows that containment of TBE is feasible by mass vaccination. In the pre-vaccination era, Austria had a very high recorded morbidity of TBE – probably the highest in Europe at the time, even despite some shortfalls in the notification system. In high-risk areas, the average annual incidence in the population exposed to ticks in their working environment was 0.9 per 1,000.[6]

The vaccination rate against TBE in the general population is 82%, which is the highest worldwide. A high awareness of TBE among the Austrian population was achieved through an annual social marketing program, and the widespread use of effective and well-tolerated vaccines has led to a successful containment of the disease. The vaccination coverage increased from 6% in 1980 to 82% in 2013 and exceeds 90% in some high-risk areas. This has led to a steady decline in the number of TBE cases from several hundred cases to roughly 50–100 cases per year (see Fig. 1).[24]

The risk of acquiring TBE in an endemic area like Austria equals 1:10,000, and this is comparable to the risk of acquiring typhoid fever for an unvaccinated tourist in a highly endemic area like India (1:3,000 to 1:25,000).[16] In fact, for an unvaccinated tourist staying in a highly endemic area in Austria for 4 weeks, the estimated risk of acquiring TBE is about 1 per 830 person-years of exposure. Based on the number of tourist overnight stays in Austria, this would equal 60 travel-associated TBE cases each summer.[17]

Residents of and travelers to TBE endemic areas who are at risk of tick bites are advised to receive TBE vaccination.[18,19]

Figure 1: TBE – annual disease numbers and vaccination rate in Austria



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

Summary and recommendations for public health

In summary and to adequately address public health issues related to TBE moving forward, the authors recommend the following:

1. Public health officials should make TBE a notifiable disease and establish appropriate tools for detection and reporting of human cases in their countries.
2. Maps indicating TBE risks should not solely be based on incidences, since these are biased due to under-diagnosis, temporal changes, reporting structures, vaccine uptake, and other factors. If incidence maps are used, maps with known areas of TBEV presence should also be published.
3. Travelers to TBE-endemic regions should be informed about TBE (even if no vaccine is available).
4. Measures on how to avoid tick exposure should be publicized.
5. In endemic areas, public health authorities need to effectively publish warnings that unpasteurized milk and dairy products may result in TBE infection. Laws for food safety must be implemented accordingly with respect to TBE risks.
6. In endemic countries awareness campaigns on TBE, as well as vaccination campaigns should be established.
7. ID specialists in non-endemic areas dealing with international travellers should update their knowledge (e.g. by reading the comprehensive chapter on TBE in Netter´s Infectious Diseases) and include TBE as a differential diagnosis whenever necessary.

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Chapter 14

Prevention:
vaccines and immunoglobulins

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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 doses) 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.
- 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.
- Recent clinical studies show long-term seropersistence of TBE antibodies after the first booster vaccination (dose 4) with the two European vaccines.
- An effectiveness of approximately 99% (years 2000–2006) and 98,7% (years 20002011) was calculated for regularly vaccinated persons in Austria, a country with established high vaccination uptake.
- Whereas in Western Europe post-exposure prophylaxis with immunoglobulins was discontinued in the late 1990s, in the highly endemic regions of Russia it continues to be common practice.
- Both - FSME-IMMUN and Encepur are well tolerated with a well-established safety profile. TBE-Moscow and EnceVir appear to be somewhat more reactogenic.

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 summarize 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.

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

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 Senzhang
Passages	PCEC	PCEC	PCEC	PCEC	PCEC	NK
Production	PCEC	PCEC	PCEC	PCEC	PCEC	PHKC
Amount of antigen	2.4 µg/1.2 µg children	1.5 µg/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	NK
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	NK
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	NK
Packaging	prefilled syringe	prefilled syringe	in ampoules	in ampoules	in ampoules	NK
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 (temperature NK)

***Abbreviations**: HSA: Human Serum Albumin; PCEC: Primary Chicken Embryonic Cells; PHKC: primary hamster kidney cells; $Al(OH)_3$: Aluminum hydroxide; NK: Not known*

From: Il'chenko, 2009[72]; Vorob'eva, 2007[3]; WHO SAGE background paper[41]

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 will be assumed immediately from 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 strain Senzhang. The vaccine is grown on primary hamster kidney cells, uses HSA as the stabilizer and aluminum hydroxide as adjuvant. This vaccine is approved for use in adults and children 8 years of age or older since 2004.[6]

Vaccination schedules

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 shot 4-12 weeks later, and a third shot is administered 5-12 months later. However, considerable differences still exist between vaccine brands. 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 recommended 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

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. Please note that „rapid schedules“ are not licensed for children.

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		2 weeks	None		1 year***

* *For regular schedules, 3rd dose immunologically appears to be a booster dose*
** *50 years (instead of 60 years) in Germany*
*** *annual dose before the start of the season*

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.

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 this 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.

Vaccine immunogenicity

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 strong immune responses 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]

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 ages 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 ages 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 ages 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

A recent randomized study compared the immunogenicity of TBE-Moscow, EnceVir, FSME-IMMUN, and Encepur Adults 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

The question of how to address prolonged intervals between the vaccinations of the primary series or between the boosters has long been debated. 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] 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]

Vaccine interchangeability

In general, it is preferred that the same vaccine brand is used for the complete primary immunization series. However, in order to not 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.

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 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 as well as 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.

Cross-protection

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 recently published systematic review[44] 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 leaving 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 statistical 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, age, and duration of immunity

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 recently published by Konior et al.[88] The study – a follow-up study of the one in 347 adults described above, 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 by a newly published study[98] 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.

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. whereby While the question if the permanent presence of protective levels of TBEV antibodies alone is responsible for the overall good effectiveness of TBE vaccines remains open, the rapid immunological memory response definitely contributes in this regard. In terms of comparability of study results, it should be mentioned that due to the different test systems used (different NT assays) the studies are not directly comparable.

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. The primary goal of this change was to increase the vaccine coverage, which was achieved only to a moderate extent in some Swiss cantons in the years thereafter.[89] Nevertheless, the increased vaccine coverage did not cause a reduction in the incidence of TBE in the country so far. Therefore, very recently, the whole territory of Switzerland except cantons of Geneva and Ticino – is now defined as a TBE risk area, which is hoped to further increase the vaccine coverage in the country.[97]

Based on the meanwhile available long-term seropersistence data after the first booster a prolongation of the booster intervals appears feasible, especially for the younger population. Primarily in countries with very low vaccination coverage this could have a positive effect. However, all data generated with respect to this issue have limitations, since the study participants were fully immunocompetent, and therefore do not entirely represent an unselected population. Moreover, it is questionable if countries with very well-established vaccination programs and high vaccination uptake would benefit from such an extension. Little clinical data exist on the seropersistence of TBE antibodies after the 3rd dose of the primary immunization. In one study investigating TBE seropositivity 2 and 3 years after the third vaccination[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 appropriateness of the existing manufacturer recommendation for the administration of the first booster dose 3 years after completion of the primary series. 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]

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] Unfortunately, few data exist on primary vaccination in individuals of more advanced age.

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 dependence of serologic results on the commercial ELISA test systems. Unfortunately, this study was not evaluated using NT. One study, which compared the primary immune response in older and younger subjects, showed that subjects 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]

A relatively recent study investigated the immune response to a conventional primary immunization schedule with FSME-IMMUN in previously unvaccinated subjects >70 years of age.[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 sufficiently increase the antibody levels, indicating an adequate immune memory response in the elderly population as well. Moreover, 1 study showed that the quality of antibodies as measured by antibody avidity were intact despite the lower antibody titers.[59] The findings described above underscore the importance of adhering to the recommended schedules, including the 3-year booster intervals in subjects age 60 years and older. Moreover, in the region of Stockholm an additional dose of the primary schedule is recommended for subjects older than 60 years of age.

Such an explicit recommendation however does not exist in other countries and existing epidemiological data do not support this recommendation.

Cellular 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.

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, 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.

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.

Vaccination 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

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

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 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, aA 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.

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]

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*

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, neither in humans nor 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 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).

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).

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 vaccination[33] 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.

Recently a cost/benefit analysis became available. In Sweden, where the area of Stockholm is highly endemic and 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 a gain 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]

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