There's no place like home: TBEV prefers its own tick population over ticks from a non-endemic area

Background

Despite the nationwide distribution of vector tick species as well as reservoir rodent hosts, risk areas for TBE are largely located in Southern Germany. Of the 1028 reported human cases in 2018 and 2019 (RKI 2020), 855 (83%) cases were attributed to the states of Bavaria and Baden-Wuerttemberg. This distribution, despite comprising similar habitats and vector/host communities in all parts of Germany, indicates that other factors apart from mere presence of vectors and host species are important for enzootic circulation of TBEV. Such factors could be environmental parameters but also intrinsic, i.e. genetic factors in tick or host populations. The current state of knowledge concerning the vector competence of *Ixodes ricinus* populations for the TBEV is incomplete due to lack of suitable infection models.

Results and discussion

To increase our understanding of TBEV-tick interactions, we established an experimental infection model using field collected ticks from different populations and low passage TBEV isolates from the endemic focus in Haselmühl (Bavaria). *Ixodes ricinus* nymphs were collected in the TBEV endemic focus Haselmühl and a non-endemic area (Hanover) and subjected to artificial blood meal using a adapted membrane feeding system (Figure 1 A) (Liebig et al. 2020). Over two seasons, the susceptibility to TBEV infection have been analyzed involving different factors as seasonality, natural co-infection, and the correlation of tick population with a respective TBEV strain.

We collected in total 2846 nymphs (Haselmühl n=1403 and Hanover n=1443) from April to October in 2018 and April to July in 2019. Feeding rates (number of engorged ticks divided by the total number of ticks tested) were calculated for every month in these two consecutive years. In Figure 1 B we present a combined dataset of 2018 and 2019 showing that despite considerable differences in feeding rates in both years (please refer to (Liebig et al. 2020)), we observe a common pattern. *Ixodes* nymphs from Haselmühl feed significantly better in early summer including April (46.7% Haselmühl versus 34.9% Hanover, p= 0.0022), May (Haselmühl 45.3% versus Hanover 10.1%, p <0, 0001) and June (Haselmühl 34.7% versus Hanover 26.0%, p= 0.0255). This observation is specifically interesting since these are the months when presumably most TBEV transmission events occur based on the TBEV case reports (ECDC 2020). The reason for this high activity of ticks from Haselmühl in April and May is not clear and warrants further investigation. One might speculate that climatic conditions in the Bavarian sampling spot Haselmühl diverge from the northern spot Hanover, resulting in earlier temperature rise accompanied higher tick activity. However, analysis of mean temperatures for the weather station Amberg (close to Haselmühl) and Hanover did not reveal specific differences. Precipitation might play a role, favoring the location with higher precipitation. However, due to lacking clear data on the influence of precipitation on tick feeding activity, this whole topic has to remain speculative. In addition, genetic differences, or differences in the ticks' microbiome, if present, could influence their feeding activity.

Next, we analyzed the engorged nymphs (n=693) for TBEV replication and found 38.38% (mean of both years and all months) of the tested ticks positive for TBEV RNA. The infection rates varied throughout the analysis, according to location,

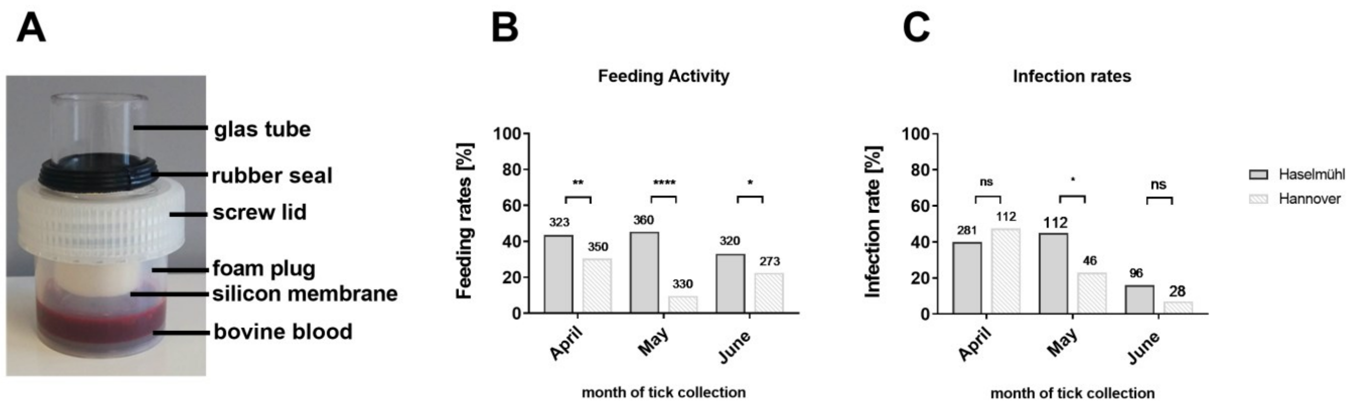

Figure 1: (A) Artificial membrane feeding system (B) Feeding rates combined 2018 and 2019 April to June. The feeding rates (number of engorged ticks divided by total number) were calculated per month. (C) The infection rates (number of positive ticks divided by the number of engorged ticks tested) after feeding of a blood meal containing 1 x 10^6 PFU of TBEV strain Haselmühl 303/16 was calculated from combined datasets for 2018 and 2019 over different months. Data were statistically compared using Chi-square test or Fisher's exact test using GraphPad Prism 8.3.1. Numbers of ticks tested are indicated above each bar plot. Significant differences are indicated by asterisks (ns; non-significant; *; p< 0.05; ** p< 0.01; *** p< 0.001).

months and years. Using different models (GLMs) with binomial error structure and logit-link function of logistic regression, we analyzed the influence of the predictor variables year, month, tick origin and co-infection (*Borrelia* spp., *Rickettsia* spp., *Anaplasma phagocytophilum*) on the likelihood of a TBEV infection. This analysis revealed that the tick origin (Haselmühl versus Hanover), year (2018 versus 2019) and *Borrelia* spp. co-infection had the strongest impact on TBEV infection in those ticks. The odds of being infected with TBEV were 2.3 higher (p= 0.0063) for nymphs from Haselmühl as compared to ticks from Hanover (Liebig et al. 2020). Again, a specific favoring of the TBEV focus Haselmühl could be observed in early summer with significant higher infection rates in May (Figure 1 C data combined from 2018 and 2019; Haselmühl 41.0% versus 27.9% Hanover, p= 0.0462). This favoring of the ticks from Haselmühl is significant, even when considering potential misidentification of TBEV infection based on natural infection of ticks derived from this endemic spot (minimum infection rate Haselmühl 2009-2019 0.16-0.45, Gerhard Dobler personal communication). Our results support the assumption that the uneven TBEV spread in Germany might be traced back to, yet unknown, variances in tick susceptibility for TBEV among tick populations.

The analysis of the annual variation of infection rates as a proxy for the influence of climatic influence on TBEV infection showed a significant impact of this predictor variable. The infection rates in the record year 2018 (warmest year since 1881) were higher compared to 2019 (32.33% Haselmühl 2019 versus 52.31% Haselmühl 2018) with 3.3-fold higher odds for TBEV infection in 2018 compared to 2019 (p< 0.0001). This influence of climatic conditions on TBEV infection rates is still not understood. Again, tick's physiology might play a role as already speculated for feeding rates. Furthermore, higher ambient temperatures could trigger higher TBEV RNA replication as has been already demonstrated for many mosquito-borne viruses including West Nile Virus (Leggewie et al. 2016; Holicki et al. 2020). Indeed, warmer weather conditions have been identified by several studies as important factor for increased TBE case numbers. This might be due to higher viral replication, higher feeding activity of ticks but also increased risk of human exposure by enhanced outdoor activities (Jaenson et al. 2012; Nah et al. 2020; Daniel et al. 2018).

Other than the tick origin and year, co-infection with *Borrelia* spp. had a significant impact on

TBEV Haselmühl infection rates. *Borrelia* infection lowered the odds of being infected with TBEV, but only in ticks from Haselmühl (OR =0.352; p= 0.0188). The reason for this, population-based difference might be differing *Borrelia* infection rates in both locations, variations of *Borrelia* species distribution or genetic differences in ticks leading to a different reaction to *Borrelia* infection. Which of these factors ultimately contributes to the observed data cannot be concluded from the current dataset. We might exclude general *Borrelia* infection rates since we found no significant differences between both locations in our dataset (30.0% Haselmühl and 34.5% Hanover mean values both years). *Borrelia* species and the ticks' response to infection remain to be investigated.

Conclusions

To summarize our current findings: we have established an artificial infection system for TBEV to study vector competence in *Ixodes ricinus* ticks. It has to be noted that the current article (Liebig et al. 2020) only gives first hints on vector competence of different *Ixodes ricinus* populations since we did not analyze two essential factor for ticks vector competence namely transstadial transmission and virus loads in ticks' saliva. However, we showed that ticks from TBEV endemic foci are more competent to allow for TBEV replication and in addition are more likely to transmit the virus due to higher feeding activities in months of high infection rates. Furthermore, our data clearly support an influence of climatic conditions and co-infection with other tick-borne pathogens such as *Borrelia* spp. on TBEV infection rates in specific populations.

Literature

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Author: Prof. Dr. Stefanie Becker

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