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RESEARCH ARTICLE

Epidemiological Investigation and Risk Assessment of Tick-Borne Encephalitis Virus in *Ixodes persulcatus*: Across Mongolia, And the China-Mongolia Border Regions

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ABSTRACT

Tick-borne encephalitis virus (TBEV) is a significant zoonotic pathogen causing central nervous system infections. This study investigates the prevalence and distribution of TBEV in *Ixodes persulcatus* ticks along the China-Mongolia border, based on large-scale surveillance conducted from 2020 to 2023. We detected TBEV RNA in 7 out of 191 tick pools, with phylogenetic analysis revealing high genetic similarity between strains from China and Mongolia, indicating potential cross-border transmission. Ecological niche modeling (MaxEnt) identified high-risk areas extending beyond current PCR-positive sites, with precipitation and temperature as key environmental drivers of TBEV distribution. Our findings highlight a broader geographical spread of TBEV than previously recognized, emphasizing the need for enhanced surveillance and targeted preventive measures in underreported hotspots.

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INTRODUCTION

Tick-borne encephalitis (TBE) is a zoonotic viral infection caused by the tick-borne encephalitis virus (TBEV), which primarily affects the central nervous system. The disease is transmitted through the bites of infected ticks, with *Ixodes persulcatus* being the primary vector in northern Asia and parts of Europe (Esser *et al.*, 2022). TBEV is a significant pathogen in regions including China, Mongolia, Russia, and parts of Europe, with an increasing incidence observed globally. The global incidence of TBE has risen from 0.4 to 0.9 cases per 100,000 individuals between 2015 and 2020 (Pustijanac *et al.*, 2023). However, the true number of clinical cases is likely higher, as many are unreported, especially in remote regions with limited surveillance. TBEV infections primarily occur through tick bites, though rare cases of transmission have been linked to consumption of unpasteurized milk from infected livestock (Elbaz *et al.*, 2022).

TBEV exists in several subtypes, including European (TBEV-Eu), Siberian (TBEV-Sib), and Far Eastern (TBEV-FE), as well as two potential new subtypes: Himalayan (TBEV-Him) (Dai *et al.*, 2018) and Baikal Lake (TBEV-Bkl) (Demina *et al.*, 2010). The Siberian and Far Eastern subtypes are primarily transmitted by *I. persulcatus*, whereas the European subtype is transmitted by *Dermacentor reticulatus*. Mortality rates vary across subtypes, ranging from 1-2% for TBEV-Eu to 6-8% for TBEV-Sib and up to 40% for TBEV-FE (Saksida *et al.*, 2018).

The disease exhibits strict geographical confinement, with the European subtype predominantly found in Central and Western Europe, the Siberian subtype in Siberia and

Northern Europe, and the Far Eastern subtype in East Asia, including China and Japan (Lu *et al.*, 2008). Rising incidence and socioeconomic impacts have heightened global concern over TBE.

In recent years, the range of tick-borne diseases, including TBE, has expanded due to climate change, urbanization, and increased human-wildlife interactions. Ticks are ectoparasites of many vertebrates and serve as vectors for several human pathogens, including TBEV, Lyme disease bacteria, and Crimean-Congo hemorrhagic fever virus (CCHFV). Ticks are the second-largest vector group after mosquitoes (Lippi *et al.*, 2021).

In Mongolia, ticks are widely distributed across diverse ecosystems, from forests to grasslands and deserts, with the Selenge Province along the Mongolia-Russia border serving as a hotspot for tick-borne diseases (Frey *et al.*, 2012). Despite its high incidence, Mongolia faces significant challenges in controlling TBE due to underdeveloped surveillance systems and limited healthcare infrastructure (Ganbold *et al.*, 2023). Annually, Mongolia reports approximately 10,000 to 12,000 clinical cases of TBE, though the actual number is likely higher, given the reporting gap and limitations in diagnosis (World Health Organization, 2020).

The China-Mongolia border region represents a unique epidemiological area, with high tick populations and diverse environmental conditions that may influence TBEV transmission. However, the distribution of TBEV in this region remains poorly understood due to the lack of comprehensive, large-scale surveillance. In this context, the study of tick populations and TBEV prevalence is crucial for assessing the true extent of the disease and for identifying underreported areas at high risk.

This study investigates the prevalence and distribution of TBEV in *I. persulcatus* ticks across the China-Mongolia border region, with the hypothesis that TBEV is widely distributed and that climate factors, such as temperature and precipitation, play a significant role in shaping its geographic spread. By combining large-scale tick surveillance, phylogenetic analysis, and ecological niche modeling (MaxEnt), this research aims to assess the prevalence of TBEV in *I. persulcatus* populations, evaluate the genetic diversity of TBEV strains, and explore evidence of cross-border transmission between Mongolia and China. Additionally, the study examines the influence of environmental factors on TBEV distribution to identify high-risk areas for future outbreaks. The integration of these methods will provide critical insights into TBEV transmission patterns, enhancing our understanding of its ecological dynamics and supporting the development of more effective surveillance and control strategies in high-risk areas of the region.

MATERIALS AND METHODS

Sampling methodology: Ticks were collected from multiple sites across the China-Mongolia border region, including Inner Mongolia (China) and various provinces in Mongolia, between April 2020 and October 2023. A stratified random sampling approach was employed to ensure representative coverage of diverse ecological zones, including forested areas, grasslands, and regions with known high tick activity. Sampling was carried out both from environmental surfaces using cloth flags for passive collection and from animal hosts using tweezers for active tick collection. Hosts were selected based on their potential exposure to ticks, including domestic livestock and wild animals commonly found in the region, allowing for a comprehensive representation of ticks from both wildlife and domesticated species. To ensure accurate spatial representation, GPS coordinates were recorded for each collection site, facilitating the mapping of tick distributions and the subsequent ecological niche modeling using MaxEnt. All spatial data were processed in ArcGIS and converted to ASCII raster format for further analysis.

RNA extraction: Tick samples were transported at 4°C, washed with PBS, labeled, and stored at -80°C in RNase-free tubes. Samples from identical locations/times were pooled (10 ticks/pool), homogenized in 600 μL PBS with beads at 4°C, and processed using the EasyPure® Virus DNA/RNA Kit for nucleic acid extraction. Extracted nucleic acid was reverse-transcribed into cDNA.

Tick species identification: Tick species identification was conducted using both morphological and molecular biological methods.

Morphological identification: Ticks were cleaned with 75% alcohol, dried, and key features (dorsal/ventral views, capitulum, palps, anal groove, spiracles, genital openings, Haller's organs, coxae) were examined under a stereomicroscope following SN/T 5337-2021 (Standardization Administration of China, 2021).

Molecular biological identification: Species-specific COI primers (Table 1) were used under PCR conditions: 95°C 5 min; 40 cycles of 95°C 30 s, 50°C 30 s, 72°C 60 s; final extension 72°C 10 min. PCR products were sequenced by Sangon Biotech (Shanghai).

Metagenomic sequencing and analysis: RNA was extracted using QIAamp Viral RNA Mini Kit. Ribosomal RNA was depleted with KAPA RNA HyperPrep Kit with RiboErase (Roche). cDNA synthesis used miScript II RT Kit. Libraries were constructed via end repair, adapter ligation, and PCR amplification. Quality was assessed by Qubit, Qseq100, and KAPA Library Quantification. Libraries were circularized into DNBs and sequenced on MGISEQ-2000 (PE150).

PCR detection: TBEV primers (Table 1) were designed based on metagenomic data and Ma Hongyu et al. (Ma, 2017). PCR conditions: 95°C 3 min; 32 cycles of 95°C 15 s, 53°C 15 s, 72°C 69 s; final extension 72°C 5 min.

Table 1: Sequences of primers used in the study

Primer Name	Sequence (5'-3')	Amplicon Size (bp)
CO I -F	GGTCAACAAATCATAAAGATATTGG	670
CO I -R	TAAACTTCAGGGTGACCAAAAAATCA	670
TBEV-F	GGTRYTGGARCTGGGDGGATGR	1150
TBEV-R	GCGTGYTCTCCKATCACTGTCA	1150

Sequence comparison and genetic evolution analysis: ORF amino acid sequences of Chinese/Mongolian TBEV E proteins were aligned against NC_001672.1 using ESPript 3.0(Robert,Gouet, 2014). Full TBEV sequences from NCBI (with time/location data) were aligned in MEGA11 (Clustal W). A maximum likelihood phylogenetic tree was constructed and visualized via iTOL (https://itol.embl.de/)(Letunic,Bork, 2024).

Spatial modeling data collection: TBEV outbreak and *I. persulcatus* distribution records in Mongolia/China-Mongolia border areas were sourced from fieldwork, literature, books, and databases (NCBI, Web of Science). Environmental predictors (climate, terrain, vegetation) were selected. All spatial data were converted to ASCII raster format (30 arc-seconds/km, UTM-WGS-1984).

Spatial distribution model analysis: Ecological niches of *I. persulcatus* and TBEV were modeled separately and overlaid. Spatial autocorrelation was minimized using SDM toolbox v1.1c (ArcGIS 10.2) for spatial thinning (gradient distances: 10–150 km). Principal component analysis (SPSS 22.0) screened predictors (eigenvalues>0.85), reducing multicollinearity. MaxEnt eliminated low-contribution/high-SD variables. Variance inflation factor (VIF<10) confirmed no multicollinearity. Distribution records and environmental data for TBEV and *I. persulcatus* were modeled in MaxEnt (75% training, 25% testing; random seed). Non-collinear low-contribution/high-SD variables were excluded. Risk layers were overlaid (ArcGIS Fuzzy Overlay) to generate the final TBE risk map. Model accuracy was evaluated by ROC curve/AUC (>0.8 = good; >0.9 = excellent; Table 2).

RESULTS

Tick collection: A total of 12,800 ticks were collected in Inner Mongolia Autonomous Region of China, and 22,000 ticks were collected from 10 provinces in Mongolia. The collection details for different locations are provided in S1.

Tick species identification: Morphologically intact ticks collected from China and Mongolia were identified according to the Chinese Technical Specification for Identification of Tick Species. Five tick species were collected in northern China's border regions: *Dermacentor silvarum*, *Haemaphysalis longicornis*, *Dermacentor japonicus*, *Haemaphysalis concinna*, and *I. persulcatus*. Six tick species were collected in Mongolia: *Dermacentor nutalli*, *Hyalomma Asiaticium*, *D. silvarum*, *Dermacentor marginatus*, *I. persulcatus*, and *Dermacentor nivenus*. Detailed morphological characteristics of ticks collected from various locations are provided in S2.

A total of 34,800 ticks were collected from northern China's border regions and Mongolia. All samples were detected using PCR, and sequencing results were verified using blast. The tick species collection is shown in Fig. 1.

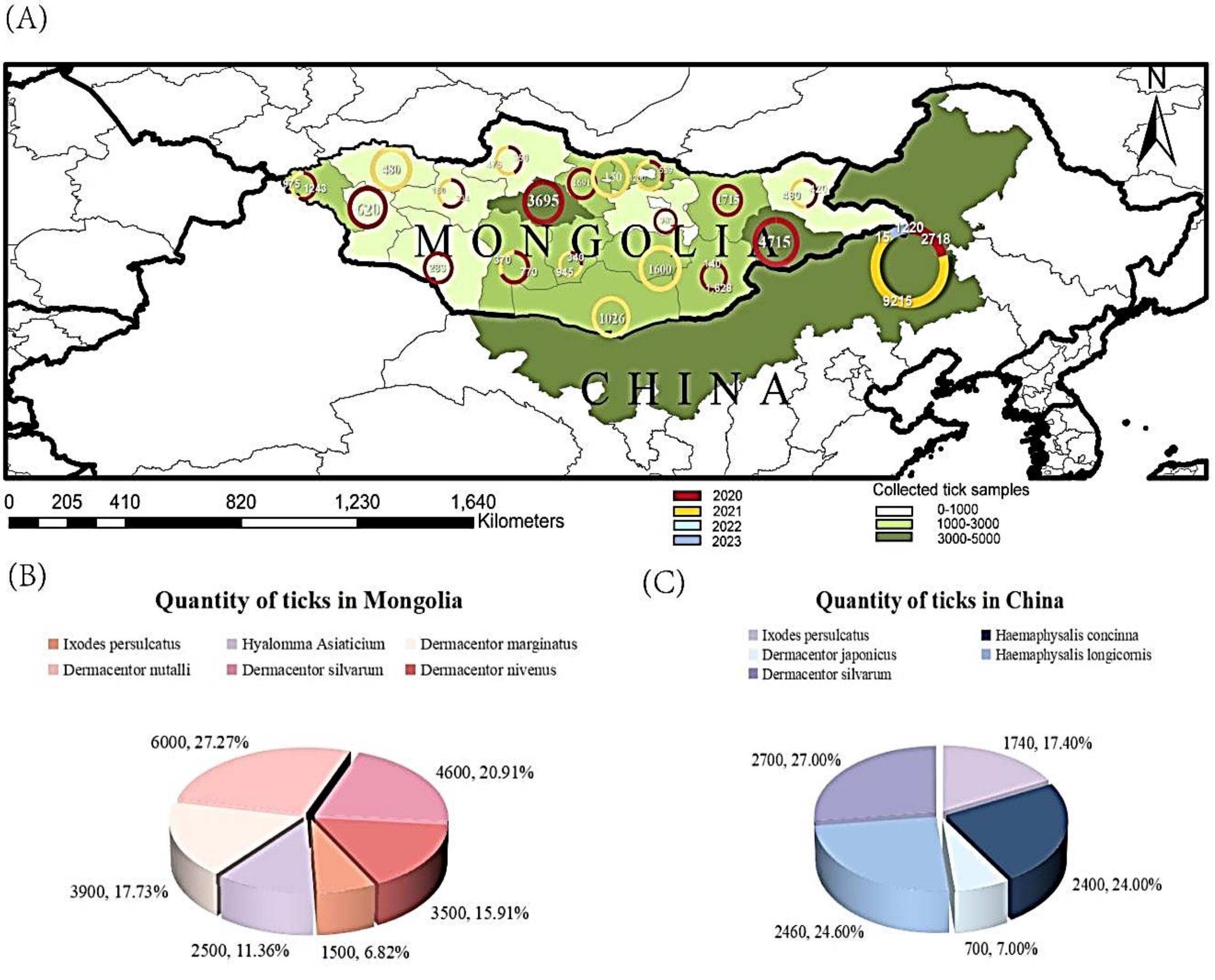


Fig. 1: Tick species collection in inner Mongolia autonomous region of China and Mongolia. Note:(A) the number of ticks collected in different regions of China and Mongolia from 2020 to 2023; (B) the species and number of ticks collected in Mongolia; (C) the species and number of ticks collected in China.

Table 2: Data layer and source, raster/vector, value range/categories (Number of subcategories in brackets) and specification of unit of measurement/impact (proxy)

Layer	Source	Type	Value/categories	Measurement unit
Climate				
Prec 1-12	worldclim	Raster	0 to 275 mm/month	Precipitation
Temp 1-12	worldclim	Raster	-32.6 to 37.3°C	Mean Temperature
Tmin 1-12	worldclim	Raster	-37.3 to 30.5°C	Minimum Temperature
Tmax 1-12	worldclim	Raster	-27.9 to 43.6°C	Maximum Temperature
Bio 1-19	worldclim	Raster		Bio 1 mean annual temperature
				Bio 2 Monthly mean value of temperature difference between day and night
				Bio 3 ratio of day / night temperature difference to annual temperature difference
				Bio 4 Seasonal change in temperature
				Bio 5 The hottest month is the hottest
				Bio 6 The coldest month is the coldest temperature
				Bio 7 Annual temperature variation range
				Bio 8 The wettest quarterly average temperature
				Bio 9 Dryer quarterly average temperature
				Bio 10 Warmest quarterly average temperature
				Bio 11 The coldest quarterly average temperature
				Bio 12 Mean annual precipitation
				Bio 13 The wettest month of precipitation
				Bio 14 Precipitation in the most dry months
				Bio 15 Precipitation seasonality
				Bio 16 The wetest quarterly precipitation
				Bio 17 Dryest quarterly precipitation
				Bio 18 The warmest quarterly precipitation
				Bio 19 Precipitation in the coldest quarter
Terrain				
Elevation	ASTER-GDEM	Raster	-10 to 8844 m a.s.l	Climbing distance
Slope	ASTER-GDEM	Raster	0 to 88.2°	
Vegetation				
Land cover	ESA	Vector	Cropland(3), Herbaceous(1), Tree(9), Shrubland(3), Grassland(1), Urban areas(1), Bare areas(2), Water bodies(1), Permanent snow and ice(1)	Animal food and refuge

Results of metagenomic sequencing: Metagene sequencing revealed 155 archaea, 3757 bacteria, 1279 eukaryotes and 354 viruses in the tick DNA samples. Seven archaea, 582 bacteria, 192 eukaryotes, and 88 viruses were present in the RNA samples. It is worth noting that high abundance of TBEV was found in tick samples from Inner Mongolia Autonomous Region of China and Selenge Province and Bulgan Province of Mongolia, with sequencing lengths of 11055 bp and 11330 bp, respectively. The e-value was 0 and the amplified sequence contrast similarity was 98%, indicating extremely high sequence comparison confidence, indicating the presence of forest encephalitis virus in ticks in China-Mongolia border areas. Therefore, in this study, PCR testing was performed for *I. persulcatus* in these two regions to determine the prevalence of TBEV.

Positive rate of TBEV in different tick species: Tick samples collected from the Aershan region of Inner Mongolia Autonomous Region and Mongolia were classified based on their sampling locations and tick species. PCR technology was used to randomly select samples for TBEV detection. A target band of nearly 1000 bp was detected, consistent with the expected 1150 bp band. Notably, all positive samples were *I. persulcatus*. DNA sequencing confirmed that the amplified fragments indeed originated from TBEV. The results showed that the TBEV positivity rate in Mongolia was 0.75% (1/133), while in the northern part of China bordering Mongolia, it was 10.34% (6/58) (Table 3). Detailed information on the positive rate in different regions is shown in Table 4.

Sequence comparison of E protein of forest encephalitis virus: This study conducted a comparative sequence analysis of the TBEV E protein from Chinese and Mongolian strains, using the NCBI reference sequence (NC_001672.1) as the standard. Alignment identified 19 amino acid substitution sites, all representing non-synonymous mutations (Fig. 2):

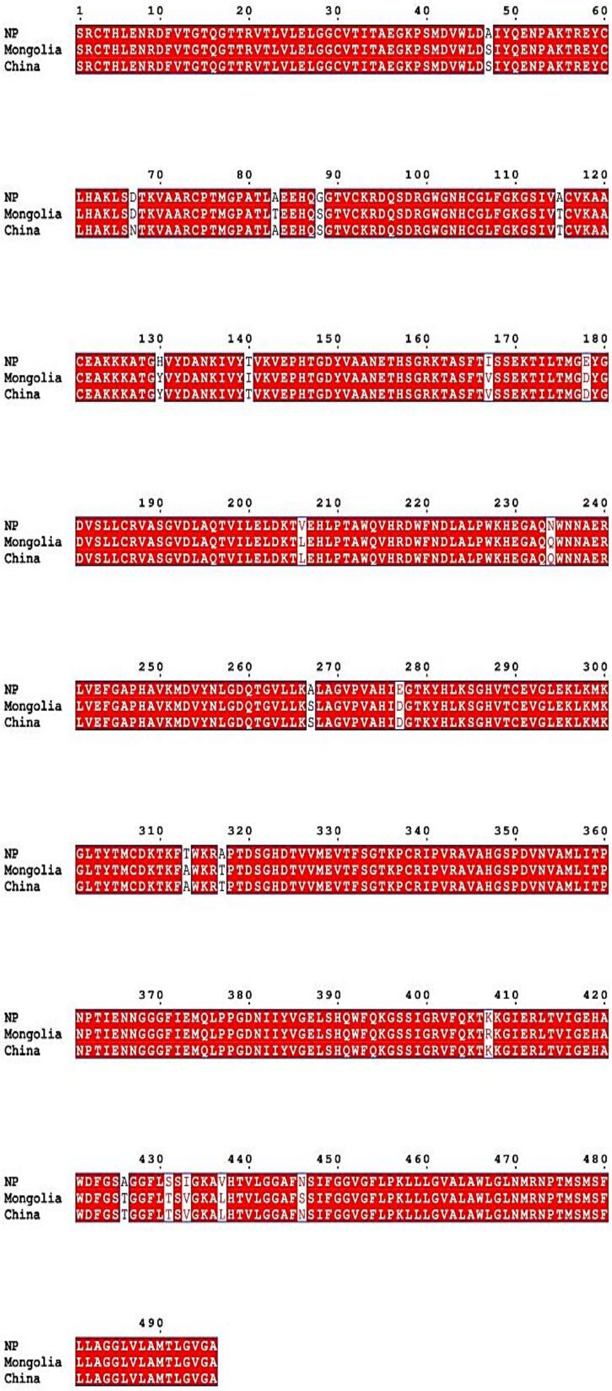
Table 3: Positive rates of different ticks

Species	Positive rates
D. nutalli	0%
H. Asiaticium	0%
D. nivenus	0%
D. marginatus	0%
I. persulcatus	3.66%
D. silvarum	0%
H. longicornis	0%
D. japonicus	0%
H. concinna	0%

Table 4: Positive rates of TBEV in various regions of China and Mongolia

Country	Province	Regions	Tick pool numbers	Positive pool	Positive rates
China	Inner Mongolia Autonomous Region	Arshan City	58	6	10.34% (6/58)
Mongolia	Bulgan	Khangal	2	0	0.75% (1/133)
	Selenge	Bugant	8	0	
		Khuder	3	0	
		Altanbulag	45	0	
		Yeroo	75	1	

The T313A (Thr→Ala) and A317T (Ala→Thr) substitutions within the IgG-like fold domain may modulate host cell binding specificity by altering surface topology. This could impact receptor-binding capacity and potentially confer cross-species transmission potential. The Mongolian strain-specific N446S (Asn→Ser) substitution occurs at the periphery of transmembrane helix TM1. Increased side-chain polarity at this position may compromise membrane-anchoring efficiency. This mutation likely represents adaptive optimization in the primary host, resulting in attenuated virulence while enhancing transmission sustainability.



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         1        10        20        30        40        50        60
NP       SRCTHLENRDFVTGTQGTTRVTLVLELGGCVTITAEGKPSMDVWLDAIYQENPAKTREYC
Mongolia SRCTHLENRDFVTGTQGTTRVTLVLELGGCVTITAEGKPSMDVWLDSIYQENPAKTREYC
China    SRCTHLENRDFVTGTQGTTRVTLVLELGGCVTITAEGKPSMDVWLDSIYQENPAKTREYC

                  70        80        90       100       110       120
NP       LHAKLSDTKVAARCPTMGPATLAEEHQGGTVCKRDQSDRGWGNHCGLFGKGSIVACVKAA
Mongolia LHAKLSDTKVAARCPTMGPATLTEEHQSGTVCKRDQSDRGWGNHCGLFGKGSIVTCVKAA
China    LHAKLSNTKVAARCPTMGPATLAEEHQSGTVCKRDQSDRGWGNHCGLFGKGSIVTCVKAA

                 130       140       150       160       170       180
NP       CEAKKKATGHVYDANKIVYTVKVEPHTGDYVAANETHSGRKTASFTISSEKTILTMGEYG
Mongolia CEAKKKATGYVYDANKIVYIVKVEPHTGDYVAANETHSGRKTASFTVSSEKTILTMGDYG
China    CEAKKKATGYVYDANKIVYTVKVEPHTGDYVAANETHSGRKTASFTVSSEKTILTMGDYG

                 190       200       210       220       230       240
NP       DVSLLCRVASGVDLAQTVILELDKTVEHLPTAWQVHRDWFNDLALPWKHEGAQNWNNAER
Mongolia DVSLLCRVASGVDLAQTVILELDKTLEHLPTAWQVHRDWFNDLALPWKHEGAQQWNNAER
China    DVSLLCRVASGVDLAQTVILELDKTLEHLPTAWQVHRDWFNDLALPWKHEGAQQWNNAER

                 250       260       270       280       290       300
NP       LVEFGAPHAVKMDVYNLGDQTGVLLKALAGVPVAHIEGTKYHLKSGHVTCEVGLEKLKMK
Mongolia LVEFGAPHAVKMDVYNLGDQTGVLLKSLAGVPVAHIDGTKYHLKSGHVTCEVGLEKLKMK
China    LVEFGAPHAVKMDVYNLGDQTGVLLKSLAGVPVAHIDGTKYHLKSGHVTCEVGLEKLKMK

                 310       320       330       340       350       360
NP       GLTYTMCDKTKFTWKRAPTDSGHDTVVMEVTFSGTKPCRIPVRAVAHGSPDVNVAMLITP
Mongolia GLTYTMCDKTKFAWKRTPTDSGHDTVVMEVTFSGTKPCRIPVRAVAHGSPDVNVAMLITP
China    GLTYTMCDKTKFAWKRTPTDSGHDTVVMEVTFSGTKPCRIPVRAVAHGSPDVNVAMLITP

                 370       380       390       400       410       420
NP       NPTIENNGGGFIEMQLPPGDNIIYVGELSHQWFQKGSSIGRVFQKTKKGIERLTVIGEHA
Mongolia NPTIENNGGGFIEMQLPPGDNIIYVGELSHQWFQKGSSIGRVFQKTRKGIERLTVIGEHA
China    NPTIENNGGGFIEMQLPPGDNIIYVGELSHQWFQKGSSIGRVFQKTKKGIERLTVIGEHA

                 430       440       450       460       470       480
NP       WDFGSAGGFLSSIGKAVHTVLGGAFNSIFGGVGFLPKLLLGVALAWLGLNMRNPTMSMSF
Mongolia WDFGSTGGFLTSVGKALHTVLGGAFSSIFGGVGFLPKLLLGVALAWLGLNMRNPTMSMSF
China    WDFGSTGGFLTSVGKALHTVLGGAFNSIFGGVGFLPKLLLGVALAWLGLNMRNPTMSMSF

                 490
NP       LLAGGLVLAMTLGVGA
Mongolia LLAGGLVLAMTLGVGA
China    LLAGGLVLAMTLGVGA
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Fig. 2: Sequence comparison of E protein of TBEV. Note: NP: NCBI reference sequence for TBEV NC_001672.1.

Results of the genetic evolution analysis: The phylogenetic tree reveals that the sequences obtained from China (PV568693) and Mongolia (PV568692) belong to the Siberian subtype of TBEV, with close genetic relationships and a similarity of 97.35%. Additionally, these sequences share an extremely close relationship with a 2011 TBEV strain (ID: KF823822) from Irkutsk, Russia, with a similarity of 97.15% (Fig. 3). The high similarity of these viral sequences suggests potential transmission across regions through animal migration, human activities, tick dissemination, or other means.

Spatial model records and selection of predictor variables: From previous studies, TBEV and *I. persulcatus* geographical distribution records were selected. After screening, 89 TBEV and 334 *I. persulcatus* records were included for subsequent analysis. The pairwise distances between records were set at 40 km and 80 km, respectively. After PCA and MaxEnt elimination of variables with low contribution values, December precipitation (prec 12) and November maximum temperature (temp 11) were ultimately used to construct the final TBEV model. January precipitation (prec 1), September precipitation (prec 9), and August minimum temperature (tmin 8) were used to construct the final *I. persulcatus* model (Table 5). Multicollinearity tests indicated VIF values of 0-2 (<10), indicating no detected multicollinearity among predictors.

Spatial models for TBEV and *I. persulcatus*: The average output of 10-fold cross-validation for the TBEV SDM indicated high training and testing AUC values with low standard deviations (0.820; 0.023). Similarly, the *I. persulcatus* SDM showed high training and testing AUC values with low standard deviations (0.809; 0.030), indicating good prediction performance and validating the robustness of the models. These models can be used for TBE risk assessment (Fig. 4).

The bell-shaped response curves indicate that the probability of TBEV occurrence is highest when the average December precipitation is 5-10 mm and the average November temperature is between -10°C and -5°C. Concurrently, the probability of *I. persulcatus* occurrence is highest when the average January precipitation is 0-10 mm, the average September precipitation is 0-50 mm, and the August minimum temperature is 15-20°C (Fig. 5).

Spatial risk of TBEV carried by *I. persulcatus*: Layer overlay analysis revealed high TBE transmission risks in Selenge, Darkhan, Uvs, and Zavkhan provinces in northern Mongolia, with Selenge Province having the highest risk level. In China, the highest TBE risks are in the northwest and northeast, primarily around the Greater and Lesser Khingan Ranges and the Changbai Mountains. There is also a risk of TBE along the China-Mongolia, Sino-Russian, and Mongolian-Russian borders. Overall, the occurrence of TBE is mainly influenced by meteorological factors (Fig. 6).

The cartographic representation was developed with spatial data outputs produced by the MaxEnt algorithm through ArcGIS 10.6 platform. Geopolitical boundaries were sourced from the Natural Earth public domain dataset (http://www.naturalearthdata. com/), serving as a generalized representation of national spatial relationships. This visualization contains no legally binding geographic information and specifically cautions against reinterpretation or republication for jurisdictional, territorial, or sovereignty-related contexts.

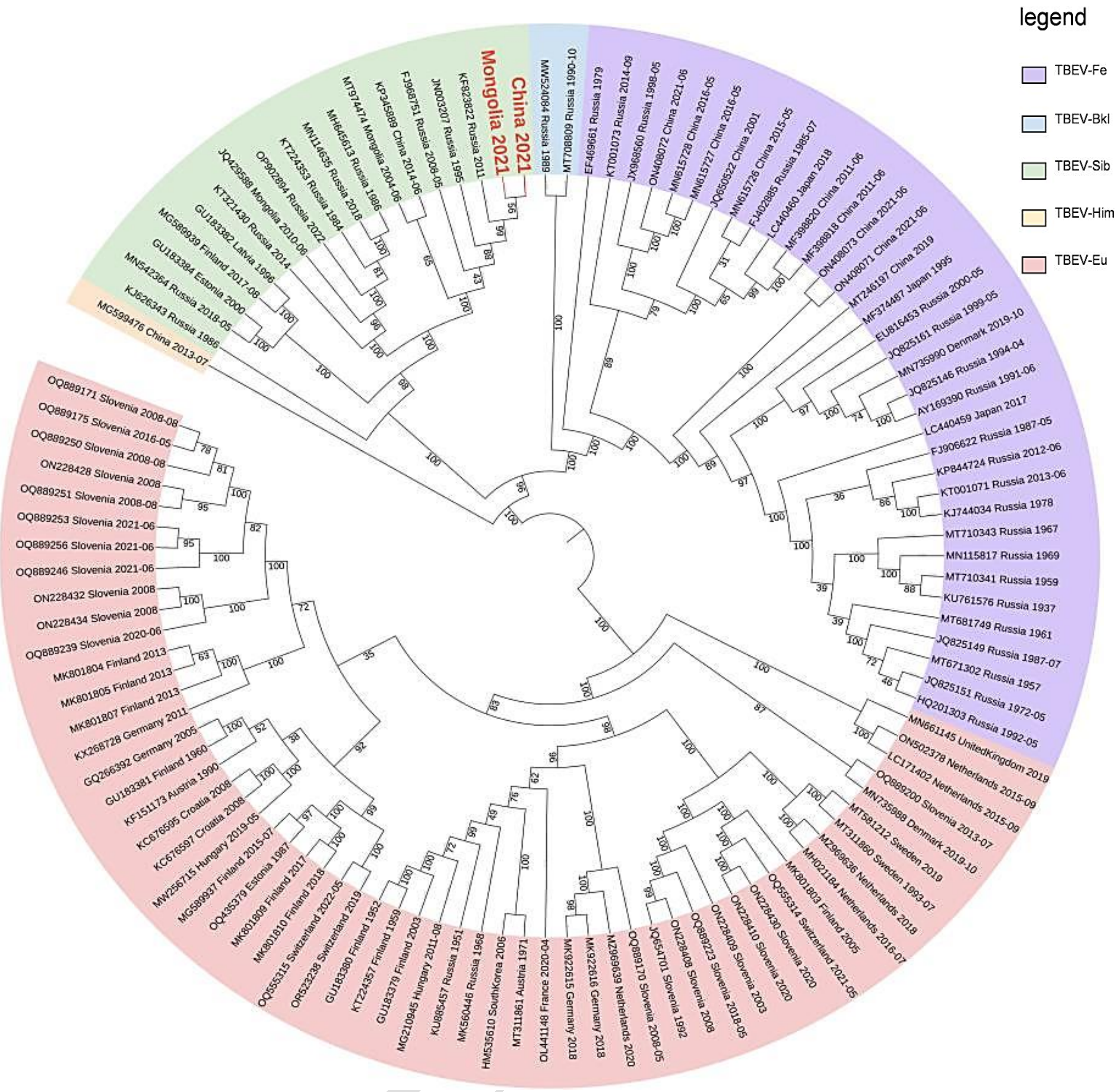

Fig. 3: TBEV phylogenetic tree with complete sequence.

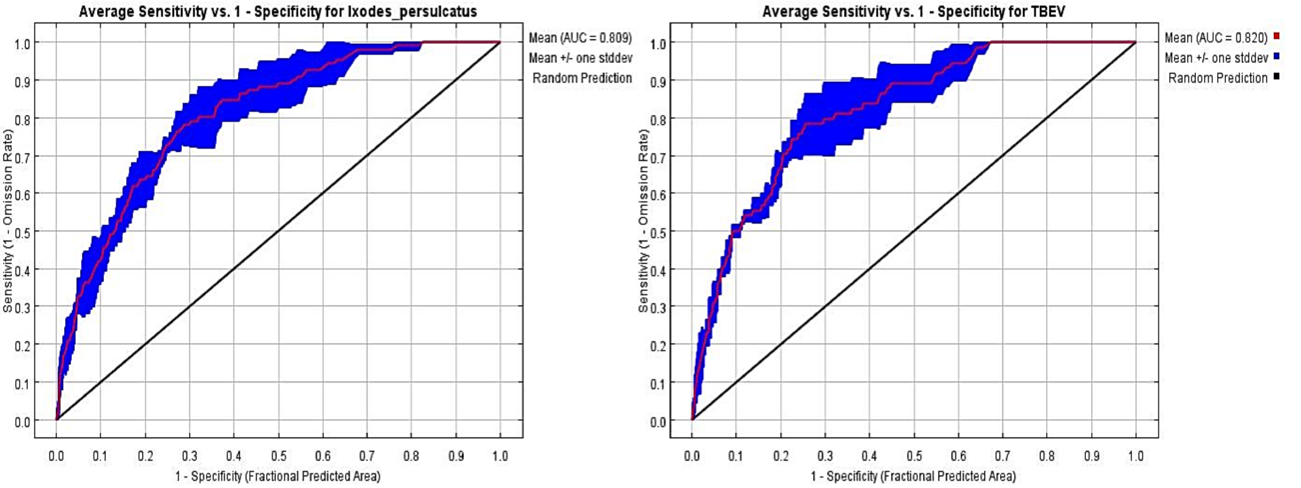

Fig. 4: Average sensitivity and specificity curves of the TBEV and *I. persulcatus* MaxEnt model. Note: The left figure shows the average sensitivity and specificity curve for the TBEV model, and the right figure shows the curve for the I. persulcatus model. The curves display the average response (red) and its average standard deviation (blue) from repeated runs in the MaxEnt model.

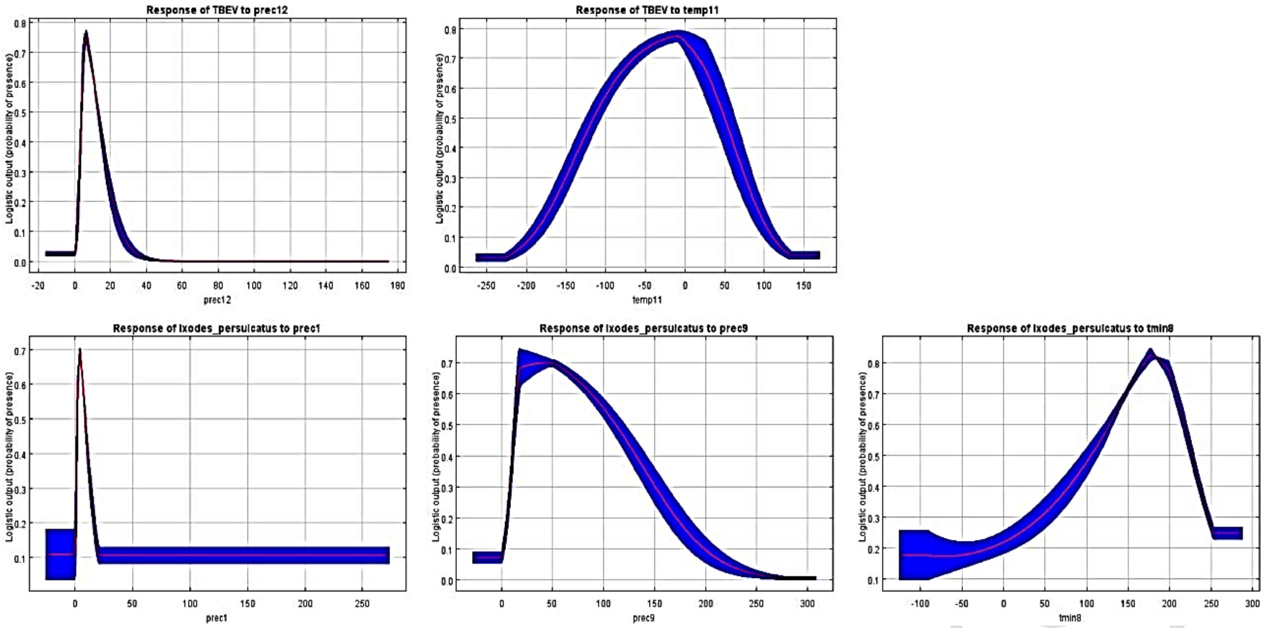

Fig. 5: Bell-shaped response curves of environmental drivers affecting TBEV and I. persulcatus. Note: (a) TBEV response curve (b) I. persulcatus response curve. The curves show the average response value (red) and its average standard deviation (blue) from repeated runs in the MaxEnt model. prec12: December precipitation, temp11: November average temperature, prec 1: January precipitation, prec 9: September precipitation, tmin 8: lowest temperature in August.

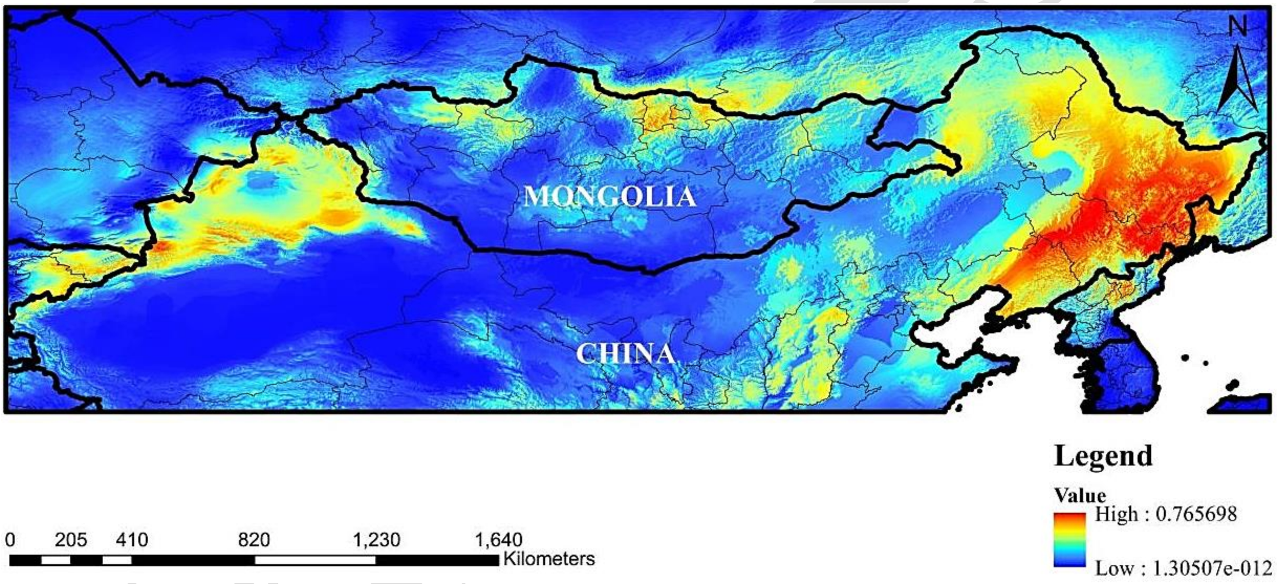

Fig. 6: Probability of TBE occurrence in high-risk areas. Note: Suitability for TBEV across the research area. The map depicts the suitability range for TBEV in China and Mongolia. Red represents the highest and blue the lowest suitability.

DISCUSSION

This study provides the first cross-border, large-scale investigation of *I. persulcatus* and TBEV in the China-Mongolia border region. By combining molecular screening, phylogenetic analysis, and ecological niche modeling, we explored the circulation patterns of TBEV in local tick populations and identified key environmental factors shaping its distribution. These findings help fill important gaps in our understanding of TBEV ecology in this region and raise new concerns about its potential spread under changing climate conditions.

Our results show that TBEV was mainly detected in *I. persulcatus* ticks and belonged to the Siberian subtype. The viral sequences shared over 97% identity with strains from Irkutsk, Russia, suggesting possible transboundary transmission, likely driven by wildlife migration, human activity, or tick dispersion(Haemig *et al.*, 2011; Li *et al.*, 2015). Sequence variation within receptor-binding regions further points to potential changes in viral fitness or host tropism. These observations provide new insight into the local evolution of TBEV and offer clues for tracking its geographic spread.

This study detected a considerable TBEV positivity rate in the China-Mongolia border region (0.75% in Mongolia and 10.34% in China). Furthermore, the region's rich wildlife diversity, coupled with the migration of migratory birds and wild animals, poses a significant risk for cross-border transmission of tick-borne encephalitis virus. Niche modeling identified December precipitation and November temperature as significant predictors of TBEV presence, consistent with previous reports linking TBEV maintenance to temperate forest environments(Wang *et al.*, 2023).

Overlaying the predicted distributions of *I. persulcatus* and TBEV revealed strong spatial overlap, underscoring the central role of this tick species in TBEV transmission. The tick' s distribution was mainly shaped by January and September precipitation and the minimum temperature in August, indicating its sensitivity to climate variables(Chen *et al.*, 2019). These findings are consistent with recent observations from Europe showing that warmer temperatures have facilitated the expansion of tick habitats and the rise of TBE incidence(Lindgren *et al.*, 2001; Randolph, 2008; Voyiatzaki *et al.*, 2022).

Given the limited healthcare infrastructure and passive surveillance in parts of Mongolia, the true extent of TBEV circulation may be underestimated(Baasandavga *et al.*, 2019; Cerny *et al.*, 2019; Ganbold *et al.*, 2023). The identification of ecologically suitable areas with limited monitoring suggests that current surveillance may be missing a significant portion of the virus's spread.

Conclusions: Our study demonstrates the critical yet underrecognized expansion of TBEV transmission risk in the China-Mongolia border region, driven by climate change, land-use shifts, and cross-border viral spread. The integration of large-scale tick surveillance with ecological niche modeling reveals wider TBEV distribution than previously documented, exposing gaps in current monitoring systems. Importantly, phylogenetic evidence confirms active cross-border TBEV circulation, emphasizing the urgent need for transnational One Health strategies to mitigate outbreak risks. Given the rapid environmental and anthropogenic changes fueling tick-borne disease emergence, sustained surveillance, real-time data sharing, and joint prevention policies must be prioritized to safeguard the public health security of the local people.

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