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Geographical Distribution of Tick-borne Encephalitis and Its Vectors in Mongolia, 2005-2016

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Objectives: In the last several years, the incidence and fatality rates of tick-borne encephalitis (TBE) in Mongolia have been increasing with new cases being registered in areas without the main tick vector. Therefore, we sought to determine the geographical distribution of TBE in Mongolia. **Methods:** Data were analyzed on TBE virus prevalence of collected ticks and seroprevalence rate of TBE and TBE incidence rate among the human population from 2005 to 2016. **Results:** During this time, TBE incidence was 0.75 per 10,000 populations in Mongolia. Among 2,182 people investigated, seroprevalence rate was 14.6±10.9% (n=312) in 10 provinces and Ulaanbaatar city. The seroprevalence rate among the populations of Selenge, Bulgan and Khuvsgul provinces, which are home to the main tick vector *I.Persulcatusis*, were 1.8 times (CI 95% 1.5-2.3, p< 0.001) higher than other provinces where the tick species *D.nuttalli* and *D.silvarumare* found. The TBE virus prevalence of *I.Persulcatus* was 3.18%, *D.nuttalli* was 0.61% and *D.silvarum* was 2.9% among the 10,464 ticks sampled. **Conclusion:** Endemic areas of TBE are expanding from northern parts of the country where the main vector I. persulcatus is more common to steppe areas where the vector *D. nuttalli* is abundant.

Keywords: Tick-borne Encephalitis, Incidence, Seroprevalence, Ticks, Mongolia

Introduction

In recent years, increasing numbers of tick-borne diseases are becoming a major public health issue due to climate change, shifting host communities, tick and human demographic patterns, pathogen diversity, deforestation, greenhouse gas emissions, human behavior, economics and regional politics [1]. Since 2005, the most commonly observed human tick-borne diseases in Mongolia include tick-borne encephalitis, tick-borne borreliosis and tick-borne rickettsiosis.

Tick-borne encephalitis (TBE) is an important viral infectious disease involving the central nervous system and is transmitted

by a bite from an infected tick. The World Health Organisation (WHO) reports (2014) that between10,000-12,000 tick-borne encephalitis cases are reported each year around the world [2]. Tick-borne encephalitis was first described by an Austrian physician (Shneider) in 1931 and the tick-borne encephalitis virus was first isolated in 1937 by the Soviet scientist Zilber, when it was understood to be transmitted by the bite of infected species of Ixodes persulcatus tick [3, 4]. In Mongolia, tick-borne encephalitis virus was first isolated (Kraminskii V.A) from marmot liver in Dornod province in 1979 while the Ixodes persulcatus tick was identified in 1987 (M.Dash) [5, 6]. Ixodes persulcatus is a taiga tick distributed in coniferous forests consisting mostly of pines, spruces and larches [7]. 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. Mongolian scientists have been conducting TBE research since the 1980s. In a 2005 study, researchers found *Ixodes persulcatus* tick counts were high in Selenge province [5].

Tick-borne encephalitis has recently attracted much attention because of the increasing incidence and consequent significant harm to human health. Since 2005, prevention measures such as vaccination, training and advocation among the population have been administered but human cases of TBE continue to increase. TBE cases are also being registered in areas without the main vector *Ixodes persulcatus* tick, thereby expanding the natural foci of disease. The goal of this research is to define geographical distribution of tick-borne encephalitis in Mongolia. Furthering knowledge of the distribution of tick-borne encephalitis in Mongolia is urgently needed for the prevention and control of this disease, as well as for reducing the disease burden among the human population.

Materials and Methods

1. Documentation of tick-borne encephalitis cases

Data on TBE cases were documented by the National Center for Zoonotic Diseases (NCZD), acting as the National Reference Laboratory for TBE virus, confirming the diagnosis through questionnaires on tick bites and visits to endemic areas from 2005 to 2016. All cases were confirmed by serological investigation using ELISA and IFA methods for the detection of IgM and IgG anti-TBEV. Confirmatory diagnostic methods were carried out according to the standards set forth by the publication, "Health care technology. Diagnosis, treatment and surveillance of Tick-borne encephalitis" MNS 5348-44:2003.

2. Sample collection

NCZD and local Center for Zoonotic Diseases (CZD) agencies implemented an active surveillance program through advertisement of TBD among at-risk populations. Teams collected 2,172 specimens of human blood serum from volunteer participants. Participant's name, province, and the sample collection date were recorded with the informed consent of the participants. Local CZD offices from the provinces of Arkhangai, Bayankhongor, Bayan-Ulgii, Dundgobi, Uvurkhangai, Selenge, Uvs, Khuvsgul, Khentii, Zavkhan, Umnugobi, Khovd, Gobi-Altai and the city of Ulaanbaatar contributed to the surveillance effort.

For tick collection, a tick drag method was implemented using a 1 square-meter strip of white cloth sized 60x100 cm. Ticks were kept in a tube until they could be investigated. Tick species were visually identified using a tick identification guide created by Russian researcher Serdyukova in 1956 [8].

3. Ethical statement

Ethical clearance was not required for this surveillance. Surveillance has been conducted among at-risk individuals since 2005 and is considered the routine work of NCZD and local CZD offices responsible for zoonotic infectious disease response and prevention measures. This practice is also mentioned in "Health care technology. Diagnosis, treatment and surveillance of Tick-borne encephalitis" MNS 5348-44:2003.

4. Laboratory methods

From the human participants, approximately 5 ml of blood was collected where 1.5 ml of serum was separated and kept at -20^0 C until analyzed. The IgG anti-TBEV levels were measured using an enzyme linked immunosorbent assay (ELISA) (EUROIMMUN, ELISA kit, Germany) and immunofluorescence assay (IFA) (EUROIMMUN, Antibody Kit, Germany). Neutralization titer of ≥1.10 was considered positive. Seroprevalence rate was the percentage of persons in the population who had detected able IgG anti-TBEV among all tested specimens based on serological investigation. It was presented as a percent of the total specimens tested. In ticks, the TBE virus RNA was detected using polymerase chain reaction (PCR). RNA was extracted from

ticks using kits (Machereynagel, Germany) according to the manufacturer's instructions. The cDNA synthesis was performed using primers (Super script® in Platinium® One step RT-PCR, Invitrogen, USA) of reverse transcription polymerase chain reaction (RT-PCR). RT-PCR for detection of the TBEV E gene was performed with primers EncE-L and 1643-R.

5. Statistical analysis

TBE incidence was calculated per 10,000 population for each province. Seroprevalence rate were calculated in the surveillance data. Prevalence data were expressed as mean ±SD. Statistical analysis was conducted using the MS Excel 2010 and Open Epi Info version. Chi-squere test was performed between the seroprevalence rate among the populations of different areas with an abundance of different tick species. Correlation analysis was performed between seroprevalence rate among population and tick infection rate in provinces. A p-value< 0.05 was considered statistically significant. A distribution of TBE incidence map was made using ArcGIS 10.1 program.

Results

1. TBE cases

A total of 225 confirmed cases were registered in 47 soums of Arkhangai, Bayankhongor, Bulgan, Darkhan-Uul, Dundgobi, Dornod, Orkhon, Uvurkhangai, Selenge, Tuv, Uvs, Khunsgul, and Khentii provinces and among 9 districts of Ulaanbaatar city. The incidence rate was 0.75 per 10,000 population in Mongolia from 2005-2016. The incidence in Selenge (11.5), Bulgan (2.7), Darkhan-uul (1.6), and Orkhon (1.1) provinces were higher than the national incidence level. In the last two years, three provinces (Uvs, Uvurkhangai and Dundgobi) were added to the

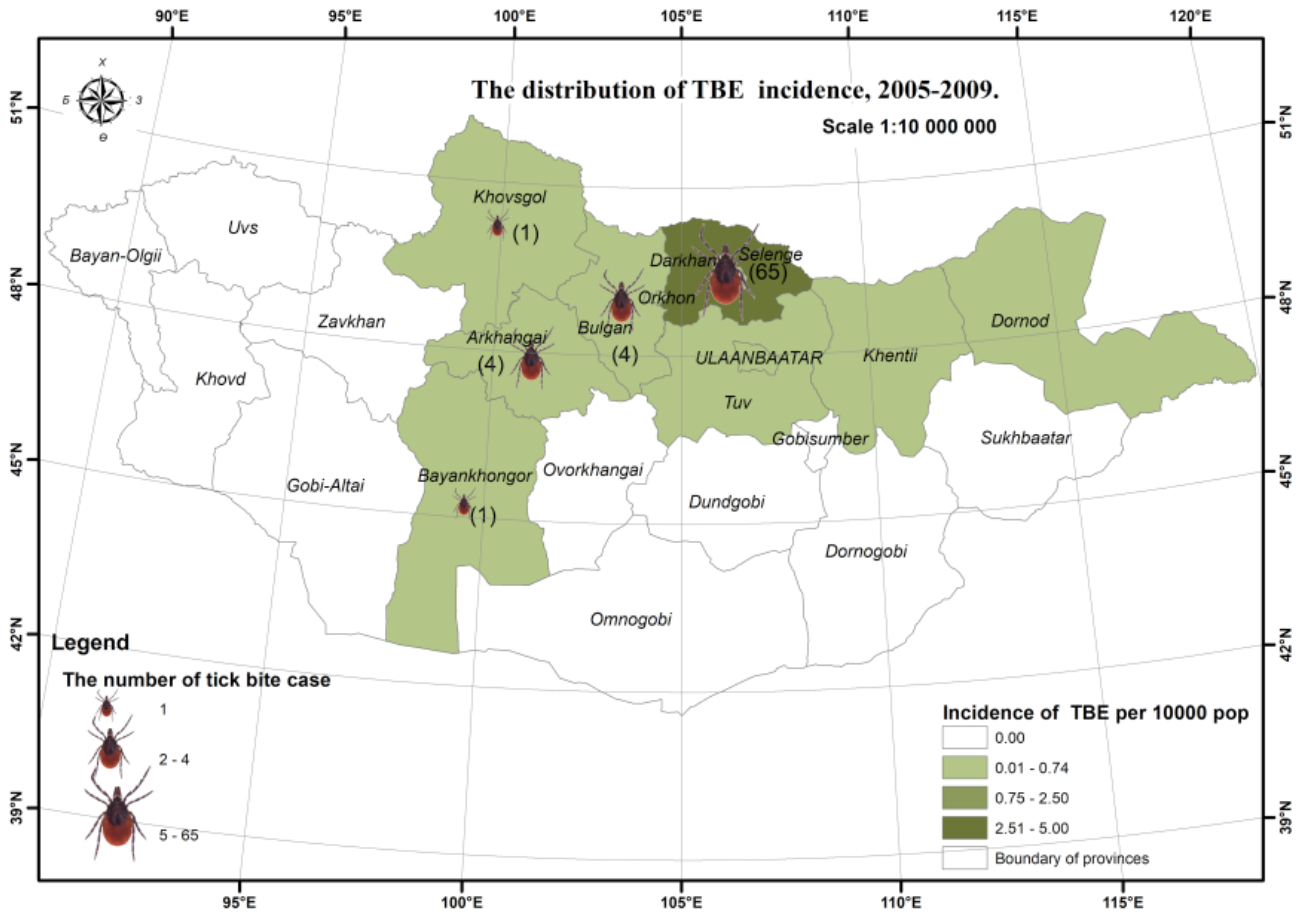

Figure 1. TBE Incidence rate and tick bites in Mongolia 2005-2009.
The tick symbol designates location of tick bite.

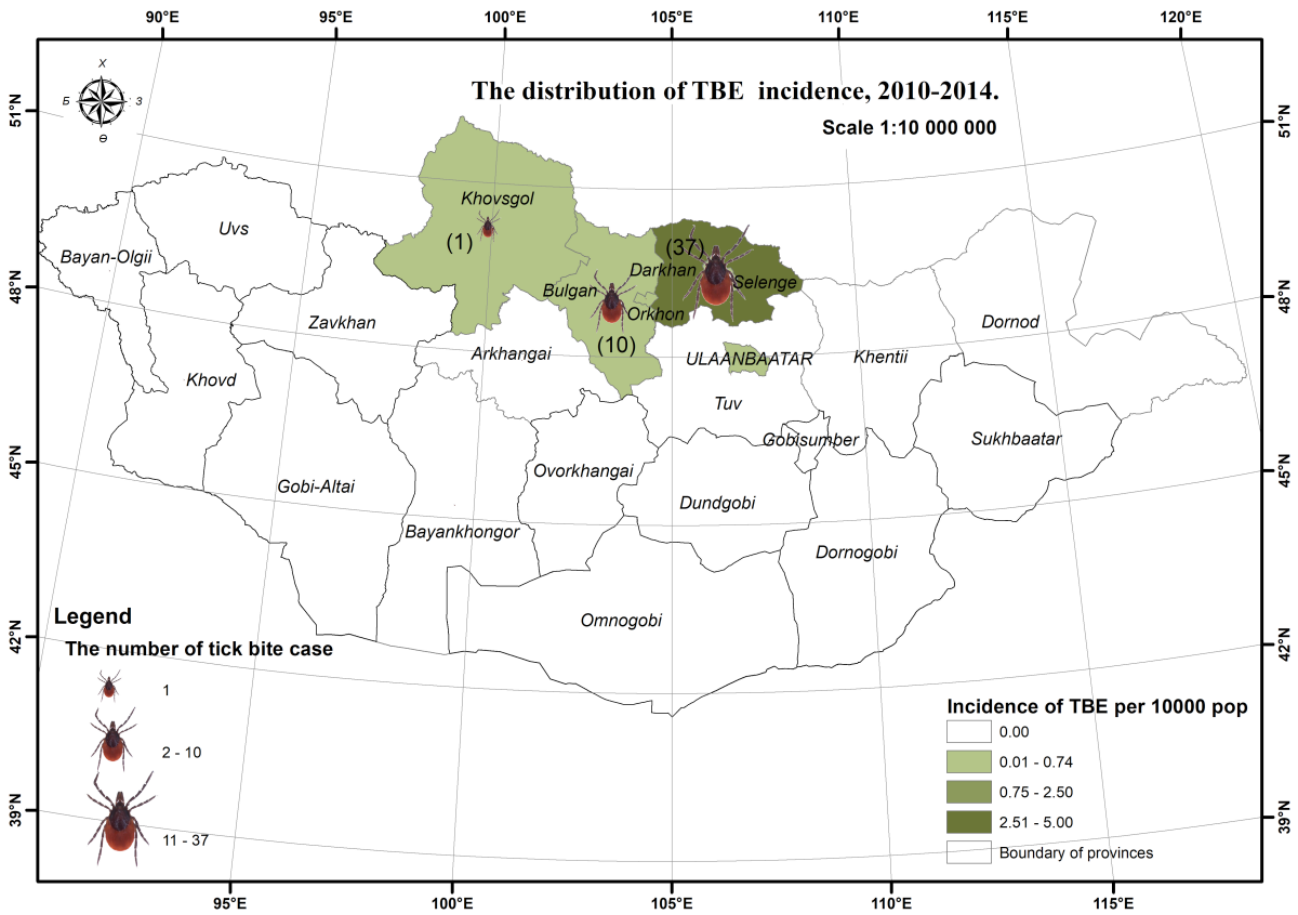

Figure 2. TBE Incidence rate and tick bites in Mongolia 2010-2014.
The tick symbol designates location of tick bite.

surveillance efforts. Questionnaire results show that most people who recalled a tick bite were from Selenge (74%) and Bulgan (12%) provinces (Figures1, 2, and 3). In addition, tick bite data from other provinces (Uvs, Uvurkhangai, Tuv, Orkhon, Darkhan-Uul and Sukhbaatar) where were added during past two years.

2. Seroprevalence rate of TBE among the population

A total of 2,182 people from 56 soums among Arkhangai, Bayankhongor, Bulgan, Gobi-Altai, Dornod, Uvurkhangai, Umnugobi, Sukhbaatar, Khuvsgul, Khentiiand Selenge provinces and 3 districts of Ulaanbaatar city involved. Approximately 318 (14.6±10.9%) of these people from 47 soums of 10 provinces and 1 district of Ulaanbaatar city had detectable of IgG anti-TBEV. Seroprevalence rates of TBE among each province are described (Table1).

The seroprevalence rate of TBE among the populations who live in provinces where the main vector tick species Ixodes persulcatusis is found (Selenge, Bulgan, and Khuvsgul provinces) was 1.8 times (CI 95% 1.5-2.3, p< 0.0001) higher than other provinces where thetick species *Dermacentor nuttalli* and *Dermacentor silvarum* are most common (Table 2).

3. Tick infection rate of TBE

According to the surveillance efforts, 10,464 tick swere collected. Following species identification, 14.7% (1,540) were classified as Ixodes persulcatus, 79.3% (8,300) were *Dermacenter nutalli,* 3.2% (341) were Dermacenter silvarum, and 2.8% (283) were *Hyalomma asiaticum* (Figure 4).

I*xodes persulcatu* stick was collected from 13 soums of Selenge, Bulgan, Orkhon, Darkhan-Uul, Khentii and Khuvsgul provinces. Mostcases were found in Selenge (66%) and Bulgan (23%) provinces. The total tick infection rate was 3.18±2.5%

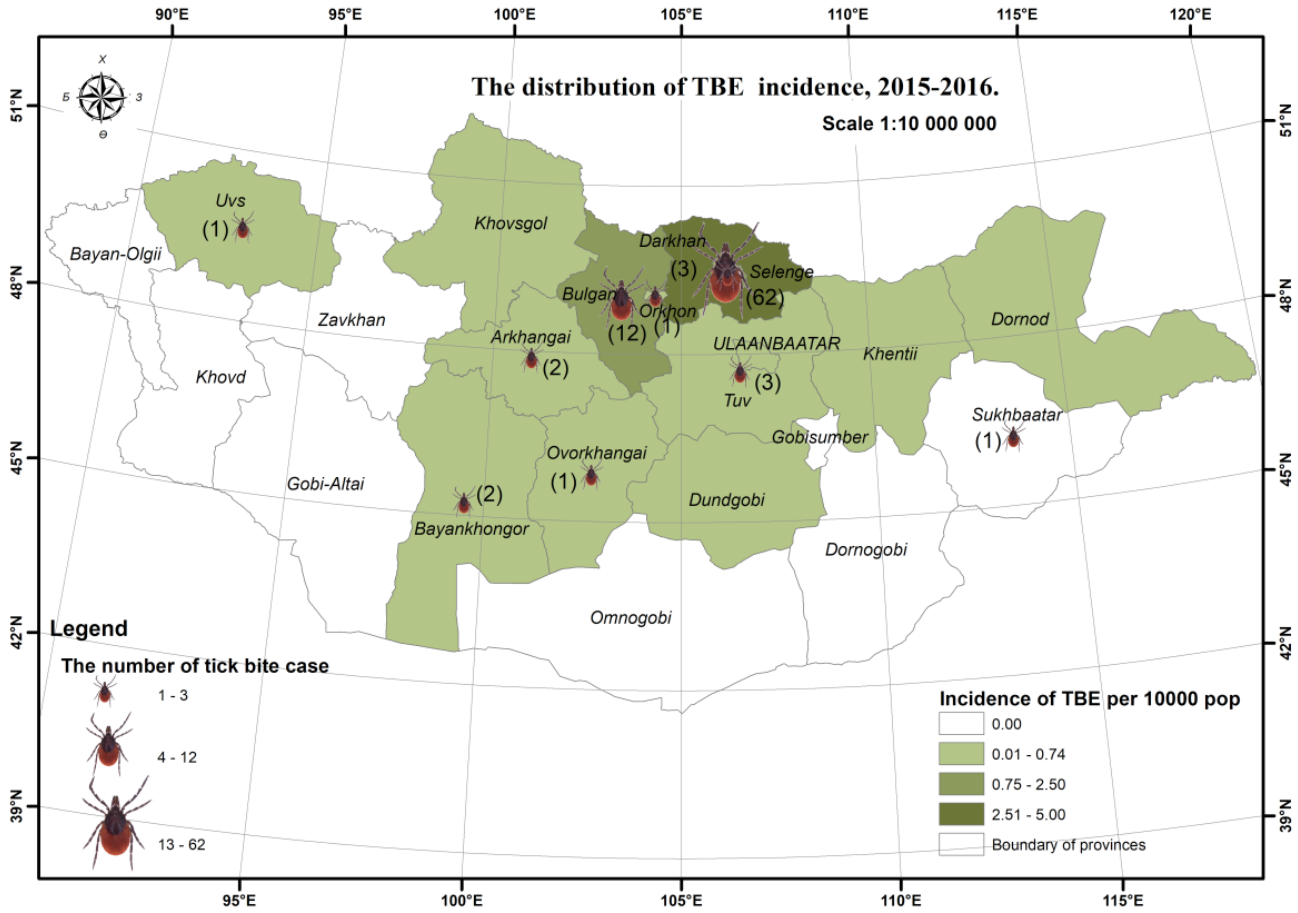

Figure 3. TBE Incidence rate and tick bites in Mongolia 2015-2016.
The tick symbol designates location of tick bite.

Table 1. Seroprevalence of TBE in Mongolia, 2005-2016

Provinces	Soum number	Seroprevalence, %	Mean±SD
Arkhangai	4	11.3(9/80)	7.1
Bayankhongor	1	2.7(2/75)	-
Bulgan	6	13.9(20/144)	11.4
Gobi-Altai	2	0 (0/22)	0
Dornod	8	14.7(33/224)	9.1
Uvurkhangai	10	20.9(27/129)	11.7
Umnugobi	3	2.5(6/239)	-
Sukhbaatar	2	3.7(2/54)	-
Khuvsgul	3	11.1(20/180)	6.8
Khentii	9	14.1(20/142)	9.2
Selenge	8	20.8(178/856)	13.2
Ulaanbaatar	3	3.7(1/27)	3
Total	59	14.6(318/2172)	10.9

Table 2. Seroprevalence of population by home to the main tick specie

Abundant tick	Provinces	Number of positive of IgGanti-TBEV of the population	Number of negative of IgG anti-TBEV of the population	RR (95 CI%)	p-value
I.persulcatus,	Selenge,Bulgan, Khuvsgul,	218	962	1.8 (1.5-2.3)	0.0001
D.Nutalli D.silvarum	Arkhangai, Dornod Bayankhongor, Gobi-Altai, Uvurkhangai, Umnugobi, Sukhbaatar, Ulaanbaatar	100	892		

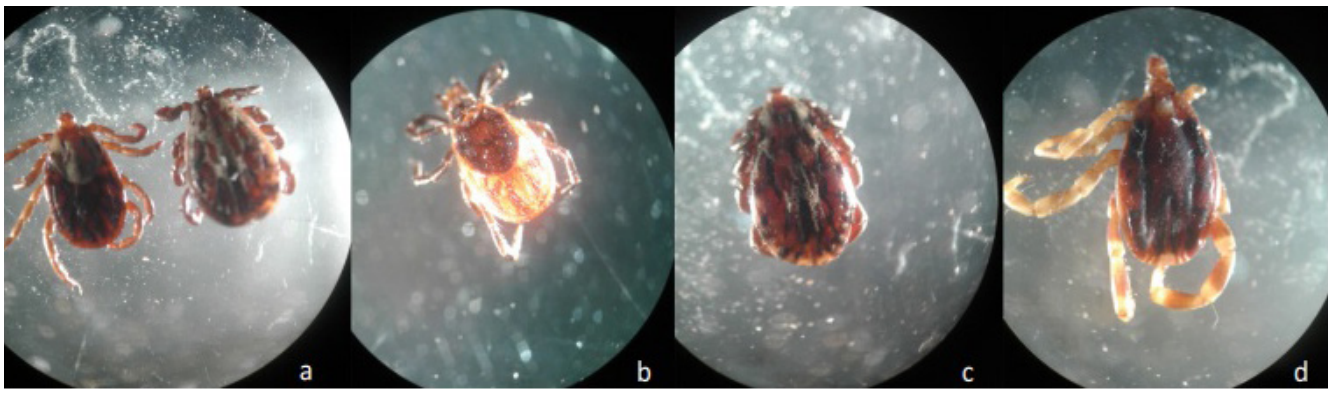

Figure 4. Ticks photo by loupe microscope. a: *D.Nutalli* from Ulaanbaatar; b: *I.Persulcatus* from Selenge; c: *D.Silvarum* from Dornod; d: *H.Asiaticum* from Umnugobi provinces.

and the highest infection rates were found in Bugat soum of Bulgan Province (7.5%) and in the Mandal soum (6.3%) and Khuder soum (3.75%) of Selenge province.

Dermacentor nuttalli ticks were collected from 43 soums of Arkhangai, Bayankhongor, Bayan-Ulgii, Gobi-Altai, Dornod, Dundgobi, Uvurkhangai, Umnugobi, Khuvsgul, Khentii, Selenge, and Zavkhan provinces and 3 districts of Ulaanbaatar city. The most ticks were found in Khuvsgul (47%), Umnugobi (11%), and Khentii (10%) provinces. The total tick infection rate for the entire country was 0.61% with the highest infection rates in Khuder soum (8.1%) of Selenge province, Chuluut soum (7.95%) and Ikhtamir soum (3.75%) of Arkhangai province, and Bayandun soum (6.76%) of Dornod province. No TBE virus was detected in the ticks from Bayankhongor, Bayan-Ulgii, Gobi-Altai, Dundgobi, Khovd, and Zavkhan provinces and Ulaanbaatar city.

Dermacentor silvarum ticks were collected from Dornod and Khentii provinces and the tick infection rate was 2.9±2.6%. *Hyalomma asiaticum* ticks were found in Umnugobi, but they were not infected with TBE virus (Table 3).

Discussion

As far as we know, our study is the most extensive investigation to date of TBE among the Mongolian population using serological methods. In 400 human serum samples from Khuvsgul, Khentii, Bulgan, Selenge, Orkhon, Tuv, Dornod, Arkhangai, and Uvurkhangai provinces, the other researchers found 4.2% (16) of the population had detectable IgG anti-TBEV [9, 10]. Several studies of tick-borne encephalitis have been conducted since then and have mostly focused on the northern parts of the country, where the main vector I*xodes persulcatus* is abundant. According to another sero survey among the population of Selenge province, the prevalence was 8% in Bugant, 6% in Khuder, and 2.1% in Zelter soums with higher levels in Khuder 26%-29.9% and Bugant 16.4%-39% years later in 2001 [11-13]. In 2003, 10% of tested residents of Khuder soum in the northern part of Selenge province had neutralizing antibodies against TBE virus [14]. Between 1998-2004, 12.5% (153/1221) of participants from 20 soums of 7 provinces had detectable TBE

Table 3. TBE infection rate of tick species collected in Mongolia, 2005-2016

Provinces	*I.persulcatus*	*D.nutalli*	*D.silvarum*	*H.asiaticum*
Arkhangai	-	28/452 (6.2%)	-	-
Bayan-Ulgii	-	0(0/165)	-	-
Bayankhongor	-	0(0/247)	-	-
Bulgan	10/357 (2.8%)	-	-	-
Gobi-Altai	-	0(0/636)	-	-
Dornod	-	3/163 (1.8%)	10/221 (4.5%)	-
Dundgobi	-	0(0/90)	-	-
Orkhon	1/94 (1.1%)	-	-	-
Darkhan	0(0/70)	-	-	-
Zavkhan	-	0(0/192)	-	-
Uvurkhangai	-	2/201 (1%)	-	-
Umnugobi	-	3/877 (0.3%)	-	0/283
Khovd	-	0/79	-	-
Khentii	0(0/1)	8/828 (1%)	0/120	-
Selenge	37/1015 (3.6%)	3/48(6.3%)	-	-
Khuvsgul	1/4 (25%)	3/3897 (0.1%)	-	-
Ulaanbaatar	-	0/402	-	-
Total	49/1540 (3.18)	51/8300 (0.61)	10/341 (2.9)	0/283

Data presented number of positive and all tested ticks and percentage of TBE infection.

antibodies [15,16]. In our study, neutralizing antibodies against TBE virus were found in 14.6±7.3% of the population from 47 soums of 10 provinces and Ulaanbaatar city. This demonstrates an increasing seroprevalence rate of TBE among the Mongolian population between 2005-2016 years. However, this could be due to the larger number of soums and provinces included in the current study compared to previous research.

The TBEV is transmitted to humans during the bite of several species of infected ticks, including *Ixodes scapularis, Ixodes ricinus* and I*xodes persulcatus* [17]. In Mongolia, *Ixodespersulcatus* tick was found in Selenge, Bulgan, Khentii, Tuv, Khuvsgul and Orkhon provinces. Testing for tick infection only detected TBE from Selenge province (Baruunburen 11.1%, Zelter 16.7%, Mandal 6%, Bugant 6.14%, Khuder 4.68%,

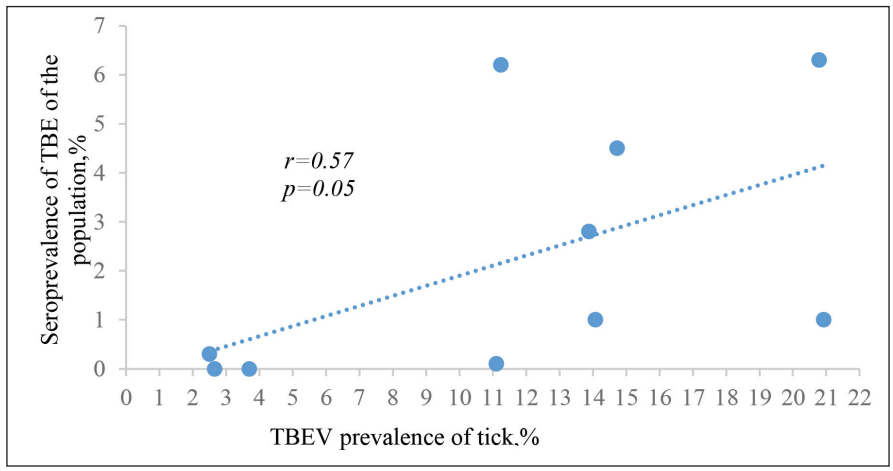

Figure 5. Correlation of the seroprevalence of TBE of the population and TBEV prevalence of tick in provinces.

and Eruu 2.4% soums) and the average prevalence was 3.4% among *Ixodes persulcatus* ticks in the forested area in 2005 year [18]. In our study, *Ixodes persulcatus* tick was found from Selenge, Bulgan, Orkhon, Khuvsgul, and Darkhan-Uul provinces. Infected ticks were detected in 10 soums of Selenge, Bulgan, Khuvsgul, and Darkhan-Uul provinces. The average infection rate of the *Ixodes persulcatus* tick was 3.18±2.5% in forested areas where the main vector is abundant. This research shows that the prevalence of infected ticks is the same as previous years. However, the seroprevalence rate among the human populations who live in Selenge, Bulgan, and Khuvsgul provinces is higher than other provinces and 88% of patients recall a tick bite from those areas (Selenge, Bulgan, Darkhan-Uul, Orkhon, Khuvsgul provinces) when traveling or visiting in endemic areas for reasons such as collecting pine nuts, fruits, herbs, gathering wood and activities related to the mining in dustry. The dominant vector of TBE in forested areas of Selenge, Bulgan, Darkhan-Uuland Orkhon, Khuvsgul provinces, where TBE is still highly endemic, remains the Ixodespersulcatus tick.

However, the distribution of TBE is expanding and the number of provinces where infected ticks are found is increasing. Of concern, the TBE virus was detected in the *Dermacentor nuttalli* tick, which is typically not considered a vector of TBE. However, provinces where the *Dermacentor nuttalli* tick is adundant are registering TBE cases in patients that have a history of a tick bite. Therefore, we consider *Dermacentor nuttallia* likely additional vector of TBE in Mongolia.

A recent study in the Altai Republic shows that changes to the leading species of tick occurs at 1,700 meters above sea level where becomes the dominant species. However, this tick species is a less effective vector than the *Ixodespersulcatus* species. Yet the TBE virus prevalence among *Dermacentorspp (Dermacentor nuttalli, Dermacentor silvarum, or Dermacentor reticulatus*) ticks was significantly higher ($p<0.001$) than that of *Ixodes persulcatus* ticks [19].

In addition, the main tick vector species in Northern China is *Ixodes persulcatus* while in Southern China *Ixodesovatus* has proven to be a vector in rare cases and *Dermacentor silvarum* has also been identified as a carrier of TBEV [20, 21]. In our study, TBE infection was detected from 2.9% of Dermacentorsilvarum ticks with a higher seroprevalence rate of TBE among the tick populations of Dornod and Khentii provinces. But very few human cases of TBE have been detected in these provinces and it was often unclear which province they were in when they were been bitten. Over the last few years, reports of human TBE cases have been increasing and are being registered in new areas such as Uvurkhangai, Dundgobi, Sukhbaatar and Uvs provinces. Seropreavalence studies have shown that people of the southern parts of Mongolia have a high prevalence of TBE. But western parts of Mongolia have not detected TBE among their populations and no TBE virus was detected from ticks from this region.

Our study has limitations. First, in some provinces only a small number of people and ticks were tested. Second, some of TBE cases did not recall the area of their tick bite and patients did not know what species of tick bit them. Third, we were unable to analyze the potential for the *Dermacentor silvarum* species to become an additional vector as human cases registered to this tick were rare, despite the fact that TBEV was detected in high rates from this tick. We recommend that in future studies, researchers should clarify the geographic area in which the tick bite occurred from patients and focus on collecting more samples to detect which province the disease originated. TBE studies should also focus in southern part of Mongolia.

In conclusion, the endemic area of TBE is expanding to the steppe area, where the vector *Dermacentor nuttalli* is abundant while northern parts of the country remain endemic with the main tick vector being *Ixodes persulcatus.* Seroprevalence studies and surveillance efforts to identify TBE in human and tick populations should continue, especially in southern parts of Mongolia such as the Gobi area. Additional studies are necessary to reveal the changing ecological relationship and climate impact of tick-borne encephalitis across the many unique ecosystems of Mongolia and to better understand how to address this public health issue to prevent future infection.

Conflict of Interest

The authors state no conflict of interest.

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