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Original article

Influence of altitude on tick-borne encephalitis infection risk in the natural foci of the Altai Republic, Southern Siberia

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ABSTRACT

The Altai Republic is a highly endemic area as far as tick-borne encephalitis (TBE) is concerned. The aim of the research was to study the effect of altitude on the risk of tick-borne encephalitis infection in the Altai Republic. The paper analyzes the following data: the study of ixodid ticks collected from the vegetation in 116 sites at the 200–2383 m elevation above sea level in 2012–2014, TBE virus prevalence of these vectors, tick-bite incidence rate, and TBE incidence rate of the population.

Species identification of 4503 specimens has shown that the most common species are *Dermacentor nuttalli* (45.3%), *Ixodes persulcatus* (33.1%), *Dermacentor silvarum* (9.4%), *Dermacentor reticulatus* (8.9%), and *Haemaphysalis concinna* (5.0%).

A total of 2997 adult ixodid ticks were studied for the presence of the TBE virus; 2163 samples were examined by ELISA, while 834 specimens were tested by PCR. The TBE virus prevalence of *Dermacentor* spp. ticks in both reactions was significantly higher than of *Ixodes persulcatus* ticks ($p < 0.001$).

The work shows that the altitude is an important factor in the development of the epidemiological situation of tick-borne encephalitis: the higher the elevation of the area above sea level, the smaller the range of vectors. There is also a change of a leading species: in middle altitude (800–1700 m above sea level) the virus is transmitted by ticks of *D. nuttalli* along with *I. persulcatus*, and in high mountains (above 1700 m above sea level) *D. nuttalli* becomes an absolute dominant species. However, these species of ticks are less effective vectors than *I. persulcatus*. With the increase of altitude the tick-bite incidence rate decreases ($r = -0.78$, $p < 0.05$), and TBE incidence also reduces ($r = -0.67$, $p < 0.05$).

Introduction

Russia possesses the most extensive geographic distribution of tick-borne encephalitis. It is believed that the intensity of the natural TBE foci determines the high abundance of the main vector species *I. ricinus* and *I. persulcatus* and their infection rate (Korenberg and Kovalevskij, 2000). The distribution of these tick species has been well studied. In recent years, some investigations have been focused on the positive effect of continuous temperature rise and humidity on the improvement of habitat conditions for ticks (Süss, 2008). In mountainous areas of Central Europe, ticks have been recorded at higher altitudes up to 1100 and 1300 m above sea level (a.s.l.) compared to the cases recorded before 1990 (at 1000 m a.s.l.) (Danielová et al., 2006, 2008; Materna et al., 2008; Daniel et al., 2009). Moreover, some cases of alimentary TBE infection caused by the consumption of nonpasteurized milk of goats, which were grazing in pastures at 1500 m a.s.l., were recorded in Austria (Holzmann et al., 2009).

In Russia, *I. persulcatus* was recorded at 1400 m a.s.l. on the northern slopes of the Western Sayan, in the Central and Eastern Altai (at 1900 m a.s.l.) and Tian Shan (at 3000 m a.s.l.) (Rubina, 1969; Sapegina, 1980; Grebenyuk, 1966).

Besides *I. ricinus* and *I. persulcatus*, other tick species can be additional vectors for tick-borne encephalitis virus (TBEV) in some areas (Danchinova et al., 2006; Verhozina et al., 2008; Zlobin, 2010). Their role in the natural TBE foci is still unclear, as well as the altitude influence on their density.

The Altai Republic is one of the areas where along with *I. persulcatus* as a dominant vector at the natural foci of TBE, there are other species of ixodid ticks. This is a mountain terrain in the south of Western Siberia, located on a junction of the Siberian taiga, Kazakh steppes, and semi-deserts of Mongolia that determines a

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surprising diversity of landscapes. Settlements are situated at 200–2200 m a.s.l. The size of the republic area is 92,903 km^2. The population is 210,344 people. The length of the terrain from the north to the south is 400 km, and from the west to the east is 360 km. Average annual temperatures in Gorny Altai fluctuate within the limits of +4° (in northern and western suburbs) to −7° (in high-mountainous regions). The fauna of the Altai Republic includes 93 species of mammals and 312 species of birds. The abundance of ixodid ticks and their widespread presence are attributed to a large number of hosts and diversity of landscapes in this territory.

Along with natural factors, social factors contribute to the spread of the TBE virus. The mountain landscape predetermines cattle-breeding in the Altai Republic. Crop lands occupy only 1.5% of the republic, whereas the largest part of agricultural lands belongs to grazing – 84.7%. Cattle breeding industry is one of the primary sources of income for the agricultural population. According to the report of the Territorial Agency of the Federal Service of State Statistics on January, 1st, 2014 in the Altai Republic the farms of all categories comprised 245,000 heads of cattle, 11,900 pigs, 477,500 sheep, 149,400 goats and 136,200 horses. Domestic animals are active hosts for ixodid ticks, sustaining a high number of vectors, and also carry ticks into settlements. The average yearly TBE incidence of the Altai Republic between 2004 and 2013 was 22.6 per 100,000 inhabitants which is almost 10 times higher than TBE incidence of Russia. However, disease levels in different districts of the Altai Republic differ considerably.

The aim of this work was to assess the influence of altitude on a tick-borne encephalitis infection risk in the natural foci of the Altai Republic.

Materials and methods

Tick collection

Questing adult ticks were collected by flagging the vegetation every 8–12 km along the highways of the Altai Republic during the peak of tick activity (from mid April till early June of 2012–2014). The sampling sites were located 30–300 m from the motorways. The preference was given to the places where earlier there had been cases of TBE infections, that is, to the tourist camps, popular vacation and recreation spots in the vicinities of settlements, and to the scenic view points at mountain passes. A total of 4503 ixodid ticks were collected in 116 sites in all districts of the Altai Republic.

Ticks were identified using a light microscope MBS-10 (LOMO, St. Petersburg) and Identification Keys (Filippova, 1977, 1997).

The abundance of ixodid ticks (an average number of ticks collected by 1 person for 1 h) was defined for each place, for each district, and for the Altai Republic as a whole.

The areas, where ticks were collected, were situated at the 200–2383 m elevation above sea level. They are considered to be lowlands (< 800 m a.s.l.), midlands (800–1700 m a.s.l.), and highlands (>1700 m a.s.l.).

Detection of TBEV

Two methods – ELISA and PCR were analyzed the ixodid ticks for TBEV presence individually.

ELISA was used to study 2163 questing adult ticks collected from 116 sites. TBEV antigens were detected with the help of the test systems produced by CJSC "Vector-Best" (Novosibirsk city, Russia) according to the manufacturer's recommendations. The absorbency measurement was carried out on a plan-table photometer "Uniplan" (manufactured by the company of "Pikon", Russia) the length of a wave is 450 nm.

In addition, 834 questing adult ticks collected from 27 sites were tested by PCR. Total RNA was extracted from ixodid ticks using the "AmpliPrime®RIBO-prep-100" kit ("AmpliPrime", Russia).

The reverse transcription was performed with "RevertaL-100" kit containing random hexanucleotides ("Amplisence", Russia).

PCR reaction was performed in 20 μl of the reaction mixture containing 67 mM Tris–HCl (pH 8.9), 16.6 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.01% Tween 20, 200 μM of each dNTP, 5% glycerol, 0.5 μM primers, 2 U of Taq DNA polymerase ("BioSan", Russia) and 3 μl of cDNA for primary reactions or 1 μl of the primary PCR products for nested reaction. The PCR conditions comprised initial denaturation for 3 min at 94 °C followed by 35 cycles of denaturation for 0.5 min at 94 °C, annealing for 0.5 min at 48 °C and elongation for 1 min at 72 °C. The primers corresponding to positions 2199–2219 and 2517–2539 nr or positions 2214–2238 and 2402–2424 nr of TBEV genome were used for primary or nested reactions, respectively. The length of final PCR-products was 211 bp.

Epidemiological data

The analysis of the TBE incidence rate of the population in the Altai Republic (during 2004–2013) was done according to the state statistical reporting: Form # 2 "Information on Infectious and Parasitic Diseases", Form # 60 "Register of Infectious Patients", Form # 003/U "Medical Record of the Inpatient", Form # 025/U "Medical Record of the Outpatient". We also analyzed TBE incidence using data from epidemiological records ($n = 2183$).

All cases of tick bites have been registered in the Russian Federation. We analyzed the tick-bite incidence rate per 100,000 inhabitants in the Altai Republic for the period of 2013.

The information on a number of the livestock was obtained from the reports of the Territorial Agency of the Federal State Statistics Service in the Altai Republic (http://statra.gks.ru).

Statistic analysis

For the graphic illustrations of the material and statistical data processing the following Microsoft programs were applied: STATISTICA-6.1 and the systems of spreadsheets of Microsoft Excel. The analysis was carried out according to the standard methods of biological statistics (mean value and standard error, regression and correlation analyses. The difference between the two groups for mean values was assessed with the help of the Student's *t*-test. To identify the relations between variables, the Pearson's coefficients of linear correlation were used. The critical level of significance when testing statistical hypotheses, $p = 0.05$.

Results

Sampling of ixodid ticks performed at 116 sites in 2012–2014 showed that in the Altai Republic there are 8 species. The most common species in this area are *Dermacentor nuttalli* – 45.3%, *Ixodes persulcatus* – 33.1%, *Dermacentor silvarum* – 9.4%, *Dermacentor reticulatus* – 6.9%, *Haemaphysalis concinna* – 5.0%. Other tick species occurred more rarely: *Ixodes pavlovskyi* – 0.09%, *Dermacentor marginatus* – 0.18%, *Haemaphysalis pospelovashtromae* – 0.02%.

The tick collection has revealed a wide distribution of ixodid ticks: they have been found in all of 10 districts of the Altai Republic and even within the boundaries of Gorno-Altaisk city. The biotopes, where ticks were collected, are classified according to elevation into 3 groups: lowlands (<800 m a.s.l.), midlands (800–1700 m a.s.l.), and highlands (>1700 m a.s.l.). The obtained information showed that the range of species was the most diverse in lowlands (Table 1).

The division of districts into 3 groups according to the altitude is a little conditional. For example, the level of elevation in the Shebalinsky district varies from 400 m up to 1100 m a.s.l. Hence,