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Original article

Tick-borne encephalitis virus in arthropod vectors in the Far East of Russia

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ABSTRACT

Isolates of tick-borne encephalitis virus (TBEV) from arthropod vectors (ticks and mosquitoes) in the Amur, the Jewish Autonomous and the Sakhalin regions as well as on the Khabarovsk territory of the Far East of Russia were studied. Different proportions of four main tick species of the family Ixodidae: *Ixodes persulcatus* P. Schulze, 1930; *Haemaphysalis concinna* Koch, 1844; *Haemaphysalis japonica douglasi* Nuttall et Warburton, 1915 and *Dermacentor silvarum* Olenev, 1932 were found in forests and near settlements. RT-PCR of TBEV RNA in adult ticks collected from vegetation in 1999–2014 revealed average infection rates of 7.9 ± 0.7% in *I. persulcatus*, of 5.6 ± 1.0% in *H. concinna*, of 2.0 ± 2.0% in *H. japonica,* and of 1.3 ± 1.3% in *D. silvarum.* Viral loads varied in a range from 10^2 to 10^9 TBEV genome-equivalents per a tick with the maximal values in *I. persulcatus* and *H. japonica*. Molecular typing using reverse transcription with subsequent real time PCR with subtype-specific fluorescent probes demonstrated that the Far Eastern (FE) subtype of TBEV predominated both in mono-infections and in mixed infection with the Siberian (Sib) subtype in *I. persulcatus* pools. TBEV strains of the FE subtype were isolated from *I. persulcatus*, *H. concinna* and from a pool of *Aedes vexans* mosquitoes. Ten TBEV strains isolated from *I. persulcatus* from the Khabarovsk territory and the Jewish Autonomous region between 1985 and 2013 cluster with the TBEV vaccine strain Sofjin of the FE subtype isolated from human brain in 1937. A TBEV strain from *H. concinna* collected in the Amur region (GenBank accession number KF880803) is similar to the vaccine strain 205 isolated in 1973 from *I. persulcatus* collected in the Jewish Autonomous region. The TBEV strain Lazo MP36 of the FE subtype isolated from a pool of *A. vexans* in the Khabarovsk territory in 2014 (KT001073) differs from strains isolated from 1) *I. persulcatus* (including the vaccine strain 205) and *H. concinna*; 2) mosquitoes [strain Malishevo (KJ744034) isolated in 1978 from *Aedes vexans nipponii* in the Khabarovsk territory]; and 3) human brain (including the vaccine strain Sofjin). Accordingly, in the far eastern natural foci, TBEV of the prevailing FE subtype has remained stable since 1937. Both Russian vaccines against TBE based on the FE strains (Sofjin and 205) are similar to the new viral isolates and might protect against infection.

1. Introduction

Tick-borne encephalitis (TBE) is the most important flavivirus infection of the central nervous system in Eurasia (Gritsun et al., 2003; Mansfield et al., 2009; Bakhvalova et al., 2016 and references therein). The number of TBE cases periodically changes with spread of its etiological agent, TBEV, to previously non-endemic countries in Europe (France) and Asia (Mongolia, Japan, and South Korea) (Gritsun et al., 2003; Mansfield et al., 2009). TBEV is listed as a category C agent on the CDC list of potential bioterrorism agents (Rotz et al., 2002).

TBEV persists in natural foci where it circulates among vertebrates (mainly small rodents and insectivorous) and arthropod hosts (principally ticks), such natural foci are very stable. Formation, development, and stability of TBE natural foci are determined by the coincidence of several ecological factors including temperature, relative humidity of air and soil, vegetation of the biotope, dynamics of population densities of ixodid ticks and their hosts along with their susceptibility to the virus, and prevalence of immune hosts and virus (Korotkov et al., 2007;

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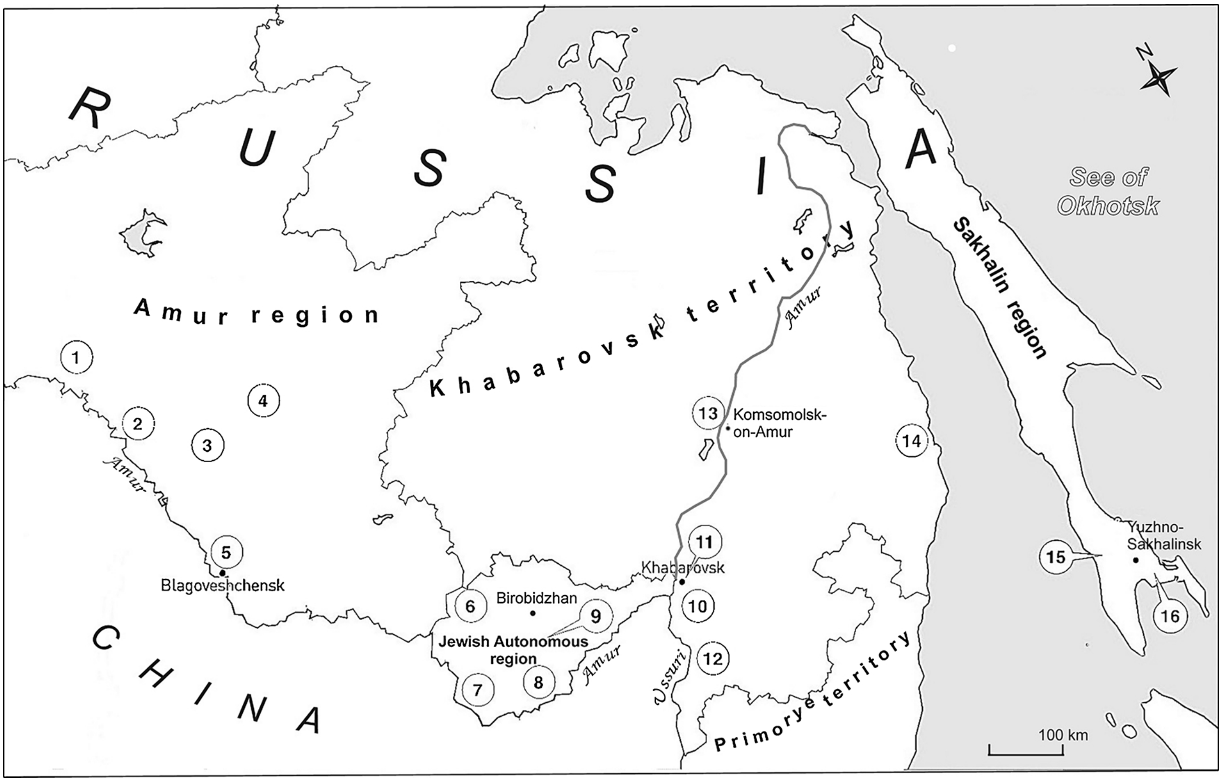

Fig. 1. Map of the arthropod collection places in the Far East of Russia.

Tick-Borne Encephalitis (TBE) and its Immunoprophylaxis, 1996; Mansfield et al., 2009; Bakhvalova et al., 2006, 2016 and references therein). Reservoir host species abundance, multiple transmission cycles, and adaptation of TBEV to different hosts facilitate long-term stability of the parasitic system (Mansfield et al., 2009; Bakhvalova et al., 2006, 2016 and references therein).

Ticks are ideal vectors of TBEV due to their ability to feed on a variety of vertebrates, intracellular digestion of blood, and their long life cycle. TBEV infection has been documented in 16 species of ixodid ticks in natural foci of Eurasia (Korenberg, 1989; Tick-Borne Encephalitis (TBE) and its Immunoprophylaxis, 1996; Mansfield et al., 2009; Bakhvalova et al., 2016). In the Far East of Russia, 22 hard tick species of the family Ixodidae have been identified among them *I. persulcatus*, *H. concinna*, *H. japonica*, *D. silvarum* predominate. In addition, *Ixodes pavlovskyi* Pomerantsev, 1946; *Ixodes maslovi* Emelyanova and Kozlovskaya, 1967, *Haemaphysalis phasiana* Saito, Hoogstraal & Wassef, 1974 have occasionally been observed (Volkov, 2005). Seasonal activity of the adult ixodids begins at soil temperature above 10 °C (Filippova, 1985). In the Far East of Russia this temperature regime is observed from April to October. First mature ixodid ticks appear on thawed patches of the Khabarovsk territory and the Jewish Autonomous region in the middle of April. The maximum number of active ticks is annually observed in May and June with a subsequent gradual decline until the end of September or beginning of October (Volkov, 2005). One should note that the peaks of activity of various tick species in the Khekhtzir mountains differ. For *I. persulcatus* and *H. japonica* the peak is May–June, for *D. silvarum* it is March–April. In contrast the number of *H. concinna* remains low during the entire season (Volkov, 2005).

On Sakhalin Island three species have been reported: 1) *I. persulcatus* in the southern part; 2) *H. concinna* in a few samples from vegetation; and 3) *Ixodes angustus* Neumann, 1899 immature ticks (larvae and nymphs), collected from wild small mammals (Pukhovskaya et al., 1991).

TBEV as a member of the family Flaviviridae possesses a single-stranded, positive-sense genomic RNA of approximately 11 kb (Gritsun et al., 2003; Mansfield et al., 2009). Molecular typing of TBEV using ELISA, molecular hybridization with radioactive oligonucleotide probes, complete genome sequencing, and phylogenetic analysis reveals three main intra-species subtypes (Pogodina et al., 1981; Zlobin et al., 1996; Ecker et al., 1999; Bakhvalova et al., 2000), namely the Far Eastern (FE) subtype, the Siberian (Sib) subtype, and the European (Eur) subtype with 15.2–16.4% nucleotide changes and 6.2–6.9% differences in amino acid sequences (Kozlova et al., 2013). The FE subtype includes mainly isolates from the Far East of Russia, China, and Japan. The Sib subtype formerly included isolates from Eastern and Western Siberia, Urals. At present it is the dominant subtype in many endemic regions of Russia and surrounding countries, gradually replacing the other TBEV subtypes (Pogodina et al., 1981; Bakhvalova et al., 2006, 2016). The Eur subtype comprises almost all known isolates from Europe (Gritsun et al., 2003; Mansfield et al., 2009). Besides the three conventional subtypes, 178–79 and "886–84 group" strains have been proposed as fourth and fifth TBEV subtypes, respectively (Kozlova et al., 2013). Any of the conventional subtypes can be found in a given geographic area (Zlobin et al., 1992).

The three main TBEV subtypes are associated with varying degrees of disease severity. Human infections with the FE subtype are usually severe, frequently have encephalitic symptoms, and carry a case fatality rate of 5–35% (Gritsun et al., 2003; Mansfield et al., 2009). The Sib subtype causes a less severe disease (fatality rate of 1–3%), with a tendency for patients to develop chronic infections (Gritsun et al., 2003; Mansfield et al., 2009). Infections caused by the Eur subtype typically take a biphasic course: the first (viremic) phase presents with fever, often followed by a symptom-free interval, and the second phase in 20–30% of infected patients is characterized by meningitis, meningoencephalitis, meningo-encephalo-myelitis or meningo-encephalo-radiculitis, and a fatality rate of less than 2% (Gritsun et al., 2003; Mansfield et al., 2009). Accordingly, changes in the proportions of the TBEV subtypes could have significant implications for public health. Therefore, the geographic distribution of the TBEV subtypes in natural