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Tick-Borne Encephalitis in the Russian Caucasus: Virus Was Detected for the First Time on the Border of Europe and Asia Minor

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Abstract

The tick-borne encephalitis (TBE) virus has been confirmed by molecular analysis in the Caucasus region for the first time. The virus obtained from a tick *Ixodes ricinus* ex Caspian green lizard belongs to the Zausaev strain of the Siberian subtype (not to a strain of the European subtype highly distributed in the territories adjacent to the Caucasus). This unusual record indicates the need to study the role of lizards in the circulation of natural focal infections.

Keywords: tick-borne encephalitis, *Lacerta strigata*, *Ixodes ricinus*, Caucasus, Zausaev strain

TICK-BORNE ENCEPHALITIS VIRUS (TBEV) is a medically important pathogen of the genus *Flavivirus*, family *Flaviviridae*. It is classified as one species with five subtypes, three of them, namely the European subtype, the Siberian subtype, and the Far Eastern subtype, inhabit Russia. The principal vector as well as reservoir of the European TBEV subtype is the ixodid tick *Ixodes ricinus* (Parasitiformes: Ixodida), and *Ixodes persulcatus* for the other two subtypes. TBEV is the causative agent of tick-borne encephalitis (TBE), a severe natural focal infection occurring in the Palearctic region mainly transmitted through *Ixodes* ticks (Filippova, 1977; Heyman et al., 2010).

In Russia, TBE is endemic in districts throughout much of the country. Most of the eleven cases of TBE described between 2011–2021 were imported (Nikitin et al. 2022).

Despite many years of large-scale epidemiological and virological research of people, livestock, and vectors (ticks,

mosquitoes), entire regions still remain unanswered to the question of whether TBEV circulation exists in them. Among them, the Caucasus is a large territory that unites several regions, republics, and states.

Single cases of TBE in humans were recorded in Kabardino-Balkaria Republic (Korenberg and Kovalevskiy, 1981), in the Republic of Dagestan (Kulichenko, private message), and in Stavropol Krai (Nikitin et al., 2022). Antibodies against TBEV have been found in the human population of the Krasnodar Krai by methods of passive hemagglutination reaction and complement fixation reaction (12.5%—Korenberg and Kovalevskiy, 1981), Stavropol Krai by method of enzyme-linked immunosorbent assay (ELISA) (1.6%—Vasilenko et al., 2013), and in the population of Georgia also by ELISA method (7% of hospitalized patients with acute febrile illness from 2008 to 2011—Kuchuloria et al., 2016). TBEV could not be detected so far in Armenia

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(Mnatsakanyan et al., 2022). Early studies of human and bovine sera for TBE by hemagglutination inhibition method in Azerbaijan (Ismailova et al., 1969) were also inconclusive.

During epizootiological monitoring in the south of Russia over the past few years, the circulation of the TBEV was recorded in ixodid ticks in the Krasnodar Krai (*Hyalomma marginatum*, *Hyalomma scupense*, *Dermacentor marginatus*, *Dermacentor reticulatus*) in the Republic of Dagestan (*H. marginatum*, *H. scupense*, *Boophilus annulatus*), *I. ricinus*, *Rhipicephalus sanguineus*, *Rhipicephalus bursa*, *Dermacentor niveus*, and *D. reticulatus* and *Haemaphysalis inermis*) and in Rostov Oblast (*H. marginatum*) (Kulichenko, private message). Antigen of TBEV has been detected in seven tick species in Stavropol Krai: *Dermacentor marginatus* (7.3%), *D. reticulatus* (2.2%), *Haemaphysalis punctata* Canestrini et Fanzago, 1878 (2.5%), *I. ricinus* (2.5%), *H. scupense* (1.7%), *Haem. inermis* (0.5%), and *D. niveus* (0.1%) (Tokhov et al., 2018).

From 2009 to 2012 as a result of the studies, the presence of IgG class antibodies against TBEV was detected in 2.7–4.7% of blood donors in the north and north-west of the Caucasus region (Vodyanitskaya et al., 2013). The detected findings of TBEV antigen in samples of the house mouse *Mus musculus* Linnaeus, 1758 (in 2010), common vole *Microtus arvalis* (in 2014), ixodid ticks *D. marginatus* (in 2013) (Dvortsova et al., 2016), *H. marginatum* (2020) (Kulichenko, private message) and serological studies data indicate the circulation of the TBEV in the Rostov Oblast, despite the fact that the territory is not endemic.

We studied 202 samples of ectoparasites (larvae, nymphs, and females of ixodid ticks belonging to four species: *I. ricinus*, *H. punctata*, *Haem. caucasica*, *H. marginatum*) removed using forceps from reptiles, collected from different years in the Caucasus region and deposited in the Reptile Collection of the Zoological institute RAS (Saint Petersburg,

Russia). Ticks were identified by the author, M.V.O., following Estrada-Peña et al. (2017).

Ticks were prepared and homogenized in 500 μ L of 0.9% sodium chloride solution using stainless steel beads 5 millimeters and TissueLyser II (Qiagen, Germany) for 5 min at 25 Hz. After centrifugation for 1 min at 10,000 rpm, 100 μ L of tick homogenate was harvested for RNA extraction. RNA from tick homogenates was extracted using RIBO-prep nucleic acid extraction kit, cDNA was synthesized using REVERTA-L RT reagents kit (Federal Budget Institute of Science “Central Research Institute for Epidemiology,” Russia) following the manufacturer’s protocols. A 250 bp fragment of the nonstructural protein 5 (NS5) gene of TBEV was amplified from samples using the primer pairs MAMD and cFD2 (Scaramozzino et al., 2001). We performed polymerase chain reaction (PCR) in a volume of 25 μ L using ScreenMix-HS (Evrogen, Russia).

Amplification was conducted at 95°C for 5 min followed by 42 cycles of 95°C for 10 s, annealing temperature 56°C for 20 s and 72°C for 30 s on Bio-Rad MyiQ thermocycler (Bio-Rad). Purified DNA fragments were obtained with Cleanup Standard (Evrogen, Russia) and fragments were subsequently sequenced in both directions using BigDye Terminator v3.1 Cycle and ABI 3500XL genetic analyzer (Applied Biosystems) according to the manufacturer’s instructions. Phylogenetic analyses were conducted using MEGA 7.0.26. The neighbor-joining and the maximum likelihood methods were used to perform the phylogenetic analysis. Bootstrap values were obtained with 1000 replicates. The sequences of Kiasanur Forest Disease Virus and Omsk Hemorrhagic Fever Virus were used as an outgroup.

One specimen of tick, *I. ricinus* (female) ex Caspian green lizard *Lacerta strigata* (Reptilia: Lacertidae) from Stavropol Krai, Sovetskiy district, Otkaznenskoye Reservoir, bank of the Kuma River, 44° 18' N 43° 52' E, 3 VI 2021, leg. I.V.

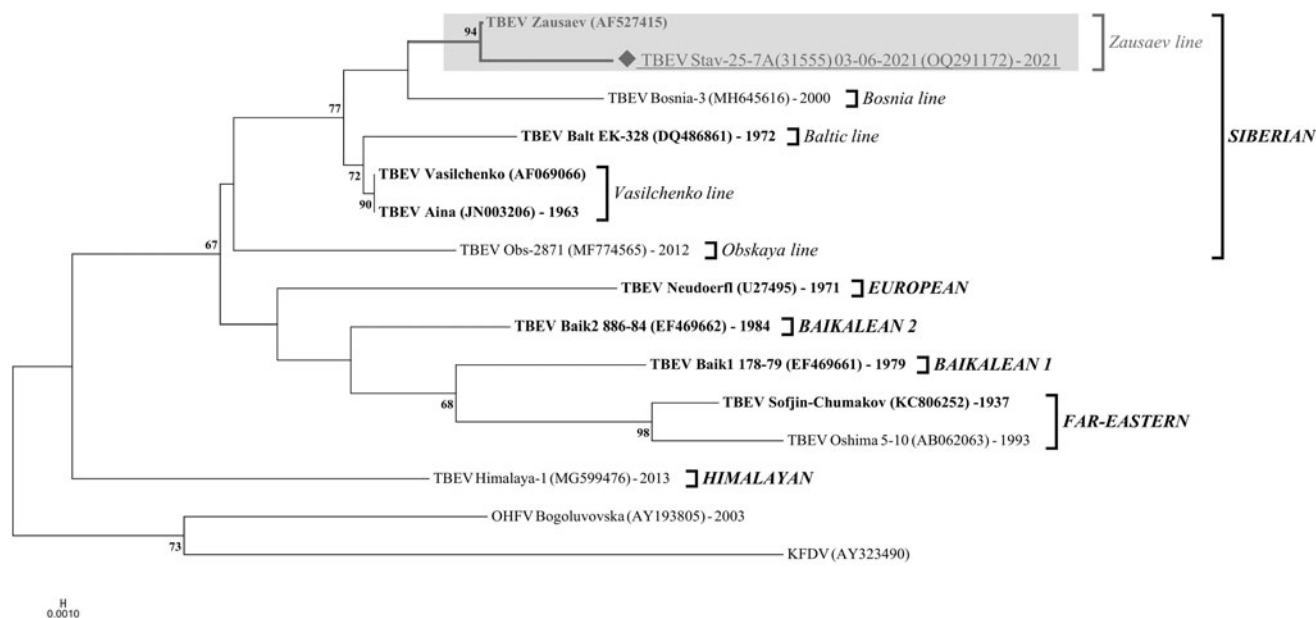


FIG. 1. Phylogenetic analysis of the TBEV NS5-protein gene (coding RdRp) based on a 263 bp fragment. Phylogenetic tree was generated using the maximum-likelihood method with the Kimura 2-parameter algorithm of distance correction, with bootstrapping over 1000 replicates. The prototype strains for subtypes and line of the TBEV are indicated in **bold black font**. NS5, nonstructural protein 5; TBEV, *TICK-BORNE ENCEPHALITIS VIRUS*.

Doronin (ZISP № 31555), was found to be positive by PCR. The sequence obtained during this study and used in the phylogenetic analysis has been deposited in GenBank under the accession number OQ291172, and this is the first TBEV sample from the Caucasus deposited in GenBank. The sequence confirmed the presence of the Zausaev group of the Siberian subtype in Caucasus (Fig. 1). The representative of the Siberian subtype could appear in the Caucasus as a result of drift with migratory birds (ducks, geese, waders, some species of cranes, and gulls), but most likely the Caucasus has its own TBE focus.

Special attention is drawn to the fact that TBE was detected in the tick collected from Caspian green lizard widely distributed in the Caucasus, Elburz, and adjacent territories within Armenia, Azerbaijan, Georgia, Iran, Russia, Turkey, and Turkmenistan. It is a common and abundant species throughout most of its range (Tuniyev et al., 2009). Participation of reptiles in circulation of TBEV remains understudied, but some literary source confirm the possibility of such circulation (Chuprikova, 1964; Grešíková-Kohútová and Albrecht, 1959; Khalansky, Polozhikhina, 1962; Kuranova et al., 2011; Morozov, 1964; Řeháček et al., 1961; Sekeyová et al., 1970). Further studies of the role of reptiles in the circulation of dangerous pathogens are necessary.

Authors' Contributions

Conceptualization, M.V.O.; methodology, M.V.Z. and I.V.V.; investigation, I.V.D.; molecular analysis, V.A.M., N.V.A., and I.V.V.; writing—original draft preparation, M.V.O., M.V.Z., and I.V.D.; writing—review and editing, M.V.O. and V.A.M.; visualization, V.A.M.

Ethical Approval

This report did not involve the use of experimental animals for research. Rather, it contains a summary of a diagnostic investigation conducted by employees from a specific production system who oversee the ethical raising of pigs in commercial facilities.

Author Disclosure Statement

No conflicting financial interests exist.

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