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Comparative analysis of complete genome sequences of European subtype tick-borne encephalitis virus strains isolated from *Ixodes persulcatus* ticks, long-tailed ground squirrel (*Spermophilus undulatus*), and human blood in the Asian part of Russia



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ABSTRACT

Tick-borne encephalitis virus (TBEV) is divided into three subtypes: European (TBEV-Eu), Siberian (TBEV-Sib), and Far Eastern (TBEV-FE) subtypes. The geographical range of TBEV-Eu dominates in Europe, but this subtype is present focally across the whole non-tropical forested Eurasian belt, through Russia to South Korea. However, the TBEV-Eu strains isolated outside Europe remain poorly characterized.

In this study, full-genome sequences of eight TBEV-Eu isolates were determined. These strains were isolated from *Ixodes persulcatus* ticks, long-tailed ground squirrel (*Spermophilus undulatus*), and human blood in the natural foci of Western and Eastern Siberia, Russia. A phylogenetic analysis of all available TBEV-Eu genomic sequences revealed that strains from Siberia were closely related to other strains from Europe and South Korea. The closest relation was identified between the Siberian strains and strains from Zmeinogorsk (Western Siberia, Russia) and strain Absettarov (Karelia, Russia), and were most divergent from strains from the Czech Republic and Norway. TBEV-Eu strains isolated in Eastern Siberia were more closely related phylogenetically to strains from South Korea, but strains from Western Siberia grouped together with the strains from Europe, suggesting two genetic TBEV-Eu lineages present in Siberia.

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1. Introduction

Tick-borne encephalitis virus (TBEV) is a member of the family Flaviviridae, genus Flavivirus, and causes a life-threatening neurological disease in humans. During the last decades, an increase of TBEV epidemic activity was seen in several European and Asian countries. According to the International Committee on Taxonomy of Viruses, TBEV is currently divided into three subtypes: Far-Eastern (TBEV-FE), Siberian (TBEV-Sib), and European (TBEV-Eu)

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http://dx.doi.org/10.1016/j.ttbdis.2017.03.002 1877-959X/© 2017 Elsevier GmbH. All rights reserved. (King et al., 2012). Moreover, it was suggested that two possibly new subtypes (with prototype strains 884-86 (EF469662) and 178-79 (EF469661)) circulate in the Baikal region of Russia (Demina et al., 2012; Zlobin et al., 2001a,b).

TBEV-FE strains cause a severe form of tick-borne encephalitis (TBE). These strains dominate in the Russian Far East, but have also been detected in China and Japan.

TBEV-Sib is the most widely distributed TBEV subtype throughout the Russian Federation, but members of this subtype have been detected also in Northern and North-eastern Europe (Golovljova et al., 2004, 2008; Jääskeläinen et al., 2006).

TBEV-Eu causes generally a mild form of TBEV. These strains dominate in natural foci of Europe; however, members of this sub-

Table 1

TBEV strains of European subtype with known full-genome sequences.

Nº	Strain	Isolation place	Isolation year	Isolation source	GenBank acc. #
1	KrM213	South Korea	2006	A.agrarius	HM535610
2	KrM93	South Korea	2006	A.agrarius	HM535611
3	Hypr	Czech Republic	1953	Human blood	U39292
4	Neudoerfl	Austria	1971	I.ricinus	TEU27495
5	AS33	Germany	2005	I.ricinus	GQ266392
6	263	Czech Republic	1987	I.ricinus	U27491
7	Toro-2003	Sweden	2003	I.ricinus	DQ401140
8	Ljubl.I	Slovenia	1992	Human blood	JQ654701
9	k23	Germany	1975	I.ricinus	AM600965
10	Salem	Germany	2006	Maccaca sylvanus	FJ572210
11	Est3476	Estonia	2000	Clinical sample	GU183383
12	84.2	Russia, Altai	2007	I.persulcatus	HM120875
13	Kumlinge A52	Finland	1959	I.ricinus	GU183380
14	Kumlinge 25-03	Finland	2003	I.ricinus	GU183379
15	Joutseno	Finland	1960	I.ricinus	GU183381
16	Absettarov	Russia, Karelia	1951	Human blood	AF091005
17	Saringe-2009	Sweden	2009	I.ricinus	KC469073
18	114	Slovakia	1980	I.ricinus	KC835595
19	285	Slovakia	1990	I.ricinus	KC835596
20	CGI223	Slovakia	1990	M.glareolus	KC835597
21	A104	Austria	1990	A.flavicollis	KF151173
22	Mandal-2009	Norway	2009	I.ricinus	KF991107
23	Vlasaty	Czech Republic	1953	Human blood sera	KJ922516
24	Tobrman	Czech Republic	1953	Human blood sera	KJ922515
25	Skrivanek	Czech Republic	1953	Human blood sera	KJ922514
26	Petracova	Czech Republic	1953	Human blood sera	KJ922513
27	Kubinova	Czech Republic	1953	Human blood sera	KJ922512
28	Sorex 18-10	Russia, Eastern Siberia	2010	Sorex sp.	KP938507
29	IrkutskBR1456-09	Russia, Eastern Siberia, Irkutsk region	2009	I.persulcatus	KP331443
30	IrkutskBR1434-09	Russia, Eastern Siberia, Irkutsk region	2009	I.persulcatus	KP331442
31	IrkutskBR99-08	Russia, Eastern Siberia, Irkutsk region	2008	I.persulcatus	KP331441
32	1G-98*	Russia, Eastern Siberia, Irkutsk region	1998	Human blood	KY069119
33	118-71*	Russia, Eastern Siberia, Irkutsk region	1971	Spermophilus undulatus	KY069120
34	126-71*	Russia, Eastern Siberia, Irkutsk region	1971	I.persulcatus	KY069123
35	163-74*	Russia, Eastern Siberia, Irkutsk region	1974	I.persulcatus	KY069121
36	262-74*	Russia, Eastern Siberia, Irkutsk region	1974	I.persulcatus	KY069122
37	Zmeinogorsk-1*	Russia, Western Siberia, Altai region	1986	I.persulcatus	KY069124
38	Zmeinogorsk-5*	Russia, Western Siberia, Altai region	1986	I.persulcatus	KY069125
39	Zmeinogorsk-9*	Russia, Western Siberia, Altai region	1986	I.persulcatus	KY069126

Note. The strains investigated in this study are marked with asterisk.

type have also been detected outside Europe. TBEV-Eu is a minor TBEV subtype on the territory of the Russian Federation, but it is widely distributed in Altai territory. TBEV-Eu has also been detected in South Korea (Kim et al., 2008; Yun et al., 2009). In Eastern Siberia, TBEV-Eu is relatively rare. Based on molecular hybridization of nucleic acids (MHNA) technique, 5/8 (62.5%) investigated TBEV strains from Altai, and 5/169 (3%) TBEV strains from Eastern Siberia were found to be TBEV-Eu (Demina et al., 2009, 2010). There have been no reports on the presence of TBEV-Eu strains in the Russian Far East so far.

It was demonstrated previously that all TBEV subtypes cocirculate in Irkutsk region and in Buryat Republic (Eastern Siberia, Russia) (Kozlova et al., 2012; Verkhozina et al., 2007) but only recently full-genome sequences of TBEV strains from Siberia have become available (Adelshin et al., 2015; Andaev et al., 2014; Kulakova et al., 2012). In this study, we present full-genome sequences of additional eight TBEV-Eu strains isolated in the Asian part of Russia. Of these, six were isolated from Ixodes persulcatus ticks, one strain was isolated from a long-tailed ground squirrel (Spermophilus undulatus). One strain (named 1G-98) was isolated from the blood of a patient with a feverish form of TBEV from Irkutsk region. Currently, the GenBank database contains 31 full-genome sequences of TBEV-Eu strains, but only nine of them were isolated from humans, mostly due to difficulties with isolation of the virus from human patients. This is the first report on a whole-genome sequence of a TBEV-Eu strain isolated from a human patient outside Europe. This study also provides important data on TBEV-Eu

genetic diversity, distribution and evolution in the Asian part of Russia.

2. Materials and methods

2.1. TBEV strains

TBEV strains used in the present study were isolated between 1971 and 1998 from the Altai and Irkutsk regions of Siberia (Table 1), using a bioassay method with intracerebral infection of 1–3 day-old white outbred suckling mice. Isolated TBEV strains were freeze-dried after 2–3 passages in mice and then stored in the lyophilized form.

2.2. RNA extraction

10% brain suspensions from infected mice were prepared and then centrifuged at 300g for 15 min. 100 μ l of the suspension was taken and then nucleic acid isolation was performed with RNA/DNA extracting kit "RIBO-prep" (InterLabService, Russia) according to the manufacturer's protocol.

2.3. DNA amplification

The reverse transcription was performed with RevertaL-100 kit containing random hexanucleotides (Amplisence, Russia). PCR reaction was performed in 20 μ l of the reaction mixture according to manufacturer's instructions (BioSan, Russia) with 3 μ l of cDNA

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