Origin and distribution of tick-borne encephalitis virus strains of the Siberian subtype in the Middle Urals, the north-west of Russia and the Baltic countries

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Tick-borne encephalitis virus (TBEV) plays an important role in infectious human morbidity, particularly in Russia and the Middle Urals. The Siberian subtype of TBEV (S-TBEV) is dominant in the Middle Urals. Determining the origin of S-TBEV strains in this territory and also in the European part of Russia and the Baltic countries is very important for understanding the cause of its distribution. The surface glycoprotein E gene was partially sequenced in 165 S-TBEV isolates collected in the Middle Urals between 1966 and 2008. Nucleotide and amino acid sequence identity of the studied isolates is 94 and 97.4 %, respectively. Eighty per cent of them are represented by six clusters with identical amino acid sequences in the glycoprotein E fragment analysed. We have determined four types of isolate distribution in the explored territory: local, split, corridor and diffuse. The average rate of nucleotide substitutions per site year \(^{-1}\) is estimated to be \(1.56 \times 10^{-4}\). The age of the S-TBEV population was evaluated to be slightly less than 400 years. Phylogenetic analysis of the data and comparison with historical events indicate that the distribution of S-TBEV strains in the Middle Urals and the European part of Russia originated twice from different foci in western Siberia. This is related to the first land road into Siberia and the Trans-Siberian Way, which functioned at different times. The main reason for such rapid distribution of S-TBEV strains is the anthropogenic factor, i.e. human economic activity during the colonization of new territories in Siberia in the recent past.

INTRODUCTION

Tick-borne encephalitis virus (TBEV) belongs to the tick-borne flavivirus group, genus Flavivirus, family Flaviviridae. TBEV causes tick-borne encephalitis (TBE) in humans after the bite of an infected tick. Two different types of host are needed for the survival of TBEV: (i) ticks, which act as virus reservoirs and vectors, and (ii) vertebrate hosts, which act as a source of blood for feeding ticks and support TBEV transmission by co-feeding of infected and non-infected ticks on the same host (Labuda et al., 1993). Two tick species, the pasture tick (Ixodes ricinus L.) and the taiga tick (Ixodes persulcatus Schulze), are the main vectors of TBEV. The geographical distribution of I. ricinus includes large areas of Europe and parts of north Africa. I. persulcatus has a vast distribution in Eurasia, from the Baltic countries, Finland, Estonia and Latvia in the west to northern Japan in the east. Natural TBE foci coincide with the distribution of these vector ticks.

Based on phylogenetic analysis, three subtypes of TBEV have been described: the Western (W-TBEV) subtype, transmitted by I. ricinus, and the Far-Eastern (FE-TBEV) and Siberian (S-TBEV) subtypes, transmitted by I. persulcatus (Ecker et al., 1999). Amino acid substitutions specific for each TBEV subtype, i.e. signature amino acids, have been identified on the E protein (Ecker et al., 1999) and, on the basis of these markers, TBEV subtypes can be distinguished easily from each other. Based on the newest information on TBEV evolution, an alternative taxonomy of TBEV was proposed for scientific discussion. This
includes four different types: loupil ill virus (LIV), including Spanish, British and Irish subtypes; Western tick-borne encephalitis virus; Eastern tick-borne encephalitis virus, including Far-Eastern and Siberian subtypes; and Turkish sheep encephalitis virus, including the Greek goat encephalitis virus subtype (Grard et al., 2007).

The W-TBEV subtype is distributed widely across Europe; strains have been isolated in Austria, Switzerland, Sweden, Germany, Hungary, Czech Republic, Slovenia, Finland, Latvia, Lithuania, Estonia, Belarus and the European part of Russia (Ecker et al., 1999; Haglund et al., 2003; Lundkvist et al., 2001). FE-TBEV and S-TBEV strains are spread from Japan and far-eastern Russia to the Baltic–Nordic countries Latvia, Estonia and Finland (Jaaskelainen et al., 2006; Lundkvist et al., 2001; Mickiene et al., 2001).

Within the S-TBEV subtype, two lineages have been reported: (i) a Baltic lineage, comprising strains from the Baltic countries, Finland and the European part of Russia, and (ii) a Siberian lineage, comprising strains from the Far East and Siberia (Golovljova et al., 2008). This geographical clustering of S-TBEV was also supported by a phylogenetic analysis at the amino acid level and by the presence of the unique signature amino acids asparagine (Asn) and threonine (Thr) at position 175 of the envelope protein for Baltic and Siberian strains, respectively.

The genome of TBEV and other flaviviruses comprises a single, positive strand of RNA, approximately 11 kb in length, that contains one open reading frame (ORF). This ORF encodes a polyprotein consisting of three structural [capsid, premembrane and envelope (E)] and seven non-structural proteins (Chambers et al., 1990). The E protein is the major structural protein and plays an important role in membrane binding and inducing a protective immune response following virus infection. Viruses of the TBE complex share 77–98% amino acid similarity in the E protein, making the E protein region a prime target for neutralizing antibodies and hence a major target for the development of effective S-TBEV vaccines. Within the E gene, three genetic markers have been identified that have been used to distinguish different flaviviruses (Gao et al., 1993; Shiu et al., 1991, 1992). Viruses in the TBE complex encode the hexapeptide marker EHLPTA at amino acid residues 207–212 in the E protein. Moreover, a characteristic tripeptide sequence has been identified at aa 232–234 that identifies flaviviruses, and a serocomplex-specific pentapeptide sequence is present at aa 320–324 in the TBEV sequence.

It was suggested that flaviviruses, including TBEV, have dispersed and evolved in a cline across the Eurasian continent in an east-to-west direction during the last few thousand years (Zanotto et al., 1995). The S- and FE-TBEV strains share a common ancestor, and are related more closely to each other than to W-TBEV (Golovljova et al., 2008). The time of divergence of the S- and FE-TBEV subtypes was estimated to be approximately 1700–2100 years ago (Hayasaka et al., 1999) and, thus, the divergence within the S-TBEV subtype should have occurred later. The mode of such a fast dispersal of TBEV through the Eurasian continent is still unclear.

In our previous study, we showed that S-TBEV is the dominant subtype of TBEV in the Sverdlovsk region, the main part of the Middle Urals, and this was the case in >95% of strains (Kovalev et al., 2008).

The generally accepted opinion is that S-TBEV originated in the east and was then distributed to the west of the Eurasian continent, and that the northern and southern boundaries of the distribution of I. persulcatus are in the Middle Urals. We assume, therefore, that TBEV could not avoid the Middle Urals in its distribution to the west and that the molecular phylogenetic analysis of strains from the Urals could be key to understanding the causes, directions and mechanisms of large-scale S-TBEV distribution in the European part of Russia and the Baltic countries.

To prove this working hypothesis, we have studied genetic variability and carried out a phylogenetic analysis of the nucleotide and amino acid sequences of the E gene fragment in 165 S-TBEV isolates that were collected in the Middle Urals during 1966–2008 and also in strains available from GenBank. We compared our results with the historical events in the Middle Urals and Siberia and came to the conclusion that the main factor in S-TBEV distribution has been human economic activity.

**METHODS**

**Virus isolates.** The 165 isolates of S-TBEV used in this study represent two groups: the first group of 35 isolates was collected during 1966–1986 and the second group of 130 isolates during 2005–2008. The isolates were collected in Sverdlovsk region (n=151), Tyumen’ region (n=12) and Chelyabinsk region (n=2).

**RNA extraction.** Viral RNA was extracted from 100 μl tick suspension, blood serum or TE-buffer solution of a lyophilized sample (5 μl) of cDNA were used for first-round PCR amplification with HotTag DNA polymerase (Interlabservice), according to the manufacturer’s instructions. Reverse transcription was done by using a REVERTA random-primer kit (Interlabservice), according to the manufacturer’s instructions.

**PCR amplification and sequencing.** A fragment of the E gene was amplified by using nested PCR with external and internal direct and reverse primers as described by Ternovoi et al. (2003), with slight modifications of the internal antisense primer, 5'-AACACYC- CAGTYTGRTCTCCRAGGGTA-T3' (1669R). Samples (5 μl) of cDNA or TE-buffer solution of cDNA were used for first-round PCR amplification with HotTag DNA polymerase (Interlabservice), using 35 cycles of denaturation at 95 °C (10 s), annealing at 55 °C (10 s) and extension at 72 °C (20 s). Final extension was continued for 5 min at 72 °C. For second-round
PCR, the conditions were the same except that annealing was done at 61 °C (10 s). Nucleotide sequences of E gene fragment PCR products (506 bp) of TBEV strains were determined by using a BigDye Terminator v3.1 cycle sequencing kit and ABI PRISM 310 Genetic Analyzer, according to the manufacturer’s instructions (ABI).

**Phylogenetic analysis.** Phylogenetic analysis of nucleotide sequences of PCR products without primer sequences (457 bp) of the gene E fragment and deduced amino acid sequences (152 aa) was conducted for all analysed isolates and also for 74 S-TBEV strains from GenBank (Supplementary Table S1). Omsk hemorrhagic fever virus (OHFV) was used as an outgroup. A phylogenetic tree based on aligned nucleotide sequences was constructed by using the neighbourhood-joining (NJ) method (Saitou & Nei, 1987) based on a p-distance matrix. Phylogenetic trees based on aligned amino acid sequences were constructed by using both minimum-evolution (ME; Rzhetsky & Nei, 1992) and NJ (Saitou & Nei, 1987) methods by MEGA version 4 (Tamura et al., 2007). In both cases, evolutionary distances were computed by using the Poisson correction method (Zuckerkandl & Pauling, 1965) and are given as the number of amino acid substitutions per site. In both cases of the analysis, bootstrap resampling (1000 replications) was used to assess the robustness of the groupings obtained.

**Estimation of nucleotide-substitution rate and lineage-divergence time.** Because approximate dates of isolation are available for the viruses included in this study, it is possible to estimate the rate of synonymous substitutions and, subsequently, to provide approximate lineage-divergence times. The rate of synonymous substitution was calculated by using the method of Li et al. (1988), in which the rate is calculated for pairs of sequences (1 and 2) with a third sequence (3) as an outgroup, so that $k = (d_{13} - d_{23})/t$, where $k$ = substitution rate, $t$ = difference in virus isolation times (years) and $d_{ij}$ = pairwise synonymous genetic distance between sequences ‘i’ and ‘j’. This equation was used to estimate the rate values for four pairs of viruses. SD values were calculated according to recognized procedures. By assuming a constant rate of substitution prior to isolation and that no nucleotide substitutions have occurred since isolation, the times when virus lineages diverged can be estimated using a method described by Li et al. (1988).

**RESULTS**

We have determined nucleotide and deduced amino acid sequences of the E gene fragment (457 bp and 152 aa, respectively) in 165 isolates of S-TBEV, information on which is available from the authors on request. Percentage identity of nucleotide and amino acid sequences of studied S-TBEV strains in the Middle Urals and the adjacent Tyumen’ region area 94.0 and 97.4 %, respectively.

Traditionally, research on the genomic diversity of TBEV has used the bioprobe method to obtain enough material for the study of viral genetic diversity. However, passage in laboratory animals brings some genetic changes into the virus genome (Lee et al., 1997; Romanova et al., 2007). In this regard, it is very important to attempt research on samples without previous passage. We have been faced with the dilemma of whether to use traditional methods that are time-consuming and costly, or to extract genomic material from ticks collected from natural populations, which costs less time and money, but allows us to analyse only a partial gene. The decision was made to conduct further research on the partial E gene for a few additional reasons. Firstly, the chosen region of the E gene comprises 457 nt and contains both conserved and variable domains, and was considered most informative for such research (Gao et al., 1993; Mandl et al., 1988). Secondly, we based our decision on the work of McGuire et al. (1998), who estimated the nucleotide-substitution rate and time of divergence by using the method described by Li et al. (1988) for strains of LIV based on the nucleotide sequence of fragment E (322 nt only). These data have been the basis for historical epidemiology of tick-borne flaviviruses until now. Thirdly, one of the objectives of our study was to compare our results with the data of other researchers; there are enough data available in GenBank for a partial E gene, but data for the whole E gene or other genes of TBEV are limited to a relatively small number of isolates from particular geographical areas that do not represent the virus population within Russia and adjacent countries. Fourthly, use of the partial E gene allowed us to enlarge the number of analysed samples. These 130 isolates represent 80 % of analysed strains and are in fact a genomic RNA that was isolated from single ticks collected in different parts of the region studied.

The phylogram based on aligned nucleotide sequences of analysed isolates and other S-TBEV strains from GenBank was very complicated for understanding their relationships (Supplementary Fig. S1, available in JGV Online). However, the phylogram based on aligned amino acid sequences of analysed isolates and also strains from GenBank (Fig. 1), although it does not show high bootstrap support, allows us to draw several conclusions because the topology of the trees constructed by using different methods (NJ and ME) remains constant. The majority (80 %) of analysed S-TBEV strains are represented by six clusters – A, B, C, D, E and F (Fig. 1; Supplementary Table S1). A cluster in this study is a set of isolates with identical amino acid sequences of the E protein fragment. The fact that the S-TBEV strains obtained in the Middle Urals show whole strain diversity of this subtype, which is distributed in the European part of Russia and the Baltic countries and also in west and east Siberia, is very important.

We have compiled a map (Fig. 2) showing the places of origin of the isolates in clusters. Analysing this information, we can recognize four types of isolate distribution. The first (local) type of distribution includes clusters B and E, isolates of which have been detected only in the Baikalovsky and Sysertsky districts, respectively. Therefore, this type of distribution, which we define as local, comprises a set of isolates restricted to a local area. The second (split) type of distribution comprises isolates from cluster C, which are common in the Baikalovsky and Verkhotursky districts and also in Yekaterinburg. This type of distribution comprises a set of isolates with local type of distribution, but from separate, non-adjacent areas. The third (corridor) type of distribution includes isolates from clusters D and F, whose origins are situated along the
Fig. 1. Phylogenetic tree (NJ analysis) of S-TBEV strains based on amino acid sequences of a fragment of the E glycoprotein (aa 104–255), with OHFV as outgroup. Bootstrap values (≥50 %) are shown above branches. The prototype strains for each cluster are shown in bold; Baltic S-TBEV, Baltic lineage Siberian subtype TBEV; W, Western subtype. The number of isolates in clusters is shown in parentheses (% of total). For information regarding TBEV strains, see Supplementary Table S1 (available in JGV Online).
Trans-Siberian Railway between Yekaterinburg and Tyumen’. This corridor type of distribution comprises a set of isolates from a strip of territory along a transport route. The fourth (diffuse) type of distribution comprises isolates from cluster A, which are distributed all over the region of the Middle Urals. We define this type of distribution as a set of isolates occurring extensively (Fig. 2).

The estimated nucleotide-substitution rates (±SD) of modern TBEV isolates (2005–2008) and strains from the collection of Yekaterinburg Research Institute of Viral Infections (1966–1986) in clusters A, B and D were \(1.35 \pm 0.39 \times 10^{-4}\), \(1.43 \pm 0.43 \times 10^{-4}\) and \(1.89 \pm 0.82 \times 10^{-4}\) per site year\(^{-1}\), respectively. As a result, the mean ± SD nucleotide-substitution rate was \(1.56 \pm 0.29 \times 10^{-4}\) per site year\(^{-1}\) that was suggested previously for FE-TBEV, full-size E gene (Suzuki, 2007). Thus, the estimated age (±1 SD) of the population of S-TBEV in the explored region is 383 (324–472) years. In other words, the S-TBEV strains arrived in the Middle Urals not earlier than during the 17th century.

At the same time, we found that 20 (12 %) analysed isolates from clusters B and D and some related solitary isolates (Fig. 1) show genetic relationships with strains that were obtained in Yaroslavl’, Vologda and Leningrad regions (Russia) and also in Estonia, Latvia and Finland. In addition, another research group (L. S. Karan and others, unpublished data) obtained five strains from the Sverdlovsk and Kurgan regions that were similar to those mentioned above: Ekaterinburg-27-11-06 (GenBank accession no. FJ214123), Kurgan-264-07 (FJ214130), Kurgan-269-07 (FJ214129), Kurgan-272-07 (FJ214128) and Kurgan-273-07 (FJ214131) (Supplementary Table S1). All of these strains are included in the so-called Baltic group S-TBEV (Golovljova et al., 2008), a distinctive characteristic of which is the presence of Asn instead of Thr in position 175 of protein E (Fig. 3). Representatives of this group of strains are still not found either in west Siberia east to Tyumen’ or in east Siberia. The estimated age of this group, estimated on the basis of the average nucleotide-substitution rate (±1 SD) for S-TBEV, is 274 (232–338) years.

Unexpectedly, 84 (50.9 %) isolates obtained in the Middle Urals have identical amino acid sequences in the analysed fragment of protein E (cluster A). This cluster includes strains from west Siberia (Kurgan, Tyumen’, Kemerovo and Novosibirsk regions) and also from east Siberia, Irkutsk region (Fig. 4; Supplementary Table S1). The typical representative of this group is strain Zausaev, which was obtained from a patient in Novosibirsk (Gritsun et al., 2003). Cluster A includes strains that are distributed in the

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**Fig. 2.** Types of distribution of S-TBEV isolates in the Middle Urals. Isolates of cluster A are represented separately (in the right-hand map) for ease of presentation. △, Cluster B (local type); ▽, cluster C (split type); ■, cluster D (corridor type); ●, cluster A (diffuse type); – – –, Trans-Siberian Way; - - -, local roads.
Asian part of Russia and are still not found in the European part of Russia or Eastern European countries.

The estimated age (±1 SD) of this group is 396 (335–488) years. However, the age of the representatives of this cluster in the Middle Urals has been restricted to 218 (184–268) years. Therefore, the strains from cluster A could have arrived into the Middle Urals not earlier than the end of the eighteenth century. Thus, the origins of the distribution were different natural foci of S-TBEV in west Siberia.

**DISCUSSION**

The problem of the origin and distribution of TBEV strains in Eurasia is one of the principles for understanding the evolution of this group of viruses. As we mentioned above, the generally accepted opinion is that TBEV strains were distributed from the east to the west of the Eurasian continent in the last 1700–2100 years (Hayasaka et al., 1999). However, the routes and dynamics of this fast spreading virus are still not fully understood.
distribution of TBEV into such a large territory have been a puzzle until now. The distribution of TBEV depends directly on its vectors and hosts. Although ticks can be carried for significant distances by animals and birds (Hayasaka et al., 1999; Hoogstraal et al., 1963; Waldenstrom et al., 2007), they do not migrate latitudinally in natural environments and therefore could not be a determining factor in the large-scale distribution of TBEV.

Our results lead us to suggest another approach to the investigation of the succession of events, which, in our opinion, could remove disagreements and answer questions about the distribution of TBEV. The main point is a comparison of the age of the separate isolates with historical events in the regions where these strains were obtained.

Our estimation of the age of the population of S-TBEV in the Middle Urals is slightly less than 400 years, which is in agreement with the time of the beginning of the colonization of Siberia by the Moscow Kingdom. In 1598, the building of the first land road into Siberia, which connected the European part of Russia with west Siberia, had just been finished. It ran across Moscow–Yaroslavl–Sol’vychegodsk–Cherdyn–Solikamsk–Verchotur’e–Tyumen’–Tobol’sk–Surgut–Narym (Fig. 4). This road existed for more than 150 years, being the only way via the Urals to the east of Russia and into Siberia, and it officially ceased to be used in the second half of the eighteenth century. Thus, we think that there is a connection between the arrival of the first strains of TBEV into the Middle Urals and the building of the first land road into Siberia. The peculiarity of this road was that it lay in the northern territories of the European part of Russia and connected with the roads to the Baltic countries.

The strains of the Baltic group of S-TBEV, the age of which was estimated in our research as nearly 300 years, and also other strains that do not belong to this group, could have been distributed exactly via this road. The origins of these strains were, probably, natural foci in the north-west territories of west Siberia in the district Tyumen’–Tobol’sk–Surgut–Narym.

The road totally lost its economic and political importance in the middle of the eighteenth century because, after 1763, all traffic into Siberia was redirected via the Trans-Siberian Way, which lay south of the first road via Moscow–Kirov–Perm’–Yekaterinburg–Tyumen’–Omsk–Krasnoyarsk–Irkutsk and which functions until the present time. The consequence of the functioning of this road was the second wave of distribution of S-TBEV into the Middle Urals, from natural foci in the south-east territories of west Siberia. The evidence for this is that strains of cluster A are distributed widely in west Siberia, but have not been detected in the European part of Russia. The age of the strains in this cluster, which we estimate to be slightly more than 200 years, confirms the above hypothesis.

The isolates from clusters D and F with a corridor type of distribution, the origins of which are situated along the Trans-Siberian Way in the district Yekaterinburg–Tyumen’ (Fig. 2), provide a forcible argument that S-TBEV strains were distributed via roads. Interestingly, the isolates from cluster D that were included in the Baltic group of S-TBEV are also common in the district Yekaterinburg–Tyumen’. This is probably an expression of secondary character of the distribution of strains of the Baltic variety of S-TBEV in the Middle Urals.

If the origins of strains are connected to the position and functioning of the roads, then the split and diffuse types of distribution of isolates in the Middle Urals are just alternatives for the corridor type. An obvious case is the allocation of isolates from cluster A, which were distributed radially from the capital of the Middle Urals, Yekaterinburg. Initially, the isolates from cluster A arrived via the Trans-Siberian Way from Tyumen’ to...
Yekaterinburg and then they were distributed via local roads all over the Middle Urals to Serov and Krasnotur’insk in the north, to Chelyabinsk in the south, to Alapaevsk in the north-east, to Shalya in the west and to Nizhnie Ser’gi in the south-west (Fig. 2).

The first road into Siberia and also the Trans-Siberian Way, which, before railways arrived, were used for commerce, postal delivery, etc., were narrow cuttings in the Taiga just wide enough for horse-drawn traffic. Ticks, *I. persulcatus*, could be carried for large distances by horses, domestic farm animals and livestock, and also by dogs that accompanied convoys of carts coming from Siberia. In addition, transfer of ticks could occur by the migration of synanthropic species of birds and mammals along these roads. The feeding time of an adult female tick, *I. persulcatus*, on a mammal is 6–10 days (Konnai et al., 2008). It is easy to estimate that this female tick, travelling 50 km a day with a convoy of carts, could be transferred 300–500 km from the place where it starts its feeding.

The significance of livestock in the distribution of TBEV was demonstrated by McGuire et al. (1998). They showed that the virus of Scottish sheep encephalitis (LIV) of Irish origin was distributed to Great Britain by the transfer of livestock via Wales and then the virus was dispersed into Scotland, northern England and Norway.

The results described above lead us to conclude that the distribution of S-TBEV strains into the Middle Urals and then into the European part of Russia took place in two phases. The first stage was in connection with the functioning of the first road to Siberia, which led to the transfer of strains of S-TBEV from the north-western part of western Siberia. The second stage was connected with the functioning of the Trans-Siberian Way, the result of which was the distribution of strains from the south-eastern part of western Siberia into the Middle Urals. The position of the Middle Urals is unique because both roads from Siberia run across its territory, diverging after Tyumen’ into north-westerly and westerly directions. Thus, the main factor in the distribution of S-TBEV around the Middle Urals is man, whose economic activity has resulted in the transfer of virus strains from the natural foci in western Siberia, which presumably were located between the rivers Tobol-Irtysh and Ob’. In some senses, the victims of TBEV in Europe are the price that the victims of TBEV in Russia pay for colonization of Siberia.

The nucleotide-substitution rate per site year$^{-1}$ for the analysed fragment of gene E of S-TBEV (1.56 × 10$^{-4}$) is very close to the rate of 1.62 × 10$^{-4}$ that was estimated previously for the full-size E gene of FE-TBEV (Suzuki, 2007). However, this rate is very different from the nucleotide-substitution rate of 2.9 × 10$^{-4}$ per site year$^{-1}$ estimated by another research group for the same genome part of FE-TBEV (Hayasaka et al., 1999). Because of the large set of analysed isolates of S-TBEV and also the good agreement with historical events in the Middle Urals, we believe that our molecular clock determines lineage-divergence time more precisely, at 13.67 years per nucleotide substitution for this part of the genome, and reflects more objectively the rate of evolution of S-TBEV. Although use of the whole genome of TBEV or whole genes is conventional for phylogenetic analysis, our research has demonstrated that the use of a comparatively small fragment of the E gene of TBEV was enough to obtain satisfactory results. This is also supported by the work of McGuire et al. (1998), as mentioned above.

We recognize that the variability of the molecular clock of S-TBEV makes it difficult to estimate divergence times precisely. Clearly, there is a need for further studies to confirm the rates estimated here. However, the phylogenetic analysis reported in this research provides strong evidence for the introduction of TBEV firstly into the Middle Urals, then at a later date into the European part of Russia, and finally the Baltic countries. Moreover, the estimates are consistent with the historical evidence of TBEV in Russia. We believe that this study represents an exciting demonstration of the complementary use of molecular biology and historical data to trace the emergence of virus infections.

More research is needed to provide additional evidence for the hypothesis concerning the distribution of S-TBEV isolates in the Middle Urals, the European part of Russia and the Baltic countries. We plan to undertake such research on the natural foci of 'TBEV in the Tyumen’ (Tobol’sk district), Perm’ (Solikamsk district) and Sverdlovsk (the territory between Verchotur’e and Baikalovo) regions.

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