Short Communication

Distribution of Far-Eastern tick-borne encephalitis virus subtype strains in the former Soviet Union

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European and Asian viruses within the tick-borne encephalitis flavivirus complex are known to show temporal, spatial and phylogenetic relationships that imply a clinal pattern of evolution. However, the isolation of recognized Far-Eastern tick-borne encephalitis virus (TBEV) strains in the European region of the former Soviet Union (SU), i.e. thousands of kilometres west of the region in which they are considered endemic, appears to contradict this concept. Here, we present a parsimonious explanation for this apparent anomaly based on analysis of the dates and regions in which these non-endemic strains were isolated, together with their phylogenetic relationships and the records of redistribution of animals under the All-Union programme for acclimatization of game animals within the former SU. Our evidence supports the concept that the anomalous distribution of Far-Eastern TBEV strains in Europe and Siberia arose primarily as the result of the large-scale westward redistribution of game animals for economic purposes.

Tick-borne encephalitis virus (TBEV), belonging to the tick-borne flavivirus group, genus *Flavivirus*, family *Flaviviridae*, is a prototype representative of the seroviruses group of the same name, which were discovered in 1937 in the Russian Far East. TBEV is the causative agent of tick-borne encephalitis in humans, usually after the bite of an infected tick. Two types of host are required for TBEV circulation in nature. The first is the tick as the reservoir and carrier of TBEV and the second the vertebrate animal whose blood is the nutrient source for ticks and also the way in which the virus is transmitted from infected to non-infected ticks by their feeding on the same animal (Labuda *et al.*, 1993). *Ixodes ricinus* L. and *Ixodes persulcatus* Schulze are the two main TBEV vectors. Small mammals are the principal hosts for pre-imaginal ticks, whereas mature ticks feed on large mammals such as lagomorphs, predators, hoofed animals and birds (Pavlovsky, 1947; Filippova, 1985). All these animals are natural reservoirs of TBEV in the infection hot spots.

According to phylogenetic analysis, there are three TBEV subtypes: Far-Eastern (FE-TBEV) and Siberian (S-TBEV), which are both transmitted by *I. persulcatus*, and European (Eu-TBEV), which is transmitted by *I. ricinus*. Each TBEV subtype has specific nucleotide substitutions in the protein E gene and their classification is based on this characteristic

The GenBank/EMBL/DDBJ accession numbers for the sequences of the 13 FE-TBEV strains reported in this study are HM008973–HM008985.

A supplementary table is available with the online version of this paper.

(Ecker *et al.*, 1999). Strains belonging to the different TBEV subtypes have their own geographical distributions. Specifically, Eu-TBEV is endemic in Europe and includes strains collected in Austria, Switzerland, Germany, Sweden, Hungary, the Czech Republic, Slovenia, Finland, Latvia, Lithuania, Estonia, Byelorussia (Belarus) and the European part of Russia (Ecker *et al.*, 1999; Lundkvist *et al.*, 2001; Haglund *et al.*, 2003), FE-TBEV is distributed mainly in the Russian Far East, the eastern part of North China and northern Japan. S-TBEV is commonest in eastern and western Siberia, in the Ural region, the European part of Russia, the Baltic countries and Finland (Lundkvist *et al.*, 2001; Mickiené *et al.*, 2001; Jääskeläinen *et al.*, 2006). However, there is also much evidence for the occurrence of FE-TBEV strains throughout the territory of the European part of the former SU, the Urals and Siberia where the dominant subtype is S-TBEV (Ecker *et al.*, 1999; Zlobin *et al.*, 2001a, b; Pogodina *et al.*, 2007). A reasonable explanation is therefore needed as to how FE-TBEV strains, which are endemic in areas thousands of kilometres away, arrived in these non-endemic territories. The Middle Urals lie on the border between the European and the Asian parts of the Russian Federation. The main part of this territory is the Sverdlovsk region and an explanation for the appearance of FE-TBEV strains here will be applicable to both the European part of the former SU and to Siberia. In the present paper, we attempt to provide a plausible explanation for these apparent anomalies in the distribution of TBEV strains, based on a comparative analysis of molecular genetic data from FE-TBEV strains collected in

the territory of the Middle Urals during different years and the peculiarities of some economic activities within this region and the whole of the former SU.

The 13 FE-TBEV strains used in this study were collected in the Sverdlovsk region and divide into two groups: the first group of 11 strains was collected during 1966–1986 and the second group, comprising two isolates, during 2005–2009 (GenBank accession nos HM008973–HM008985). Data on the place and time of collection and on the host species from which they were taken are given in Supplementary Table S1 (available in JGV Online). The virus isolates of the first group were passaged in suckling mice, 10 % (w/v) brain suspensions of which were lyophilized and stored without further passages in the collection of the Yekaterinburg Research Institute of Viral Infections (YekRIVI). For isolates of the second group, which were obtained directly from ticks without prior passage in mice, only genomic RNA was available and this was converted into cDNA. The methods used for extraction of viral genomic RNA for both groups and its conversion into cDNA, PCR amplification, the sequencing of a 454 nt fragment of gene E (nt 309–762) and phylogenetic analysis have been described by Kovalev et al. (2009).

Retrospective analysis of the TBEV strains that were collected during the period 1966–1986 and that were kept in the YekRIVI showed that S-TBEV and FE-TBEV subtypes were present in the territory of the Middle Urals. The proportion of FE-TBEV strains in this group was 18.6 % (11/59 strains) while the proportion of FE-TBEV isolates collected during 2005–2009 was only 1.3 % (2/151 isolates). Thus, there has been a more than tenfold reduction in the relative number of FE-TBEV isolates occurring in this area over an approximately 40-year period. A similar large-scale reduction in the proportion of FE-TBEV strains during the 70-year period 1939–2006 was shown by an independent research group on a different sample collection in the Middle Urals (Pogodina et al., 2007).

During the period 1966–1986, FE-TBEV strains were found in five districts of the Sverdlovsk region, whereas the two FE-TBEV isolates that were collected in 2007 were found in only one district (Supplementary Table S1). The latter two isolates, which were extracted from I. persulcatus that had been collected in the same place, had identical nucleotide sequences in the gene E fragment and thus are clones. Our attempts to find FE-TBEV in this particular area in subsequent years were unsuccessful. A similar situation was registered for other districts of the Sverdlovsk region in which FE-TBEV was found during the 1960s, 1970s and 1980s. Thus it seems that in areas where S-TBEV is endemic, the FE-TBEV foci are not stable and have a relatively short existence.

Considering that FE-TBEV strains have been found in the territory of the Middle Urals since the end of the 1930s (Pogodina et al., 2007) and the high speed of elimination of these strains from the viral population (as shown above), we assume that the FE-TBEV strains which appeared in this

territory in 1930s should have already disappeared by the 1970s. Therefore, there should have been some factor that provided further inflow of FE-TBEV strains from the Far East into the Sverdlovsk region, a factor that, it is interesting to note, ceased to exist in the 1990s.

Comparative analysis of the nucleotide sequences of the gene E fragment for FE-TBEV isolates collected in the Sverdlovsk region, the European part of Russia, Ukraine and the Baltic countries (Latvia, Estonia), as well as in Siberia, shows that these isolates form clusters with other FE-TBEV isolates that were collected in the Russian Far East and in the eastern part of North China (Fig. 1). The reason why this does not also apply to the Oshima group of strains from Japan (Fig. 1; Supplementary Table S1), is probably because there is no land connection between Japan and the rest of Eastern Asia. The distribution of FE-TBEV strains in the Middle Urals and in the European part of the former SU has a mosaic character without any reasonable connection between the points where the isolates were collected (Fig. 1). The time of collection for most of the isolates (approx. 90 %) falls in the period of the existence of the SU, before its disintegration in 1991. Another important peculiarity of the distribution of the FE-TBEV isolates collected in Europe is that their geographical distribution is limited in the west by the European part of the former SU and does not include the adjacent European countries.

The mosaic character of the distribution of FE-TBEV isolates in non-FE-TBEV-endemic territories is not in agreement with the hypothesis of continuous evolution of TBEV and its persistent expansion into new geographical regions (Zanotto et al., 1995). It is very unlikely that the parallel and similar evolution of TBEVs occurred in restricted geographical areas, and we suggest that FE-TBEV foci appeared in the non-endemic regions relatively recently. The two most likely mechanisms of redistribution of FE-TBEV strains are human economic activity and the migration of birds and mammals. Some authors have shown that birds can carry infected ticks and thereby distribute TBEV over long distances (Hayasaka et al., 1999; Hoogstraal et al., 1963; Waldenström et al., 2007). However, seasonal bird migration occurs mainly in longitudinal, not latitudinal, directions, and it is improbable that migrant birds from the Russian Far East or East Siberia would appear in the Urals. It is even more unlikely that they would appear in Ukraine or Estonia. A more compelling argument against birds being the primary cause of FE-TBEV occurrence in the west is that, if migratory birds were largely responsible for this movement of FE-TBEV, a much wider distribution of the viruses would be expected, and TBEV clines would not be evident. Further support for this idea that birds are not the primary cause comes from the very widespread distribution of West Nile virus, which is transmitted by ornithophilic mosquitoes.

In a previous publication we showed that the decisive role of human economic activity in the distribution of S-TBEV
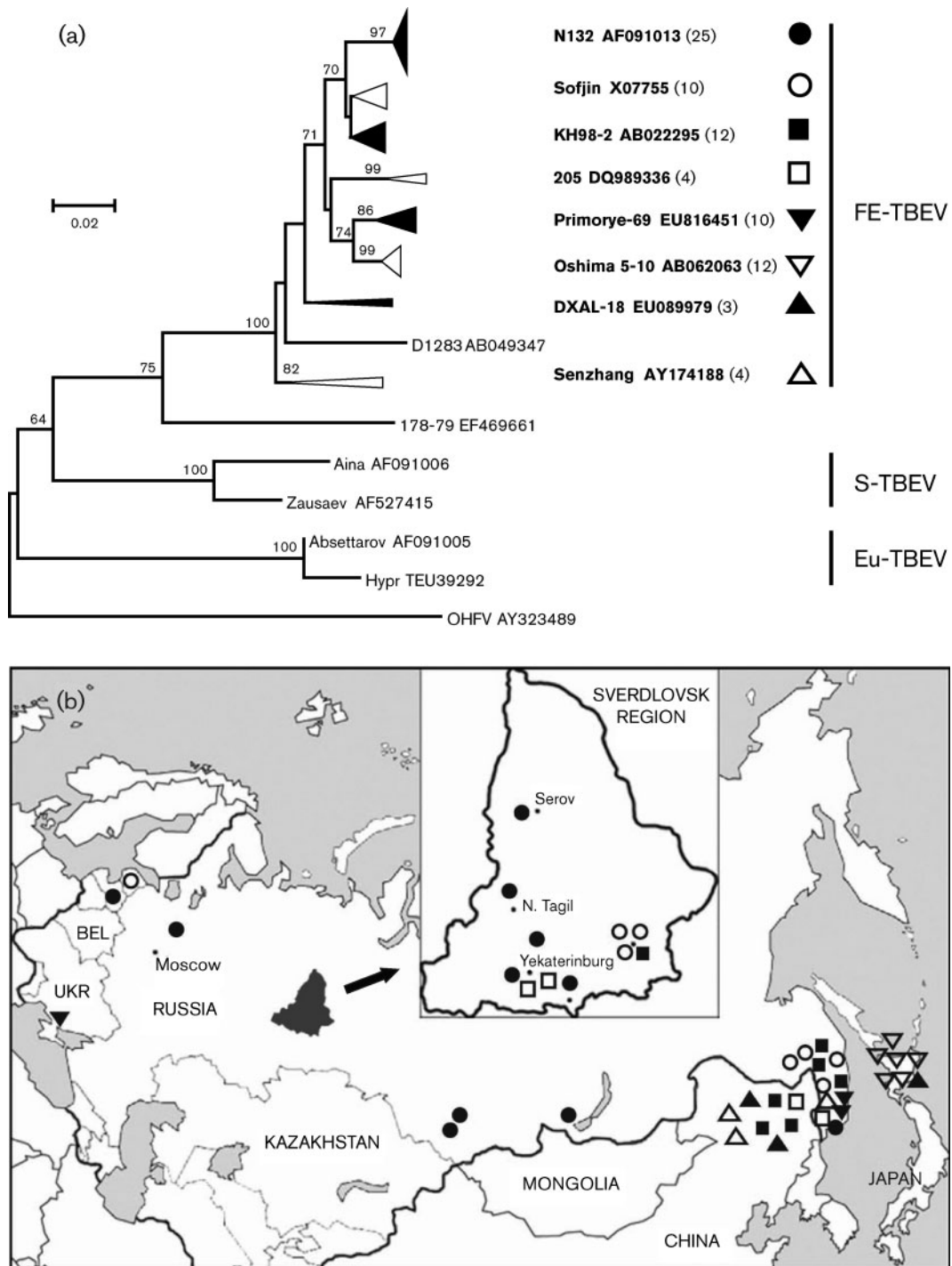
Fig. 1. Distribution of FE-TBEV strains in the territory of the former SU. (a) Phylogenetic tree (neighbour-joining analysis) of FE-TBEV strains based on nucleotide sequences of an E gene fragment (nt 309–762) with Omsk hemorrhagic fever virus as out-group. Bootstrap values ($\geqslant$50 %) are shown above branches. The prototype strains and their GenBank accession numbers are given in bold for each cluster. The number of strains that are known at present is given in parentheses. Bar, 0.02 substitutions per nt position. (b) Location of FE-TBEV strains in the territory of the former SU. Bold line, borders of the former SU. The inset area shows an expanded view of Sverdlovsk region. BEL, Belarus; UKR; Ukraine.

in the Middle Urals, the European part of Russia and in the Baltic countries was connected with the colonization of Siberia by European people in recent times (Kovalev *et al.*, 2009). It is logical to suggest that anthropogenic factors were also determinative in the occurrence of FE-TBEV strains in eastern Europe, the Urals and Siberia.

Thus, the main purpose of the current research was to find the human activity most likely to have led to the distribution of FE-TBEV strains over enormous distances. Any explanation for this redistribution should be able to answer the following questions. Firstly, why should it be only within the borders of the former SU, include the whole territory of the SU and have occurred in the period of the existence of the SU? Secondly, why should this distribution have an unpredictable character and, thirdly, how must it be related to the movement of ticks or their hosts?

The most probable human activity that answers the above-mentioned questions was the long-term All-Union programme of acclimatization of game mammals and birds ('Program') (Pavlov *et al.*, 1973). This 'Program' continued for more than 60 years, from the beginning of the 1930s until the middle of the 1990s, which was the time of the disintegration of the SU. The scale of the 'Program' was impressive. Its purpose was the targeted rearrangement of game fauna to produce fur and meat more effectively (Pavlov, 1999). From the beginning of the 1930s until 1974, 45 species of wild mammal (lagomorphs, carnivores and ungulates) and eight species of bird (grouse, partridge, pheasant and waterfowl) were resettled. This involved moving approximately 430 000 fur-bearing mammals and more than 140 000 other wild mammals intended for amateur hunting and fowling. It is difficult to tell the exact number of animals resettled during the whole period of this 'Program' but the approximate numbers are more than 1 000 000 individual mammals and 400 000 individual birds (Pavlov, 1996, 1999; Pavlov *et al.*, 1973, 1974). Most of the resettled species of mammals and birds are natural hosts for ticks. They could have transmitted infected ticks and/or could have been the source of TBEV for ticks in the new areas, which is more likely, being infected themselves.

This 'Program' operated only in the territory of the former SU and included the majority of regions and all republics of the former SU. It had practically no influence on other countries. Predominantly, the resettling of mammals and birds had a directional character, mainly from east to west, from the less-populated territories of Eastern Siberia and the Russian Far East into Western Siberia, the Urals and especially into the European part of the former SU including Estonia, Latvia, Lithuania, Byelorussia and Ukraine. These were regions that had small numbers of game animals. There was some resettling in the reverse direction but on a much smaller scale. It is possible that the occurrence of the Eu-TBEV strains (GenBank accession numbers FJ214154, FJ214155 and AF231806) in Eastern Siberia was a result of resettling of some species of

mammals from the European part of the Soviet Union (SU). For example, 225 European brown hares (*Lepus europaeus* Pall.) were taken from the forest-steppe area of Bashkortostan to the Irkutsk region in 1938–39 (Pavlov *et al.*, 1974). The Middle Urals was significantly involved in the 'Program'. For example, 61 axis deer (*Cervus nippon* Temm.) (Pavlov *et al.*, 1974), 123 wild boars (*Sus scrofa* L.) and 43 roe deer (*Capreolus capreolus* L.) (Pavlov, 1999) were brought there at different times from the Russian Far East (Primorye and Khabarovsk Territories). A similar situation also existed in the European part of Russia and in the former republics of the SU (Estonia, Latvia, Lithuania, Byelorussia and Ukraine) where large numbers of different species of mammals and birds from Eastern Siberia and the Russian Far East were introduced (Pavlov, 1996, 1999; Pavlov *et al.*, 1973, 1974).

It is still not clear why the FE-TBEV strains did not form stable foci in non-endemic territories and disappeared from the viral population even though they were transmitted by *I. persulcatus* just as they were in the Far East. Probably, the number of FE-TBEV strains present was too small to form a stable viral population and there was competition from endemic S-TBEV strains. Evidence for the long-term persistence of TBEV in populations of small rodents without ticks was provided by the vertical transmission of TBEV between generations of these animals (Bakhvalova *et al.*, 2006, 2009). Comparable data are missing for large mammals and birds. Although it is known that the main source for the maintenance of viral populations is small vertebrates, it is our opinion that the role of large mammals and birds in this process has not been evaluated sufficiently and needs further research.

This hypothesis, that the distribution of non-endemic TBEV strains occurred as a result of the resettlement of mammals and birds by man, could explain both the occurrence of the FE-TBEV strains in Western Siberia, the Middle Urals and the European part of former SU, and the occurrence of the Eu-TBEV subtype in Eastern Siberia. It could also provide a possible explanation for the appearance of Eu-TBEV strains in South Korea (Kim *et al.*, 2008, 2009). These Korean strains have the same ancestral form and constitute an isolated phylogenetic branch of the Eu-TBEV subtype (Yun *et al.*, 2009). Assuming that the rate of nucleotide substitution per site per year for gene E of FE-TBEV of 1.62×10^{-4} (Suzuki, 2007) and for the fragment of the gene E of S-TBEV of 1.56×10^{-4} (Kovalev *et al.*, 2009) are also applicable for Eu-TBEV, the age of the Korean strains would be estimated at 83 and 95 years, respectively. Thus, the possible time of appearance for Eu-TBEV strains on the Korean peninsula is the first half of the 20th century and could be connected with the possible importation of livestock or game animals from Europe.

There are several published examples of equivalent, apparently anomalous movements of TBEV-related viruses; for example, Negishi virus [which is actually louping ill

virus (LIV) (Venugopal *et al.*, 1992)] was mysteriously reported to have been isolated in Japan during the late 1940s (Ando *et al.*, 1952). Venugopal *et al.* (1992) offer the suggestion that animals introduced from Scotland to Japan (immediately following the Second World War) could have been the source of this virus. Similarly, LIV was shown to have been redistributed from Scotland to south-west England (Devon) as the result of the introduction of animals and birds onto the Devon moors (McGuire *et al.*, 1998). The same publication also demonstrates that a UK strain of LIV was isolated in Norway. This was traced to sheep introduced into Norway from northern England. McGuire *et al.* (1998) also propose (on the basis of phylogenetic evidence and evidence of animal movements) that LIV might have been introduced into the UK by animals imported from Europe a few centuries ago.

Every occurrence of non-endemic TBEV strains can be explained, but such explanations may not be obvious. Our hypothesis led us to look at the same problem from different angles and to expand the research field. It is clear that most occurrences of non-endemic TBEV-subtype strains are connected with human activity rather than the natural migration of mammals and birds. Taking into account anthropogenic factors it should be possible not only to determine the mechanisms of distribution of TBEV but also to predict the locations of their possible occurrence.

Acknowledgements

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