

Zoonoses

367

Next generation sequencing of a longitudinal tick-borne encephalitis virus study in a micro-focus in Central EuropeS. Frey¹, D. Höper², M. Beer², G. Dobler¹, S. Essbauer¹¹Institut für Mikrobiologie der Bundeswehr, Virologie & Rickettsiologie, München, Germany²Friedrich Löffler Institut, Institut für Virusdiagnostik, Greifswald-Riems, Germany

Tick-borne encephalitis virus (TBEV) is a member of the genus *Flavivirus* in the family *Flaviviridae* and is transmitted by ticks. The temporal dynamics of the genome structure of TBEV within a single natural focus is poorly understood. In our study we analysed for the first time TBEV full-genomes from a single TBEV focus in Central Europe over a time period of 4 years.

From 2009 to 2012 a total of 5787 *Ixodes ricinus* ticks were collected monthly by flagging in a micro-focus which was detected on the appearance of human cases. In real-time RT-PCR 24 ticks were tested positive for TBEV RNA and the E-genes of these viruses were sequenced. These E-gene sequences suggest multiple and continuous introductions of TBEV strains from Eastern Europe to Central Europe. For detailed analysis of the TBEV genomes cDNA libraries of 9 selected TBEV isolates were made and sequenced using 454 pyrosequencing. For these selected isolates complete genomes were assembled from roughly 3100 - 35,000 reads (representing 5.8 - 35.0 % of the reads).

Comparison of the open reading frames revealed a strong conservation both at the nucleotide (nt) and amino acid (aa) level. A single aa exchange was deduced in 7 out of the 9 strains in the NS5 region encoding the RNA-dependent RNA polymerase. In one strain an additional aa substitution was deduced in the NS3 region encoding the helicase. Analysis of the 3' and the 5' non-coding regions which are important for virus replication also showed several nt-exchanges. We show first data which imply that TBEV within one micro-focus has a highly conserved genome for at least 4 years. Changes in nt-sequences which could be acquired by spontaneous mutations may have implications for the replication and are subject of further investigations.

Corresponding author:

Stefan Frey

stefanfrey@gmx.com