

# Complete Genome Sequence of Tick-Borne Encephalitis Virus Strain A104 Isolated from a Yellow-Necked Mouse (*Apodemus flavicollis*) in Austria

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This work is dedicated in memoriam to our friend, a great and honorable scientist, Milan Labuda.

**Tick-borne encephalitis virus (TBEV) strain A104 was isolated from the brain of a yellow-necked mouse in Austria in 1990. The complete genome sequence was 11,097 nucleotides long. Comparison with TBEV prototype strain Neudoerfl showed 32 amino acid exchanges and the absence of an internal poly(A) stretch within the 3' noncoding region.**

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Tick-borne encephalitis virus (TBEV) is a human-pathogenic flavivirus within the family *Flaviviridae* (1). The genome of TBEV consists of a single-stranded 11-kb (+) RNA coding for one large polyprotein, yielding three structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) (2). Three subtypes of TBEV with different phylogenetic and pathogenic characteristics have been distinguished, the Western subtype (W-TBEV), the Siberian subtype (S-TBEV), and the Far-Eastern subtype (FE-TBEV) (3). In the past decade Europe has suffered an increase of tick-borne encephalitis (TBE), which can cause a disease of the central nervous system (4). In the unvaccinated population of Austria, a total of 8,493 cases of TBE have been reported from 1972 to 2011, with an average incidence rate of about 6 cases per 100,000 population (5). Meanwhile, vaccination has reached >85%, thereby dropping the Austrian incidence rate to 0.9 per 100,000 population (5). Here we describe a new complete genome sequence of a TBEV strain from Austria, which is the first isolate since strain Neudoerfl (1970; accession number U27495) and also the first TBEV strain isolated from a free-ranging mouse for which the complete sequence was determined.

The TBEV strain A104 was isolated from the brain of a yellow-necked mouse (*Apodemus flavicollis*) trapped in Wagnitz (Austria) in 1990 (6). The virus was passaged two times in mouse brains. After one passage in VeroB4 cells, nucleic acid was extracted from the infected cell culture supernatant. Full-genome sequencing was performed using protocols described elsewhere (S. Frey, S. Essbauer, G. Zöller, B. Klempa, M. Weidmann, G. Dobler, and M. Pfeffer, submitted for publication). Nucleotide sequences were determined by using an ABI PRISM 3130 genetic analyzer.

Strain A104 had 97.6% nucleotide identity to the Western TBEV prototype strain Neudoerfl. The most remarkable nucleotide heterogeneity was shown in the 3' noncoding region (NCR). At position 10485 of strain A104, a poly(A) tail of A<sub>3</sub>-C-A<sub>6</sub> was

observed, which is much shorter than that from strain Neudoerfl, A<sub>3</sub>-C-A<sub>48</sub> (7). The origin of heterogeneity in the 3' NCR was considered to be associated with virus propagation *in vitro* as well as polymerase stumbling across extensive secondary structures of the viral RNA (8, 9). The deduced polyprotein of strain A104, with a length of 3,414 amino acids (aa), differed by 32 aa from the polyprotein of strain Neudoerfl. Positions with aa changes were not equally scattered along the viral genome. NS2a (4 aa exchanges), NS3 (8 aa exchanges), and NS5 (9 aa exchanges) showed the highest relative aa change rates, whereas preM and NS4a were 100% identical. Phylogenetic analysis of A104 with other available complete TBEV genomes from the W-TBEV showed that the Austrian strain grouped together with strain AS33 (GQ266392) (10) from southeastern Germany and three TBEV strains from Slovakia (KC835595 to KC835597) (Frey et al., submitted). Interestingly, strain A104 showed a closer phylogenetic relationship with the German strain AS33 than with the geographically closer Austrian strain Neudoerfl. The latter finding is concordant with data from neighboring Slovenia, Slovakia, and Germany showing that geographically close does not necessarily mean phylogenetically close (11, 12; Frey et al., submitted). Although this would be expected in a rodent-associated tick-borne virus, other factors will likewise drive geographical distribution of TBEV (11, 12).

**Nucleotide sequence accession number.** The complete genome sequence of TBEV strain A104 has been deposited in GenBank under the accession number [KF151173](https://www.ncbi.nlm.nih.gov/nuccore/KF151173).

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## REFERENCES

1. Fauquet CM, Fargette D. 2005. International Committee on Taxonomy of Viruses and the 3,142 unassigned species. *Virology* 2:64.

2. Chambers TJ, Hahn CS, Galler R, Rice CM. 1990. Flavivirus genome organization, expression, and replication. *Annu. Rev. Microbiol.* **44**: 649–688.
3. Ecker M, Allison SL, Meixner T, Heinz FX. 1999. Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia. *J. Gen. Virol.* **80**:179–185.
4. Donoso Mantke O, Escadafal C, Niedrig M, Pfeffer M, Working Group for Tick-Borne Encephalitis Virus. 2011. Tick-borne encephalitis in Europe, 2007 to 2009. *Euro. Surveill.* **16**:pii=19976. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19976>.
5. Heinz FX, Stiasny K, Holzmann H, Grgic-Vitek M, Kriz B, Essl A, Kuni M. 2013. Vaccination and tick-borne encephalitis, Central Europe. *Emerg. Infect. Dis.* **19**:69–76.
6. Labuda M, Stünzner D, Kozuch O, Sixl W, Kociánová E, Schäffler R, Výrosteková V. 1993. Tick-borne encephalitis virus activity in Styria, Austria. *Acta Virol.* **37**:187–190.
7. Mandl CW, Kunz C, Heinz FX. 1991. Presence of poly(A) in a flavivirus: significant differences between the 3' noncoding regions of the genomic RNAs of tick-borne encephalitis virus strains. *J. Virol.* **65**:4070–4077.
8. Wallner G, Mandl CW, Kunz C, Heinz FX. 1995. The flavivirus 3'-noncoding region: extensive size heterogeneity independent of evolutionary relationships among strains of tick-borne encephalitis virus. *Virology* **213**:169–178.
9. Mandl CW, Holzmann H, Meixner T, Rauscher S, Stadler PF, Allison SL, Heinz FX. 1998. Spontaneous and engineered deletions in the 3' noncoding region of tick-borne encephalitis virus: construction of highly attenuated mutants of a flavivirus. *J. Virol.* **72**:2132–2140.
10. Kupča AM, Essbauer S, Zoeller G, de Mendonça PG, Brey R, Rinder M, Pfister K, Spiegel M, Doerrbecker B, Pfeffer M, Dobler G. 2010. Isolation and molecular characterization of a tick-borne encephalitis virus strain from a new tick-borne encephalitis focus with severe cases in Bavaria, Germany. *Ticks Tick Borne Dis.* **1**:44–51.
11. Fajs L, Durmiši E, Knap N, Strle F, Avšič-Županc T. 2012. Phylogeographic characterization of tick-borne encephalitis virus from patients, rodents and ticks in Slovenia. *PLoS One* **7**:e48420. doi:10.1371/journal.pone.0048420.
12. Weidmann M, Frey S, Freire CCM, Essbauer S, Růžek D, Klempa B, Zubrikova D, Vögerl M, Pfeffer M, Hufert FT, Zanotto PM, Dobler G. 19 June 2013. Molecular phylogeography of tick-borne encephalitis virus in central Europe. *J. Gen. Virol.* [Epub ahead of print.] doi:10.1099/vir.0.054478-0.