

Centre for EBOV diagnostics. Real-time reverse transcription PCR (RT-PCR) was positive for Zaire EBOV; viral load was 2.04×10^4 genome copies/mL. ELISA of the same sample detected Zaire EBOV-specific IgM (titer 1:400) and IgG (titer 1:3,200). This case of EVD in Senegal was reported to WHO on August 29. The patient received supportive care, and his clinical course progressed well; on August 31, he was afebrile and his asthenia had decreased. In terms of virus evolution, a second blood sample tested on day 18 after illness onset showed diminution of viral load (4.96×10^3 genome copies/mL) and an IgG titer increase to 1:6,400. A third blood sample collected on day 20 showed a negative RT-PCR result, but a urine sample collected the same day showed a positive result with a viral load of 2.04×10^4 genome copies/mL. RT-PCRs of blood and urine collected on days 24 and 34 were negative, and serologic analyses showed a high IgG titer (1:12,800).

The patient was declared cured on September 18, 2014. Epidemiologic investigations revealed a total of 74 contacts in Senegal, including 41 healthcare workers (from the suburban medical center and Fann Hospital). Symptoms developed in 5 of these contacts, but their test results were negative for EBOV. No secondary case was detected after 42 days of monitoring, and the outbreak in Senegal was declared over on October 17, 2014, with only 1 confirmed case reported.

The case-patient's low viral load, detected during the first RT-PCR 10 days after illness onset, probably explains the absence of secondary cases in Fann Hospital. However, the absence of secondary cases in the suburban medical center that the patient had visited on days 3–4 after illness onset and among the family members in Dakar is a rare feature of EVD. The preparedness and surveillance established in Senegal after announcement of EVD in Guinea led to training of healthcare workers for proper use of protective equipment and security procedures with any patient, which probably prevented virus spread in the suburban medical center. This case of EBOV importation from Guinea to Senegal confirms the problems encountered with Ebola outbreak management, including the roles of nonsecure funerals and travel in virus spread.

Acknowledgments

We thank Moussa Dia, El Hadji Abdourahmane Faye, Ousmane Kébé, Khardiata Mbaye, Davy Evrard Kiori, and Oumar Ndiaye for their excellent technical assistance in laboratory diagnosis.

This work was supported by grants from the Institut Pasteur de Dakar, Senegal, and the Ministry of Health, Senegal.

Dr. Ka is an infectious disease physician who works in the Infectious and Tropical Diseases Clinic, Fann Hospital, Dakar, Senegal. His research interests are EVD, HIV, and hepatitis.

Dr. Fall is a virologist who works at Arbovirus and Viral Hemorrhagic Fever Unit, Institut Pasteur de Dakar, Senegal.

Her research interests include arbovirus–vector interactions, mechanisms of arbovirus transmission, and public health activities such as diagnosis of arboviruses and hemorrhagic fever viruses.

References

1. Cenciarelli O, Pietropaoli S, Malizia A, Carestia M, D'Amico F, Sassolini A, et al. Ebola virus disease 2013–2014 outbreak in west Africa: an analysis of the epidemic spread and response. *Int J Microbiol.* 2015;2015:769121. <http://dx.doi.org/10.1155/2015/769121>
2. Feldmann H, Klenk HD. Filoviruses. In: S. Baron, editor. *Medical Microbiology*, 4th ed. Galveston (TX): University of Texas Medical Branch; 1996.
3. World Health Organization. Report of an International Commission: Ebola haemorrhagic fever in Zaïre, 1976. *Bull World Health Organ.* 1978;56:271e93.
4. World Health Organization/International Study Team. Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. *Bull World Health Organ.* 1978;56:247–70.
5. Centers for Disease Control and Prevention. Outbreaks chronology: Ebola virus disease [cited 2015 Aug 2]. <http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html>
6. World Health Organization. Ebola virus disease [cited 2015 Nov 28]. <http://www.who.int/mediacentre/factsheets/fs103/en/>
7. Colebunders R, Borchert M. Ebola haemorrhagic fever—a review. *J Infect.* 2000;40:16–20. <http://dx.doi.org/10.1053/jinf.1999.0603>
8. World Health Organization. Ebola virus disease in Guinea [cited 2015 Aug 18]. http://www.who.int/csr/don/2014_03_23_ebola/en
9. Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, et al. Emergence of Zaire Ebola virus disease in Guinea. *N Engl J Med.* 2014;371:1418–25. <http://dx.doi.org/10.1056/NEJMoa1404505>
10. World Health Organization. Ebola virus disease update—Senegal [cited 2014 Nov 15] http://www.who.int/csr/don/2014_08_30_ebola/en/

Address for correspondence: Ousmane Faye, Virology Pole, Institut Pasteur de Dakar, BP 220 Dakar, Senegal; email: ofaye@pasteur.sn

Tick-Borne Encephalitis Virus in Ticks and Roe Deer, the Netherlands

Setareh Jahfari, Ankje de Vries, Jolianne M. Rijks, Steven Van Gucht, Harry Vennema, Hein Sprong, Barry Rockx

Author affiliations: National Institute for Public Health and the Environment, Bilthoven, the Netherlands (S. Jahfari, A. de Vries, H. Vennema, H. Sprong, B. Rockx); Utrecht University, Utrecht, the Netherlands (J.M. Rijks); Scientific Institute of Public Health, Brussels, Belgium (S. Van Gucht)

DOI: <https://dx.doi.org/10.3201/eid2306.161247>

We report the presence of tick-borne encephalitis virus (TBEV) in the Netherlands. Serologic screening of roe deer found TBEV-neutralizing antibodies with a seroprevalence of 2%, and TBEV RNA was detected in 2 ticks from the same location. Enhanced surveillance and awareness among medical professionals has led to the identification of autochthonous cases.

Tick-borne encephalitis virus (TBEV) can infect humans, causing febrile illness; neurologic complications include encephalitis (1). TBEV is transmitted through bites of infected ticks to many animals, including deer, which serve as feeding hosts for ticks (2,3). Expansion of TBEV subtypes has been reported (4). Reports of TBEV-neutralizing antibodies in wildlife and cattle in Belgium prompted us to reinvestigate the presence of TBEV in the Netherlands (5,6).

During January–September 2010, hunters collected 297 blood samples from roe deer (*Capreolus capreolus*) from locations across the Netherlands. We used a commercial ELISA to detect TBEV-reactive antibodies in roe

deer serum samples. Serologic screening of all 297 samples by ELISA yielded 6 positive and 8 borderline results. All positive, 7 borderline, and 3 negative serum samples were confirmed by testing in a TBEV serum neutralization test (SNT), with the Neudörfel strain as the accepted prototype TBEV-EU, formerly called central European encephalitis virus (5). Five of 6 ELISA positive samples and 1 of 7 borderline samples were confirmed positive by SNT. Five of the 6 SNT-confirmed roe deer were shot at or near a popular recreation area, the National Park Sallandse Heuvelrug (Figure, panel A).

In response to the serologic findings, we collected 1,160 nymph and 300 adult *Ixodes ricinus* ticks by blanket dragging in 7 locations at the national park in September 2015. We extracted RNA from pools of 5 nymphs or 2 adults (7) and tested for flavivirus by using a reverse transcription quantitative PCR. We detected flavivirus RNA in 1 nymph pool and 1 pool of adult female ticks.

To obtain sequences of the 2 reverse transcription quantitative PCR-positive samples, we used primers and protocols as described (8). Both sequences obtained from

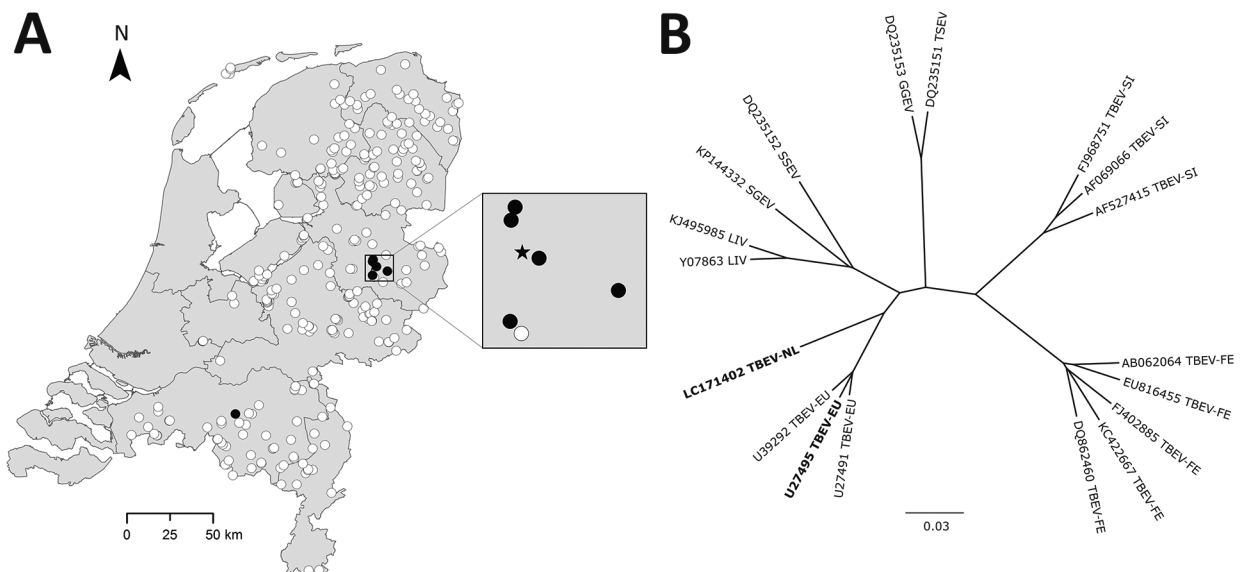


Figure. Spatial distribution of TBEV-positive roe deer and genetic cluster analysis of TBEV sequences from the Netherlands. A) Spatial distribution of serologic test results (solid black circle, SNT positive; open white circle, ELISA and/or SNT negative) for 297 serum samples from roe deer collected according to a sampling scheme designed to obtain a representative sample of the roe deer population from locations across the Netherlands. Enlargement of the National Park Sallandse Heuvelrug area indicates the locations of the TBEV serologically positive roe deer (solid black circle) in relation to the site with reverse transcription quantitative PCR-positive ticks (solid black star) from 2015. B) Genetic cluster analysis of TBEV-NL sequences obtained from tick pools in the Netherlands with other tickborne viruses (indicated by GenBank accession number). Bold indicates the TBEV-NL sequence, which consists of 10,242 nt of the genome (GenBank accession no. LC171402), and the TBEV-EU strain with which TBEV-NL clusters. Where available, representatives of the subtypes are included. We conducted distance-based analyses using Kimura 2-parameters distance estimates and constructed the trees using the neighbor-joining algorithm, implemented in Bionumerics 7.1 (Applied Math, Sint-Martens-Latem, Belgium). We calculated bootstrap proportions by analyzing 1,000 replicates for neighbor-joining trees. Scale bar indicates nucleotide substitutions per site. GGEV, Greek goat encephalomyelitis virus; LIV, Louping ill virus; SSEV, Spanish goat encephalitis virus; TBEV, tickborne encephalitis virus; TBEV-EU, TBEV European subtype; TBEV-FE, Far Eastern subtype; TBEV-NL, TBEV Netherlands subtype; TBEV-SI, TBEV Siberian subtype; TSEV, Turkish sheep encephalomyelitis virus.

the tick pools were identical. The sequences obtained in this study were designated TBEV-NL and clustered within the TBEV-EU subtype complex (Figure, panel B), with a 91% sequence identity with the currently known TBEV-EU sequences.

TBEV-EU RNA in 2 pools of ticks collected through surveillance in 1 national park confirms the presence of TBEV-EU in the Netherlands. Serologic evidence that roe deer from the same location had been infected with a flavivirus, most probably a TBEV, 5 years before the detection of TBEV RNA in ticks suggests that TBEV has been endemic to the Netherlands for at least 5 years.

The concentration of serologically positive roe deer is striking and remains unexplained. One explanation could be that this area has dense beech tree coverage, and beechnuts are a major food source for roe deer and the bank vole (*Myodes glareolus*). These host species play a pivotal role in the TBEV enzootic cycle; a habitat suitable for both may have enhanced the local establishment and spread of TBEV. In addition, the finding of a serologically positive roe deer in a southern province of the Netherlands (Figure, panel A), also known for the presence of beech trees, suggests that TBEV is distributed more widely within the Netherlands.

Dissemination of information about the occurrence of TBEV in ticks and wildlife is needed for medical professionals and the general public. In response to our findings, 2 autochthonous TBEV infections were reported in the Netherlands (9,10). At least 1 of these autochthonous cases was infected with a TBEV strain showing 99% homology with the Neudörfel strain, suggesting the presence of multiple TBEV-EU strains in the Netherlands. Our findings indicate that clinicians should be aware of the possibility for TBEV infection in humans in the Netherlands.

Acknowledgments

We thank Fedor Gassner, Gilian van Duijvendijk, Ryanne Jaarsma, Aleksandra Krawczyk, and Miriam Maas for performing fieldwork; Daan Vreugdenhil and Tom Klomphaar for access to the nature reserves; Natashja Buijs, Ewa Frazer, Najima Lamkaraf, and Sophie Lamoral for technical support in the laboratory; and Marion Koopmans for critically reading this manuscript.

This study was supported by the Netherlands Ministry of Health, Welfare, and Sport and performed under the frame of EurNegVec Cost Action TD1303. The collection of roe deer sera in 2010 was financed by the Netherlands Ministry of Economic Affairs (former LNV; verplichtingnummer 1400004212).

Ms. Jahfari is a PhD candidate at the Dutch National Institute for Public Health and the Environment (RIVM) and Erasmus Medical Center. Her primary research interest is tickborne diseases.

References

- Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet*. 2008; 371:1861–71. [http://dx.doi.org/10.1016/S0140-6736\(08\)60800-4](http://dx.doi.org/10.1016/S0140-6736(08)60800-4)
- Bakhvalova VN, Dobrotvorskoy AK, Panov VV, Matveeva VA, Tkachev SE, Morozova OV. Natural tick-borne encephalitis virus infection among wild small mammals in the southeastern part of western Siberia, Russia. *Vector Borne Zoonotic Dis*. 2006;6:32–41. <http://dx.doi.org/10.1089/vbz.2006.6.32>
- Gerth HJ, Grimshandl D, Stage B, Döller G, Kunz C. Roe deer as sentinels for endemicity of tick-borne encephalitis virus. *Epidemiol Infect*. 1995;115:355–65. <http://dx.doi.org/10.1017/S0950268800058477>
- Donoso Mantke O, Schädler R, Niedrig M. A survey on cases of tick-borne encephalitis in European countries. *Euro Surveill*. 2008; 13:18848.
- Roelandt S, Suin V, Van der Stede Y, Lamoral S, Marche S, Tignon M, et al. First TBEV serological screening in Flemish wild boar. *Infect Ecol Epidemiol*. 2016;6:31099. <http://dx.doi.org/10.3402/iee.v6.31099>
- Roelandt S, Suin V, Riocreux F, Lamoral S, Van der Heyden S, Van der Stede Y, et al. Autochthonous tick-borne encephalitis virus—seropositive cattle in Belgium: a risk-based targeted serological survey. *Vector Borne Zoonotic Dis*. 2014;14:640–7. <http://dx.doi.org/10.1089/vbz.2014.1576>
- Klaus C, Hoffmann B, Hering U, Mielke B, Sachse K, Beer M, et al. Tick-borne encephalitis (TBE) virus prevalence and virus genome characterization in field-collected ticks (*Ixodes ricinus*) from risk, non-risk and former risk areas of TBE, and in ticks removed from humans in Germany. *Clin Microbiol Infect*. 2010;16:238–44. <http://dx.doi.org/10.1111/j.1469-0691.2009.02764.x>
- Kupča AM, Essbauer S, Zoeller G, de Mendonça PG, Brey R, Rinder M, et al. 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*. 2010;1:44–51. <http://dx.doi.org/10.1016/j.ttbdis.2009.11.002>
- de Graaf JA, Reimerink JHJ, Voorn GP, bij de Vaate EA, de Vries A, Rockx B, et al. First human case of tick-borne encephalitis virus infection acquired in the Netherlands, July 2016. *Euro Surveill*. 2016;21:30318. <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.33.30318>
- Weststrate AC, Knapen D, Laverman GD, Schot B, Prick JJ, Spit SA, et al. Increasing evidence of tick-borne encephalitis (TBE) virus transmission, the Netherlands, June 2016. *Euro Surveill*. 2017;22:30482. <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.11.30482><jrn>

Address for correspondence: Hein Sprong, Center for Infectious Disease Control, National Center for Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, 3721 MA, Bilthoven, the Netherlands; email: hein.sprong@rivm.nl