CASE REPORT

A 43-year-old male patient from a rural region near Cottbus (on the Northern German Plain) was admitted to the hospital. Since the previous day, he had suffered from fever (40.0°C), chills, headache, and left-thoracic, breathing-associated pain. Since the previous day, he had suffered from fever (40.0°C), unproductive cough, and pain in the left thorax. Diagnostic radiology then demonstrated an infiltration within the left pulmonary midzone. The laboratory tests detected elevated levels of serum creatinine (116 µmol/liter ≈ 1.3 mg/dl), C-reactive protein (91.55 mg/liter), and a left shift in the differential blood count. Platelet count, serum bilirubin, and transaminase values were found to be normal. However, proteinuria, erythrocyturia, urobilinogenuria, and bilirubinuria were detected in urine. During the clinical course, the level of proteinuria reached a value of 4.55 g/day. No oliguria was observed, but a moderate polyuria did occur at day 7 and on the following days. With routine diagnostic methods, no evidence for acute bacterial, fungal, or viral infections (with the exception of elevated hantavirus antibody titers; see below) was found to explain the nephritis and pneumonia. A spontaneous remission of clinical symptoms and laboratory values was observed during supportive treatment of the patient. The man had not visited other countries in previous years. However, he reported that he had frequently seen and trapped rodents in a barn near his house.

At day 15, the patient was discharged from the clinic; however, at day 21 he was hospitalized again with symptoms of fever (up to 40°C), unproductive cough, and pain in the left thorax. Diagnostic radiology then demonstrated an infiltration in the basolateral segment of the lower-left lobe, whereas the primary infiltration in the left pulmonary midzone was no longer detectable. Again, elevated levels of serum creatinine and C-reactive protein were found, but this time no proteinuria occurred. Ribavirin treatment (1 g/day; Rebetol) was started and maintained over 2 weeks. Within 1 week after the second admission (day 28 after the onset of illness), the patient was free of fever and biochemical values gradually returned to normal. At day 36 after onset, the patient was finally discharged from the clinic. In the follow-up period, no clinical or laboratory deviations were observed. In particular, there was a complete remission of the pulmonary infiltration. The results of subsequent laboratory diagnostics demonstrated an acute hantavirus infection of the patient.

Hantaviruses are rodent-borne viruses which cause hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome in the Americas (9, 11, 15). There is a strong association of the various hantaviruses with certain reservoir host species; for example, in Eurasia, Puumala virus (PUUV) is carried by voles, Hantaan virus (HTNV) and Dobrava virus (DOBV) are carried by Apodemus mouse species, and Seoul virus is carried by rats. Hantaviruses in the Americas are carried by New World mice, the best known examples being Sin Nombre virus and Andes virus. There is an association of renal failure with hantavirus infections in Eurasia and of lung disease with infections by American hantaviruses; however, hantavirus pulmonary syndrome cases in the Americas with renal and/or hemorrhagic involvement and HFRS cases in Europe affecting the lungs have been observed (for a short overview, see reference 9).

In the 1990s, a new PUUV-related hantavirus called Tula virus (TULV) was found in European common voles (Microtus spp.) (12, 18, 22). Since that time, TULV has been detected in voles from several European regions (1, 5, 13, 14, 19). The extent to which the virus is able to infect humans and cause disease remains unclear. A single case of (anamnestic) human infection could be found by serological evidence in a healthy blood donor, thus indicating that TULV (or a TULV-like virus) can be transmitted to humans (22). In addition, one acute case of TULV infection of a patient with fever, paronychia, and exanthema but without renal or pulmonary affection has been reported (17) and recently commented upon (2).

Here we describe the first case of HFRS associated with...
TULV infection. The hantavirus antibody titers against PUUV antigen as determined by immunofluorescence assay (IFA) (Progen GmbH; Heidelberg, Germany) increased from 1:128 (day 4 after onset), through 1:256 (day 15) and 1:512 (day 27), to 1:1,024 in a later serum, thereby demonstrating an acute hantavirus infection. The first serum (no. 1) available for more detailed studies was taken 27 days after the onset of symptoms. The patient gave his informed consent for participation in these investigations. Immunoglobulin G (IgG) antibodies were tested for by nucleocapsid protein-specific enzyme-linked immunosorbent assays (ELISAs) that were carried out with PUUV and HTNV antigens (Progen GmbH); results were positive. Failure of IgM-specific ELISAs to detect IgM can be explained by the disappearance of IgM within 4 weeks after onset of disease. Such disappearance of detectable IgM has also been described for other cases of hantavirus infection (3, 6). Antibody titers against PUUV (1:512), TULV (1:512), HTNV (1:32), and DOBV (1:256)-infected cells were measured by in-house IFA. A second serum sample (no. 2) taken 7 months later confirmed these findings (Table 1). The slightly higher IFA antibody titers against the viruses of the PUUV- TULV group compared with those against the DOBV-HTNV group may indicate an infection by PUUV or a related hantavirus. However, IFA detects mainly antibodies directed against the immunodominant nucleocapsid protein and cannot be used for serotyping since anti-nucleocapsid protein antibodies exhibit cross-reactivities against the different hantavirus types (see reference 9).

The focus reduction neutralization test (FRNT), which is performed under biocontainment level 3 conditions, is the only method for fine-typing sera that are positive for hantavirus antibodies. Typing of neutralizing antibodies directed against glycoproteins in the virus envelope is most representative when late acute- and convalescent-phase sera are investigated (10). Using a chemiluminescence-based FRNT (4), we have determined the neutralizing activities of serum samples 1 and 2 against PUUV, TULV, HTNV, DOBV, and Seoul virus. As shown in Table 1, an at least fourfold-higher reciprocal antibody titer was found against TULV than that found for the other viruses. This supports the assumption that TULV was the virus responsible for the infection of the patient. Nevertheless, we could not completely rule out the possibility that the infection was caused by a TULV-related, unidentified virus that was not present in the virus collection used for FRNT. To support evidence for the involvement of TULV, it was important to show that the virus is endemic to this region of Germany.

In the course of our epidemiological studies on hantavirus distribution in Germany, rodents were trapped and screened for hantavirus infections according to standard procedures (20). Infected Microtus arvalis rodents were captured in northeast Germany at two sites, both of which were a few kilometers from the home village of the patient. Hantaviral RNA was detected by reverse transcriptase PCR in tissues from 3 out of 18 animals tested. The nucleotide sequence of the complete S segment was determined by following the procedure described previously by Sibold et al. (19). In all three cases (samples D5, D17, and D63), the S segments were found to be 1,852 nucleotides in length (1,828 nucleotides if excluding the primer sequences) and were identified as belonging to the TULV species. There is no direct evidence that one of these TULV strains had caused the infection of our patient; however, these data clearly demonstrate the circulation of TULV in the area surrounding his village.

A maximum-likelihood phylogenetic tree that included the known complete S-segment sequences of other TULV strains was constructed by using the TREE-PUZZLE package (16, 21) on the basis of the Tamura-Nei evolutionary model (Fig. 1). The results show that the TULV strains can be divided into three distinct, well-supported lineages. The first lineage is represented by strains from Russia (East Europe), the second is represented by strains from the Czech Republic and West Slovakia, Croatia, and South Germany (Central and Southeast Europe), and the third is represented by strains from northeast Germany and Poland (Central Europe). Regarding the third genetic lineage, it seems reasonable to conclude that its distribution follows the Northern German Plain, which extents to Poland in the east.

The clustering of TULV strains in three different genetic lineages correlates well with the geographical clustering of the locations of virus identification in three different regions of Europe. One could speculate that the host species, M. arvalis, also forms different genetic lineages. To the best of our knowledge, this has not been investigated yet on a molecular phylogenetic level; however, a study of the phylogenetic relationships within the M. arvalis species that was based on phenotypic parameters has described a postglacial remigration of three M. arvalis subspecies into Central Europe: M. arvalis arvalis from the west, M. arvalis levis from the south, and M. arvalis duplicatus from the east (8).
Our data let us conclude that TULV is circulating in northeast Germany and that the virus can be associated with both renal and pulmonary affection in humans. Clinical courses involving more patients need to be evaluated in order to define the principal signs that characterize infection by TULV. In addition to PUUV and the Apodemus agrarius-derived lineage of DOBV (references 7, 9, 20, and our unpublished data), TULV should be considered as the third Central European hantavirus with potential pathogenicity for humans.

**Nucleotide sequence accession numbers.** The complete S segment sequences of the TULV strains D5-98, D17-98, and D63-98 have been deposited in the GenBank sequence database under the accession numbers AF289819, AF289820 and AF289821, respectively.

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**FIG. 1.** Maximum-likelihood phylogenetic tree of TULV strains based on complete S-segment nucleotide sequences. The TREE-PUZZLE package (16, 21) was used to reconstruct the phylogenetic tree on the basis of the Tamura-Nei evolutionary model. Missing parameters were reconstructed from the data set. The values at the tree branches represent the PUZZLE support values. TULV lineages are marked by gray background, Northeast German strains are shown in boldface type. ILVV, Isla Vista virus; PHV, Prospect Hill virus; TOPV, Topografov virus; KBRV, Khabarovsk virus; ELMCV, El Moro Canyon virus; BAYV, Bayou virus; ANDV, Andes virus; SNV, Sin Nombre virus; NYV, New York virus; SEOV, Seoul virus.
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REFERENCES


