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Jean-Marc Reynes, Damien Carli, Nourredine Boukezia, Monique Debruyne, Samir Herti

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JM Reynes [1], D Carli [1], N Boukezia [2], M Debruyne [3], S Herti [4]
1. Centre National de Référence des Hantavirus, Unité de Biologie des Infections Virales Emergentes, Institut Pasteur, Centre International de Recherche en Infectiologie, Lyon, France
2. Laboratoire, Centre Hospitalier de Coulommiers, Coulommiers, France
3. Laboratoire Cerba, Cergy Pontoise, France
4. Service de Médecine Interne, Centre Hospitalier de Coulommiers, Coulommiers France

Correspondence: Jean-Marc Reynes (jean-marc.reynes@pasteur.fr)

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We report an infection with Tula virus in June 2015, leading to hospitalisation, in a patient living approximately 60 km east of Paris with no previous remarkable medical history. Clinical symptoms were limited to a fever syndrome with severe headache. The main laboratory findings included thrombocytopenia and elevated transaminase levels. Based on S (small) gene sequence analysis, the strain affecting the patient was closely related to strains detected in Central Europe, especially to a south-east German strain.

Case report

In June 2015, man in his mid-thirties presented to hospital, three days after the appearance of symptoms (day 3) including sudden fever onset, diffuse pain including back pain, headache and weakness. His previous lifetime medical history was unremarkable with no reported alcohol dependence. His body temperature was 39.6 °C and he reported a severe headache. Physical examination did not reveal any further abnormalities. Blood pressure, and heart and respiratory rate measures were normal. Blood test results however revealed thrombocytopenia, leucopenia, and elevated transaminase and C-reactive protein values (Table).

Results of a chest X-ray and magnetic resonance imaging of the brain found no abnormality. However, abdominal ultrasound demonstrated moderate enlargement of the liver and spleen (lengths 144 mm and 128 mm respectively). The patient was hospitalised and symptomatic treatment was carried out. Serological investigations were requested, to test for cytomegalovirus, Epstein–Barr virus, hantavirus, viral hepatitis, human immunodeficiency virus and parvovirus B19 infection. A microscopic haematuria was observed on day 6 (20,000 red blood cells/mL) but renal function remained unaltered (Table). Symptoms disappeared during the hospitalisation. Blood parameters returned to normal, in particular liver parameters and platelet count (Table). The patient was discharged on day 16.

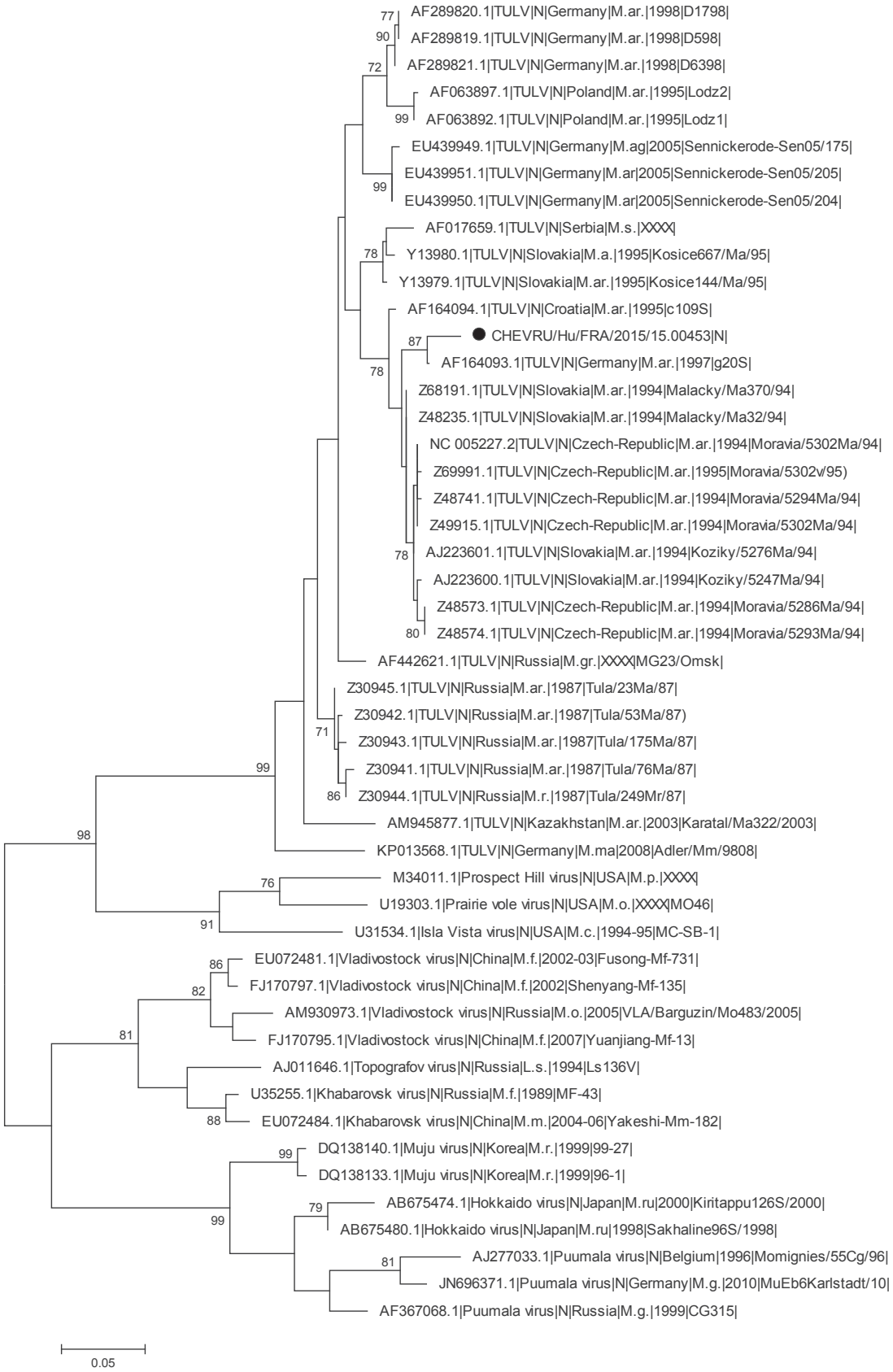
Aetiological investigation

Serological tests were negative, except for tests for the detection of IgM and IgG against hantaviruses (Hantavirus Pool 1 'Eurasia' IgG and Hantavirus Pool 1 'Eurasia' IgM; Euroimmun), including a mixture of purified recombinant nucleocapsid proteins from Hantaan, Dobrava, and Puumala virus (PUUV). These tests were positive on a serum sample collected on day 4 (ratios 1.8 and 4.7 for IgM and IgG respectively, both above the cut-off value of 1.1). As usual in France for surveillance purposes, the sample with positive results was then transferred to the National Reference Centre for Hantavirus. The acute hantavirus infection was serologically confirmed using PUUV native antigen in enzyme-linked immunosorbent assays and immunofluorescence assay, the results being negative using Seoul virus (SEOV) native antigen (both antigens are routinely used). The serum sample was subsequently tested for the presence of hantavirus RNA. The assay was negative using a real-time reverse transcription-polymerase chain reaction (RT-PCR) targeting part of the small (S) genome segment of PUUV, but positive using a pan-hantavirus nested RT-PCR targeting part of the large (L) segment, and a Arvicolinae-borne hantavirus nested RT-PCR targeting part of the S segment, PUUV being used as positive control [1-3].

Amplicons were sequenced and an analysis by basic local alignment search tool indicated that both sequences were very similar to those of Tula virus (TULV) strains, especially to that of the south-east German rodent strain GER/152/Arv (GenBank accession numbers: HQ728459 and HQ697350). Compared to this strain, the patient strain had 89.5% and 90.2% respective nucleotide (nt) sequence identities to the partial

Figure

Phylogenetic analysis of the Tula hantavirus strain found in an infected patient in France, June 2015



The phylogenetic analysis is based on the entire nucleotide (nt) coding sequence of the small (S) genome segment. Sequences from strains of Tula virus and other Arvicolinae-borne hantaviruses are included in the phylogenetic tree and the French Tula virus strain CHEVRU/Hu/FRA/2015/15.00453 retrieved in this study is indicated by a full circle. Bootstrap percentages ≥ 70%, from 500 re-samplings are indicated at each node. The scale bar indicates nt substitutions per site. Sequences were aligned by Muscle, and the tree was constructed using molecular evolutionary genetics analysis (MEGA) version 5.1 with the maximum likelihood method. According to the best fit substitution model proposed, analyses were performed applying the Tamura Nei model using a gamma distribution (+G) with five rate categories.

Table

Haematological and biochemical findings of a Tula hantavirus-infected patient, France, June 2015

Parameters measured on blood specimen	Unit	Norm	Day of sampling[a]					
			Day 3	Day 6	Day 9	Day 11	Day 16	Day 25
White cells	10^9/L	4–10	2.1	6.4	4.3	5.4	4.5	4.6
Platelets	10^9/L	150–450	100	31	88	177	300	254
Haemoglobin	g/dL	13–17	15.7	16.5	14.0	14.9	14,5	13.8
C-reactive protein	mg/L	‹5	17	19	4	ND	ND	ND
Aspartate aminotransferase	IU/L	10–50	114	174	106	188	55	43
Alanine transaminase	IU/L	10–50	163	232	223	322	162	78
Gamma-glutamyltransferase	IU/L	8–61	112	273	228	236	153	121
Prothrombin ratio	%	70–100	88	97	ND	ND	ND	ND
Creatinine	µmol/L	62–106	93	72	81	ND	80	80

IU: international unit; ND: not done.

[a] The sampling day refers to the number of days after symptom onset.

L (n=347 nt) and S segments (n=307 nt). This corresponded, at the amino acid (aa) level, to 99.1% (n=115) and 100% (n=102) aa identity (partial L sequence deposited in GenBank database under accession number: KU297981).

The complete S coding DNA sequence (CDS) (GenBank accession number: KT946591) was recovered via three nested RT-PCRs using primers reported elsewhere [4], producing three overlapping amplicons. The aa sequence (n=429 aa) was similar to those of TULV strains reported in GenBank (divergence 0.2 to 4.9%), and presented highest similarity at the nt and aa levels with the sequence of the rodent Bavarian German strain g20 (GenBank accession number: AF164093). Using molecular evolutionary genetics analysis (MEGA) version 5.1 [5], a phylogenetic analysis based on the S segment coding domain sequence confirmed that the strain – named CHEVRU/Hu/FRA/2015/15.00453 – belonged to the TULV species, and was most closely related to the g20 south-east German strain (Figure).

Sequence comparison was also performed with a reduction of the S CDS dataset to 297 nt (positions 865–1,161 according to the numbering of our sequence) in order to include the only two TULV partial sequences reported from France and detected in *Microtus arvalis* [6]. Divergence at the aa level was 4.0% with these two sequences (compared to only 1.0% with the g20 sequence). The phylogenetic analysis was also performed with this dataset. The French human and animal strains were not closely related but the statistical support was low (data not shown).

Background

Five zoonotic hantaviruses have been described in Europe: Dobrava-Belgrade (DOBV), PUUV, Saaremaa, SEOV and TULV. Among these, PUUV and DOBV are responsible for most human infections, causing mild to severe haemorrhagic fever with renal syndrome [7-9]. The pathogenic potential of TULV in humans is not well known. Although, this virus was found in rodent samples from numerous European countries (including France) after its first identification in 1994 from *Microtus* spp. rodents sampled in 1987 in Tula (Russia), it has only been reported once in humans, from an immunocompromised patient [7-11].

Epidemiological investigation

The investigation was limited to an interview of the patient. The patient lived in a small rural village, surrounded by flat open fields of corn, wheat and sugar beet, in the west part of the Seine-et-Marne department (ca 60 km east of Paris). He was working as an aircraft engine technician. During the six weeks before disease onset, he had often thrown away, barehanded, voles (unidentified species) taken back home by his pet cat. He reported during that period one bite by a live vole. Other potential sources of contamination were not reported.

Discussion

TULV infection in humans without symptoms has been serologically documented [12]. However, evidence of disease in patients is rare with only three such cases being reported (see [7,10] for review). Among these, one, which occurred after a wild rodent bite remained controversial, as clinical symptoms were more compatible with rat-bite fever and late seroconversion suggested that although TULV infection may have occurred, it was perhaps not responsible for the symptoms [13]. From the three reported symptomatic cases, TULV was detected in only one, which was immunocompromised. The molecular evidence of TULV infection in our patient confirms the pathogenic potential of TULV, as this lead to hospitalisation. Furthermore, we mainly observed a fever syndrome with an alteration of the liver function, whereas the two previous non-controversial cases reported, both exhibited a renal and pulmonary syndrome [10,14]. Reported cases are too rare to draw any conclusions about the main tropism of TULV.

Routine hantavirus diagnosis in France is based on commercial serological assays that do not allow discrimination between different hantavirus infections, and consequently diagnosed infections are mostly attributed to PUUV, the main prevalent hantavirus in Europe. Using serological and molecular diagnostic assays as confirmation tests, we recently confirmed virologically for the first time in Europe a human SEOV infection [15]. The diagnostic of this TULV and SEOV infection indicate that molecular diagnostics of hantavirus should be promoted in order to discriminate between hantaviruses involved in human diseases.

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Conflict of interest

None declared.

Authors' contributions

Samir Herti and Nourredine Boukezia took care of the patient. Monique Debruyne performed hantavirus serological analysis. Damien Carli and Jean-Marc Reynes performed the molecular detection and analysis of the Tula virus strain. Jean-Marc Reynes and Samir Herti wrote the manuscript. All co-authors reviewed the manuscript.

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