Genetic detection of Dobrava/Belgrade virus in a Czech patient with Haemorrhagic fever with renal syndrome

A. Papa1, H. Zelena2, D. Barnetova3 and L. Petrousova4
1) First Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece, 2) Department of Virology, Institute of Public Health Ostrava, 3) Department of Paediatrics, University Hospital Ostrava and 4) Department of Infectious Diseases, University Hospital Ostrava, Ostrava, Czech Republic

Abstract

In the summer of 2008, a 15-year-old boy was hospitalized in a paediatric intensive care unit in the Czech Republic. Laboratory diagnosis of hantavirus infection was established by serological and molecular methods. Sequence and phylogenetic analyses showed that the causative strain was Dobrava/Belgrade virus, which is genetically closer to strains associated with Apodemus flavicollis rodents.

Keywords: Czech Republic, Dobrava/Belgrade virus, hantavirus, haemorrhagic fever with renal syndrome, PCR

Original Submission: 9 July 2009; Revised Submission: 31 August 2009; Accepted: 7 September 2009
Editor: E. Gould
Article published online: 14 October 2009

Clin Microbiol Infect 2010; 16: 1187–1190
10.1111/j.1469-0691.2009.03075.x

Corresponding author and reprint requests: A. Papa,
Department of Microbiology, Medical School, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
E-mail: annap@med.auth.gr

Hantaviruses (genus Hantavirus, family Bunyaviridae) cause human haemorrhagic fever with renal syndrome (HFRS) in Asia and Europe, and hantavirus pulmonary syndrome in the Americas. Similar to other bunyaviruses, hantaviruses are enveloped single-stranded RNA viruses with a genome consisting of three segments: S (small), which encodes the nucleocapsid (N) protein; M (medium), which encodes a polyprotein that gives rise to the envelope glycoproteins G1 and G2; and L (large), which encodes the L protein. Five hantavirus genotypes are known to circulate in Europe: Puumala virus (PUUV), Dobrava/Belgrade virus (DOBV), Saar- ema virus, Tula virus and Seoul virus [1,2]. The most pathogenic is DOBV carried by Apodemus flavicollis, which causes HFRS, mainly in the Balkans, with a fatality rate of up to 10% [3,4].

In former Czechoslovakia, the first report of hantavirus antigen detection in rodents (Myodes glareolus, A. flavicollis, Apodemus agrarius) was in 1984, representing the first report on hantaviruses in Central Europe [5]. Hantavirus antigen was detected in 6.8% of small mammals, with a highest rate of 14.1% in Mycrotus arvalis [6]. The first HFRS cases in the Czech Republic were reported in 1992, whereas PUUV nephropathy was serologically diagnosed in 2002 in three children [7,8]. A seroprevalence of 0.93% in the human population was reported, with a higher percentage among military personnel (1.66%) [9]. A similar seroprevalence has been observed in neighbouring Slovakia (0.5–2%), Germany (1–3%) and Austria (1–2%) [1,2]. TULV was detected and isolated in M. arvalis captured in the Czech Republic, representing the first report in Central Europe, whereas PUUV was detected in M. glareolus in Moravia, and DOBV was detected in rodents in the southern Bohemia [10–12]. During the period 1998–2006, 23 HFRS cases were reported in the Czech Republic [2]. The number of cases is very low, probably because clinicians are not familiar with the disease, and cases remain unrecognized.

On 11 August 2008, a 15-year-old hitherto healthy boy from Ostrava presented to a pediatrician, after 4 days of high fever (39°C) and vomiting. He had spent the summer holidays in Ostravice village, in the foothills of the Morava–Silesian Beskydy mountains, 35 km from Ostrava, and close to the borders of the Czech Republic, Slovakia and Poland (altitude 415 m above sea level; 49°32’23”N, 18°23’29”E) (Fig. 1). One week before the onset of disease, he had swum in the river and helped his grandparents to pull down some old cowsheds. Physical examination did not reveal any abnormality, and only antipyretics were prescribed. Two days later (day 6), his condition deteriorated and he presented weakness, malaise, headache, nausea and facial paresthesia. Because meningitis was suspected, the patient was admitted to the Infectious Diseases Unit of the University Hospital of Ostrava. Serum samples were negative for herpes- and hepatitis A, B and C viruses. Biochemical parameters of the cerebrospinal fluid were in the normal range, and culture for bacteria was negative. PCRs for enteroviruses and herpes-viruses were negative. He had leucopenia (2.8 × 10^9/L) and thrombocytopenia (45 × 10^9/L), elevated liver enzymes [alanine aminotransferase 10.7 ukat/L (normal range 0.15–0.75 ukat/L), aspartate aminotransferase 14.43 ukat/L (normal range 0.1–2.5 ukat/L), and C reactive protein (CRP) 40 mg/L (normal range < 0.6 mg/L)]. The patient was suspected to have hantavirus infection and was treated with corticosteroids. He recovered from the disease with no sequelae.
range 0.15–0.65 ukat/L), lactate dehydrogenase 17 ukat/L (normal range 0.15–0.65 ukat/L), and his C-reactive protein value was repeatedly low. Thromboplastin time, activated partial thromboplastin time and fibrinogen were at normal levels, and there was no clinical sign of bleeding. Within the first 3 days of hospitalization, he developed renal failure, with oliguria, proteinuria and macroscopic haematuria, and, on the fourth day of hospitalization (day 9), he was transferred to the paediatric intensive care unit. Serum urea increased to 30.8 mmol/L (normal range 2.5–7.2 mmol/L) and creatinine to 424 μmol/L (normal range 44–88 μmol/L). Renal biopsy revealed acute tubulointerstitial nephritis and hantavirus infection was suspected. Serum samples taken on days 6 and 10 were tested in parallel for the detection of IgG and IgM antibodies to Hantaan virus and PUUV using ELISA (PROGEN Biotechnik GmbH, Heidelberg, Germany); index values of IgG were 2.26 and 2.86, and those of IgM were 4.31 and 3.75, respectively (values >1.5 for IgG and >2 for IgM are considered positive). Antibodies to PUUV were not detected. It was suggested that the causative agent was DOBV. Despite the relatively serious renal failure, it was considered that haemodialysis was not necessary. High fever lasted up to day 9. Complete recovery was achieved within 1 month (total hospitalization time of 23 days). Renal function returned to normal, whereas mild hypertension persisted.

A serum sample on day 6 was sent for molecular analysis to the Aristotle University of Thessaloniki in Greece. Viral RNA was extracted using the QIAamp Viral RNA kit (Qiagen, Hilden, Germany) and two RT-nested PCRs were performed using two sets of primers designed to detect hantaviruses associated with rodents of the *Muirinae* subfamily (HTN, DOB, SEO) [4]. Briefly, one primer set (MS120C: GGATGCAGAAAAICAGTATGA – MS1170R: AGTTGTATI CCCATIGATTGT and MS364C: GAIATTGATGAACCTAC AG – MS963R: ACCCAIATTGATGTTGA) was used for the detection of a 590-bp fragment of the N coding region, and a second primer set (MM1470C: CCIGGITTI CATGGITGGGC – MM2029R: CCATGICITITCIGT/G/T TCCA and MM1674C: TGTGAMAR/AGIT/AATAIAGATG TGA – MM1990R: TCIGA/C/TGCI(G/C)TGCI(G/C)CCCA) was used to detect a 317-bp fragment of the partial G1 coding region [4]. Each reaction was performed in a 50-μL volume, containing 1 μM of each primer. The annealing temperature for the first RT-nested PCR (N coding region) was 45°C, whereas it was 40°C for the second RT-nested PCR (G1 coding region). Sequencing of the PCR products revealed that the causative agent was DOBV (GenBank Accession numbers: FJ986109 and FJ986110 for S and M segment sequences, respectively). Sequences were aligned by CLUSTAL W and phylogenetic trees based on S and M segment sequences were constructed by the Neighbour-joining method using PHYLIP software. In both trees, the Czech strain (strain Beskydy/08) clusters together with DOBV strains associated with *A. flavicollis* (Fig. 2). The genetic distances with other DOBV strains are higher in the M segment. The genetically closest strains in the S segment are the DOBV strains from Slovenia and Greece, with both differing by 8.5%, and the strain 400Af/98 from East Slovakia (9%), whereas, in the M segment, the closest strains are
400Af/98 from East Slovakia, differing by 15.5%, and the Greek (16.7%) and Slovenian (18.6%) strains.

The laboratory findings of the present case, especially the extensive leucopenia and the highly elevated liver enzymes, are not characteristic of hantavirus infection, and rather resemble a tick-borne infection, such as anaplasmosis or ehrichiosis. Elevation of liver enzymes was observed in HFRS cases; however, they were approximately two-fold greater than the normal limits [13]. In addition, liver involvement appears to be an ominous prognostic factor because it was correlated with severe renal failure and thrombocytopenia [13]. The young age of the patient might explain this unusual presentation, probably as a result of a variational innate immune response. The negative CRP was exceptional. Although the PUUV viraemia lasts for a short time, DOBV RNA can be detected even after 17 days after the onset of the disease (4). In the present case, the sample was taken the sixth day of illness and was easily detected in both RT-nested PCRs.

The present study is the first genetic report of a HFRS case caused by DOBV in the Czech Republic. The fact that the boy was most probably infected when present in a region that borders several other countries, together with the results obtained in previous studies conducted in East Slovakia [14], suggests that the likelihood that DOBV might present a risk to humans in those countries neighbouring the Czech Republic could be relatively high. Further studies on clinical cases and rodents are needed to elucidate hantavirus epidemiology in the Czech Republic and, at the same time, attention should be paid to public health authorities of Central European countries when assessing health risk issues.

Acknowledgements

We are grateful to I. Matsini and E. Papadimitriou for their technical assistance.

Transparency Declaration

The authors declare no conflicts of interest.
References