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

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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

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Hantaviruses (genus Hantavirus, family *Bunyaviridae*) cause human haemorrhagic fever with renal sydrome (HFRS) in Asia and Europe, and hantavirus pulmonary sydrome in the Americas. Similar to other bunyaviruses, hantaviruses are enveloped single-stranded RNA viruses with a genome consisting of three segments: S (small), which enodes 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), Saarema 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 neigbouring 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 herpesviruses 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

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FIG. 1. Map of the Czech Republic. The city of residence (Ostrava) and the location of infection are marked.

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 transfered 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 umol/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 *Murinae* subfamily (HTN, DOB, SEO) [4]. Briefly, one primer set (MS120C: GGATGCAGAAAAICAGTATGA – MS1170R: AGTTGTATI CCCATIGATTGT and MS364C: GAIATTGATGAACCTAC AG – MS963R: ACCCAIATTGATGATGGTGA) was used for the detection of a 590-bp fragment of the N coding region, and a second primer set (MM1470C: CCIGGITTI CATGGITGGGC – MM2029R: CCATGIGCITTITCI(G/T)T CCA and MM1674C: TGTGAI(A/G)TITGIAAITAIGAGTG TGA – MM1990R: TCIG(A/C)TGCI(G/C)TIGCIGCCCA) 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

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