CASE STUDY

Two clinical cases of renal syndrome caused by Dobrava/Saaremaa hantaviruses imported to the Netherlands from Poland and Belarus, 2012–2014

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We report the rare event of two imported cases in the Netherlands presenting with renal syndrome caused by Dobrava (DOBV)/Saaremaa (SAAV) hantaviruses. DOBV/SAAV hantaviruses are not circulating in the Netherlands and their clinical manifestation is typically more severe than that of the endemic Puumala virus (PUUV). This report aims to increase awareness among healthcare professionals and diagnostic laboratories to consider different hantaviruses as a cause of renal failure.

Keywords: hantavirus infections; Dobrava; Haemorrhagic fever with renal syndrome; rodent-borne; zoonosis

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We report two cases of Dobrava (DOBV)/Saaremaa (SAAV) hantaviruses imported to the Netherlands from 2012 to 2014. Both patients presented with renal syndrome following travels to Belarus and Poland, respectively, where DOBV is known to be circulating. This report serves to create awareness among clinicians and diagnostic laboratories to consider different hemorrhagic fever-causing hantaviruses in patients with renal syndrome.

Case 1

In July 2012, a previously healthy 54-year-old Dutch woman was referred to the emergency department because of acute kidney injury. She suffered from confusion, persisting after a period with fever (up to 40°C), vomiting and oliguria 10 days before referral. Temperature (36.8°C) and blood pressure (145/87 mmHg) were normal upon admission but a slight abdominal pain and a slight edema at the ankles were noticed. Laboratory investigation showed a mild normocytic anemia with a Hb of 7.0 mmol/l (n = 7.5–9.5); acute renal failure with a serum creatinine of 810 µmol/l (n = 55–90); and elevated liver enzymes [ASAT 52 U/l (ULN < 31), ALAT 236 U/l (ULN < 34), LDH 444 U/l (ULN < 247), alkaline phosphatase 165 U/l (ULN < 115); γ-GT 61 mmol/l (ULN < 38)]. The C-reactive protein of 13 mg/l and erythrocyte sedimentation rate of 53 mm/hr (n = 0–20) were modestly elevated. Ultrasonography showed two edematous kidneys and a normal liver. The patient had travelled to the Republic of Belarus on a bird-watching trip 3 weeks before the onset of symptoms. During her stay in a holiday home located in a woody area, she had cleaned the floor of dust and mouse

These authors have contributed equally for this work.
droppings. Infection with a hantavirus was immediately suspected upon anamnesis and thus part of the initial differential diagnosis. Both IgM and IgG antibodies were detected by Puumala virus (PUUV) ELISA screening (Progen) on a serum sample taken 10 days upon onset of illness. Comparative serology using an immunofluorescent antibody mosaic assay (IIFA, Euroimmuun) demonstrated high titers of both IgM and IgG against Dobrava (DOBV) and Saaremaa (SAAV), but no detectable antibodies against PUUV (Table 1). The patient was treated with saline infusion upon which renal function improved; serum creatinine dropped to 174 μmol/l in 4 days and later completely normalized. At a follow-up visit 6 weeks later (52 days after onset of symptoms), convalescent serum was sent to the lab and was again tested positive for both DOBV and SAAV by comparative IFA (Table 1). Virus neutralization as described previously (1, 2) confirmed these results and showed a two-fold higher titer to DOBV than to SAAV. This indicated, but did not confirm, a DOBV infection.

Case 2
In November 2014, a previously healthy 26-year-old Polish seaman visited an outpatient clinic in the Netherlands with symptoms of diarrhea and vomiting since 4 days and fever since 2 days. There was no previous history of disease and no reports of further cases among the patients’ crewmates. The ship was reported to navigate exclusively in northern European waters. Besides the travels on the ship, his travel history contained a family trip to the southwest of Poland 3 weeks before presentation, where he had visited a farm with chickens and pigs.

During physical examination, an acutely ill patient was observed; he was agitated and his movements were uncoordinated. He was disoriented in place, his consciousness was slow, and he had word finding difficulties in his own language. Physical examination revealed a temperature of 40°C, normal blood pressure (123/70), and tachycardia of 130 beats per minute. There was no evidence of neck stiffness; lymph nodes were palpable, occipital, and cervical; there was conjunctivitis in both eyes and petechiae on the thorax. Laboratory results showed a normal Hb of 10.9 mmol/L (n = 8.5–11), thrombocytopenia of 50 × 10⁹ (n = 150–370 × 10⁹) with leucocytes of 10.8 × 10⁹ (n = 3.5–10 × 10⁹). He had an elevated creatinine of 167 (n = 65–115 μmol/L) with an estimated glomerular filtration rate (eGFR) of 43 (n > 60 mL/min) and slightly elevated liver enzymes. Cerebrospinal fluid (CSF) examination showed no signs of (meningo-)encephalitis and the CT cerebrum showed no intracerebral pathology.

Serological testing for viral hepatitis, cytomegalovirus, Epstein-Barr virus, Mycoplasma, parvovirus B19, and adenovirus did not show any evidence for a recent infection.

Table 1. Hantavirus diagnostics performed in convalescent sera of two patients in the Netherlands with HFRS after travelling to Belarus and Poland, 2012–2014

|                  | Patient 1 | Patient 2 |
|------------------|-----------|-----------|
|                  | Day 10    | Day 52    | Day 3     | Day 9     |
|                  | After symptom onset | After symptom onset |
| ELISA<sup>a</sup> | PUUV      | IgM 17.475 17.728 | IgM 1.159  1.146 |
|                  |           | IgG 11.96  12.104 | IgG 0.295  2.392 |
|                  | DOBV      | IgM 4,000  4,000 | IgM 100   2,000 |
|                  |           | IgG 128,000 64,000 | IgG 4,000 ≥16,000 |
|                  | SAAV      | IgM 16,000 1,000 | IgM 1,000 2,000 |
|                  |           | IgG 4,000 64,000 | IgG 4,000 ≥16,000 |
|                  | PUUV      | IgM <100  <100 | IgM <100  <100 |
|                  |           | IgG <100  <100 | IgG <100  100 |
| FRNT<sup>c</sup> | DOBV      | 1:320 1:160 | 1:160 |
|                  | SAAV      | 1:160 1:160 | 1:160 |
|                  | PUUV      | <40       <40 |
| PCR<sup>d</sup>  | DOBV      | Negative  Negative |
|                  | SAAV      | Negative  Negative |
|                  | PUUV      | Negative  Negative |
|                  | SEOV      | Negative  Negative |

PUUV, Puumula virus; DOBV, Dobrava virus; SAAV, Saarema virus; SEOV, Seoul virus. Bold figures indicate positivity. <sup>a</sup>Enzyme-linked immunosorbent assay (Progen), values <1.0 were considered negative (4). <sup>b</sup>Indirect immunofluorescence assay (Euroimmuun), titers <100 were considered negative (5). <sup>c</sup>Virus neutralization test, titers <40 were considered negative (1). <sup>d</sup>In-house real-time reverse transcriptase-polymerase chain reaction.
Due to the renal dysfunction and travel history, an acute infection with a pathogenic hantavirus was considered as a differential diagnosis and ascertained in the acute serum sample taken upon admission. DOBV RNA was detected (Ct 32) using an internally controlled in-house real-time reverse transcriptase-polymerase chain reaction (RT-PCR, Table 2), in combination with one-step fast virus mastermix (Applied Biosystems, Bleiswijk, the Netherlands), according to manufacturer’s protocol. IgM antibodies were detected in the acute serum sample by the PUUV ELISA (Progen) upon which a mosaic IIFA (Euroimmun) was performed demonstrating the presence of IgM and IgG antibodies against DOBV and SAAV in both the acute serum and in a serum sample 9 days later (upon discharge). Only a borderline reactivity was observed for anti-PUUV IgG antibodies in the serum at Day 9 (Table 1).

Upon admission, the patient’s renal function deteriorated but the patient was hemodynamically stable and improved neurologically. On Day 4, his renal impairment was most severe with an eGFR of 14 (n > 60 mL/min) and a creatinine level of 440 (n = 65–115 μmol/L). After 1 week of supportive care, his renal function started to improve slightly and the patient requested to be dismissed. A follow-up serum sample for hantavirus serology was taken at discharge, Day 9 after onset of symptoms. Virus neutralization on both sera confirmed the presence of neutralizing antibodies against both DOBV and SAAV but could not distinguish between both viruses based on a four-fold titer difference as the second serum was taken too early after onset of symptoms (1).

**Background**

Hantaviruses are enveloped viruses with a segmented negative-strand RNA genome belonging to the family *Bunyaviridae*. Within Europe, at least four hantavirus species cause infections in humans. Puumula (PUUV) and the closely related Dobrava-Belgrade virus (DOBV) and Saaremaa (SAAV) are mostly reported, while recently the first cases of Seoul virus (SEOV) infection have been described (7, 8). The close relationship between DOBV and SAAV is reflected by a high level of serological crossreactivity between these viruses. A further hantavirus, Tula virus (TULV), has been detected in vole species and its rodent reservoir in several European countries (9). The pathogenicity of TULV in humans is considered to be low, but has been described in an immunocompromised patient (10).

The total recorded number of hantavirus infections in Europe has been steadily increasing during the past years; an average of 3,138 cases were recorded from 2000 to 2009 versus 1,671 in the period 1990–1999 (11). The highest number of reported cases is found in Finland, Sweden, Germany, France, and Belgium, but severe underdiagnosis is suspected in several other European countries (12). The majority of clinically apparent human hantavirus infections in Western Europe are caused by PUUV (6, 11). In the Netherlands, from 2008 to 2013, the number of yearly reported PUUV cases varied between 4 and 24 cases per year, but seroprevalence studies suggest that this number is an underestimation as well with a peak of 36 notified cases in 2014 (13–15). DOBV cases have thus far mostly been reported in the Balkan countries and the Alpe-Adrian region (16).

Each hantavirus is associated with a specific natural reservoir (17, 18). For the hantaviruses causing hemorrhagic fever with renal syndrome (HFRS), these include the *Apodemus*, *Rattus*, *Myodes*, and *Microtus* species. Hantaviruses infect humans primarily from aerosolized rodent excreta. Particular risk has been associated with opening, occupying, and cleaning structures, such as summer cottages, barns, and cellars, which have been infested by rodents. There is no specific vaccination or antiviral therapy in use and so infection should be avoided by not inhaling unventilated air in such structures, using personal protection such as surgical masks and by rodent control measures (12). As humans are generally considered dead-end hosts for hantaviruses, there is no risk of ongoing transmission of DOBV upon import of infected patients.

Diagnosis of HFRS/NE currently relies on serology. The viremic stage in hantavirus infections is short and diagnostic requests for HFRS/NE are often too late in the course of disease to enable a diagnosis by RT-PCR, outside of endemic areas. The most commonly used methods for verifying a hantavirus infection are indirect IgG- and IgM-enzyme-linked immunosorbent assays (ELISA), IgM capture ELISAs, or IIFAs. With these routine serology

| Table 2. Primer and probes used for internally controlled DOBV real-time RT-PCR |
|--------------------------|--------------------------|--------------------------|
| Name                     | Sequence 5’–3’            | Conc (pmol/μl)            | References         |
| Dobrova                  |                         |                          |                    |
| Dobrova-rev-TM           | AGACATTTCAGGAAGCATAATAGA | 30                       | In-house          |
| Dobrova-fwd-short        | GGTGGTTTAGGAYGTACCTTAAGTG | 45                       |                    |
| Dobrova-probe-new        | FAM-ACAACAACAYCTACC*CAAAACAACACTACCTCA | 10    |                    |
| PDV                      |                         |                          |                    |
| PDV fwd                  | CGGTTGCCCTTACCAAGAAC     | 30                      | (3)               |
| PDV rev                  | TTCTTTCTCAACCTGCCTCC     | 7.5                      |                    |
| PDV probe               | CY5-ATGCAAGGGCCAAATT-MGB | 10                      |                    |

*BHQ1, internally coupled quencher. PDV, phocine distemper virus, used as an internal process control.
DOBV, clinical data indicate that SAAV infections are characterized by more severe HFRS, with a case fatality rate of 0.1 to 0.4% (6). More recently, PUUV has been demonstrated as a cause of non-endemic NE (NE). The course of NE/HFRS has been divided into febrile, hypotensive, oliguric, diuretic, and convalescent phases, but these phases are not always clinically evident (6). HFRS is characterized by fever and renal failure associated with hemorrhagic manifestations. In NE, renal failure and fever (above 38.5°C) dominate, and hemorrhagic signs are rare. The described case fatality rate of NE is low and varies from 0.1 to 0.4% (6). More recently, PUUV has been demonstrated as a cause of hantavirus pulmonary syndrome as well, which was previously thought to be caused solely by American hantaviruses (22). DOBV, in contrast to PUUV, is usually characterized by more severe HFRS, with a case fatality rate up to 12% (23). Although genetically resembling DOBV, clinical data indicate that SAAV infections are much less severe than DOBV (24–26).

During the acute phase of HFRS, mild neurological symptoms such as headache, vertigo, and nausea are common and direct PUUV infection of CNS has been demonstrated (27). Data on the occurrence of severe, potentially life-threatening neurological manifestations is rather scarce; since only a few cases of severe neurological manifestation in DOBV have been described (28–30).

Discussion and conclusion
Routine diagnostics of NE/HFRS in the Netherlands and the majority of northwestern European countries are based on serology and relies on the assumption that PUUV is the most common causative agent. Even during the first days of a hantavirus disease, IgM and usually even IgG antibodies are present. Extensive crossreactivity of antibodies to the various hantaviruses species circulating in Europe complicates interpretation of routine serology tests. However, the cases presented here illustrate that the crossreactivity of antibodies to PUUV and DOBV/SAAV is sometimes weak or completely absent (1, 21). This reflects their antigenic distance and also emphasizes the need for use of multiplex antigens (at least PUUV and DOBV/SAAV) to cover HFRS diagnostics within Europe. Possible SEOV infections will usually be detected by this panel as well, as SEOV is antigenically similar to DOBV (5) which is of importance seeing the increasing evidence for SEOV circulation in wild and pet rats in Europe (31). Definite serological diagnosis of a specific hantavirus infection can be established only by virus neutralization assay. The neutralizing antibody titer to the causative hantavirus should be four-fold higher in comparison to all other relevant hantaviruses. Alternatively, RT-PCR and subsequent sequencing can be performed on acute phase samples, although viral genome sequences can be detected in less than two-thirds of the acute serum samples of suspect cases (30, 32).

The two presented cases emphasize the challenge of definitely diagnosing a specific hantavirus infection based on serology, as both patients were reactive in common routine PUUV diagnostic tests and we could not unambiguously conclude whether infection was caused by DOBV or SAAV (although they are sometimes considered to be lineages of one hantavirus species). In the acute serum of the second patient, hantavirus genome was detected at the same end-point dilution for DOBV and SAAV (1:160).

The presented cases furthermore underscore that health care workers in regions where typically PUUV related, and possibly SEOV, infections may be detected should be aware of other circulating hantaviruses that may be imported to non-endemic regions. DOBV is known to cause severe and fatal HFRS and requires more intensified supportive care than the less severe PUUV- and SAAV-infected patients. For an adequate diagnosis, multiplex testing targeting different strains of hantavirus is required in clinical suspect cases. Recently published quality assessments have demonstrated that within Europe, the overall specificity and sensitivity of detecting hantavirus infection is acceptable but that the exact serotyping can be problematic with currently available serodiagnostic methods. Especially, the misclassification of DOBV cases by routine diagnostics based on ELISA warrants vigilance and more studies into the burden of DOBV infections in Europe (33, 34).

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