Network for wildlife health surveillance in Europe

Diagnosis Card

Hantavirosis

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Etiology

Hantaviruses are members of a separate genus *Hantavirus* within the family *Bunyaviridae*. Enveloped viruses with a single stranded, trisegmented RNA genome of negative polarity. Species definition according to the association to a certain reservoir, level of sequence divergence in the nucleocapsid and glycoprotein precursor, the level of cross-neutralisation and absence of reassortment processes.

Affected species (wildlife, domestic animals, humans)

Natural reservoirs of the hantaviruses in Europe are rodents and insectivores. Puumala virus (PUUV) is harbored by the bank vole (*Myodes glareolus*). Tula virus has been molecularly detected in the common vole (*Microtus arvalis),* thought to be the main reservoir, but also in the southern vole (*M. levis*, previously *M. rossiaemeridionalis*), the field vole (*Microtus agrestis*) and the water vole (*Arvicola amphibius*). Four different genotypes of Dobrava-Belgrade virus (DOBV) have been detected in different *Apodemus* species, i.e. “Dobrava” in the yellow-necked field mouse (*Apodemus flavicollis*), “Saaremaa” and “Kurkino” in the striped field mouse (*Apodemus agrarius*) and “Sochi” in the Black Sea Field mouse (*Apodemus ponticus*). In addition rat-associated Seoul virus (SEOV) was detected to be present in Europe. Seewis virus and Asikkala virus have been detected in their reservoirs in different regions of Europe, i.e., in common shrew *Sorex araneus* and in pygmy shrew *Sorex minutus*, respectively. Nova virus has been reported so far for common mole (*Talpa europaea*) samples from few parts of Europe. In addition to infections of natural reservoirs, hantavirus infections have rarely been detected in non-reservoir small mammal species. Most mammals, including humans, are potential targets for spillover infections. Serological findings demonstrated natural infections of cats, dogs, foxes, moose and non-human primates. Domestic animals have not been investigated for the susceptibility to hantavirus infection. Human hantavirus infections have been reported for almost all European countries.

Epidemiological characteristics and disease course

The hantavirus epidemiology is mainly determined by the reservoir hosts. The reservoir hosts are persistently infected without obvious pathological consequences and shed the virus in urine, feces and saliva. The transmission occurs indirectly through inhalation of virus-contaminated aerosols or by biting. Depending on the geographical distribution of the reservoir, hantaviruses are endemic in certain regions of Europe. PUUV outbreaks have been reported in several European countries and are mainly driven by a mass reproduction of the bank vole. Human disease is a notifiable disease in several European countries. The oscillation of the number of reported human cases is also influenced by the awareness of the physicians. The seasonal pattern of human infections vary between the geographical regions in Europe.

Transmission to humans is also mediated by virus-contaminated aerosols, very rarely transmission by biting have been reported. Hantavirus infections in Europe are characterized by a broad spectrum of clinical symptoms ranging from non-symptomatic infections to severe cases with lethal outcome. The severity of disease and the case fatality rate depends on the hantavirus causing the human infection with the highest for DOBV genotypes “Dobrava” and “Sochi” reaching 12-15%. For PUUV and the DOBV genotype “Kurkino” a very low case fatality rate has been found. In addition, the gender and other genetic factors seem to influence the disease outcome in humans. With regards to other

hantaviruses, knowledge of human infections is very limited until now. For insectivore-borne hantaviruses it is currently not known if they may cause disease in humans. After virus clearance the patients recover completely, although some long-term sequelae are increasingly discussed.

Clinical signs

Humans: After an incubation period of normally 2 to 3 weeks typical signs of disease are high fever, headache, gastrointestinal symptoms, abdominal pain, nausea and vomiting. In more severe cases renal dysfunction and haemorrhagies will occur. Therefore all human hantavirus disease cases are summarized as haemorrhagic fever with renal syndrome (HFRS). PUUV infections cause a milder form of HFRS, also designated nephropathia epidemica. For typical HFRS cases five distinct disease stages have been reported: febrile phase, hypotensive phase, oliguric phase, polyuric phase, convalescent phase. Laboratory parameters of HFRS are thrombocytopenia, as well as markers for renal dysfunction. i.e. increase of serum creatinine, microhaematuria, proteinuria.

Other non-reservoir mammals: It is not known if natural infection of non-reservoir mammals results in the development of clinical symptoms. Few infection experiments of non-human primates resulted in the development of disease similar to that observed in human.

Reservoir: In general, rodent reservoirs are believed to be infected without obvious clinical signs, but some studies reported histopathological lesions and reduced fitness.

Gross lesions

HFRS patients: post mortem detection of haemorrhages in the kidney

Histological lesions

HFRS patients: some cell destruction in tubular epithelial cells found in kidney biopsies

Reservoir: pulmonary oedema and periportal hepatitis reported for New York virus-infected white-footed mouse *Peromyscus leucopus* and Sin nombre virus-infected deer mouse *P. maniculatus*.

Differential diagnosis

Leptospirosis, other diseases with “flu”-like symptoms.

Criteria for diagnosis

Human: RT-PCR or serological detection is needed for diagnosis, as clinical signs in humans are usually unspecific; for differentiation of the causative agent nucleotide sequence determination or focus neutralization assay required.

Reservoir: RT-PCR and sequence determination The reservoir species appear to be asymptomatic. Diagnosis can be made either by detection of antibodies (ELISA, see below) or specific molecular nucleic acid detection (RT-PCR, see below)

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

(a) **Serological detection of hantavirus reactive antibodies**

- indirect immunofluorescence assay (IFA): commercially available and in-house assays based on Vero E6 cells infected by selected hantaviruses
- rapid immunochromatographic assay: commercially available assay for bed-side diagnosis in patients and for on-side diagnosis in rodents
- immunoblot assay: commercially available line assay using recombinant proteins of selected hantaviruses or in-house assays using viral or recombinant antigens
- ELISA: commercially available and in-house assays using viral or recombinant antigens. Usually bacterial, yeast or baculovirus-expressed nucleocapsid protein is used as antigen
- focus reduction neutralization assay: the assay is time consuming and requires a BSL-3 biocontainment laboratory. Allows a serological differentiation of hantavirus infections based on the hantaviruses used in the assay
- Samples: blood, serum (from alive individuals) or chest cavity fluid (from dead animals), stored at -20 °C. The minimum amount needed for a single assay is 50 µl for blood and 10 µl for serum.

(b) **Detection of hantavirus antigen**

- antigen ELISA: in-house capture ELISA for detection of viral nucleocapsid protein in tissue samples of reservoirs
- Samples: lung tissue, stored at -20 °C

(c) **Molecular detection of hantaviruses**

- conventional RT-PCR: mostly RT-PCR assays targeting the S-segment are used, for detection of PUUV/TULV a S-segment specific RT-PCR was developed, for detection of other European

hantaviruses and novel unknown hantaviruses a nested RT-PCR assay targeting the L-segment was developed

- real-time RT-PCR: both Sybr Green- and probe-based real-time RT-PCR assays have been developed, the high sequence divergence between the hantaviruses requires the use of a RT-PCR assay adapted for the virus expected in the sample (or a parallel use of different primer combinations).
- Microarray: microarray technology might also be used for hantavirus diagnostics; an array has been developed for identification and discrimination of hantaviruses.
- Samples: A piece of at least 3 mm diameter lung or kidney tissue is needed for RNA isolation. The tissue should be stored at -20 °C and the RNA at -70 °C.

(d) **Virus isolation**

Virus isolation from human patient material has only rarely been successful; isolation approaches from rodent tissue material resulted in the generation of virus isolates of several hantavirus species.

APHAEA protocol (for harmonization at large scale)

The protocol depends on the diagnostic or research question. For standardized serological investigations in humans, reservoirs and spillover-infected sentinels the use of commercial ELISAs, IFAs or rapid immunochromatographic tests is recommended. Virus RNA detection should use real-time or conventional S-segment specific RT-PCR assays; for molecular epidemiological investigations conventional RT-PCR assays and sequence analysis should be performed. When searching for novel hantaviruses, a generic L-segment RT-PCR or a Next Generation Sequencing approach is recommended.

Laboratories that can be contacted for diagnostic support

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