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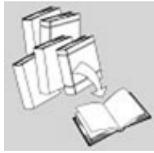
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Hantavirus infections in Europe and their impact on public health

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SUMMARY

Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) are enveloped tri-segmented negative-stranded RNA viruses each carried by a specific rodent or insectivore host species. Several different hantaviruses known to infect humans circulate in Europe. The most common is Puumala (PUUV) carried by the bank vole; another two important, genetically closely related ones are Dobrava–Belgrade (DOBV) and Saaremaa viruses (SAAV) carried by *Apodemus* mice (species names follow the International Committee on Taxonomy of Viruses nomenclature). Of the two hantaviral diseases, hemorrhagic fever with renal syndrome (HFRS) and hantaviral cardiopulmonary syndrome, the European viruses cause only HFRS: DOBV with often severe symptoms and a high case fatality rate, and PUUV and SAAV more often mild disease. More than 10,000 HFRS cases are diagnosed annually in Europe and in increasing numbers. Whether this is because of increasing recognition by the medical community or due to environmental factors such as climate change, or both, is not known. Nevertheless, in large areas of Europe, the population has a considerable seroprevalence but only relatively few HFRS cases are reported. Moreover, no epidemiological data are available from many countries. We know now that cardiac, pulmonary, ocular and hormonal disorders are, besides renal changes, common during the acute stage of PUUV and DOBV infection. About 5% of hospitalized PUUV and 16%–48% of DOBV patients require dialysis and some prolonged intensive-care treatment. Although PUUV–HFRS has a low case fatality rate, complications and long-term hormonal, renal, and cardiovascular consequences commonly occur. No vaccine or specific therapy is in general use in Europe. We conclude that hantaviruses have a significant impact on public health in Europe. Copyright © 2012 John Wiley & Sons, Ltd.

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INTRODUCTION

Hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are tri-segmented negative-stranded enveloped RNA

viruses carried by rodents and insectivores. They cause two diseases, hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) [1–3]. Humans get mainly infected from aerosolized rodent excreta but HCPS may be also transmitted from person-to-person and HFRS from blood transfusions [4,5]. Several hantaviruses cause HFRS in Europe, an endemic zoonosis, diagnosed in more than 10,000 individuals in Europe annually. The principal HFRS-inducing hantaviruses in Europe are Puumala (PUUV) carried by *Myodes* voles and two interrelated viruses carried by *Apodemus* mice, Dobrava–Belgrade

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Abbreviations used

CRP, C-reactive protein; DOBV, Dobrava–Belgrade virus; HCPS, hantavirus cardiopulmonary syndrome; HFRS, hemorrhagic fever with renal syndrome; IDO, indoleamine 2,3-dioxygenase; NE, nephropathia epidemica; PUUV, Puumala virus; SAAV, Saaremaa virus; SEOV, Seoul virus; TULV, Tula virus.

virus (DOBV), and Saaremaa virus (SAAV). These are the species listed by the International Committee on Taxonomy of Viruses, but the nomenclature of the European *Apodemus*-derived hantaviruses has been and still is, under debate and revision: in literature DOBV variants in *Apodemus flavicollis* are also referred to as DOBV-Af, and variants in *Apodemus ponticus* as DOBV-Ap. Some strains recovered from *Apodemus agrarius* are described as a genotype DOBV-Aa. Seoul virus (SEOV) is the causal virus for medium severe HFRS in Asia and in many cities worldwide but has been detected only once with certainty as the cause of HFRS in Europe [6,7]. Similarly, Tula virus (TULV) although common in *Microtus* voles in Central and Eastern Europe, has been associated with HFRS in one patient [8]. No specific antiviral therapy or vaccine is in general use in Europe. Recently, several complications and long-term consequences have been associated with HFRS. In the following, we will evaluate the disease burden of hantavirus infections and HFRS in Europe.

Hantavirus infections in Europe

European human-pathogenic hantaviruses form phylogenetically and serologically two distinct groups (a separate antigen is preferentially needed

for the diagnosis), dependent on whether they are carried by the distinct rodent groups, either voles or by (Old World) mice and rats. Viruses carried by voles, mice, and rats are found in Europe (Table 1, Figures 1–3). In addition to the two rodent-borne virus clusters, an even larger or enlarging variety of hantaviruses is found within insectivores.

Viruses carried by voles (family Cricetidae, subfamily Arvicolinae)

Puumala virus, a causative agent of hemorrhagic fever with renal syndrome (PUUV–HFRS) or nephropathia epidemica (NE), has been detected widely in Europe, excluding British Isles, southern Mediterranean areas, and the very northernmost tundra regions. This parallels (except for the British Isles) with the distribution of the main carrier of PUUV, the bank vole (*Myodes glareolus*, previously known as *Clethrionomys glareolus*). Tula virus, carried by *Microtus arvalis*, *M. levis*, and by some other *Microtus* species [9], is found widely in Central and Eastern Europe and can rarely infect humans or cause disease – only a single case has been reported [8,10,11].

Table 1. Hantaviruses circulating in Europe

Virus	Carrier	Disease
Viruses carried by voles		
Puumala*	<i>Myodes glareolus</i> (bank vole)	HFRS (mild)
Tula*	<i>Microtus arvalis</i> , <i>M. levis</i> (common vole, sibling vole) other <i>Microtus</i>	Infects humans, HFRS in one case reported
Viruses carried by mice or rats		
Dobrava–Belgrade* (or DOBV-Af) (or DOBV-Ap)	<i>Apodemus flavicollis</i> (yellow-necked mouse) <i>Apodemus ponticus</i> (Black Sea field mouse)	HFRS (severe) HFRS (medium severity)
Saaremaa* (or DOBV-Aa)	<i>Apodemus agrarius</i> (striped field mouse)	HFRS (mild)
Seoul*	<i>Rattus norvegicus</i> , <i>R. rattus</i> (rat)	HFRS (medium severity)
Viruses carried by insectivores (Seewis, Nova)	No known human infection to date	

*Officially recognized virus species by International Committee on Taxonomy of Viruses. The nomenclature of the *Apodemus*-carried hantaviruses is under discussion.

HFRS, hemorrhagic fever with renal syndrome; DOBV, Dobrava–Belgrade virus.

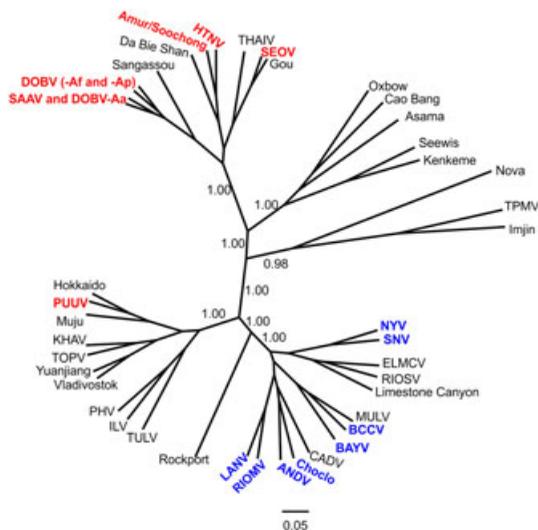


Figure 1. Phylogenetic tree on the basis of S segment ORF sequences. Bayesian maximum clade credibility tree with Bayesian posterior probabilities is given at main nodes. International Committee on Taxonomy of Viruses-approved hantavirus species are shown as abbreviations and tentative species with full names. Hantavirus species associated with HFRS are shown in red and with HCPS in blue

Viruses carried by Old World mice and rats (family Muridae, subfamily Murinae)

Saaremaa (SAAV or DOBV-Aa) has been found in striped field mice (*A. agrarius*) in Estonia, Russia, southeastern Finland, Germany, Denmark, Slovenia, Croatia, and Slovakia. There are no well-documented SAAV-HFRS cases but serology including neutralizing antibodies indicates that human SAAV (DOBV-Aa) infections are common [1–3,12]. Dobrava–Belgrade virus (DOBV or DOBV-Af), which is associated with considerable up to 12% case fatality rate, is carried by yellow-necked mice (*A. flavicollis*) and DOBV–HFRS has been found in Slovenia, Serbia, Croatia, Greece, Albania, Hungary, and Bosnia–Herzegovina [7,11]. In southern Russia (Sochi district), where HFRS is endemic, severe to moderate HFRS cases were found to be caused by a new DOBV variant, DOBV-Ap or Sochi subtype, carried by *A. ponticus* (Black Sea field mouse), a novel hantavirus natural host [3,12]. The severity of disease associated with DOBV-Ap seems to be less than for DOBV-Af

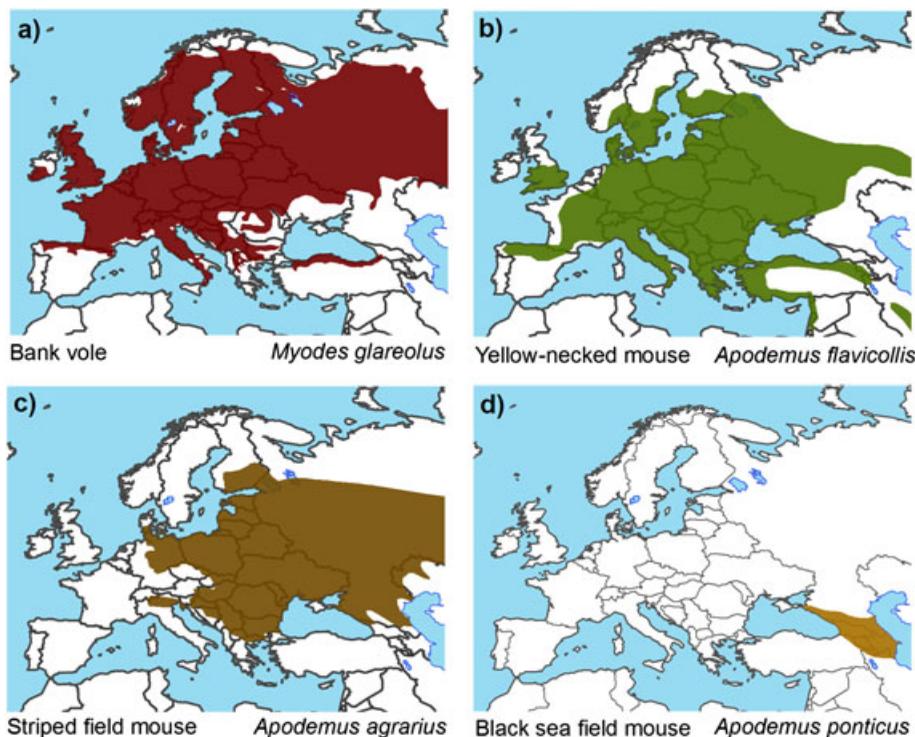


Figure 2. Distribution of rodent hosts of pathogenic hantaviruses in Europe: (a) *Myodes glareolus*, host of Puumala virus. (b) *Apodemus flavicollis*, host of Dobrava–Belgrade virus (DOBV-Af). (c) *Apodemus agrarius*, host of Saaremaa virus (and DOBV-Aa). (d) *Apodemus ponticus*, host of DOBV-Ap. Please note that the geographic areas where virus-carrying rodents have been found are different from the overall distribution of the rodents (see text). The distribution of rodents is based on the IUCN Red List of Threatened Species (update 2010.4, <http://i.iucnredlist.org/spatial-data/2010.4/GISData/MAMMTERR.zip>)

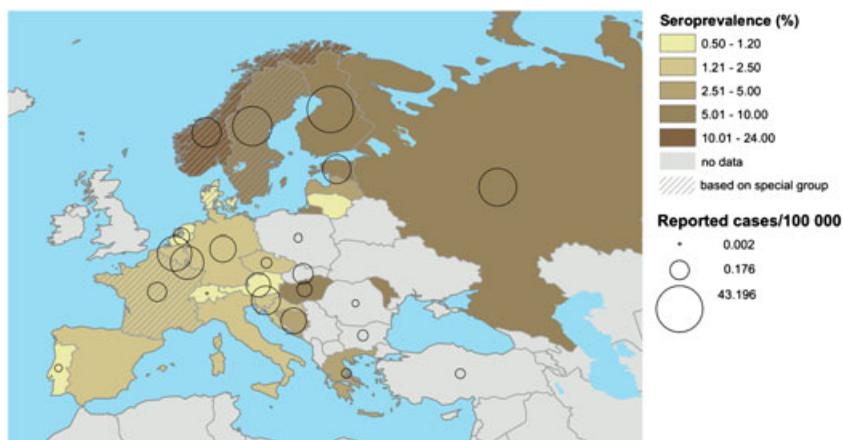


Figure 3. Seroprevalence and incidence of hemorrhagic fever with renal syndrome (HFRS) in different European countries. The area of the circles refers to the number of reported cases. In the “striped” countries, the seroprevalences are based on restricted populations: In Denmark, the Island of Fyn, in France, foresters in île-de-France, in Norway, endemic areas, and in Sweden, Northern part of the country. For references, see text and in the case of the seroprevalences in restricted populations, see Olsson *et al.* [23]

but more than for PUUV. Altogether, while the nomenclature of the European *Apodemus*-derived hantaviruses is still under revision, it is evident that *A. flavicollis* (DOBV-Af) and *A. ponticus* (DOBV-Ap)-derived viruses cause severe and life-threatening infections, whereas *A. agrarius*-derived hantavirus infections (DOBV-Aa) are mild, which is in contrast to the high pathogenicity of the Asian prototype Hantaan virus carried by local *A. agrarius*.

In Europe, Seoul virus (SEOV) has been found in a few laboratory rat-derived outbreaks and in a few wild rats (*Rattus*) in France. Other than these findings, SEOV infection has been detected only in a single human case in France [7]. Tula virus is known to infect humans and has been associated with a clinical HFRS case [8,11].

Other hantaviruses

With travelers, imported human cases to Europe of other hantaviruses may occur, including American viruses causing HCPS, which are carried by sigmodontine and neotomine rodents (rats and mice of the New World, family Cricetidae, subfamilies Sigmodontinae and Neotominae), which only occur in North and South America [1–3].

The first hantavirus ever discovered, Thottapalayam, was isolated from a species of the order Soricomorpha, the Asian house shrew (*Suncus murinus*) in southern India more than four decades ago [13]. Recently, several new hantaviruses have been found by RT-PCR; some also isolated in cell culture: Imjin from a crocidurine shrew in

Korea [14] and two new viruses from Finland [our unpublished results] from soricine shrews. In addition, new hantaviruses have been detected in moles (*Talpidae*), such as Nova virus in Europe [15–17]. Interestingly, a hantavirus, related to Thottapalayam and Nova viruses, was recently detected by RT-PCR in an African insectivorous bat, *Nycteris hispida* [18]. However, it is not known whether any of these shrew-associated, mole-associated or bat-associated hantaviruses can infect humans or cause illnesses.

Epidemiology of hantavirus infections in Europe

Nephropathia epidemica/HFRS is a notifiable disease in most European countries. Hantavirus infections are very common, for example, in Finland (especially central and eastern areas), Northern Sweden, Ardennes forest region (Belgium, France), parts of Germany and especially in its southwestern part, the Balkans and in parts of European Russia (e.g. Bashkortostan and Udmurtia regions and Republic of Mari). Also notably, in large areas of Europe (e.g. Estonia, Latvia, Hungary, and Greece), the population has a high seroprevalence (Figure 3), but only relatively few HFRS cases are reported. Moreover, comprehensive epidemiological data are not available from many countries (e.g. UK, Poland, Ukraine, and Greece). Thus, hantavirus infections are heavily underdiagnosed in Europe and even more so in most of Asia [2,7,10,19].

In Finland, the overall seroprevalence in the total population is highest in central and eastern

Finland; males contract NE at the mean age of 40 years, females at 44 years. From the seroprevalence (5% in Finland) and incidence (Figure 3), it may be calculated that only 20%–30% of infected humans experience clinical problems severe enough to seek medical attention leading to serological confirmation [20–22].

The epidemiological pattern has a particular temporal cyclicity and can change geographically. In Northern Europe, there are 3- to 4-year cycles of *M. glareolus*, and up to the late 1990s many parts of Finland were in non-synchronous phases of vole cycles. More recently, the whole southern part of the country has been synchronous leading to a simultaneous epidemic peak in a large area instead of smaller local non-synchronous peaks every year [23]. Consequently, in 1999, 2002, and 2005, Finland had about 2500 serologically diagnosed HFRS cases, and in 2008, a record year, 3259 PUUV–HFRS cases. Belgium had peak years in 2007 (298 cases) and 2008 (336 cases), Sweden in 2007 (2195 cases), and Germany in 2007 (1688 cases) and in 2010 (>2000 cases) [2,7,10,24].

Environmental factors predicting HFRS epidemics

Hemorrhagic fever with renal syndrome epidemics is spatially associated with the natural habitats of hantavirus carrier rodents. As the bank vole is a forest-dwelling species, the risk of PUUV infection increases with the proportion of forested land cover [23,25–27], the vicinity of forests [28], and green biomass [29]. Within boreal forested habitats, increased bank vole abundance and hence the abundance of PUUV-infected bank voles appears to be associated with characteristics of old-growth moist forest [30]. *A. flavicollis*, carrier of DOBV, prefers temperate deciduous forests. *A. agrarius*, carrier of SAAV/DOBV-Aa viruses on the other hand, is connected to agricultural habitats [23].

The human epidemiology of PUUV–HFRS follows the local rodent dynamics, that is, human cases occur in the same rhythm as the rodent fluctuations [31,32]. In temperate Europe, HFRS follows mast years of deciduous trees (beech and oak), which in turn tend to follow warm summers [32,33]. A heavy crop of beech mast and acorns induces an outbreak of *M. glareolus* and *A. flavicollis* [9,33–36]. Interestingly, although bank voles and climate-driven mast years also occur on British Isles, no hantavirus

infections have been reported in humans or rodents [37]. Human epidemics typically occur in summer of the rodent peak year. In the North, bank voles undergo 3- to 4-year population cycles and human HFRS epidemics coincide with vole peaks in late autumn and winter; there are annually two seasonal peaks: a minor peak in August (urban people are infected during their summer vacations in July) but a major peak in November–February (after the major bank vole density peak, and when rodents typically have entered human dwellings) [6,20,23]. The large outbreak of PUUV infections in Northern Sweden in 2007 was preceded not only by an increase in rodent population but also by unusual weather conditions: mild early winter with rain and melting snow followed by heavy frost and ice on the ground, which presumably forced rodents to human dwellings [38]. It is of particular interest here that even though rodent peaks superficially look similar in temperate and boreal Europe, the underlying causes are very different. In the north, it is primarily a question of top-down ecological processes (predation) causing the cyclicity while in temperate zone bottom-up processes (masting) govern the outbreaks of forest rodents. It is also worth remembering that it takes 1–2 years for a rodent peak to develop, and therefore, current climatic conditions during an epidemic are not the primary cause even though they may contribute to it.

Hantaviruses, despite being enveloped RNA viruses, are unexpectedly stable, >10 days at room temperature and >18 days at +4 °C and –20 °C [39,40]. The considerably colder conditions in Northern Europe, particularly during the human epidemic peak, could therefore contribute to the high disease burden. PUUV has also an impact on its carrier rodent: no visible disease but notably impaired winter survival [41].

Risk factors to catch hantavirus infections

The incidence of PUUV infection varies geographically considerably between countries and within each country. The male gender is a clear risk factor with a male/female ratio of, for example, 1.67 in Finland and 1.52 in Sweden [24,42]. It is evident that rodent contact, “seeing rodents”, marks an increased risk. The most important risk factors include smoking and condition of the housing (whether there are holes allowing rodents to enter) and opening closed buildings/premises suggesting that hantavirus infections occur mainly indoors

and by inhalation and are therefore affected by condition of the respiratory tract [43–45]. Further risk factors include use of rodent traps instead of poison in rodent control, and risk has been attributed also to woodcutting and house warming with firewood and spending time and working in the forest. Increased incidence or occupational risk is attributed also military activity and crises, farming, forestry, camping, and summer cottages [24,45,46].

Clinical picture

The course of PUUV and DOBV infection is highly variable ranging from asymptomatic to lethal outcome. The most common clinical findings are fever, headache, abdominal pains, backache, and nausea/vomiting [6]. Patients usually do not have remarkable respiratory tract symptoms. Slovenia has both DOBV-infected and PUUV-infected HFRS patients that has made comparison of the clinical presentations possible [47]. Hemorrhagic complications, pleural and abdominal effusion, shock and case-fatality rate were found to be all more common in DOBV-HFRS. Similarly, thrombocytopenia is more severe, and alanine aminotransferase as well as serum creatinine levels is higher in DOBV-HFRS.

Ocular findings are very common (70%) in acute PUUV-HFRS. A total of 87% had reduced visual acuity, 78% had myopic shift, 88% had decreased intraocular pressure, and 88% thickening of the lens [48]. Thus, ocular findings combined with fever, headache, and thrombocytopenia may be pathognomonic. PUUV-related CNS symptoms seen in magnetic resonance imaging and electroencephalography and as signs of inflammation and PUUV-IgM in cerebrospinal fluid are common in acute PUUV-HFRS [49]. Lethal cases, in which the pituitary gland was invaded by PUUV resulting in local hemorrhages and necrosis, have been described [50].

The most typical laboratory findings in the acute phase are leukocytosis, thrombocytopenia, increased serum C-reactive protein (CRP), and creatinine levels as well as proteinuria and hematuria. The clinical picture and laboratory findings are basically similar in PUUV and DOBV-induced HFRS, but in DOBV infection the findings are commonly more severe [6,47]. Thus, the serum levels of IL-10, IFN- γ , TNF- α , and of procalcitonin were higher in patients infected with DOBV than PUUV [51,52].

The clinical course of HFRS in Central-European and Balkan DOBV infections varies from mild to moderate to severe [53–55]. The severity of the disease caused by DOBV-Aa resembles that of HFRS caused by PUUV, and the severity of the disease caused by DOBV-Ap infections is more often moderate to severe [12,56,57].

Typical renal histological finding in PUUV-HFRS is acute tubulointerstitial nephritis. An immunocytochemical study indicated that TNF- α is strongly expressed in the peritubular area of the kidneys [58]. The level of IL-6 in urine correlates with the amount of proteinuria in acute PUUV-HFRS suggesting local production of this cytokine in the HFRS kidneys [59].

Severe clinical course of PUUV-HFRS is strongly associated with HLA-B8 and mild with HLA-B27 [60–63]. In a recent study, Slovenian DOBV-infected patients had a significantly higher frequency of HLA-B*35 than PUUV-infected patients [64]. According to preliminary evidence, the same HLA haplotype may be associated with a severe course of Sin Nombre HCPS infection [65]. Interestingly, in *M. glareolus*, the DQA MHC class II gene and TNF- α polymorphism are associated with PUUV infections [66–68]. Could rodent host genetics explain why only some European *A. flavicollis* populations carry DOBV?

There is good evidence that complement activation contributes to the pathogenesis of PUUV infection [69,70]. Levels of the soluble terminal SC5b-9 complex were higher, and C3 levels were lower in the acute stage than during convalescence, especially in patients with chest x-ray abnormalities. These changes had a significant correlation with clinical and laboratory parameters reflecting disease severity. These results suggest that complement activation via the alternate pathway contributes to the pathogenesis of acute PUUV [70], and we have obtained further support for these findings from analysis of lethal PUUV-HFRS cases [Sironen *et al.*, in manuscript]. It was concluded that in lethal PUUV-HFRS, pulmonary involvement is critical—in addition to multiorgan failure (liver, pituitary gland) and that complement activation leading to vascular leakage, especially in the lungs may contribute to the pathogenesis.

Acute-phase complications

Several severe complications have been described both in PUUV-caused and DOBV-caused HFRS

(Table 2). Many of them are rare but can lead to intensive care treatment of the patients and overall long hospital treatment or even a lethal outcome. Case fatality in PUUV–HFRS is very low ranging from 0.08% [24] to 0.4% [42]. In contrast, the case-fatality rate of DOBV infections has been reported up to 12% for DOBV-Af [6,47], and about 6% for DOBV-Ap. For SAAV/DOBV-Aa, the case-fatality rate is 0.6%–0.9% [12,57]. Fatal cases have been due to fluid imbalance after shock, hemorrhages and necrosis in the pituitary gland, and encephalitis. Severe complications and deaths in PUUV–HFRS have been reported only in adult patients [101].

Dialysis treatment is needed in 0%–6% of PUUV-infection-induced acute renal failure [73,93,97]. In DOBV infection, the corresponding figures have been 16%–48% [47,97].

A third of acute-stage PUUV–HFRS patients have abnormal chest radiography findings but almost all have lung parenchymal abnormalities when studied by high-resolution computed tomography [88,90,104]. The most severe abnormality, pulmonary edema is a rare complication. These

findings indicate that HFRS is a general disease and not so different from HCPS [92]. The most severe abnormality, pulmonary edema is a rare complication, but it has been observed in both PUUV and DOBV infections [54,87,88,102]. These findings indicate that HFRS is a general disease and not so different from HCPS [92,103].

About half of the PUUV and DOBV infection patients have abnormal cardiac findings [85,86]. Most common are electrocardiographic changes, but also abnormal echography findings have been observed [86]. All these cardiac changes reverted to normal during the follow-up.

Long-term consequences

Hormonal deficiencies are common during and after PUUV infection. More than 50% of patients had abnormalities of the gonadal and/or thyroid axis during acute PUUV–HFRS [105]. Notably, 17% had a chronic overt hormonal deficit 5 years after acute PUUV–HFRS. In several cases, continuous hormone-replacement therapy (hydrocortisone, thyroxin, and testosterone) was required. Chronic hormonal defects could not be predicted by the severity of acute PUUV–HFRS [105]. Hypopituitarism is the most common endocrinological complication of HFRS (Table 3), but in our recent study, also several cases of primary hormonal defects (hypothyroidism and testicular failure) were observed [105].

Acute tubulointerstitial nephritis caused by hantaviruses has a good prognosis. In most patients, a total recovery of the renal function is observed. Depressed renal tubular function, however, has been reported in many studies (Table 3). This manifests

Table 2. Severe complications of acute Puumala and Dobrava–Belgrade infection

<i>Neurological</i>
Meningoencephalitis [71–74]
Acute disseminated encephalomyelitis [75,76]
Generalized seizure [77]
Pituitary hemorrhage [49,50,78–81]
Guillain–Barré syndrome [72,83]
Urinary bladder paralysis [80]
Epileptic seizures and hemiparesis due to focal encephalitis [84]
<i>Cardiopulmonary</i>
Shock [6]
Perimyocarditis [71,73,74,81,85,86]
Pulmonary edema [54,87–92,102]
<i>Hematological</i>
Disseminated intravascular coagulopathy [81,87,93–95]
Multiple bleedings [47,82]
<i>Others</i>
Need for dialysis [47,73,93,96,97]
Pancreatitis [98]
Multiorgan failure [99,100]
Lethal outcome [6]

Table 3. Long-term consequences of Puumala and Dobrava–Belgrade infection

<i>Nephrological</i>
Depressed tubular function [71,93,106–111]
Glomerular hyperfiltration [108,110,111]
Chronic glomerulonephritis [112,113]
<i>Cardiovascular</i>
Hypertension [71,108,110,111,114–117]
<i>Endocrinological</i>
Hypopituitarism [49,50,81,82,98,105,118–122]
Primary hypothyroidism [105]
Testicular failure [105]

itself as increased tubular proteinuria several years after acute PUUV or DOBV infection.

In rare cases, glomerulonephritis may complicate the convalescent phase of PUUV–HFRS [112,113]. The clinical manifestation has been the nephrotic syndrome, and the renal histopathological finding has usually been membranoproliferative glomerulonephritis. The long-term outcome has been favorable in most patients.

In our two independent series, PUUV–HFRS patients had higher glomerular filtration rate, more proteinuria and higher blood pressure than healthy controls 5–6 years after acute disease [108,111]. After 10 years of follow-up, the effect had largely, but not totally, disappeared. It seemed possible that PUUV–HFRS may predispose some patients to the development of hypertension [123]. Hypertension, first observed for the ratborne Seoul virus in Baltimore [124], has been reported in several other studies as a consequence of PUUV or DOBV infection (Table 3).

Finally, our unpublished work based on a large serum bank shows that in Finland PUUV seropositivity is associated with increased tendency to cardiovascular disease (myocardial infarction) in men aged ≥ 50 years.

New markers for severe course of hantavirus infection

Thrombocytopenia is a known hallmark of hantaviral disease, both HFRS and HCPS. Although the mechanism of thrombocytopenia is obscure, it is now known to be associated with increased thrombin formation and fibrinolysis [95]. It is also known that the circulating adhesive platelet ligands are altered in acute PUUV–HFRS. Fibrinogen and von Willebrand factor antigen are markedly upregulated, and fibronectin is decreased [125]. These findings imply several rearranged interactions between platelets and their ligands. It is possible that the interaction of platelets with endothelium could provide the mechanism of thrombocytopenia. [95,125].

High plasma IL-6 levels are associated with severe renal failure and thrombocytopenia in PUUV–HFRS and can be used as a marker of the severity of the disease [126]. Interestingly, high plasma CRP may have a protective effect on renal function [126]. Pentraxins are a family of acute-phase proteins with a cyclic multimeric structure.

They are related to the short pentraxins, CRP, and serum amyloid P. We recently found that high plasma pentraxin-3 levels associate with overall clinical severity of PUUV–HFRS so that pentraxin-3 may even be involved in the pathogenesis of thrombocytopenia [127].

Indoleamine 2,3-dioxygenase (IDO) is an immunomodulatory enzyme produced by, for example, activated macrophages. IDO is involved in tryptophan catabolism leading to tryptophan depletion and halted growth of microbes as well as inhibition of T-cell responses. IDO is induced by IFN- γ . High serum IDO levels are associated with increased disease severity, especially renal impairment in PUUV infection [128].

At the acute stage, the degree of leukocytosis and of GATA-3 mRNA in urinary cells is a risk factor for severe acute kidney injury in PUUV–HFRS [129]. GATA family transcription factors play multiple vital roles in hematopoiesis in many cell lineages, and in particular, T cells require GATA-3 for execution of several developmental steps. Thus, it seems possible that the elevated GATA-3 mRNA in urinary sediment reflects kidney injury in distal tubular or collecting duct cells in PUUV-infected kidneys.

Increased levels of Mac-2 binding protein (Mac-2BP; also known as tumor-associated antigen 90K or galectin-3 binding protein) have been detected in the circulation of patients with certain tumors and patients with chronic virus infections (HIV-1, HBV, and HCV) in which the levels correlate with the severity of the disease [for review on Mac-2BP, see Hepojoki J, PhD thesis, available at <http://ethesis.helsinki.fi>]. When purifying Tula hantavirus, we found that it copurifies and binds to Mac-2BP. The results indicated that hantavirus binds Mac-2BP. We found high Mac-2BP levels in acute-stage PUUV–HFRS and that the levels correlate with disease severity and increased complement activation [Hepojoki *et al.* submitted]. The physiological functions of Mac-2BP are linked with immune defense against invading microbes and tumors, but this is the first time Mac-2BP has been shown to bind a microbe. The results suggest a role for Mac-2BP in the recognition of an invading virus and activation of the innate-immune response.

Diagnostics

Hemorrhagic fever with renal syndrome should be suspected if high fever is accompanied with head/

backache, thrombocytopenia, acute renal deficiency, and ocular findings. However, because the symptoms are so variable, the diagnosis should be confirmed serologically [22]. When the patient seeks medical attention ordinarily, both IgM and IgG antibodies are found in more than 95% of the cases, and on day 6 after onset of symptoms at the latest. Acute HFRS is diagnosed by detection of IgM antibodies, most commonly today by enzyme immunoassay on the basis of recombinant nucleocapsid protein. Many laboratories also use immunofluorescence on acetone-fixed virus-infected cells since the early IgG antibodies are primarily targeted to the nucleocapsid protein, which gives a granular staining pattern; during convalescence antibodies to envelope proteins (Gn and Gc) also appear, which are seen as diffuse cytoplasmic staining. Commercially-available user-friendly immunochromatographic tests are available for detection of IgM antibodies within 15 min. Although the hantaviral antibodies show antigenic cross-reactions, two tests should be used in many areas of Eurasia, one detecting antibodies to the vole-borne Puumala virus and another one to a mouse/rat borne virus such as Hantaan, Seoul, and Dobrava–Belgrade viruses.

As serology is usually diagnostic in the beginning, RT-PCR may not be needed for diagnosis. The extent of viremia (and of viral RNA) varies in HFRS and HCPS and depends largely on the hantavirus type. In general, viral RNA is readily detected or a high viral load is found in severe hantavirus infections (caused, for example, by Hantaan, Dobrava, Sin Nombre, or Andes viruses. Both classical and real-time RT-PCR methods have been developed that accurately provide the diagnosis from blood, serum, urine, cerebrospinal fluid, or saliva even before IgM antibodies [130–134]. Notably, in the case of HFRS, no evidence exists that hantaviruses are transmitted from person-to-person, for example, by kissing. Similar to Sin Nombre HCPS, a high DOBV viral RNA load may be associated with severe HFRS [133,135]. The different hantavirus infections can be sero/genotyped by neutralization/RT-PCR-sequencing tests.

In many endemic areas in Europe, diagnostics are available and used. However, there are regions where the medical community does not recognize HFRS, and diagnostics are not used [6]. For example, in France, HFRS is recognized in the Ardennes and northeastern regions but not around Orleans, 100 km south of Paris, where a large

proportion of bank voles carry PUUV [Noël Tordo, personal communication]. In short, if there are no diagnostics, there is no HFRS. Moreover, because the incubation time is quite long and variable, 2–6 weeks, it may sometimes be difficult to know where the infection was caught.

Prevention

Vaccines have been used in the Republic of Korea and in China for a number of years but not outside Asia. Hantavax, derived from formalin-inactivated Hantaan virus infected suckling mouse brain has been widely used, but frequent booster doses are needed to develop neutralizing antibodies and protective immunity [136]. Jay Hooper and Connie Schmaljohn at the United States Army Medical Research Institute for Infectious Diseases have developed promising DNA vaccines encoding separately Hantaan and Puumala virus glycoproteins that induce neutralizing antibodies [137]. These vaccines have potential use both in Asia and Europe. No specific antiviral therapy is in general use in Europe but both interferon- α and ribavirin have been administered in trials in China with promising results if the drugs can be applied early enough [138–140].

Rodents secrete hantaviruses in their urine, feces, and saliva for many months after infection [141]; and the viruses, as mentioned earlier, are surprisingly stable. Hantaviruses infect humans primarily from aerosolized rodent excreta. In colder climates, rodents enter human dwellings when winter is arriving. Particular risk has been associated with opening, occupying and cleaning structures, such as woodsheds, summer cottages, cowhouses, cellars or granaries, that have been unoccupied by humans but by rodents for a longer time. Infection may be avoided by not inhaling unventilated air in such structures and/or using personal protection. Continuous rodent control in buildings prevents them from shedding virus that could remain infectious for weeks.

Considerations of the disease burden

The average annual total number of reported HFRS cases in Europe (excluding Russia) in 2000–2009 was 3138 but the numbers varied greatly from country to country in different years [10]. Thus, in 2007 and 2010, Germany alone reported 1688 and 2015 cases, respectively. Finland reported 3259 cases in 2008, Sweden 2195 cases in 2007 and

Belgium 372 in 2005 and 336 cases in 2008 [10,142]. Russia reported 7256 and 7157 cases in 2005 and 2006 and a total of 89,162 cases in 1996–2006, but these numbers include also a minority of cases from Asian Russia [10]. Most of the aforementioned HFRS cases were caused by Puumala virus. As indicated earlier, no data are available from several European countries and, judging from the seroprevalence in several countries, HFRS is heavily underdiagnosed. This is true even considering the fact that most PUUV infections are inapparent and maybe only 20% of PUUV infections have symptoms leading to medical attention and diagnostics.

In Finland, which has the highest number of reported HFRS cases in European Union (Figure 3), the disease burden of Puumala virus infections in 1995–2008 was recently estimated based on data reported by laboratories to the National Infectious Disease Registry. Of a total of 22,681 cases, 52% were hospitalized, 85% were in persons aged 20–64 years, and there were 13 deaths (0.08%) [24]. When estimating the disease burden, the following aspects need to be considered: the patients stay in hospital on average 7 days, and as mentioned earlier, up to 5% of them need dialysis and some patients need prolonged intensive-care treatment. Moreover, as mentioned earlier, there are multiple long-term consequences (such as hormonal changes, need for hormone-replacement therapy, and increase in blood pressure and proteinuria).

In case effective antiviral therapy and/or vaccine with properties acceptable for EU standards would become available, a cost-benefit analysis would be needed when considering vaccination of different populations or risk groups. No such analysis is available.

Concluding remarks and future prospects

Hantavirus infections and HFRS are a growing public-health problem in Europe. No specific therapy or vaccine is in use in Europe. Four different inactivated vaccines based on rodent brains or cultured rodent cells were developed in Korea and China and are used locally. Nevertheless, there is a need to develop more advanced vaccines for the European market as well, which could be based on DNA or recombinant proteins. For European use, the vaccine probably should contain components from a vole-derived virus (PUUV) and from a mouse-derived virus (DOBV/SAAV), because

viruses between these two groups cross-react only weakly. In addition, more research is definitely needed on the pathogenesis of HFRS to understand the mechanism of shock and vascular leakage to treat properly the severe forms of HFRS. More detailed studies of the closely related *Apodemus*-carried viruses, some causing life-threatening, others mild infection, could reveal the molecular determinants of pathogenicity. Well-planned controlled prospective studies are needed to define the role of hantavirus infections in the development of chronic kidney diseases, hypertension, hormonal disorders, and other possible chronic diseases. Notably, in many countries and regions, HFRS is not a recognized entity by the medical community. This is also because diagnostics are not available throughout Europe. Very simply, if there are no diagnostics, there is no HFRS. Detection of pathogenic hantavirus infections in rodents or serosurveys of selected patient populations such as dialysis patients provide feasible approaches to detect new endemic areas. However, it seems that some areas of Europe are lacking hantavirus circulation, although the carrier rodents are there—some areas lack also the carrier rodents mentioned here. Anyhow, ecological cycles cause rodent populations to fluctuate strongly, and climate—and climate change—affect these cycles. Monitoring rodent population densities, and preferably also hantavirus infections in rodent host populations, can predict onset of local HFRS outbreaks. In temperate Europe, temperature models could be used to predict masting events and consequent rodent outbreaks and HFRS epidemics [33,34,143].

CONTRIBUTORS

All six authors contributed significantly in preparation of this review.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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