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# The broad spectrum of hantaviruses and their hosts in Central Europe

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**Summary. –** Hantaviruses are considered to be emerging viruses due to their increasing significance as human pathogens and their cyclic reappearance during outbreaks. Central Europe is an important endemic region for hantavirus infections. Reflecting the presence of all relevant small mammals serving as reservoir hosts, close to all recognized European hantaviruses occur also in Central Europe. Important human pathogens, Puumala and Dobrava-Belgrade viruses, are present and cause hemorrhagic fever with renal syndrome of various severities. Moreover, several of the newly recognized shrew- and mole-borne hantaviruses are present. In this review, we summarize current data on molecular detection of hantaviruses in reservoir hosts as well as on molecular epidemiology of human hantavirus infections in Central Europe.

**Keywords:** hantavirus; hemorrhagic fever with renal syndrome; rodent; shrew; Central Europe

**Contents:**



## 1. Introduction

Hantaviruses (the genus *Hantavirus*, the family *Bunyaviridae*) are considered as emerging viruses due to their increasing significance as human pathogens and their cyclic reappearance during outbreaks. They cause two human zoonoses; hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome. Prominent examples of hantaviruses that cause human disease are Hantaan virus, Seoul virus, Dobrava-Belgrade virus (DOBV), and Puumala virus (PUUV) causing HFRS in Eurasia while Sin Nombre virus and Andes virus cause hantavirus cardiopulmonary syndrome in the Americas (Kruger *et al*., 2011). Recently, hantaviruses have also been found in Africa (Klempa *et al*., 2006, 2007, 2012; Kang *et al*., 2011; Weiss *et al*., 2012; Sumibcay *et al*., 2012; Meheretu *et al*., 2012) where they may also represent a significant public health threat (Klempa *et al*., 2010, 2012b).

Hantaviruses are transmitted to humans by aerosolized excreta of their natural hosts, mainly rodents of the family *Muridae*. Recently, several other small mammal groups were shown to be hosts of distinct hantaviruses, including shrews (the order *Soricomorpha*, the family *Soricidae*) (Klempa *et al*., 2007; Arai *et al*., 2007; Song *et al*., 2007a,b), moles (the order *Soricomorpha*, the family *Talpidae*) (Arai *et al*., 2008; Kang *et al*., 2009b,c), and most recently even bats (the order *Chiroptera*) (Weiss *et al*., 2012; Sumibcay *et al*., 2012; de Araujo *et al*., 2012).

Hantaviruses are considered host-specific, usually being associated with a single or a few closely related species as their reservoir hosts. This strong association between hantaviruses and their reservoir hosts is consequently reflected in their geographical distribution. In Europe, two geographical regions are usually considered as typical

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**Abbreviations:** ASIV = Asikkala virus; DOBV = Dobrava-Belgrade virus; HFRS = hemorrhagic fever with renal syndrome; NVAV = Nova virus; PUUV = Puumala virus; SWSV = Seewis virus; TULV = Tula virus

hantavirus endemic regions; Fenno-Scandinavia (due to highest annual numbers of human PUUV infections) and the Balkans (due to the most severe HFRS cases in Europe associated with DOBV). However, Central Europe is also a particularly important "hantavirus region" for several reasons: a) both European pathogenic hantaviruses, PUUV and DOBV are present, b) the number of human hantavirus infections has recently increased to an alarming number (particularly in Germany), c) two genotypes (lineages) of DOBV associated with different mice species are present, d) several novel hantaviruses associated with shrews and moles were recently described here.

The scope of this review is to summarize current data on the molecular detection of hantaviruses in reservoir hosts and molecular epidemiology of human hantavirus infections in Central Europe. Although broader definitions of the regions encompassing Central Europe exist, this review focuses on the following countries: Austria, Czech Republic, Germany, Hungary, Poland, Slovakia, and Switzerland (Fig. 1).

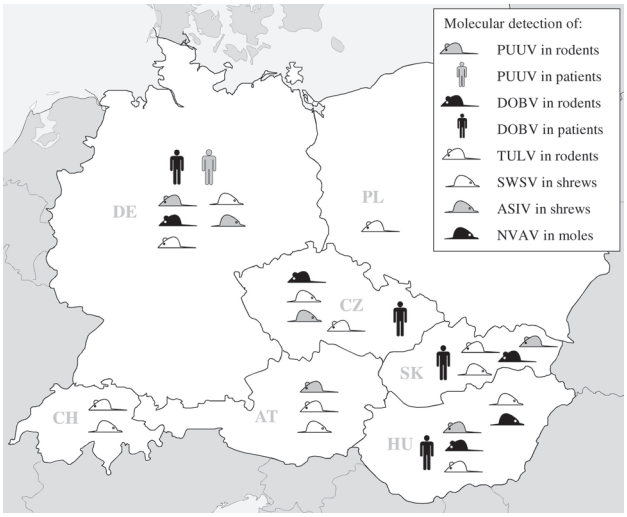


**Fig. 1**

**Summarizing map of Central Europe indicating countries with molecularly documented presence of hantaviruses**

AT, Austria; CH, Switzerland; CZ, Czech Republic; DE, Germany; HU, Hungary; PL, Poland; SK, Slovakia; ASIV, Asikkala virus; DOBV, Dobrava-Belgrade virus; NVAV, Nova virus; PUUV, Puumala virus; SWSV, Seewis virus; TULV, Tula virus.

## 2. Molecular evidence for rodent-borne hantaviruses in Central Europe

### *2.1 Puumala virus*

Bank voles (*Myodes glareolus*, formerly *Clethrionomys glareolus*) are the natural host of PUUV. Interestingly enough, the first fragment of a PUUV genome in Central Europe was amplified not from this natural reservoir but from a human source (Pilaski *et al.*, 1994). The first partial PUUV sequence from a vole trapped in Austria was published 3 years later (Bowen *et al.*, 1997). Meanwhile more extensive PUUV S and M segment sequences were characterized from Austrian voles (Aberle *et al.*, 1999; Plyusnina *et al.*, 2006). PUUV in *M. glareolus* has also been molecularly detected in Slovakia (Leitmeyer *et al.*, 2001) and Hungary (Plyusnina *et al.*, 2009).

In Germany, the detection of PUUV in a vole from the North-Western part of the country was reported in 1999 (Heiske *et al.*, 1999). From various *M. glareolus* specimens sampled during or after a PUUV outbreak in the Bavarian Forest (South-East Germany) in 2004, molecular phylogenetic analyses have been carried out (Essbauer *et al.*, 2006; Schilling *et al.*, 2007; Mertens *et al.*, 2011). At the same time, vole-derived PUUV nucleotide sequences were also collected from other parts of Germany (Essbauer *et al.*, 2007; Schilling *et al.*, 2007). During the large PUUV outbreaks in 2007 and 2010, comprehensive molecular analyses of PUUV strains from voles and patients were carried out which led to the definition of various molecular PUUV clades corresponding to different geographical regions in Germany (Hofmann *et al.*, 2008; Ettinger *et al.*, 2012; see chapter 4 of this review).

Molecular comparisons of the different Central European PUUV strains are usually based on comparative analysis of partial S segment sequences. The strains fall into two main clades. First, PUUV strains from Germany and Slovakia form a clade which also includes strains from neighbouring Western European countries such as Belgium. The second clade consists of strains from Austria and Hungary which are phylogenetically related to PUUV strains from South-East Europe. As pointed out very recently, the molecular strain differentiation within these clades and their different subclades can be further advanced by detailed vole surveillance in the different geographical regions. The strict local distribution of bank voles enables an allocation of particular PUUV strains to defined geographical areas (Ettinger *et al.*, 2012).

### *2.2 Dobrava-Belgrade virus*

DOBV is hosted by mice of at least three species of the genus *Apodemus*. Molecular phylogenetic analyses have shown that DOBV forms four evolutionary lineages. One of these lineages, Saaremaa virus, is currently recognized as an independent virus species on the ICTV species list. Unfortunately, all strains associated with striped field mice (*A. agrarius*) are designated as Saaremaa virus by some authors regardless of their phylogenetic relationship with other DOBV lineages which often leads to confusion. In accordance with the four phylogenetic lineages, we have

recently proposed a subdivision of the DOBV species into 4 genotypes, named Dobrava, Kurkino, Saaremaa, and Sochi according to the geographical place where the first strain of the genotype was molecularly detected (Klempa *et al.*, 2013a) and will use this classification throughout this review.

Two of the defined genotypes, Dobrava (associated with yellow necked mouse, *A. flavicollis*) and Kurkino (associated with *A. agrarius*) have been molecularly detected in Central Europe. The first molecular evidence of the presence of DOBV in Central Europe was obtained from *A. agrarius* mice caught in Slovakia (Sibold *et al.*, 1999b). In the follow-up study, *A. agrarius*- and *A. flavicollis*-specific virus lineages, now recognized as Kurkino and Dobrava genotypes, respectively, were found to be present sympatrically in Eastern Slovakia (Sibold *et al.*, 2001) and thorough phylogenetic analyses suggested putative genetic interactions (homologous recombination and reassortment) in the evolution of the genotypes (Klempa *et al.*, 2003b).

Two Central European DOBV-Kurkino cell culture isolates from mice allowing *in vitro* studies currently exist, one from Slovakia (Klempa *et al.*, 2005) and the other from Germany (Popugaeva *et al.*, 2012). *In vitro* studies with the German isolate showed that the virus uses cellular β3 integrins and Decay Accelerating Factor as entry receptors as has also been shown for the highly pathogenic Hantaan virus. Recently, multiple spillover infections of *A. agrarius*-associated Kurkino genotype (=DOBV-Aa lineage) to *A. flavicollis* mice were reported in Germany (Schlegel *et al.*, 2009) which might have important consequences in terms of putative genetic reassortment or stable host switch and spread of the virus to new regions where *A. agrarius* mice are absent.

The first molecular evidence that Kurkino genotype causes human HFRS cases was obtained in Germany when the phylogenetic analysis of DOBV sequence obtained from HFRS patient material showed that it belonged to the cluster of *A. agrarius*-associated strains (Klempa *et al.*, 2004). In addition to Slovakia and Germany, in Central Europe the DOBV-Kurkino genotype has so far been detected only in Hungary (Scharninghausen *et al.*, 1999; Jakab *et al.*, 2007a; Plyusnina *et al.*, 2009).

It is important to note that the Dobrava genotype of DOBV, associated with *A. flavicollis* mice and known to cause severe and fatal HFRS cases in South-East Europe, is also present in Central Europe. In Slovakia (Sibold *et al.*, 2001) and Hungary (Plyusnina *et al.*, 2009), Dobrava genotype was found to co-circulate with the Kurkino genotype. Most importantly, Dobrava genotype was shown to cause severe HFRS cases in the Czech Republic (Papa *et al.*, 2010), Slovakia (Zelená *et al.*, 2011), and in Hungary (Jakab *et al.*, 2007b).

### *2.3 Tula virus*

Tula virus (TULV) was initially associated with European common voles *Microtus arvalis* and *M. rossiaemeridionalis* caught in Russia (Plyusnin *et al.*, 1994). In parallel, the virus was also found in *M. arvalis* voles caught in Slovakia (Sibold *et al.*, 1995). Soon after, the first and so far only TULV cell culture isolate was obtained from *M. arvalis* vole trapped in the Czech Republic (Vapalahti *et al.*, 1996).

Besides the aforementioned Slovakia and Czech Republic, TULV has also been molecularly detected in Austria (Bowen *et al.*, 1997), Germany (Klempa *et al.*, 2003a), Poland (Song *et al.*, 2004), Hungary (Jakab *et al.*, 2008), and Switzerland (Schlegel *et al.*, 2012a). Interestingly, phylogenetic analysis of TULV strains from Western and Eastern Slovakia revealed the existence of two distinct virus lineages and indicated putative homologous recombination event in the evolution of the Eastern Slovakian strains (Sibold *et al.*, 1999a). In general, TULV strains from Central Europe show a remarkable degree of divergence and can be assigned to several clades across the TULV phylogenetic tree (as shown in, e.g., Schlegel *et al.*, 2012a) suggesting multiple introductions and long term survival of the virus in the region.

Recent studies from Germany and Switzerland have indicated that TULV is less host-specific than for hantaviruses generally assumed and that several other species of voles from *Microtus* genus, such as *M. agrestis* (Schmidt-Chanasit *et al.*, 2010), but also from different genus, such as *Arvicola amphibious* (Schlegel *et al.*, 2012a), might serve as TULV reservoir hosts.

## 3. Newly recognized hantaviruses associated with shrews and moles

Since 2007, knowledge on hantavirus host range has been notably revised through the identification of numerous new shrew- and mole-associated hantaviruses. Several of them were identified or later detected also in Central Europe. These new hantaviruses share very low sequence similarity with rodent-borne viruses. There is most likely no serological cross-reactivity with the 'old' hantaviruses explaining why these viruses remained undetected for such a long period of time. The human pathogenic potential of these viruses is therefore currently unknown and remains to be determined.

Seewis virus (SWSV), the first European shrew-borne hantavirus was identified in the European common shrew (*Sorex araneus*) caught near Seewis village in Switzerland (Song *et al.*, 2007a). Later on, SWSV was detected in *S. araneus* shrews captured in Finland and Hungary (Kang *et al.*, 2009a) as well as and in Austria and Germany (EU418604-16; Nowotny *et al.*, unpublished data). Extensive phylogenetic study of Schlegel *et al.* (2012b) focusing on Germany, the Czech Republic and Slovakia confirmed a wide geographic distribution of SWSV across Europe and indicated high genetic divergence and strong geographical

clustering of the virus in local shrew populations. European common shrew is regarded as the main reservoir of SWSV, however other species such as Pygmy shrew (*S. minutus*) and Mediterranean or Miller´s water shrew (*Neomys anomalus*) have been shown to carry SWSV, too, probably only in the form of random and transient, so called "spill-over" infections (Schlegel *et al.*, 2012b).

Very recently, Asikkala virus (ASIV) has been described in pygmy shrews (*S. minutus*) as a second shrew-borne hantavirus in Central Europe. Currently available genomic data is derived from two shrew samples from the Czech Republic and one from Germany (Radosa *et al.*, 2013; Fig. 2).

In 2009, a new and highly divergent hantavirus was described in samples from European common mole (*Talpa europaea*) from the Zala region of Hungary and was designated as Nova virus (NVAV) (Kang *et al.*, 2009c). NVAV is the first and, until now, only mole-borne hantavirus present in Europe. European common mole is widely dispersed throughout Europe and covers the complete area of Central Europe. Phylogenic analysis revealed that NVAV belongs to the highly divergent group of shrew-, bat-, and mole-borne hantaviruses with the bat-associated Mouyassué virus from Africa currently recognized as the most closely related virus (Sumibcay *et al.*, 2012). Available amino acid sequences of the nucleocapsid and polymerase proteins show remarkably low similarity (50–60%, respectively) with other hantaviruses (Kang *et al.*, 2009c).

Nucleotide sequences of yet another putative new Central European shrew-borne virus recently appeared in GenBank (Acc. No. JX990964-66; Gu *et al.*, unpublished data). The virus, designated as Boginia virus, was found in two Eura-

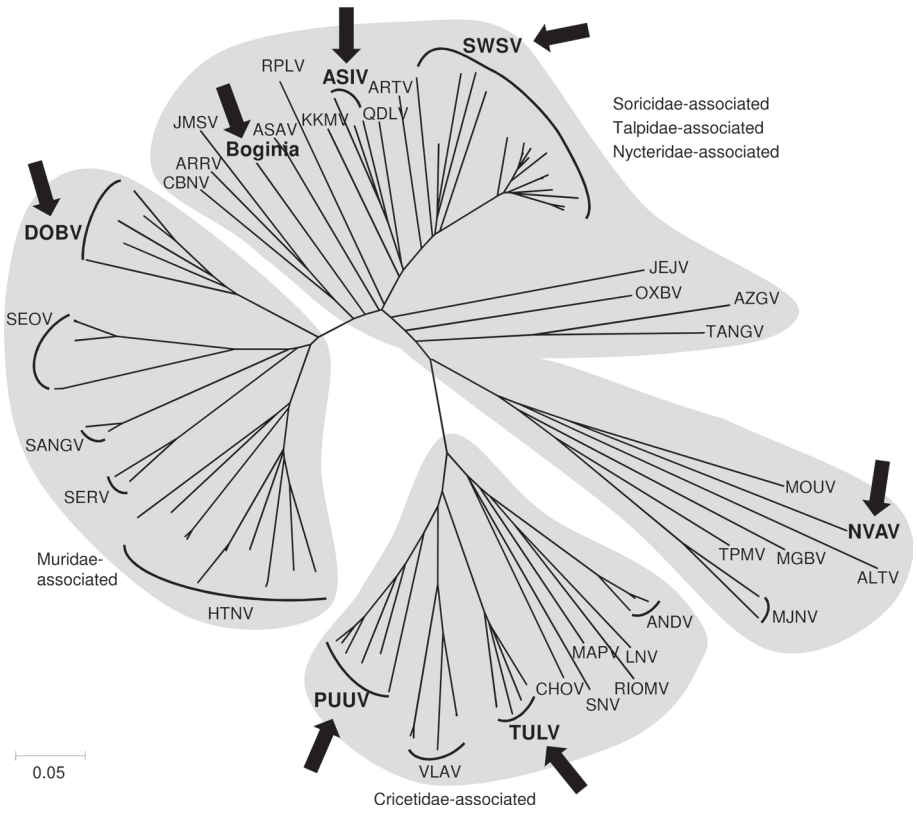


**Fig. 2**

**Hantavirus phylogenetic tree illustrating high divergence of hantaviruses detected in Central Europe**

Hantaviruses detected in Central Europe are marked by black arrows. The grey shaded areas indicate association of hanta viruses with reservoir host families. The phylogenetic tree was constructed on the basis of partial L segment sequences (352 nucleotides) in the MEGA5 program (Tamura *et al.*, 2011) by using the Neighbor-Joining method with Maximum Composite Likelihood method applied to calculate the evolutionary distances. Scale bar indicates an evolutionary distance of 0.05 substitutions per position in the sequence.

ALTV, Altai virus; ANDV, Andes virus; ARRV, Ash River virus; ARTV, Artybash virus; ASAV, Asama virus; ASIV, Asikkala virus; AZGV, Azagny virus; CBNV, Cao Bang virus; CHOV, Choclo virus; DOBV, Dobrava-Belgrade virus; HTNV, Hantaan virus; JEJV, Jeju virus; JMSV, Jemez Springs virus; KKMV, Kenkeme virus; LNV, Laguna Negra virus; MAPV, Maporal virus; MGBV, Magboi virus; MJNV, Imjin virus; MOUV, Mouyassué virus; NVAV, Nova virus; OXBV, Oxbow virus; PUUV, Puumala virus; QDLV, Qiandao Lake virus; RIOMV, Rio Mamore virus; RPLV, Camp Ripley virus; SANGV, Sangassou virus; SEOV, Seoul virus; SERV, Serang virus; SWSV, Seewis virus; SNV, Sin Nombre virus; TGNV, Tanganya virus; TPMV, Thottapalayam virus; TULV, Tula virus; VLAV, Vladivostok virus.

**Table 1. Hantaviruses molecularly demonstrated to be present in Central Europe**

| Virus | Puumala virus (PUUV) | Dobrava Belgrade virus (DOBV) | Tula virus (TULV) | Seewis virus (SWSV) | Asikkala virus (ASIV) | Nova virus (NVAV) |
|---|---|---|---|---|---|---|
| Host(s)* | *Myodes glareolus* | *Apodemus agrarius*<br>*A. flavicollis* | *Microtus arvalis*<br>*M. agrestis*<br>*Arvicola amphibius* | *Sorex araneus*<br>*S. minutus*<br>*Neomys anomalus* | *Sorex minutus* | *Talpa europea* |
| Detection in Central Europe (First report for a given country) | | | | | | |
| Austria | Bowen *et al.* (1997) | – | Bowen *et al.* (1997) | EU418604-6 | – | – |
| Czech Republic | – | Papa *et al.* (2010) | Plyusnin *et al.* (1995) | Schlegel *et al.* (2012b) | Radosa *et al.* (2013) | – |
| Germany | Pilaski *et al.* (1994) | Klempa *et al.* (2004) | Klempa *et al.* (2003) | Schlegel *et al.* (2012b) | Radosa *et al.* (2013) | – |
| Hungary | Plyusnina *et al.* (2009) | Scharninghausen *et al.* (1999) | Jakab *et al.* (2008) | Kang *et al.* (2009a) | – | Kang *et al.* (2009c) |
| Poland | – | – | Song *et al.* (2004) | – | – | – |
| Slovakia | Leitmeyer *et al.* (2001) | Sibold *et al.* (1999b) | Sibold *et al.* (1995) | Schlegel *et al.* (2012b) | – | – |
| Switzerland | – | – | Schlegel *et al.* (2012a) | Song *et al.* (2007a) | – | – |

*Virus detection in these species has been reported in Central Europe but they are not necessarily the virus primary reservoir hosts in all cases.

sian water shrews (*Neomys fodiens*) in Poland but further information is currently not available.

## 4. Clinical epidemiology

Hantavirus infections in their respective host animals seem to be persistent and without obvious harm to the animal. In contrast, "spill-over" infections of humans proceed as acute disease in 10–20 % of cases after primary infection of immune-naïve persons (clinical manifestation index). The pathogenesis of hantavirus disease is characterized by vasodilatation and barrier impairment of the vascular endothelium as well as disturbances in blood coagulation (Kruger *et al.*, 2011). Consequently, inflammatory processes occur in organs such as kidney und lung. In continents outside America, hantavirus disease is also named hemorrhagic fever with renal syndrome (HFRS). In addition, the term nephropathia epidemica is sometimes used for mild forms of HFRS caused by PUUV infections.

Of the Central European countries, the following numbers of HFRS cases per year were reported between 2005 and 2009 (minimum-maximum); Austria 12–78 cases / Czech Republic 2–7 cases / Germany 72–1,688 cases / Hungary 6–16 cases / Poland 3–17 cases / Slovakia 3–22 cases / Switzerland 0–1 case (Heyman *et al.*, 2011). However, one can expect serious underreporting since in many clinical cases, doctors are not aware of this disease and do not initiate specific virological diagnostics. Moreover, the assays for primary and confirmatory serodiagnostics are not standardized and the results are not comparable Europe-wide. This is also true for the diagnostic methods used for serosurveillance studies in the different countries.

It is known that antibody cross-reactivity between different hantaviruses complicates the typing of the hantavirus which infected patients (Schilling *et al.*, 2007). Therefore seroassays based on the detection of (non-neutralizing) antibodies against the immunodominant nucleocapsid protein do not always allow an unequivocal statement about the hantavirus species involved in the infection. Only molecular genetic methods enable the clear identification of hantavirus species and strains. Unfortunately, the use of those methods based on a RT-PCR approach is hampered by the fact that virus RNA is present in patients′ blood only during the first few weeks after the onset of disease.

The first "Central European" PUUV-specific nucleotide sequence in a patient was detected about 20 years ago in Germany (Pilaski *et al.*, 1994). More comprehensive PUUV sequence data from human sources was collected in Germany during outbreaks in 2004 (Schilling *et al.*, 2007) and 2007 (Hofmann *et al.*, 2008). Over the last few years, HFRS cases in Germany have reached new record values with more than 2,000 reported cases in 2010 and 3,000 cases in 2012 (Robert Koch-Institut, http://www3.rki.de/SurvStat). A country-wide alert network was established and enabled the assessment of serum samples from PUUV-infected patients during the early (viraemic) clinical phase. The data showed that amplified nucleotide sequences of human origin from the different outbreak regions in Germany resemble sequences derived from the local *M. glareolus* animals. Coinciding sequences of human and vole origin

formed different molecular PUUV clades corresponding to the different outbreak regions. These findings allow for the establishment of a molecular registry of PUUV strains in Germany, the exact allocation of the geographic site where a certain patient became infected, and the generation of risk maps for infection (Ettinger *et al.*, 2012).

From Austria, there are two anecdotal reports about detection of PUUV nucleotide sequences in patients with acute renal failure and pulmonary oedema (Fakhrai *et al.*, 2011) and another patient suffering from multiorgan failure (Hoier *et al.*, 2006). Since the natural PUUV host, *M. glareolus*, is present in all Central European countries, one can expect human PUUV infections in these countries, too.

In general, the case fatality of PUUV-HFRS is thought to range between 0.1–0.4% (Hjertqvist *et al.*, 2010; Kruger *et al.*, 2011). About 5% of hospitalized patients require temporary haemodialysis (Krautkramer *et al.*, 2012).

Human infections by DOBV in Central Europe are caused by two DOBV lineages; genotype Dobrava carried by the yellow-necked mouse, *Apodemus flavicollis*, and genotype Kurkino, hosted by the striped field mouse, *A. agrarius* (Klempa *et al.*, 2013; Kruger and Klempa, 2011; Papa, 2012). Most human infections by DOBV-Dobrava occur in South-East Europe, however they have also been occasionally reported from Central European countries. Molecular evidence in HFRS patients exists from the Czech Republic (Papa *et al.*, 2010), Slovakia (Zelena *et al.*, 2011; our unpublished data) and Hungary (Jakab *et al.*, 2007b). Human infections by DOBV-Kurkino in Central Europe were molecularly demonstrated in North-East Germany (Klempa *et al.*, 2004; Hofmann *et al.*, unpublished data). The natural host of DOBV-Kurkino, *A. agrarius*, is prevalent in Central Europe with North-East Germany as the western border of its distribution. This explains why DOBV-Kurkino infections are registered in this region but not in South or West Germany. Since DOBV-Kurkino has been found in *A. agrarius* animals from different Central European countries (see above), one can also expect its future molecular detection in patients outside of Germany.

From the clinical point of view, different human virulence of the two DOBV genotypes must be mentioned. Infections by DOBV-Dobrava in the Balkans cause moderate to severe disease with case fatality rates of 10–12%, whereas our investigations of large HFRS outbreaks in Russia due to DOBV-Kurkino infections showed mild to moderate courses with 0.3–0.9% case fatality (Dzagurova *et al.*, 2009; Klempa *et al.*, 2013a; Papa, 2012).

The pathogenicity towards humans of the third rodent-borne hantavirus from Central Europe, Tula virus (TULV), is less clear. One human HFRS case has been reported with neutralizing antibodies best reactive against TULV and molecular detection of TULV sequences in *M. arvalis* mice from the environment of the patient, however no direct molecular evidence exists for human disease caused by TULV infection (Klempa *et al.*, 2003a).

## 5. Concluding remarks

Central Europe is undoubtedly an important endemic region for hantavirus infections. Practically all European hantaviruses described so far also occur in Central Europe (Table 1) which is directly connected with the presence of all relevant small mammals as hantavirus hosts. Both important human pathogens, PUUV and DOBV are present and cause HFRS of various severities. In the case of DOBV, two distinct host-specific genotypes exist sympatrically and are shown to cause human diseases of distinct clinical severity. Based on recent epidemiological trends, it might be expected that Central Europe will face increasing numbers of HFRS cases in the form of disease outbreaks (as seen in recent years in Germany) and one can anticipate the identification of new hantaviruses in non-rodent hosts. The public health relevance of these newly recognized shrew- and mole-borne hantaviruses, not only in Central Europe, still needs to be determined. The progress in Central Europe in terms of development and research of hantavirus diagnostics, molecular epidemiology, and pathogenesis, treatment will advance the field of hantavirus research in general.

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