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Co-circulation of Soricid- and Talpid-borne Hantaviruses in Poland

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Abstract

Previously, we reported the discovery of a genetically distinct hantavirus, designated Boginia virus (BOGV), in the Eurasian water shrew (*Neomys fodiens*), as well as the detection of Seewis virus (SWSV) in the Eurasian common shrew (*Sorex araneus*), in central Poland. In this expanded study of 133 shrews and 69 moles captured during 2010–2013 in central and southeastern Poland, we demonstrate the co-circulation of BOGV in the Eurasian water shrew and SWSV in the Eurasian common shrew, Eurasian pygmy shrew (*Sorex minutus*) and Mediterranean water shrew (*Neomys anomalus*). In addition, we found high prevalence of Nova virus (NVAV) infection in the European mole (*Talpa europaea*), with evidence of NVAV RNA in heart, lung, liver, kidney, spleen and intestine. The nucleotide and amino acid sequence variation of the L segment among the SWSV strains was 0–18.8% and 0–5.4%, respectively. And for the 38 NVAV strains from European moles captured in Huta Dłutowska, the L-segment genetic similarity ranged from 94.1–100% at the nucleotide level and 96.3–100% at the amino acid level. Phylogenetic analyses showed geographic-specific lineages of SWSV and NVAV in Poland, not unlike that of rodent-borne hantaviruses, suggesting long-standing host-specific adaptation. The co-circulation and distribution of BOGV, SWSV and NVAV in Poland parallels findings of multiple hantavirus species coexisting in their respective rodent reservoir species elsewhere in Europe. Also, the detection of SWSV in three syntopic shrew species resembles spill over events observed among some rodent-borne hantaviruses.

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Keywords

Hantavirus; *Sorex*; *Neomys*; *Talpa*; Shrew; Mole; Phylogeny; Poland

1. Introduction

Hantaviruses (genus *Hantavirus*), the causative agents of hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS), are unique among the approximately 400-member Bunyaviridae family in that they have no known arthropod hosts, and instead are harbored by small mammals. Initially, hantavirus reservoirs were thought to be restricted to rodents (order Rodentia, family Muridae and Cricetidae), despite the detection of hantavirus antigens in tissues of shrews and moles (order Eulipotyphla, family Soricidae and Talpidae), such as the Eurasian common shrew (*Sorex araneus*), Eurasian pygmy shrew (*S. minutus*), Eurasian water shrew (*Neomys fodiens*) and European mole (*Talpa europaea*) in Russia and the former Yugoslavia more than two decades ago (Gavrilovskaya et al., 1983; Gligic et al., 1992; Tkachenko et al., 1983). Also, Thottapalayam virus (TPMV), originally an unclassified virus isolated from the Asian house shrew (*Suncus murinus*) in India (Carey et al., 1971), was later shown to be a hantavirus (Zeller et al., 1989) that occupied a separate evolutionary lineage from rodent-borne hantaviruses (Chu et al., 1994; Song et al., 2007a; Xiao et al., 1994; Yadav et al., 2007).

Guided by these reports, and empowered by the generosity of museum curators and field mammalogists who provided access to their archival tissue collections, an aggressive and opportunistic search, employing reverse transcription-polymerase chain reaction (RT-PCR), has resulted in the molecular identification of multiple genetically distinct hantaviruses across space and time (Bennett et al., 2014; Yanagihara et al., 2014), including Seewis virus (SWSV) and Nova virus (NVAV), in the Eurasian common shrew and European mole, respectively (Kang et al., 2009b; Song et al., 2007b).

Subsequent studies have indicated that SWSV is widespread throughout Europe and Asia across the vast distribution of its soricid reservoir, in Austria, Czech Republic, Finland, Germany, Hungary, Russia, Slovakia and Slovenia (Kang et al., 2009a; Klempa et al., 2013; Korva et al., 2013; Resman et al., 2013; Schlegel et al., 2012b). SWSV has also been detected in the Siberian large-toothed shrew (*S. daphaenodon*) and tundra shrew (*S. tundrensis*) in Russia (Yashina et al., 2010). And in a comprehensive phylogeographic study of SWSV in *Sorex* species in Central Europe, SWSV exhibited distinct geographic-specific clustering in Eurasian common shrews (Schlegel et al., 2012b). Moreover, a genetically related but distinct hantavirus, named Asikkala virus (ASIV), has been found recently in the Eurasian pygmy shrew (Radosa et al., 2013).

Previously, in an exploratory study of soricine shrews in central Poland, we reported the discovery of a novel hantavirus, designated Boginia virus (BOGV), in the Eurasian water shrew, as well as the detection of SWSV in the Eurasian common shrew (Gu et al., 2013). The overlapping geographic ranges of these shrew species and the European mole prompted the present expanded study on the genetic diversity of SWSV and NVAV in central and southeastern Poland. Since NVAV had been detected in archival liver tissues of a single

European mole captured in Zala County, Hungary, in 1999 (Kang et al., 2009b), our investigation also sought to ascertain the prevalence and tissue distribution of NVAV in the European mole. Moreover, because SWSV exhibits host sharing among genetically related soricine shrew species (Schlegel et al., 2012b; Yashina et al., 2010), a separate aim was to determine the host restriction of NVAV in the European mole. Definitive data are presented showing the high prevalence and widespread tissue distribution of NVAV in the European mole. Moreover, SWSV, BOGV and/or NVAV were found to co-circulate in different regions of central Poland, but no spill-over infection from shrews to moles, or vice versa, was evident.

2. Materials and methods

2.1. Trapping and specimen processing

Shrews were captured, using wooden live traps and pitfall traps, consisting of cones of galvanized steel, measuring 40 cm high and 15 cm in diameter at the top, placed 5 m apart and baited with raw bacon or beef. Moles were trapped using PVC pipes (25 cm long and 5 cm in diameter, equipped at both ends with flat aluminum latches). Trapping was conducted during the month of September in 2010, 2011, 2012 and 2013, with each session covering 120 to 210 trap nights. Coordinates of each trap site are provided in Table 1. Although the traps for shrews were checked very four hours and those for moles three times a day, approximately 50% of shrews and 40% of moles were found dead. Live-caught shrews and moles were euthanized by cervical dislocation, and stored at 4°C or -20°C for several hours or days before harvesting of tissues. Lung, heart, liver, kidney, spleen and intestine tissues, dissected using separate alcohol-cleaned instruments, were preserved in RNeasy Lysis Reagent (Qiagen, Valencia, CA).

2.2. Ethics statement

All trapping and experimental procedures on shrews were approved by the Łódź Ethical Committee on Animal Testing (14/LB/511/2010 and 29/LB/548/2011) and the General Directorate for Environmental Protection (DOP-OZGiZ.4200/N2732/10/JRO, DOP-OZGiZ.6401.05.25.2011kp.3 and DOP-OZGiZ.6401.05.28.2011kp.1). The Łódź Regional Directorate for Environmental Protection approved protocols for moles (WPN-I6631.2010.MS and WPN-L6400.59.2011.MS).

2.3. RNA extraction, cDNA synthesis and RT-PCR amplification

Total RNA was extracted from 20–50 mg of tissue, using the PureLink Micro-to-Midi total RNA purification kit (Invitrogen, San Diego, CA). cDNA, synthesized using the SuperScript III First-Strand Synthesis Systems (Invitrogen), were analyzed for hantavirus RNA by RT-PCR, using oligonucleotide primers designed from highly conserved regions of hantavirus genomes (Table 2) (Song et al., 2007b, 2007c, 2009; Kang et al., 2009a, 2009b; Gu et al., 2013, 2014a, 2014b).

Nested or hemi-nested PCR was performed in 20-μL reaction mixtures, containing 250 μM dNTP, 2.5 mM MgCl₂, 1 U of Takara LA Taq polymerase (Takara, Shiga, Japan) and 0.25 μM of each primer (Table 2). Initial denaturation at 94°C for 2 min was followed by two

cycles each of denaturation at 94°C for 30 sec, two-degree step-down annealing from 46°C to 38°C for 40 sec, and elongation at 72°C for 1 min, then 30 cycles of denaturation at 94°C for 30 sec, annealing at 42°C for 40 sec, and elongation at 72°C for 1 min, in a GeneAmp PCR 9700 thermal cycler (Perkin-Elmer, Waltham, MA) (Arai et al., 2008b). PCR products were separated, using MobiSpin S-400 spin columns (MoBiTec, Goettingen, Germany), and amplicons were sequenced directly using an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.4. Genetic and phylogenetic analyses

Sequences were aligned using Clustal W (Thompson et al., 1994). Unrooted phylogenetic trees were generated by maximum likelihood and Bayesian methods, implemented in PAUP* (Phylogenetic Analysis Using Parsimony, 4.0b10) (Swofford, 2003), RAxML Blackbox webserver (Stamatakis et al., 2008) and MrBayes 3.1 (Ronquist and Huelsenbeck, 2003), under the best-fit GTR+I+ Γ model of evolution selected by hierarchical likelihood-ratio test in MrModeltest v2.3 (Posada and Crandall, 1998) and jModelTest version 0.1 (Posada, 2008). Two replicate Bayesian Metropolis–Hastings Markov Chain Monte Carlo runs, each comprising six chains of 10 million generations sampled every 100 generations with a burn-in of 25,000 (25%), resulted in 150,000 trees overall. Each genomic segment (S and L) was treated separately in phylogenetic analyses. The posterior node probabilities were based on 2 million generations and estimated sample sizes over 100 (implemented in MrBayes).

3. Results and discussion

3.1. Co-circulation of genetically distinct hantaviruses

As determined by RT-PCR and verified by DNA sequencing, we previously demonstrated BOGV in Eurasian water shrews and SWSV in Eurasian common shrews and in Eurasian pygmy shrews from central Poland (Gu et al., 2013). In this expanded study of lung tissues from 53 Eurasian common shrews, 52 Eurasian pygmy shrews, 12 Mediterranean water shrews, 16 Eurasian water shrews and 69 European moles, we documented SWSV in six of 18 Eurasian common shrews in Chmiel, BOGV in four of 14 Eurasian water shrews from Boginia, Kurowice and Huta Dłutowska, and the co-circulation of SWSV, BOGV and NVAV and Huta Dłutowska (Table 1, Fig. 1).

SWSV has been detected in a small proportion of Eurasian pygmy shrews in Germany and the Czech Republic (Schlegel et al., 2012b), as well as in Finland (Ling et al., 2014). Similarly, in this study, SWSV RNA was found in tissues of two of 17 Eurasian pygmy shrews from Kurowice, as well as in one of 12 Mediterranean water shrews from Chmiel (Table 1, Fig. 1). Since there is only one other SWSV sequence from the Mediterranean water shrew in Austria (GenBank EU418604), further research is necessary to determine if this shrew species serves as a true reservoir host of SWSV. Whether the Mediterranean water shrew also harbors a BOGV-like hantavirus, rather than only SWSV, is unclear. Nevertheless, neither physical proximity with sharing of habitats nor genetic relatedness of shrew host species allows prediction of the hantavirus species in a particular reservoir.

This study provides clear evidence of SWSV infection in the Eurasian common shrew in central and southeastern Poland, as well as the co-existence of BOGV and NVAV in their shrew and mole reservoir host species inhabiting the same ecological niche. Similar observations have been reported for rodent-borne hantaviruses. That is, co-circulation of hantaviruses is exemplified by bank voles (*Myodes glareolus*), yellow-necked field mice (*Apodemus flavicollis*) and striped field mice (*Apodemus agrarius*), trapped in the Transdanubian region of Hungary, harboring Puumala virus (PUUV), Dobrava virus (DOBV) and Saaremaa virus, respectively (Plyusnina et al., 2009). Also, the simultaneous occurrence of PUUV, DOBV and Tula virus (TULV) has been reported in Slovakia (Sibold et al., 1999b), and two distinct hantaviruses associated with *Apodemus* mice have been reported in Croatia (Plyusnina et al., 2011). Recently, hantavirus RNA was detected in *My. glareolus*, *Microtus agrestis* and *S. araneus*, found dead in an area exposed to flooding in eastern Poland (Wójcik-Fatla et al., 2013), and the first molecular evidence of PUUV has now been reported from bank voles in Poland (Ali et al., 2014).

Extensive host sharing is evidenced by TULV infection in the common vole and field vole in different regions of Germany (Schmidt-Chanasit et al., 2010), and TULV has also been detected in the Eurasian water vole (*Arvicola amphibius*) (Schlegel et al., 2012a). Mention has been made about the circulation of SWSV in several soricine shrew species in Siberia (Yashina et al., 2010). Similarly, Jemez Springs virus appears to be harbored by the dusky shrew (Arai et al., 2008) and other related *Sorex* species (such as *S. palustris*, *S. trowbridgii* and *S. vagrans*) in North America (unpublished observations).

3.2. High prevalence of NVAV infection and virus distribution in tissues of European moles

In Huta Dłutowska, the overall prevalence of NVAV infection in European moles was 65.5% (38/58) during the four-year period, 2010–2013 (Table 1), resembling that reported recently from two capture sites frequented by humans in France (Gu et al., 2014a). Such high prevalences, exceeding 50%, are likely indicative of a long-standing reservoir host-virus relationship and raise questions about factors that drive transmission. While it may be assumed that NVAV is widespread throughout the geographic distribution of the European mole, which extends from the United Kingdom across Scandinavia into European Russia, longitudinal studies are warranted to ascertain temporal fluctuations of NVAV transmission and the influence of insect and predator abundance (Gu et al., 2014a).

As determined by RT-PCR, NVAV RNA was detected in multiple tissues, besides lung, in a high proportion of European moles (Table 2), suggesting widespread and persistent infection. NVAV sequences within tissues of a single mole were identical, but varied by 0–5.9% at the nucleotide level between moles. Although similarly extensive studies have not been performed on other non-rodent species, hantavirus RNA has also been demonstrated in multiple organs of the Ussuri white-toothed shrew (*Crocidura lasiura*) (Song et al., 2009) and the Chinese mole shrew (*Anourosorex squamipes*) (Song et al., 2007c). Overall, the tissue distribution was not unlike that found in rodents either naturally or experimentally infected with Hantaan virus (HTNV) (Lee et al., 1978, 1981) or PUUV (Yanagihara et al., 1985a, 1985b). Notably the presence of NVAV RNA in the kidney and intestine (Table 2) would be consistent with viral shedding in urine and feces, which is believed to be important

in the transmission of rodent-borne hantaviruses to humans. Since European moles often reside near human habitation, human exposure to their excretions is plausible. As such, febrile illnesses or unusual clinical syndromes, occurring among individuals with known exposure to moles, should be thoroughly investigated.

A weakness of the study was the unavailability of appropriately fixed tissues for histopathological examination and immunocytochemical staining. As such, we were unable to determine if endothelial cells and/or other specific cell types were infected with NVAV. Also, logistical challenges of maintaining an adequate cold chain precluded the collection of frozen tissues for assessment of virus infectivity in different tissues. Future studies are being planned to address this shortcoming.

3.3. Genetic analysis

S-, M- and/or L-segment sequences of SWSV, BOGV and NVAV were analyzed. GenBank accession numbers are provided for 60 L-, three M- and 42 S-segment sequences (Supplementary Table 1). At the nucleotide level, the partial sequences of SWSV from Poland showed considerable divergence from the SWSV prototype mp70 strain, differing by 15.0–17.3% and 14.8–22.0% in the S and L segment, respectively. However, nucleotide sequence variation of SWSV strains within a specific geographic region was low, ranging from 0–2.9% in Chmiel and 0% in Huta Dłutowska. Moreover, the encoded nucleocapsid protein varied by 6.9% among SWSV strains from Poland and elsewhere in Europe.

BOGV, detected in four of 14 Eurasian water shrews captured in three separate villages in central Poland (Boginia, Huta Dłutowska and Kurowice), was genetically distinct from SWSV and other representative soricine shrew-borne hantaviruses, with M- and L-segment sequence similarity of only 64.8–77.5% and 84.7–87.3% at the nucleotide and amino acid levels, respectively (Gu et al., 2013).

The full-length 1,825-nucleotide S-genomic segment of NVAV strains 1135, 1136, 2095, 2097, 3328, 3329 and 3350 from Huta Dłutowska and strain 2086 from Bronisin contained a single open reading frame, encoding a predicted N protein of 428 amino acids (nucleotide positions 53 to 1336), and 52- and 486-nucleotide 5'- and 3'-noncoding regions (NCR), respectively. The sequence variation in the coding region of the entire S-genomic segment between the eight NVAV strains was 0.1–3.3% and 0–0.9% at the nucleotide and amino acid levels, respectively. Comparison of the S segment to the prototype NVAV strain MSB95703 showed a nucleotide and amino acid sequence similarity of 85.8–86.1% and 96.0–96.5%. A similar degree of nucleotide sequence divergence was found between the NVAV strains from Poland and France, but there was high amino acid sequence similarity of >96%.

Using software available on the @NPS structure server (Combet et al., 2000), the predicted secondary structure of the NVAV N protein was nearly identical to that reported earlier for the prototype strain (Kang et al., 2009b). Similarly, alignment and comparison of a 788-nucleotide (262-amino acid) region of the L-genomic segment of NVAV strains from Poland showed approximately 15% difference at the nucleotide level but less than 2% difference at the amino acid level from prototype NVAV MSB95703 from Hungary.

Moreover, SWSV strains from Poland varied from those from other geographic areas, but there was high conservation at the amino acid level.

3.4. Phylogenetic analysis

Phylogenetic analyses of the partial L-segment sequences of SWSV strains, generated using maximum-likelihood and Bayesian methods, showed two distinct lineages with evidence of geographic-specific clustering (Fig. 2). SWSV strains from Eurasian common shrews captured in Chmiel (southeastern Poland) and in Boginia and Huta Dłutowska (central Poland) were positioned on separate branches. High genetic diversity of SWSV was also found in common shrews from Germany, the Czech Republic and Slovakia (Schlegel et al., 2012b). Moreover, divergent lineages of the same hantavirus species within the same geographic region have been reported for HTNV in the striped field mouse (Song et al., 2000) and PUUV in the bank vole (Plyusnin et al., 1994; Razzauti et al., 2009, 2012). Homologous recombination events have been suggested to account for the genetic variation of TULV in Slovakia (Sibold et al., 1999a).

Phylogenetic analyses, using PAUP*, RAxML and MrBayes, based on the full-length S-segment sequences of five NVAV strains from Poland showed a highly divergent clade, comprising prototype NVAV from Hungary and newfound NVAV strains from France, showing segregation according to geography (Fig. 2). Similarly, tree topologies, well supported by bootstrap analysis (data not shown) and posterior node probabilities (Fig. 2), were generated from maximum-likelihood and Bayesian analysis of the partial L-segment sequences of NVAV strains from Poland, France and Hungary.

Recent discovery of genetically distinct hantaviruses in shrews and moles, and the detection of a highly divergent lineage of hantaviruses in insectivorous bats from sub-Saharan Africa (Gu et al., 2014b; Sumibcay et al., 2012; Weiss et al., 2012) and Asia (Arai et al., 2013; Gu et al., 2014b; Guo et al., 2013), have forced a reframing of their evolutionary origins and phylogeography. That these newfound hantaviruses are genetically more diverse than those hosted by rodents suggests a complex evolutionary history. Moreover, ancestral eulipotyphlans and chiropterans in Asia were the likely early mammalian hosts of primordial hantaviruses, predating their appearance in rodents (Bennett et al., 2014). That is, in keeping with other members of the Bunyaviridae family, which are transmitted by insects and arthropods, the evolutionary origins of hantaviruses may have entailed insect-borne viruses, which initially infected insectivorous ancestral hosts, with subsequent host switching and species-specific adaptation in the distant and more recent past.

3.5. Conclusions

While outbreaks of HFRS have long been known to occur in central Europe (Klempa et al., 2013), including Germany (Hofmann et al., 2008; Krüger et al., 2001), the Czech Republic (Pejcoch et al., 2010) and Slovakia (Sibold et al., 1999b), reports of hantavirus infection and disease in Poland have been conspicuously uncommon, despite the existence of the same reservoir rodent species. Previously, TULV was isolated from the common vole in central Poland (Song et al., 2004), and anti-hantavirus antibodies were reported among Polish mammalogists (Sadkowska-Todys et al., 2007) and forestry workers (Grygorczuk et al.,

2008). The first serologically confirmed case of HFRS, occurring in May 2005, was reported in 2006 (Knap et al., 2006). Recently, an outbreak of hantavirus infection occurred between August and December 2007 in the Carpathian mountains in southeastern Poland, in close proximity to an HFRS focus in the Ruska Poruba region of neighboring Slovakia (Nowakowska et al., 2009). Of the 13 serologically confirmed HFRS cases, 10 were caused by DOBV and three by PUUV. Most patients had moderate to severe disease, and several required hemodialysis (Nowakowska et al., 2009). Other suspected HFRS cases, albeit unconfirmed by serological tests, have been described in southeastern Poland (Gut et al., 2013).

HFRS cases are probably being misdiagnosed and under reported in Poland. Apart from improving primary-care physician education and heightening their awareness about HFRS throughout Poland, standardized serodiagnostic tests must be made more widely available in clinical laboratories. Also, surveys of rodent and non-rodent reservoir host populations and virus isolation attempts are urgently needed to gain more in-depth knowledge about the distribution and pathogenic potential of hantaviruses, particularly in southeastern Poland.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Three genetically distinct hantaviruses co-circulate in central Poland
- Seewis virus in three syntopic shrew species suggests spill-over infection
- High prevalence of Nova hantavirus is found the European mole
- Nova virus RNA is widespread in tissues of infected European moles

Fig. 1.

Map of Poland, showing trap sites in central and southeastern Poland, where hantavirus-infected shrews and moles were captured. GPS coordinates are provided in Table 1.

Fig. 2.

Phylogenetic trees generated by the maximum-likelihood and Bayesian methods, using the GTR+I+ Γ model of evolution as estimated from the data, based on the alignment of the coding regions of the (S) partial and full length S segment and (L) partial L segment of soricid- and talpid-borne hantaviruses in Poland. Because the unrooted phylogenetic trees using both methods were very similar, the trees generated by MrBayes were displayed. The phylogenetic positions of SWSV (blue) and BOGV (green) from Poland are shown in relationship to the prototype SWSV strain from Switzerland (SWSV mp70, S: EF636024; L: EF636026). NVAV (red) strains from Poland are shown in relationship to the prototype NVAV strain from Hungary (MSB95703, S: FJ539168; L: FJ593498) and to representative NVAV strains from France (YA0067, S: KF010576; L: KF010544; and YA0069, S: KF010575; L: KF010542). Other hantaviruses harbored by shrews and moles include Thottapalayam virus (TPMV VRC66412, S: AY526097; L: EU001330), Imjin virus (MJNV CI05-11, S: EF641804; L: EF641806), Cao Bang virus (CBNV CBN-3, S: EF543524; L: EF543525), Qian Hu Shan virus (QHSV YN05-284, S: GU566023; L: GU566021), Jeju virus (JJUV 10-11, S: HQ834695; L: HQ834697), Asama virus (ASAV N10, S: EU929072; L: EU929078), Oxbow virus (OXBV Ng1453, S: FJ5339166; L: FJ593497), Asikkala virus (ASIV CZ/Drahany/420/2010/Sm, S: KC880342; L: KC880348), Bowé virus (BOWV

VN1512, S: KC631782; L: KC631784), Kenkeme virus (KKMV MSB148794, S: GQ306148; L: GQ306150) and Rockport virus (RKPV MSB57412, S: HM015223; L: HM015221). Also shown are representative Murinae rodent-borne hantaviruses, including Hantaan virus (HTNV 76-118, S: NC_005218; L: NC_005222), Soochong virus (SOOV SOO-1, S: AY675349; L: DQ056292), Dobrava virus (DOBV Greece, S: NC_005233; L: NC_005235) and Seoul virus (SEOV HR80-39, S: NC_005236; L: NC_005238); Arvicolinae rodent-borne hantaviruses, including Tula virus (TULV M5302v, S: NC_005227; L: NC_005226), Puumala virus (PUUV Sotkamo, S: NC_005224; L: NC_005225) and Prospect Hill virus (PHV PH-1, S: Z49098; L: EF646763); and Neotominae rodent-borne hantaviruses, Sin Nombre virus (SNV NMH10, S: NC_005216; L: NC_005217) and Andes virus (ANDV Chile9717869, S: AF291702; L: AF291704). The numbers at each node are posterior node probabilities based on 150,000 trees. The scale bar indicates nucleotide substitutions per site.

RT-PCR detection and sequence confirmation of hantaviruses in lung tissues of shrews and moles from Poland.

Table 1

Genus species	Capture Site *	Trapping Year	Number Tested	Number Positive†	Hantavirus
<i>Sorex araneus</i>	Boginia	2010	3	1	SWSV
		2011	6	0	
		2013	1	0	
	Chmiel	2010	11	4	SWSV
		2011	6	2	SWSV
<i>Sorex minutus</i>		2013	1	0	
	Huta Dłutowska	2011	9	2	SWSV
		2011	1	0	
	Kurowice	2013	13	5	SWSV
	Nowosolna	2011	1	0	
	Pabianice	2011	1	0	
	Boginia	2011	2	0	
		2013	2	0	
	Chmiel	2010	7	0	
		2011	2	0	
<i>Neomys anomalus</i>		2012	4	0	
		2013	8	0	
	Huta Dłutowska	2011	4	0	
		2012	1	0	
	Kurowice	2012	10	1	SWSV
		2013	7	1	SWSV
	Nowosolna	2012	5	0	
		2010	3	0	
	Chmiel	2011	8	1	SWSV
		2013	1	0	
<i>Neomys fodiens</i>	Boginia	2011	1	1	BOGV
		2012	1	0	
	Huta Dłutowska	2011	1	1	BOGV

Genus species	Capture Site*	Trapping Year	Number Tested	Number Positive [†]	Hantavirus
<i>Talpa europaea</i>	Kurowice	2012	4	1	BOGV
		2013	8	1	BOGV
	Pabianice	2012	1	0	
	Boginia	2011	4	0	
	Brosinin	2011	3	1	NVAV
	Huta Dłutowska	2010	11	4	NVAV
		2011	15	13	NVAV
		2012	3	3	NVAV
		2013	29	18	NVAV
	Łódź	2011	1	0	
	Pawlikowice	2011	1	0	
	Ro niałów	2013	1	0	
	Wi czy	2013	1	0	

* Coordinates of capture sites: Boginia (N51°20'26.8, E19°36'41.36); Bronisin (N51°41'36.69, E19°32'41.66); Chmiel (N49°13'25.67, E22°36'49.82); Chojnowo (N53°21'55.12, E22°37'27.37); Huta Dłutowska (N51°35'49.51, E19°22'46.8); Jura (N50°44'7.9, E19°15'58.58); Kurowice (N51°39'48.03, E19°42'20.92); Łódź (N51°45'32.39, E19°29'34.98); Nowosolna (N51°47'14.85, E19°34'14.85); Pabianice (N51°39'8.79, E19°18'47.45); Pawlikowice (N51°36'53.89, E19°20'22.8); Ro niałów (N52°02'46, E18°50'04); Wi czy (N51°46'41, E19°36'36).

[†] RT-PCR L-segment amplicons were confirmed as hantavirus by DNA sequencing.

Table 2

Oligonucleotide primers used to detect hantaviruses in shrew and mole tissues.

Nested or Hemi-nested Primer Pairs			
Hantavirus	Forward: sequence (5'-3')	Reverse: sequence (5'-3')	Position
Shrew-borne			
S	Han-5'-end-EcoRI: CTC GAA TTC TAG TAG TAG AC	Shrew-S777R: AAN CCD ATN ACN CCC AT	1-720
	Han-5'-end-EcoRI: CTC GAA TTC TAG TAG TAG AC	Shrew-S764R: CCA TNA CWG GRC TNA TCA	1-710
	OSM55: TAG TAG TAG ACT CC	Han-S6: AGC TCN GGA TCC ATN TCA TC	1-1260
	Cro-2F: AGY CCN GTN ATG RGW GTN RTY GG	Cro-2R: ANG AYT GRT ARA ANG ANG AYT TYT T	700-1140
L	Han-L2520F: ATN WGH YTD AAR GGN ATG TCN GG	Han-L2970R: CCN GGN GAC CAY TTN GTD GCA TC	2520-2970
	Han-L2520F: ATN WGH YTD AAR GGN ATG TCN GG	Han-L2935R: GTN GCR TCN GCA CTN ACA TAC AT	2520-2957
	HAN-L-F1: ATG TAY GTB AGT GCW GAT GC	HAN-L-R1: AAC CAD TCW GTY CCR TCA TC	2935-3354
	HAN-L-F2: TGC WGA TGC HAC NAA RTG GTC	HAN-L-R2: GCR TCR TCW GAR TGR TGD GCA A	2946-3337
Mole-borne			
S	OSM55: TAG TAG TAG ACT CC	Mole-S732R: GRA AKC CDA TVA CTC CCA T	1-730
	OSM55: TAG TAG TAG ACT CC	Mole-S712R: CAT HAC AGG ACT WAT CA	1-710
	Tal-S696F: TGA TNA GYC CTG TNA TGG GAG T	NVAV-3'endR: TAG TAG TAT ACT CCT TGA AAA GC	696-end
	Tal-S710F: ATG GGA GTG ATA GGC TTT CA	Han-S6: AGC TCN GGA TCC ATN TCA TC	710-1266
L	NVAV-S1143F: ACA TGA GAA GGA CTC AGT C	NVAV-3'endR: TAG TAG TAT ACT CCT TGA AAA GC	1143-end
	NVAV-S1169F: ATG CAG ATG GAT CAG CGA	NVAV-3'endR: TAG TAG TAT ACT CCT TGA AAA GC	1169-end
	Han-L2520F: ATN WGH YTD AAR GGN ATG TCN GG	Han-L2970R: CCN GGN GAC CAY TTN GTD GCA TC	2530-2987
	Han-L2520F: ATN WGH YTD AAR GGN ATG TCN GG	Han-L2935R: GTN GCR TCN GCA CTN ACA TAC AT	2530-2972
HAN-L-F1: ATG TAY GTB AGT GCW GAT GC		HAN-L-R1: AAC CAD TCW GTY CCR TCA TC	2950-3373
HAN-L-F2: TGC WGA TGC HAC NAA RTG GTC		HAN-L-R2: GCR TCR TCW GAR TGR TGD GCA A	2960-3356

Table 3
NVAV distribution in tissues of European moles, as determined by DNA sequencing of RT-PCR products.*

Year	Capture Site	NVAV Strain	GenBank Accession Numbers					
			Lung	Heart	Liver	Kidney	Spleen	Intestine
2010	Huta Dlutowska	1129	JX990946	KF663714	KF663752	KF663751	KF663750	N.A.
		1132	JX990947	KF663749	KF663748	N.D.	KF663747	N.A.
		1135	JX990948	KF663746	KF663745	KF663744	KF663743	N.A.
		1136	JX990949	KF663742	KF663741	KF663740	KF663739	N.A.
2011	Huta Dlutowska	2088	JX990950	N.D.	N.D.	N.D.	N.D.	N.D.
		2090	JX990951	N.D.	N.D.	KF663738	KF663737	N.D.
		2091	JX990952	N.D.	N.D.	KF663736	N.D.	N.D.
		2093	JX990953	N.D.	KF663735	KF663734	KF663733	KF663732
		2095	JX990954	N.D.	KF663731	KF663730	KF663729	KF663728
		2097	JX990955	N.D.	KF663727	KF663726	KF663725	KF663724
		2099	JX990956	N.D.	N.D.	KF663723	KF663722	KF663721
		2100	JX990957	N.D.	N.D.	N.D.	N.D.	N.D.
		2101	JX990958	N.D.	N.D.	N.D.	N.D.	N.D.
		2102	JX990959	KF663720	N.D.	N.D.	N.D.	N.D.
		2103	JX990960	N.D.	N.D.	N.D.	N.D.	N.D.
2104	JX990961	N.D.	KF663719	KF663718	KF663717	KF663716		
2105	JX990962	N.D.	N.D.	N.D.	KF663715	N.D.		

* GenBank accession numbers are provided for NVAV sequences amplified from tissues of European moles captured in Huta Dlutowska in 2010 and 2011. N.D.: NVAV not detected; N.A.: tissue not available for testing.