Isolation and characterization of Dobrava hantavirus carried by the striped field mouse (Apodemus agrarius) in Estonia

Kirill Nemirov,1 Olli Vapalahti,1 Åke Lundkvist,2,3 Vera Vasilenko,4 Irina Golovljova,4 Angelina Plyusnina,4 Jukka Niemimaa,5 Juha Laakkonen,6 Heikki Henttonen,5 Antti Vaheri1 and Alexander Plyusnin1, 3

1 Department of Virology, Haartman Institute, PO Box 21, University of Helsinki, FIN-00014 Helsinki, Finland
2 Swedish Institute for Infectious Disease Control, S-105 21 Stockholm, Sweden
3 Microbiology and Tumour Biology Centre, Karolinska Institute, S-171 77 Stockholm, Sweden
4 Institute of Experimental and Clinical Medicine, EE-0016 Tallinn, Estonia
5 Finnish Forest Research Institute, FIN-01301 Vantaa, Finland
6 Section of Anatomy, Faculty of Veterinary Medicine, PO Box 57, University of Helsinki, FIN-00014 Helsinki, Finland

Dobrava hantavirus (DOB) was isolated from the striped field mouse (Apodemus agrarius) trapped on Saaremaa Island, Estonia, and its genetic and antigenic characteristics were subsequently analysed. Phylogenetic analysis showed that the Estonian DOB strain, together with several wild strains carried by Apodemus agrarius, forms a well-supported lineage within the DOB clade. The topography of the trees calculated for the S, M and L nucleotide sequences of the Estonian DOB suggests a similar evolutionary history for all three genes of this virus and, therefore, the absence of heterologous reassortment in its evolution. A cross-neutralization comparison of the Estonian virus with the prototype DOB, isolated from a yellow-necked mouse (A. flavicollis) in Slovenia, revealed 2- to 4-fold differences in the end-point titres of rabbit and human antisera. When studied with a panel of 25 monoclonal antibodies (MAbs), the Estonian and Slovenian DOB isolates showed similar antigenic patterns that could be distinguished by two MAbs. Genetic comparison showed sequence differences in all three genome segments of the two DOB isolates, including an additional N-glycosylation site in the deduced sequence of the G2 protein from the Estonian virus. Whether any of these mutations relates to the different rodent hosts rather than to the distant geographical origin of the two isolates remains to be resolved. Taken together, our observations suggest that A. agrarius, which is known to harbour Hantaan virus in Asia, carries another hantavirus, DOB, in north-east Europe.

Introduction

Hantaviruses (family Bunyaviridae) are negative-strand RNA viruses with a tripartite genome that consists of three segments, large (L), medium (M) and small (S), encoding four structural proteins: a viral polymerase, the surface glyco-proteins G1 and G2 and a nucleocapsid protein (N) (for reviews see Plyusnin et al., 1996; Schmaljohn, 1996). Hantaviruses are rodent-borne agents that are maintained in nature in persistently infected rodent hosts. Humans are thought to be infected by inhalation of aerosolized rodent excreta and, with rare exceptions (Padula et al., 1998), do not spread the virus further. Hantaan (HTN), Seoul (SEO), Puumala (PUU) and Dobrava (DOB) hantaviruses cause haemorrhagic fever with renal syndrome (HFRS) in Eurasia, while Sin Nombre (SN) and related viruses are indigenous to the New World where they cause hantavirus pulmonary syndrome (HPS) (for reviews see Lundkvist & Niklasson, 1994; Nichol et al., 1996).

DOB was originally discovered in Slovenia; the prototype
strain was isolated from a yellow-necked mouse (*Apodemus flavicollis*) trapped near Dobrava village (Avsic-Zupanc et al., 1992). This strain, passaged in Vero E6 cell culture, was used for serological characterization and sequence analysis of the S and M genome segments that identified DOB as a distinct hantavirus type (Avsic-Zupanc et al., 1995; Lundkvist et al., 1997c). DOB has caused severe HFRS in Slovenia (T. Avsic-Zupanc, M. Petrovec, P. Furlan, R. Kaps & Å. Lundkvist, unpublished results), Greece, Albania (Antoniadis et al., 1996; Papa et al., 1998) and Bosnia-Herzegovina (Lundkvist et al., 1997c).

In 1996, DOB was found in the striped field mouse (*A. agrarius*) in Estonia (Plyusnin et al., 1997). This confirmed the earlier observations on the circulation of an HTN-like hantavirus in *Apodemus* mice in Estonia (Vasilenko et al., 1987) and suggested *A. agrarius* as an alternative host for DOB. By retrospective analysis, DOB was shown to have caused the HFRS outbreaks in the Tula and Ryazan regions of Central Russia in 1991–1992, at a time when population densities of the striped field mouse were extremely high (Lundkvist et al., 1997a). Here we report the isolation and subsequent characterization of DOB from *A. agrarius* collected in Estonia.

**Methods**

- **Trapping of rodents.** As our main targets were *A. agrarius* and *A. flavicollis*, trapping efforts in 1996 concentrated on field edges, where the highest densities of *A. agrarius* are found after crop harvesting, and in 1997 concentrated on deciduous forests with hazel and oak, which *A. flavicollis* prefers. In 1996, trapping was performed on Saaremaa Island between 28 September and 1 October and on Vormsi Island on 2–4 October. In 1997, trapping was done on Saaremaa Island on 11–14 November. Trapping in 1996 was performed soon after harvesting and the densities were higher than in 1997, when trapping took place almost 1 1/2 months later.

Rodents were caught in multiple-capture live traps (model Ugglan), which were checked two or three times per 24 h. The rodents were kept for no more than 24 h individually in the live traps or mouse cages, with food ad libitum, until euthanized with ether. Altogether, 287 *A. agrarius*, 57 *A. flavicollis*, 103 Clethrionomys glareolus (bank voles), 105 Microtus arvalis, six M. agrestis (field voles) and seven Microtus minutus (harvest mice) were caught. Samples of lung, and alternatively blood serum (1996) or the whole heart (1997), were taken with sterilized instruments into tubes and stored in liquid nitrogen. Some samples of Estonian *A. agrarius* were collected to be deposited later in the Zoological Museum, University of Helsinki.

- **Screening of rodent specimens.** Lung samples were screened by immunoblotting for HTN/DOB/SEO-like nucleocapsid protein antigen (N-Ag), essentially as described previously (Plyusnin et al., 1994a, b) with the primer TTCTGCAGTAGTAGTA(G/T)(A/G)CTC-CCTAA(G/A)AG, which was designed based on the known sequences of HTN (Schmaljohn et al., 1986) and DOB (Avsic-Zupanc et al., 1995). Partial sequences of the S segment (nucleotides 4–357 and 384–757) from wild DOB strains were obtained by RT–nested PCR. The entire M segment from the Estonian DOB isolate was amplified in three overlapping parts, nucleotides 1–1251, 1182–3039 and 2870–3644. The last part of the M segment (nucleotides 2870–3644) was recovered from the wt DOB strains Saaremaa/111Aa/96 (Sa111), Saaremaa/171Aa/96 (Sa171), Saaremaa/90Aa/97 (Sa90) and Saaremaa/91Aa/97 (Sa91). In addition, a partial M segment sequence (nucleotides 2101–2583) was obtained for strains Sa111, Sa171 and Sa90. Partial sequences of the L segment (nucleotides 173–530) were amplified from both the prototype strain DOB/Slovenia and the Estonian strain DOB/Saarema/160V. PCR amplification products were separated in agarose gels and purified with the QIAquick kit (QIAGen). Direct sequencing was performed with the ABI PRISM Dye Terminator sequencing kit (Perkin--
Dobrava hantavirus carried by Apodemus agrarius

Results

Screening of rodents and isolation of the virus

The results of the screening of Apodemus mice are summarized in Table 1. The population density of A. agrarius was high in 1996 and this species represented 95% of all Apodemus mice trapped. However, only three hantavirus N-Ag-positive A. agrarius were found, all in one location. None of the 11 A. flavicollis were positive. In 1997, the trapping effort concentrated more on A. flavicollis. Consequently, A. flavicollis represented 37% of all Apodemus mice trapped on Saaremaa island. However, none of the 48 A. flavicollis were positive for the hantavirus N-Ag, while two A. agrarius from Saaremaa island contained viral antigen. The overall prevalence of DOB N-Ag in A. agrarius in the material collected from Saaremaa over 2 years was 1.7% (5/287). However, in the two positive foci, the prevalences were 2.5- to 5-fold higher: 4.5% (3/66) and 8.3% (2/24), respectively.

N-Ag was also found in Clethrionomys glareolus, Microtus arvalis and M. agrestis, suggesting the presence of Puumala- and Tula-related hantaviruses in these rodent species (data not shown).

Attempts were made to isolate virus from the three hantavirus N-Ag-positive lung samples from A. agrarius (nos 111, 160 and 171) trapped in 1996. The lung tissues were homogenized and inoculated onto confluent Vero E6 cells, and the cells were trypsinized and passaged with fresh uninfected Vero E6 cells every 3 weeks. After the second passage at 46 days, hantavirus antigen-positive cells were found by IFA in all three cell cultures, and after 54 days, most cells were positive. The cell culture supernatants were harvested at 59 days post-infection and were used to infect fresh Vero E6 cell cultures, which became infected, with 100% of cells positive in 8 days. N-Ag from Saaremaa/160V (DOB/Saar) was further passaged and selected as the reference strain, DOB/Saaremaa/160V (DOB/Saar).

Antigenic characterization of the Estonian DOB isolate by cross-FRNT and Mabs

To evaluate the serological relationship between the prototype DOB strain from Slovenia (DOB/Slo) and DOB/Slo).
Table 2. Antigenic characterization of DOB/SaaV by cross-FRNT

Reactivities of sera against DOB/SaaV, DOB/Slo or HTN strain 76-118 (HTN/76-118) were measured by FRNT as end-point titres. Rabbit sera were obtained from rabbits immunized with either DOB/SaaV or DOB/Slo. Human sera were obtained from individuals infected with DOB hantavirus (according to results of FRNT with all hantaviruses known to cause HFRS).

<table>
<thead>
<tr>
<th>Serum</th>
<th>End-point titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOB/Slo</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
</tr>
<tr>
<td>DOB/Slo</td>
<td>160</td>
</tr>
<tr>
<td>DOB/SaaV-a</td>
<td>80</td>
</tr>
<tr>
<td>DOB/SaaV-b</td>
<td>160</td>
</tr>
<tr>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>Slo-1</td>
<td>1280</td>
</tr>
<tr>
<td>Slo-2</td>
<td>1280</td>
</tr>
<tr>
<td>Est-1</td>
<td>640</td>
</tr>
<tr>
<td>Est-2</td>
<td>640</td>
</tr>
</tbody>
</table>

SaaV antisera were raised in rabbits by intranasal immunization. Cross-FRNT comparison showed 2- or 4-fold higher end-point titres to the homologous strain compared with the heterologous strain, while reactivities to HTN virus were low or absent. The results showed that the Estonian strain belongs to the DOB serotype but also indicated significant antigenic differences between the two DOB virus strains (Table 2). To clarify the serological relationship further, four human sera from previously confirmed DOB convalescents were analysed. Two sera from Slovenia reacted with 4-fold higher titres to DOB/Slo. One serum from Estonia also reacted with a 4-fold higher titre to the local virus strain, while the other Estonian serum instead reacted with a 2-fold higher titre to DOB/Slo.

Antigenic characterization of the Estonian DOB isolate was performed by using a panel of 25 MAbs against HTN glycoproteins and N proteins of HTN, PUU and TUL. The patterns obtained in IFA with this panel were most similar for DOB/Slo and DOB/SaaV and clearly distinguished the DOB isolates from HTN, SEO and PUU (Table 3). However, two of the MAbs showed different reactivities to the two DOB strains, namely (i) the PUU N-specific MAb 4E5 (Lundkvist et al., 1991), for which the titres were < 1:5 and 1:16 to DOB/SaaV and DOB/Slo, respectively, and (ii) the anti-HTN-G1 MAb 8B6 (Arikawa et al., 1989), with corresponding titres of 1:64 and 1:8. These results suggest that the gross antigenic structure of DOB is similar in both isolates. In addition, we noted some differences in reactivity of these MAbs to those reported previously (Lundkvist et al., 1996), as the anti-TUL-N MAb 1C8 reacted with SEO and also weakly with HTN virus. Also, in contrast to previously reported data (Arikawa et al.,...
Dobrava hantavirus carried by *Apodemus agrarius*

Fig. 1. Phylogenetic trees of hantaviruses based on (a) the complete sequences of the S segment coding region, (b) the complete sequences of the M segment coding region and (c) partial sequences of the L segment (nucleotides 173–530). Trees calculated for the partial S segment sequences (nucleotides 377–934) and partial M segment sequences (nucleotides 1701–1969) of the DOB strains are inset in (a) and (b), respectively. The following abbreviations are used for DOB strains: DOB-SaaV, strain SaaV; DOB-Saa90, strain Saaremaa/90Aa/97; DOB-Slo, the prototype strain from Slovenia (Avsic-Zupanc et al., 1995); DOB-GreGA and DOB-GreHA, Greek strains GA and HA (Papa et al., 1998); DOB-Slv862 and DOB-Slv872, strains Slovakia/Aa862/97 and Slovakia/Aa872/97 (C. Sibold, personal communication). For other virus abbreviations, see Methods.
We were not isolated, have been designated Saa111, DOB Haeska locality and two from the Loode Tammik locality, both (Plyusnin from another Estonian Island, Vormsi, was recovered earlier from each other. One partial S segment sequence originating strains recovered from Loode Tammik were indistinguishable sequences of wild strains originating from Haeska were DOB strains form a well-supported sublineage within the DOB DHG

Genetic analysis

The complete sequences of the S and M segments of the Estonian DOB isolate were determined. Also, partial L segment sequences (nucleotides 173–530) of both the prototype DOB isolate from Slovenia and the Estonian isolate were obtained. In addition, complete or partial S segment sequences and partial M segment sequences (see Methods) were recovered from lung specimens of three N-Ag-positive strains (positions 28 and 32) also, five one-nucleotide gaps were introduced to allow the annealing of amplification primers and, therefore, were not found, excluding the first and the last 22 nucleotides of the S segment and the first and the last 19 nucleotides of the M segment, which were involved in the annealing of amplification primers and, therefore, were not determined directly. Two substitutions (positions 28 and 32) were located within the 5′ non-coding region (NCR) of the S segment and 25 substitutions were found within the 3′ NCR. Also, five one-nucleotide gaps were introduced to allow the proper alignment of the two 3′ NCR sequences. The 5′ NCR of the M segment contained only one substitution, A for G at position 23. The 3′ NCR of the M segment was more variable, showing 22.9% difference over the 214 nucleotide alignment.

The coding region of the S segment was found to contain 158 nucleotide substitutions, 144 of them being silent. Of 13 deduced amino acid substitutions in the N protein, 11 were conservative (two nucleotide substitutions were located within the same codon). All three cysteine residues conserved in all hantavirus N sequences were present in both DOB isolates.

Comparison of the M segment coding regions revealed a deletion of one triplet, encoding Ile\(^{1113}\) of the G1–G2 of DOB/Slo. Notably, an amino acid residue is present in this position in all other hantaviruses, including DOB/SaaV. The

| Table 4. Comparison of the nucleotide and deduced amino acid sequences of DOB/SaaV with those of other hantaviruses |
|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sequence    | Saa90 | Vor | Slv | Slo | Gre | JTN | SEO | PUU | TUL | SN |
| S. nt*      | 98.3  | 90.3 | 86.2 | 87.8 | 86.6 | 73.4 | 72.1 | 62.0 | 62.7 | 63.8 |
| N. aa       | 99.3  | 97.7 | 96.2 | 97.0 | 96.2 | 82.1 | 79.5 | 61.0 | 62.3 | 62.6 |
| M. nt†      | 98.1  | –   | –   | 81.2 | 81.0 | 71.2 | 70.5 | 58.8 | 59.2 | 58.7 |
| G1–G2. aa   | 98.9  | –   | –   | 93.8 | 95.5 | 76.2 | 76.3 | 53.1 | 54.6 | 54.2 |
| L. nt‡      | –     | –   | –   | 86.0 | –   | 72.4 | 74.0 | 64.2 | 65.9 | 62.0 |
| L. aa       | –     | –   | –   | 96.6 | –   | 82.9 | 80.3 | 63.2 | 68.4 | 66.6 |

* Complete sequence available for all but Vor (4–357), Slv (378–935) and Gre (377–934).
† Complete sequence available for all but Saa90 (2101–2583) and Gre (1701–1969).
‡ Partial sequence available from DOB/SaaV and DOB/Slo, nucleotides 173–530.
number of cysteine residues and their localization were identical for both DOB strains. Comparison of potential asparagine-linked glycosylation sites (Asn–X–Ser/Thr) has shown an additional one (Asn–Trp–Ser) located in G2 of the Estonian isolate (amino acids 762–764). Identical sites were found in all sequenced wild strains from Saaremaa (Saa111, Saa171 and Saa90), suggesting that this feature is a hallmark of, at least, the local strains of DOB carried by A. agrarius.

The nucleotide and deduced amino acid sequences of the Estonian strains were compared with those of other known DOB strains and selected members of other hantavirus sero/genotypes (Table 4). The closest relatives among the other hantaviruses were HTN and SEO (both carried by Murinae rodents in Asia), followed by other viruses carried by Arvicolinae (PUU, TUL) and Sigmodontinae (SN) rodents. These data are in agreement with the results obtained by cross-FRTN (Table 2) and IFA with a panel of monoclonal antibodies (Table 3). Comparative analysis (Table 4) revealed different levels of genetic diversity of DOB strains: (i) up to 1-9% between the two strains from the Saaremaa locality; (ii) up to 9-7% within the Estonian sublineage; and (iii) up to 19% within the DOB genotype. These findings are also consistent with the geographical clustering of the DOB genetic variants.

Discussion

Taken together, our data suggest that A. agrarius, which is known to harbour HTN in Asia, carries another hantavirus in Europe, DOB. The results of our trapping expeditions confirmed the previous observations that A. agrarius is the predominant rodent species on Saaremaa Island (U. Timm, personal communication). The frequency of hantavirus N-Ag-positive A. agrarius (4-5–8-3%) suggested a moderate intensity of infection in the natural foci of DOB on the island. Recently, antibodies to DOB were found in human sera from Saaremaa (Lundkvist et al., 1998), suggesting that this intensity is high enough to cause human infections. No DOB-infected A. flaviocollis were found on the island, suggesting that the virus is carried permanently by A. agrarius and is not a spill-over from the other Apodemus species. The distribution ranges of A. agrarius and A. flaviocollis overlap over most of Europe, including Estonia (Wilson & Reeder, 1993). Thus, additional studies (e.g. evaluations of the rate of seropositivity) will need to be performed to show which, if either, of the two species is the predominant host for DOB in Europe.

There are several examples of hantaviruses associated with closely related host species: SEO in Rattus rattus and R. norvegicus (Lee & Johnson, 1982), TUL in Microtus arvalis and M. rossiaemeridionalis (Plyusnin et al., 1994a), SN in Peromyscus maniculatus, P. leucopus, P. boylii and P. truei (Childs et al., 1994; Mills et al., 1997; Ollenson et al., 1996; Morzunov et al., 1998) and PUU in Clethrionomys glareolus (Brummer-Korvenkontio et al., 1982) and C. rufocanus (Kariwa et al., 1995). In several cases, however, it is difficult to judge whether this represents a genuine alternative host rather than spill-over of the virus from its primary host. As current knowledge on the properties of hantaviruses carried by alternative hosts is limited, comparison of the two DOB isolates from Slovenia and Estonia provides an opportunity to gain insight into this problem.

The present cross-neutralization comparison revealed a close antigenic relationship between DOB/SaaV and DOB/Slo. On the other hand, 2- or 4-fold higher titres were observed for the homologous DOB strain, similar to results described for PUU strains from widely separated regions (Niklasson et al., 1991; Lundkvist et al., 1997b). In line with the data on rabbit antisera, the analysis of human sera revealed antigenic differences between the two DOB strains; three of the four sera reacted with a 4-fold higher titre to the DOB strain isolated from the same geographical region. However, one serum from Estonia reacted with a higher titre to DOB/Slo. Whether this result reflects individual differences in antibody cross-reactivity or that more than one DOB type is circulating in Estonia, including perhaps DOB carried by A. flaviocollis, remains to be studied. Larger serum panels from both regions are at present under investigation. Whether DOB virus, as well as PUU virus, should be divided into subtypes remains to be defined.

As there are no available MAbs raised against DOB as yet, antigenic characterization of the Estonian isolate had to be performed with MAbs generated against heterologous hantaviruses, limiting the possibility of detecting differences between the Estonian and Slovenian DOB isolates. However, different reactivities to one N- and one G1-specific MAb were observed. More detailed studies, using homologous MAbs, will probably disclose additional epitope differences between these two DOB isolates, as indicated by the cross-neutralization data.

Genetic comparison has revealed sequence differences in all three genome segments of the DOB isolates from Estonia and Slovenia (Table 3). However, at present it seems impossible to judge whether any of these mutations reflect the distinct nature of the rodent hosts rather than the distant geographical origins of the two strains. Along these lines, results obtained recently on two wild strains of DOB, one obtained from A. flaviocollis and another from A. agrarius trapped at the same locality in Slovenia (T. Avsic-Zupanc, personal communication), are of particular interest. Partial DOB M segment sequences (nucleotides 1314–1590) that were recovered from the two rodent specimens differed from each other by 13%, suggesting the possibility of co-circulation of two distinct variants of DOB in two Apodemus species within the same area, thus supporting the hypothesis of virus–host co-speciation.

As shown for other enveloped RNA viruses, such as influenza virus and human immunodeficiency virus type 1, an altered pattern of glycosylation of surface proteins may change the virus phenotype substantially (Kawaoka et al., 1984; Lee et al., 1992). Accordingly, it has been proposed that PUU variants that have lost one of the glycosylation sites in the G2
glycoprotein possess higher virulence for humans (Pilaski et al., 1994; Bowen et al., 1995). The putative N-glycosylation site (Asn\(^{62}\)-Trp-Ser) that was found in the deduced G2 sequence of DOB/SaaV but not of DOB/Slo could be considered as a candidate for a host-specific marker in DOB. However, the two following reservations should be noted. Firstly, according to the data obtained for rabies virus, tryptophan in the middle position may lead to inefficient glycosylation (Shakin-Eshleman et al., 1996). Second, a cysteine located next to the site may prevent glycosylation because of disulphide bridging (Mellquist et al., 1998). Although the secondary structure of G2 has not yet been determined, the fact that Cys\(^{765}\) is conserved in all hantaviruses suggests its involvement in disulphide binding. Whether this site is indeed used for glycosylation remains to be studied.

There are reasons to believe that the DOB variants circulating in some areas show higher virulence for humans than those found in other areas. For instance, a fatality rate of 9% was recently reported for HFRS caused by DOB in Greece (Papa et al., 1998), although this figure was based on the most severe cases that were hospitalized and the overall case fatality rate was lower when milder cases were also included. A fatality rate of 12% was observed among hospitalized DOB cases in the Dolnenjska region of Slovenia (T. Avsic-Zupanc, M. Petrovec, P. Furlan, R. Kaps & A. Lundkvist, unpublished results). In contrast, no casualties were reported during large HFRS outbreaks caused by DOB and associated with abundance of A. agrarius in Russia in 1991–1992 (Lundkvist et al., 1997a). Similarly, our data on the screening of Estonian blood donors for the presence of DOB-specific antibodies have demonstrated that the virus causes human infections all over the country (Lundkvist et al., 1998). However, no severe or fatal HFRS cases, which might be associated with DOB infections, have so far been reported from Estonia. The situation is further complicated by the observation that no fatal cases were observed among more than one hundred serologically confirmed HFRS cases from the 1995–1996 outbreak in Bosnia-Herzegovina, of which many were caused by DOB (Lundkvist et al., 1997c). It should be noted, however, that the military crisis at this time might have severely hampered the recognition of many HFRS cases. It remains unclear whether these differences indicate a variation in the pathogenicity of DOB strains determined by distinct rodent hosts (A. flavicollis for more-virulent variants in the Balkans and A. agrarius for less-virulent ones in Russia and Estonia) or occur simply because of incomplete diagnosis and/or biased reporting of only severe and fatal cases.

Definitive hantavirus typing by neutralization tests or by genetic analysis has so far confirmed only PUU and DOB as causative agents of HFRS in Europe. Another hantavirus, TUL, is widespread throughout the continent and has in one case been shown to have caused human infection (Vapalahi et al., 1996); however, it has not so far been associated with any human disease. The European map of hantavirus distribution still seems to be incomplete, and whether other members of the genus are present awaits further study.

We thank Tatjana Avsic-Zupanc, Anna Papa and Claus Sibold for sharing sequencing data with us before publication. Valuable information on Saaremaa rodents given by Uudo Timm is greatly appreciated. Tytti Manni and Katarina Brus Sjölander are acknowledged for excellent technical assistance. This work was supported by the EC (Biomed-2), the Academy of Finland and the Swedish Medical Research Council (grants 12177 and 12642).

References


Received 24 August 1998; Accepted 5 October 1998