Distribution and genetic heterogeneity of Puumala virus in Sweden

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Small mammals trapped in Sweden were analysed for specific antibody responses against three hantavirus serotypes and for the presence of viral antigen. To determine the genetic identity of viral RNA in lungs of seropositive bank voles (Clethrionomys glareolus), polymerase chain reactions and subsequent partial sequencing of both the M and S segments were employed. The sequences obtained were all identified as Puumala (PUU) virus, with a high degree of heterogeneity between the different geographical localities. Alignment of nucleotide and deduced amino acid sequences, together with phylogenetic analysis, showed that PUU viruses circulating in central Sweden were distinct from those in the northern region. The localization of the two distinct PUU virus genotypes was shown to correlate with the postglacial recolonization of Sweden by bank voles.

Introduction

Several of the viruses in the genus Hantavirus of the family Bunyaviridae are important human pathogens. Puumala (PUU), Hantaan (HTN) and Seoul (SEO) viruses are associated with haemorrhagic fever with renal syndrome (HFRS), a group of widely distributed diseases characterized by fever, renal failure and, in severe cases, haemorrhagic manifestations (Yanigahara & Gajdusek, 1988). In North America, Sin Nombre (SN) virus has been identified as the causative agent of hantavirus pulmonary syndrome (HPS) (Nichol et al., 1993b).

The hantaviruses can be divided into at least nine serotypes: HTN, SEO, PUU, SN, Dobrava (DOB), Prospect Hill (PH), Thailand (THAI), Thottapalyam (THOT) and Khabarovsky (KBR) (Chu et al., 1994; Hör ling et al., 1996; Nichol et al., 1993b; Xiao et al., 1993). In addition, several viruses, potentially representing new serotypes, have recently been reported, including El Moro Canyon (ELMC) (Hielle et al., 1994), Tula (TUL) (Plyusnin et al., 1994a), Black Creek Canal (BCC) (Rollin et al., 1995) and Bayou (BAY) (Khan et al., 1995; Morzunov et al., 1995).

Each hantavirus is primarily associated with a single rodent species and the distribution appears to be species-restricted. The main reservoirs for HTN, SEO, PUU, SN and PH viruses are Apodemus agrarius, Rattus norvegicus, Clethrionomys glareolus, Peromyscus maniculatus and Microtus pennsylvanicus, respectively (Lundkvist & Niklasson, 1994). In the European parts of Russia, and in central Europe, TUL virus has been detected in M. arvalis and M. rossiaemeridionalis (Plyusnin et al., 1994a; Sibold et al., 1995). The rodents are persistently infected with virus (Yanigahara et al., 1985) and transmission occurs via aerosolized excretions (Tsai, 1987; Nuzum et al., 1988).

In common with other members of the Bunyaviridae, hantaviruses have a single-stranded, tripartite RNA genome, packaged in enveloped helical nucleocapsids (Elliott, 1990). The negative-stranded genome, of approximately 12 kb, encodes four structural proteins; an RNA dependent RNA polymerase, two glycosylated envelope proteins (G1 and G2) and the nucleocapsid protein (N) (Schmaljohn et al., 1985; Elliott, 1990, Antic et al., 1991).

PUU virus is a causative agent of nephropathia epidemica...
The bank vole belongs to the subfamily Arvicolinae of the family Muridae (Wilson & Reeder, 1993), and is distributed over most of Europe (Stenseth, 1985). In Fennoscandia, a northern and a southern population of bank voles can be distinguished by mitochondrial (mt) DNA variation (Tegelström, 1987). The two populations, estimated to have been separated for 30,000–60,000 years, have a parapatric distribution. The contact zone between the two populations runs through central Sweden and is about 50 km wide (Jaarola & Tegelström, 1995). A similar contact zone has been demonstrated in central Finland (Tegelström et al., 1995). A similar recolonization scenarios have been suggested for many mammals, like the brown bear (Ursus arctos) (Taberlet & Bouvet, 1994) and several species of shrews and voles, including the field vole (Microtus agrestis) (Jaarola & Tegelström, 1995).

To investigate circulation and genetic heterogeneity of hantaviruses in the two different bank vole populations, rodents from several geographical locations were examined for antibodies reactive with PUU, KBR and HTN viruses, and for virus antigen and genomes by sensitive ELISAs followed by polymerase chain reaction (PCR) and subsequent nucleotide sequencing.

**Methods**

**Animals.** Small mammals were collected with live-traps in the summer and fall of 1994 and 1995 at seven localities, all with a radius of approximately 30 km (see Table 1). The locations were numbered from north to south (Fig. 1). In addition, lung tissue from a previously confirmed serologically-positive bank vole (Niklasson & LeDuc, 1987), trapped at locality 7, was included in the study. The animals were either transported alive or sacrificed on location and transported frozen on dry ice to the Swedish Institute for Infectious Disease Control. The animals were bled for serological analysis, sacrificed, and dissected for lung tissue. The lung tissues were ground in 1 ml PBS, aliquoted, and stored at -70 °C until analysed.

**Serological screening.** The rodent sera were examined for the presence of antibodies reactive with either PUU, KBR or HTN viruses. Antigens for the serological assays were prepared by infecting Vero E6 cells (CRL 1586; ATCC) with PUU virus (P360) (Niklasson et al., 1991), KBR virus (MF43) (Dzagurova et al., 1995; Hörling et al., 1996) or HTN virus (76-118) (Lee et al., 1978). After incubation for 14 days, the cells were centrifuged at 2000 g for 10 min and disrupted in RIPA buffer (0-01 M-Tris-HCl pH 7-8, 2% Triton X-100, 0-15 M-NaCl, 0-6 M-KCl, 5 mM-EDTA, 1% aprotinin and 1 mM-PMSE).

A sandwich ELISA for the detection of hantavirus-specific antibodies was used. Rabbit anti-PUU virus immunoglobulin (Niklasson & Kjellson, 1988) was adsorbed to microtiter plates at 4 °C overnight. After blocking of non-saturated binding sites with 3% BSA in PBS, virus antigens were incubated for 1 h at 37 °C. Serum samples, diluted 1:200, were also incubated for 1 h at 37 °C. Specific antibody binding was detected either by alkaline phosphatase (ALP)-conjugated goat anti-mouse IgG (y-specific) antibodies (Sigma), diluted 1:2000, or ALP-conjugated rabbit anti-mouse IgG (H+L chain) antibodies (Jackson ImmunoResearch, Avondale, Penn. USA) diluted 1:1000, followed by p-nitrophenyl phosphate substrate (Sigma) as described by the manufacturer. The plates were washed five times between each step in 0-9% NaCl with 0-05% Tween 20. Optimal dilutions of antigens and test samples were determined by box titrations. The reactivities of the anti-mouse conjugates to immunoglobulins from different rodent species were determined by titration. A pool of bank vole monoclonal antibodies (MAb; 1C12, 2E12 and 4C3), directed to epitopes conserved in the different hantaviruses, was used as a positive, internal control (Lundkvist et al., 1991; Dzagurova et al., 1995).

The absorbances (A) of the samples were calculated as the average absorbance minus the average absorbance with negative control antigen. The absorbance of the positive control was recalculated to 1.000 on each
plate, and the absorbance values of the samples were adjusted correspondingly. The sensitivities of the assays with different antigens were determined by titration of the MAb pool and a pool of sera from experimentally infected bank voles. The cut-off value was set to 0.100.

**Hantavirus antigen-ELISA.** Ground lung suspensions from animals where sera were not available, and from serologically positive animals, were analysed for the presence of virus antigen by a hantavirus N-antigen ELISA, constructed with protein G purified MAbS directed to different epitopes on the nucleocapsid protein, as previously described (Lundkvist et al., 1995).

**Immunofluorescence assay (IFA).** Animal sera that scored positive by ELISA were confirmed by titration in IFA. The sera were titrated in fourfold steps and incubated on slides with acetoine-fixed, virus-infected Vero E6 cells, as previously described (Lundkvist et al., 1991).

**PCR amplification and sequencing.** RNA from the ground lung suspensions was extracted by the acid guanidinium thiocyanate-phenoI-chloroform method (Chomczynski & Sacchi, 1987).

To assess samples for the presence of viral RNA, short fragments of 210 bp from the S segment [nucleotides (nt) 799–1008] were amplified using PUU-virus-specific primers and a nested strategy as previously described (Höring et al., 1995a). To generate longer fragments of the S segment (nt 791–1139), the outer primer pair described above were used as inner primers for primers PUU 0.1 and PUU 5 (Höring et al., 1996) and amplified under the same conditions as described below for the M segment PCR, except using an annealing temperature of 53 °C.

Amplification of the M segment was performed using 50 μM of each primer A1 and C2 (Höring et al., 1995b), 5 U of Rous-associated virus 2 reverse transcriptase (Amersham), 10 U of placent al ribonuclease inhibitor (Gibco BRL), 2 U Taq polymerase (Perkin Elmer Cetus) in 100 μl of PCR buffer and incubated at 42 °C for 1 h and at 95 °C for 2 min followed by 40 cycles at 94 °C for 1 min, 46 °C for 1 min, and 72 °C for 1.5 min. Five μl of this amplification mixture were added to 45 μl of PCR buffer with 1 U of Taq polymerase and 50 μM each of either primers HG2F1 and HG2R1 (Xiao et al., 1994) for nt 2147–2610, or primers B1 and B2 (Höring et al., 1995b) for nt 2014–2346, and amplified for 30 cycles at 94 °C for 1 min, 46 °C for 1 min and 72 °C for 1.5 min. Amplified products were gel-purified using a kit (Jetsorb; Genomed) as described by the manufacturers.

Dideoxy sequencing (Sanger et al., 1977) in both directions was performed with primers either labelled with 5'-biotin or with adenosine 5'-[α-32P]triphosphate as previously described (Höring et al., 1996).

**Phylogenetic analysis.** The PHYLIP program package (Felsenstein, 1993) was used to make 200 bootstrap replicates of the sequence data (SEQBOOT), distance matrices were calculated using Kimura’s 2-parameter model (DNADIST), with a transition:transversion value of 4, and analysed using the Fitch–Margoliash tree fitting algorithm (FITCH) with global arrangements option set. The occurrence ratios of particular branchings were calculated from these trees (CONSENSE).

**Mitochondrial DNA analysis.** To determine the population pertinence of each bank vole positive for PUU virus by PCR, mtDNA was subjected to restriction endonuclease digestion as described (Jääola & Tegelström, 1995). Briefly, mitochondria were isolated from hearts and spleens by differential centrifugation. MtdNA was prepared by phenol-chloroform extractions and digested with HpalI. Restriction fragments were resolved by electrophoresis in 5% polyacrylamide gels and visualized by silver-staining. The resulting fragment patterns were compared to previously obtained patterns (Tegelström, 1987; Tegelström et al., 1988).

**Results**

**Serology and antigen detection**

A total of 561 rodents (482 C. glareolus and 79 M. agrestis) were analysed for the presence of PUU-, KBR- or HTN-virus-reactive antibodies by ELISA. The ELISA was found to be equally sensitive for all three antigens, as determined by endpoint titrations of the pool of MAbS. The γ-chain-specific anti-mouse conjugate used for detection of antibody binding was shown to bind specifically to IgG from C. glareolus, but not from M. agrestis. For these animals, specific antibody binding was determined using a more broadly reactive conjugate directed to the whole IgG molecule.

In the four localities north of the bank vole contact zone (localities 1–4), nine out of a total of 306 bank voles were positive for antibodies to PUU virus or for antigen in the lungs. In the contact zone (locality 5), and immediately south (locality 6), seven out of 47, and four out of 69 bank voles, respectively, scored as positive. None of the 79 M. agrestis from any locality scored as positive, nor did any of the bank voles trapped south of the endemic region (locality 8). In total, 21 bank voles scored as positive, including the one from locality 7. The total number of trapped and positive animals at each location is summarized in Table 1. All sera had higher absorbances to PUU virus than to KBR or HTN viruses. The results were confirmed by endpoint titration in IFA, where most of the bank voles had significantly higher titres against PUU virus than to KBR or HTN viruses (data not shown).

**PUU RNA detection and sequencing**

All serologically or antigenically positive animals were analysed for the presence of viral RNA in lung tissue. Twenty of the 21 animals had detectable PUU virus genomes with the short S segment PCR. The PCRs employed to generate larger fragments of the S and M segments were found to be less sensitive. In total, 11 bank voles scored as positive in the

<table>
<thead>
<tr>
<th>Locality</th>
<th>C. glareolus</th>
<th>M. agrestis</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>75 (2)</td>
<td>18</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>63 (1)</td>
<td>16</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>108 (4)</td>
<td>30</td>
<td>138</td>
</tr>
<tr>
<td>4</td>
<td>60 (2)</td>
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<td>60</td>
</tr>
<tr>
<td>5</td>
<td>47 (7)</td>
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<td>50</td>
</tr>
<tr>
<td>6</td>
<td>40 (4)</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>7</td>
<td>1 (1)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>88 (0)</td>
<td>11</td>
<td>99</td>
</tr>
<tr>
<td>Total</td>
<td>482 (21)</td>
<td>79</td>
<td>561</td>
</tr>
</tbody>
</table>

*No. of animals (n) serologically or antigenically positive for PUU virus.
Table 2. Serological and PCR data of PUU-virus-positive bank voles

<table>
<thead>
<tr>
<th>Locality and bank voles</th>
<th>PUU ELISA*</th>
<th>Antigen ELISA</th>
<th>S segment PCR nt 819-988</th>
<th>S segment PCR nt 791-1139</th>
<th>M segment PCR nt 2014-2346</th>
<th>M segment PCR nt 2147-2610</th>
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</thead>
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<tr>
<td>Locality 1, Hundberg 36</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Locality 2, Åmsele 242</td>
<td>+ + + +</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Locality 3, Tavelsjö 81</td>
<td>+ + + +</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Locality 4, Mellansel 47</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sollefteå 31</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Lungvik 72</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sundsvall 105</td>
<td>+ + +</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sundsvall 126</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sundsvall 255</td>
<td>+ + +</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Locality 7, Gräsmark 67</td>
<td>+ + + +</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* + + + +, A > 0.701; + + +, 0.501–0.700; + +, 0.301–0.500; +, 0.101–0.300; -, A < 0.100; NA, not analysed (serum not available).

Table 3. Nucleotide and deduced amino acid divergence between PUU strains

<table>
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<tr>
<th>Locality 1–4</th>
<th>N. Sweden Locality 1–4</th>
<th>C. Sweden Locality 5–7</th>
<th>N. Sweden PUU 83-L20</th>
<th>Finland PUU Sokkamo</th>
<th>Germany PUU Berkel</th>
<th>France PUU 90-13</th>
<th>Russia PUU CG-1820</th>
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</thead>
<tbody>
<tr>
<td>S segment, nt</td>
<td>0.3–10.6</td>
<td>14.0–17.5</td>
<td>1.4–8.6</td>
<td>14.6–16.3</td>
<td>16.9–18.1</td>
<td>20.1–20.4</td>
<td>18.2–19.5</td>
</tr>
<tr>
<td>aa</td>
<td>0.0–3.5</td>
<td>3.5–9.6</td>
<td>0–3.5</td>
<td>6.1–9.6</td>
<td>4–7.8</td>
<td>6.1–9.6</td>
<td>6–9.6</td>
</tr>
<tr>
<td>M segment, nt</td>
<td>0.0–11.1</td>
<td>17.1–22.2</td>
<td>6.9–9.1</td>
<td>18.9–20.4</td>
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<td>15.9–17.4</td>
<td>21.3–23.7</td>
</tr>
<tr>
<td>aa</td>
<td>0.0–5.5</td>
<td>6.4–11.8</td>
<td>1.8–5.5</td>
<td>6–10.0</td>
<td>NA</td>
<td>5.5–9.1</td>
<td>8.2–11.8</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Locality 5–7</th>
<th>N. Sweden Locality 1–4</th>
<th>C. Sweden Locality 5–7</th>
<th>N. Sweden PUU 83-L20</th>
<th>Finland PUU Sokkamo</th>
<th>Germany PUU Berkel</th>
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<td>S segment, nt</td>
<td>14.0–17.5</td>
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<td>aa</td>
<td>3.5–9.6</td>
<td>0.9–2.6</td>
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<td>M segment, nt</td>
<td>17.1–22.2</td>
<td>0.3–17.1</td>
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<td>17.1–19.5</td>
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<td>18.3–19.5</td>
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<tr>
<td>aa</td>
<td>6.4–11.8</td>
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<td>5.5–7.3</td>
<td>7.3–9.1</td>
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</tbody>
</table>

* NA, Data not available.
to 0-3.5% aa differences. Similar differences (nt 0.3-9.2% and aa 0.9-2.6%) were observed between locality 5 (the locality situated in the border) and the southern localities 6 and 7, 80 and 430 km away, respectively. The nt divergence between the strains from localities 4 and 5, only 55 km apart, was considerably higher, 14-9-17.5%, corresponding to aa differences of 3.5-6.1%. These results and the nt and aa differences between other PUU strains are summarized in Table 3. In general, the nt divergence between strains from localities 1-4 and strains from localities 5-7, was 14.0-17.5%, corresponding to aa differences of 3.5-9.6%. Thus, two separate virus populations were identified, one with a northern distribution and one with a more southern distribution. A similar pattern was observed for the partial M segment, except for the two strains from locality 6 (Sundsvall 126 and 255), which differed by 15.0-17.1% from the strains from localities 5 and 7 at the nt level, but only by 2.7-4.5% at the aa level.

### Phylogenetic analysis

In the phylogenetic tree based on the partial S segment sequence (nt 791-1139), the strains from the northern bank vole population were clearly separated from their southern counterparts (Fig. 2a). All strains from localities 1, 3 and 4 (Hundberg, Vindeln and Mellansel) were shown to branch closely to each other, but separate from the strains from localities 5-7 (Lungvik, Sollefteå, Sundsvall and Gräsmark). A similar branching pattern was observed for the partial M segment sequence (nt 2014-2610), with the northern and southern strains clearly separate from each other (Fig. 2b). The bootstrap probabilities of the two distinct phylogenetic branches in the S and M trees were 93% or higher, indicating a different evolutionary history for the two groups of Swedish PUU strains. Notably, the Vranica strain was located on the M tree within the cluster formed by the northern Swedish strains.
The relationships between all different groups of PUU strains were not completely resolved in either of the trees due to low probabilities (30–62%).

**Mitochondrial DNA analysis**

All virus-carrying bank voles were subjected to mtDNA analysis with restriction endonuclease digestion to confirm their population origins. All bank voles trapped in the northern localities 1–4 were shown to belong to the northern mtDNA lineage. In the contact zone (locality 5) a mixed population was found: six of the bank voles belonged to the northern mtDNA lineage, and one to the southern (Lungvik 72), whereas the bank voles from locality 6 all belonged to the southern mtDNA lineage. The bank vole from locality 7 could not be analysed, but previous data from the distribution analysis of the two lineages suggest that this bank vole also belonged to the southern mtDNA lineage.

**Discussion**

Twenty-one bank voles from northern and central Sweden were found to be positive for hantavirus-specific antibodies. The titres in most of the positive animals were higher for PUU virus than for KBR or HTN viruses, but in some instances no significant differences could be observed. To deduce the serotypic origin of these hantaviruses, more accurate assays have to be employed, either neutralization tests, or, as in this study, PCR and subsequent genotyping by nucleotide sequencing.

All serologically positive animals except one (locality 2) were positive by the short S segment PCR assay. Thus, the genetic identity of the strain carried by this single animal could not be assessed. In the serological analysis, this serum had a ninefold higher titre to PUU than to KBR or HTN viruses, which makes it reasonable to assume that this strain was also PUU-virus-like. The high ratios of antibody-positive animals with detectable viral antigen and RNA (Table 2) indicate that the previous findings of persistent or prolonged infection of bank voles under laboratory conditions (Yanigahara et al., 1985) are also valid under natural conditions.

Sequence analysis of the PCR-generated S fragments showed that strains from animals trapped in close proximity (within a radius of 3 km) had a genetic difference of 0–3–4.6% base substitutions within all localities, which is consistent with the high mutation rate of RNA viruses (Holland, 1992). Between more distant localities, the observed nt difference was 8.6–10.6%, which is in agreement with previous studies where the intra-local nt diversities were found to be 1–2% and the diversities between localities were 6–8%, respectively (Plyusnin et al., 1995 a, b).

The PUU strains from bank voles trapped south of, and within, the contact zone (localities 5–7), were found to be substantially different from their northern counterparts; the nt differences of the partial S segment between strains from localities 4 and 5, situated only 55 km apart, were 14.9–17.5%, corresponding to an aa difference of 3.5–6.1%, which is considerably higher than would be expected for such closely situated localities. In general, overall nt differences between geographically distant PUU strains are 15–17%, corresponding to 3–5% aa differences (Plyusnin et al., 1994 b). The differences in the partial M segment between localities 4 and 5 were even more prominent, with nt differences of 18–21.9% and aa differences of 6.4–9.2%. Thus, our results show that the PUU strains present in the northern bank vole population are clearly different from those circulating in the more southern bank vole populations (Table 3).

The two distinct PUU virus lineages were confirmed by phylogenetic analysis where it was shown that the northern and central Swedish strains were clearly separate from each other with bootstrap probabilities of more than 93% in both the S and M segment trees (Fig. 2 a, b). The branching pattern of the two trees indicates that the differences between the northern and southern virus lineages have not evolved from interchange of gene segments, as shown for SN virus (Li et al., 1995), but rather indicates different evolutionary origins. The strain Vranica was found to branch closely to the northern Swedish strains, confirming that the previously reported uncertainty regarding its origin from former Yugoslavia (Reip et al., 1995) is well-grounded.

The Swedish virus strain 83-L20, isolated from a bank vole trapped in Vindeln in 1983 and passaged 11 times in cell culture, differed by 3–3.5% at the nt level from the three PUU strains from bank voles trapped in the same area in 1994 (locality 3). Thus, the evolutionary pattern of PUU virus strains resembles that of vesicular stomatitis virus (Nichol et al., 1993 a), also expressing a correlation to geographical origin rather than time of virus isolation (Plyusnin et al., 1995 b). In addition, it appears that several passages of PUU virus in cell culture do not alter the genome significantly, at least not in the parts sequenced in the present study. Similar data have been generated for SN virus, where only 16 nt substitutions were detected between the entire genomes of the isolated virus and the original autopsy tissue, even after several passages (Chizhikov et al., 1995).

The two Swedish PUU lineages appear to have been separated from each other for a long time. Evolutionary studies of mtDNA from bank voles indicated that Sweden, after the retreat of the Weichselian ice sheet about 10000 years ago, was recolonized by bank voles from two directions; from Russia via northern Finland to northern Sweden, and from Denmark to southern Sweden until the straits of Denmark opened up about 8500 years ago (Tegelström et al., 1988). This hypothesis is supported by the observation that the bank vole populations in Great Britain, Holland, Denmark, Poland and southern Finland belong to the same mtDNA lineage as the bank voles in southern and central Sweden, whereas the bank vole populations in northern Finland and northern Sweden belong to a distinct mtDNA lineage (H. Tegelström, un-
published observation). The contact zone in Sweden between the two populations is situated just north of Sundsvall. In Finland, the contact zone is positioned north of Sortakamo. To investigate whether the different PUU strains could be correlated to the northern and southern bank vole populations, each PCR-positive animal was subjected to mtDNA analysis. The results were in concordance with the previous study (Tegelström et al., 1988); all bank voles from localities 1–4 were of the northern mtDNA lineage, and the animals from locality 6 were all of the southern type. The bank voles trapped in the contact zone (locality 5) were shown to be of mixed origin (six belonged to the northern mtDNA lineage and one to the southern). In spite of this, all PUU strains in this locality were of the southern type. Thus, in the contact zone, the different PUU lineages do not appear to be restricted in each bank vole population, since some transfer must obviously have occurred. The co-existence of rodents in the same geographical zone carrying genetically distinct hantaviruses has also been observed for PH.ELMC and different lineages of SN viruses in North America (Rowe et al., 1995).

In conclusion, the close relationship between bank voles colonizing Sweden from two directions and the two distinct Swedish PUU lineages suggests that the two genetic variants of PUU virus may not have evolved in situ, but rather reflects the origin of two different bank vole populations. Further studies to elucidate the evolutionary origin of the Swedish PUU viruses, e.g. investigation of PUU virus in other parts of Fennoscandia, are needed to substantiate the hypothesis. The recent biogeographical history of Fennoscandia provides a potential model for further studies on virus and host animal co-evolution.

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References


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