SHORT REPORT: SIMULTANEOUS OCCURRENCE OF DOBRAVA, PUUMALA, AND TULA HANTAVIRUSES IN SLOVAKIA

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Abstract. The prevalence of antibody to hantaviruses in Slovakia (serum panel n = 2,133) was lower in the western part (0.54%) and higher in the eastern part (1.91%) of the country and was found to be significantly enhanced in a group of forest workers from eastern Slovakia (5.88%). One-third of the IgM-negative convalescent phase sera from patients with hemorrhagic fever with renal syndrome exhibited antibodies reacting predominantly with Puumula virus antigen, while two-thirds had antibodies directed mainly against Hantaan virus antigen. Fine analysis of two Hantaan virus–reactive sera by a focus reduction neutralization test showed that Dobrava hantavirus was the source of these human infections. Initial results of rodent screening indicated the circulation of Dobrava virus in populations of striped field mice (Apodemus agrarius) in eastern Slovakia.

Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome.1-2 The natural reservoirs of hantaviruses are small rodents, and each of the various virus types is associated primarily with a single host species. Hantavirus serotypes previously reported to be endemic in Europe are Puumala (PUU, carried by the bank vole, Clethrionomys glareolus), Hantaan (HTN, carried by the striped field mouse, Apodemus agrarius), and Seoul (SEO, carried by rats).

Dobrava virus (DOB), recently isolated from tissues of a yellow-necked field mouse (A. flavicollis) trapped in the former Yugoslavia, has been characterized as an unique hantavirus.3,4 This virus is related to the HTN and SEO virus types, with all of them carried by different hosts of the subfamily Murinae.5-7 An association of DOB virus with HFRS types, with all of them carried by different hosts of the subvirus.8 These specific features made Slovakia an interesting area to evaluate the significance of infections by different hantavirus types in the human population, in risk groups, in clinically suspected cases, as well as in the rodent population.

For seroepidemiologic studies, nucleocapsid proteins of PUU (strain Vranica-Hállás13), TUL (strain Malacky12), and HTN (strain Fojnica14, exhibiting only 3 nucleotide differences in the small [S] segment and 6 nucleotide differences in the medium [M] segment [Sibold C and others, unpublished data]) were cloned into the pQE30 vector (Qiagen, Düsseldorf, Germany), expressed as N-terminal, His-tagged proteins in Escherichia coli, purified by Ni-chelate chromatography, and used as solid-phase antigens in ELISAs (Cifire F and others, unpublished data)16 and Western blots.

The specificities of the ELISAs were determined using sera from 250 healthy blood donors (all were negative for antibodies to HTN and PUU viruses by immunofluorescent assays [IFAs] and by Western blots using chromatographically purified HTN and PUU virus nucleocapsid antigens). The specificities were 99%, 98%, and 99% for PUU, TUL, and HTN viruses, respectively. The sensitivities of the ELISAs were determined using sera from 25 patients with HFRS (all were positive for antibodies to PUU or HTN viruses by IFAs/Western blots). The sensitivities were 100% and 100%, respectively. The sensitivity for TUL virus could not be determined because of the absence of clinical cases infected with this virus.

To determine the seroprevalence of antibodies to hantaviruses in the human population, sera from 2,133 residents from western-central Slovakia (n = 1,661) and eastern Slovakia (n = 472) were examined (Table 1). Informed consent

<table>
<thead>
<tr>
<th>Region</th>
<th>Group</th>
<th>No. of sera tested</th>
<th>No. (%) of sera predominantly reactive with N protein</th>
<th>Ratio HTN:PUU</th>
<th>No. (%) of reactive sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HTN-like</td>
<td>PUU-like</td>
<td></td>
</tr>
<tr>
<td>West/Central Slovakia</td>
<td>Average population</td>
<td>1,661</td>
<td>5 (0.30)</td>
<td>4 (0.24)</td>
<td>1.25</td>
</tr>
<tr>
<td>East Slovakia</td>
<td>Average population</td>
<td>472</td>
<td>4 (0.85)</td>
<td>5 (1.06)</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>Average population</td>
<td>2,133</td>
<td>9 (0.42)</td>
<td>9 (0.42)</td>
<td>1.00</td>
</tr>
<tr>
<td>East Slovakia</td>
<td>Forest workers</td>
<td>153</td>
<td>5 (3.27)</td>
<td>4 (2.61)</td>
<td>1.25</td>
</tr>
</tbody>
</table>

* HTN = Hantaan; PUU = Puumala.
was obtained from all serum donors. The study was approved by the Central Ethical Committee of the Ministry of Health of the Slovak Republic. The average seroprevalence was 0.84% but varied between the different geographic regions, ranging from 0.54% (western/central) to 1.91% (eastern). The number of sera reacting with HTN or PUU virus antigens were nearly equal. Forest workers are known as a risk group for acquiring hantavirus infections.15 Indeed, the investigated forest workers from eastern Slovakia showed a significantly higher seroprevalence (5.88%, Table 1) when compared with the general population of the respective area ($\chi^2 = 6.34, P = 0.012$).

Sera from 102 patients with the tentative clinical diagnosis of HFRS (collected between 1988 and 1996) were analyzed for hantavirus-specific antibodies. Twenty-eight (27.5%) of the suspected HFRS cases were found to be positive for antibody to hantavirus (Table 2). Since it has been shown that acute phase or early convalescent phase sera are difficult to type for the causative hantavirus,6 in a second step of the analysis we excluded the IgM-reactive sera. The remaining sera (n = 14) presumably revealed a more reliable picture of the distribution of the various hantavirus types causing disease in Slovakia and showed a predominance of HTN virus antigen reactivity (Table 2).

Despite the distribution of TUL virus in the common vole (Microtus arvalis) throughout Slovakia (Sibold C and others, unpublished data), no current indication for infections of humans with TUL virus in this country could be found. None of the PUU virus–reactive sera tested showed a significantly higher ELISA or focus reduction neutralization test (FRNT) endpoint titer to TUL virus in comparison with PUU virus. We conclude that the human infections detected by PUU virus antigen in the ELISA, Western blots, and IFAs are in fact caused by PUU virus. In the neighboring Czech Republic, one case of human TUL virus infection (a healthy forest worker) has been identified.18

The question arose which hantavirus caused the reactivity of human sera towards the HTN virus antigen. Two HTN virus–reactive sera, one from a healthy forest worker and the other from a convalescent phase patient with HFRS, were further characterized by FRNT. In both cases DOB virus was clearly identified as the causative agent of infection (Table 3).

Between 1994 and 1997, 1,974 small rodents were trapped in Slovakia (western area, n = 1,572; eastern area, n = 402). Among the species identified, A. flavicollis, C. glareolus, and M. arvalis were found in all investigated parts of Slovakia, whereas A. agrarius was identified exclusively in the eastern part of the country. Whereas all the A. flavicollis and C. glareolus studied were hantavirus-negative, 12 M. arvalis and four A. agrarius were found to be hantavirus antibody- and RNA-positive when tested by an ELISA, an IFA and a reverse transcription–polymerase chain reaction. In two A. agrarius (Apa862 and Apa872, Table 4) the nucleotide sequence of a 558-basepair S segment region was identified after amplification using S segment-specific DOB virus primers. The sequence determined showed a high sequence identity of 86% at the nucleotide level (96–98% at the amino acid) to DOB virus strains from the former Yugoslavia4 and Estonia (Plyusnin A, unpublished data), but significantly less sequence identity to the HTN virus76–118 sequence15 (nucleotide = 72%, amino acid = 76–77%). These results demonstrate that in eastern Slovakia A. agrarius rather than A. flavicollis carries DOB virus. Further studies should clarify the nature of human infections leading to sera reacting predominantly with HTN virus antigen as identified in the population of western and central Slovakia (Table 1) where A. agrarius is thought to be absent. We have recently iden-
HANTAVIRUSES IN SLOVAKIA

Comparison of hantavirus small (S) segment sequences (558 base-pairs) amplified from *Apodemus agrarius* (East Slovakia) with known DOB and HTN sequences*

<table>
<thead>
<tr>
<th></th>
<th>Apa682</th>
<th>Apa872</th>
<th>DOB (Yugoslavia)</th>
<th>DOB (Estonia)</th>
<th>HTN76-118</th>
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<tbody>
<tr>
<td>Apa682</td>
<td>99.6</td>
<td>99.6</td>
<td>85.5</td>
<td>86.2</td>
<td>71.8</td>
</tr>
<tr>
<td>Apa872</td>
<td>93.5</td>
<td>97.8</td>
<td>85.5</td>
<td>86.2</td>
<td>71.8</td>
</tr>
<tr>
<td>DOB (Yugoslavia)</td>
<td>97.3</td>
<td>97.8</td>
<td>87.8</td>
<td>69.7</td>
<td></td>
</tr>
<tr>
<td>DOB (Estonia)</td>
<td>95.2</td>
<td>95.7</td>
<td>96.2</td>
<td>71.3</td>
<td></td>
</tr>
<tr>
<td>HTN76-118</td>
<td>76.3</td>
<td>76.3</td>
<td>76.3</td>
<td>75.8</td>
<td>–</td>
</tr>
</tbody>
</table>

* The upper right triangle shows the percentage of nucleotide identity and the lower left triangle shows the percentage of identical amino acid residues. DOB = Dobrava; HTN = Hantaan.

Our data show a geographically varied distribution of at least three hantaviruses in Slovakia. We have demonstrated the circulation of DOB virus in *A. agrarius*, as well as the occurrence of human DOB virus infections in this part of Europe.

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