Saaremaa Hantavirus Should Not Be Confused with Its Dangerous Relative, Dobrava Virus

In a recent paper, Klempa and coauthors described an isolate from a striped field mouse (Apodemus agrarius) captured in Slovenia (4). They concluded that this hantavirus, which they call Dobrava virus (DOBV)-Aa, is responsible for most of the DOBV-caused cases of hemorrhagic fever with renal syndrome (HFRS) in central Europe. While we appreciate this contribution, we also feel that in that paper some opinions on the controversial issue of DOBV and Saaremaa virus (SAAV) have not been represented. We wish to correct these in the following paragraphs.

First, we will discuss nomenclature. The name SAAV, first suggested in 2000 (1), is in line with the old tradition in arbovirus and robovirus (for rodent borne) taxonomy; discoverers of a new virus name it after the geographic area where it was first found. Had we named the newly discovered virus properly at the beginning, i.e., in 1997 (13), there would not be any problem. But the name SAAV was not coined until 1999, when, in a collaborative study, we detected the cocirculation of DOBV and SAAV in Slovenia in association with their respective rodent hosts, A. flavicollis and A. agrarius. Reproductive isolation of these two hantaviruses—one is allowed to use such a term after the virus species concept has been introduced (see, e.g., reference 16)—was a crucial piece of information to draw a demarcation line. These results were published in 2000 (1). By that time, the first SAAV isolate had been reported from Estonia (9) and the virus was also found in Russia (12). In a paper published in the next year (14), SAAV was reported in Slovakia and it was suggested to call it “DOBV-Au” (from A. agrarius). We feel that this name is not correct, and not for priority reasons only; it is also misleading. DOBV is known to be one of the most dangerous European viral pathogens, with a fatality rate of associated HFRS of around 10% (Table 1). In contrast, no fatalities have so far been associated with SAAV, not even in Estonia and Latvia, where human anti-SAAV seroprevalence is about 3% (6, 17). In addition to the paper by Klempa et al. and other studies from the same laboratory (e.g., reference 14), our unpublished results on HFRS patients in Germany, Slovakia, and Estonia confirm the low pathogenicity of SAAV for humans. Clinical data indicate that SAAV infections are much less severe than DOBV infections, perhaps even milder than Puumala virus infections. In line with this, our studies with animal models clearly distinguished SAAV from DOBV; i.e., SAAV infections did not harm mice, while DOBV killed 100% of the animals infected (5). It obviously makes a major difference for patients to be diagnosed with SAAV and not DOBV infection.

In their paper, Klempa et al. stated that the SAAV isolate from Estonia is “apparently a reasortant.” This conclusion is based on the conflicting phylogenies inferred for the S/L and M segment sequences of the SAAV (DOBV-Aa) and DOBV (DOBV-Af) strains. We have discussed this specific issue in detail elsewhere (15). Here we wish to emphasize that there is an alternative point of view (7, 11, 15) which is based on the results of in-depth phylogenetic analyses using not only the Tree-Puzzle method (which Klempa et al. used) but also the classic maximum-likelihood, maximum-parsimony, and distance matrix methods. The important conclusion from these studies is that all SAAV (DOBV-Aa) S sequences are monophyletic; i.e., they share a most recent common ancestor which is different from the most recent common ancestor shared by all DOBV strains (DOBV-Af).

The main reason for difficulties in inferring correct S segment-based phylogeny might be a host switch which probably occurred in the evolution of DOBV and SAAV (8, 11). A likely scenario is that pre-DOBV colonized another host, A. agrarius, establishing pre-SAAV. In the course of evolution, the house-keeping N and L proteins (and the encoding S and L genome RNA segments) of the two viruses have been diverging more slowly than surface glycoproteins G1 and G2 (and the encoding M segment), which are involved in the recognition of a host cell receptor(s). Consequently, M/G1G2 sequences have accumulated more mutations than S/N and Lsegm/Lprot sequences, making phylogenetic reconstructions easier. By the way, the L segment-based phylogeny inferred by Klempa et al. is currently based on a short part of the sequence (541 out of 6,500 nucleotides) and therefore awaits further investigation.

In spite of this controversy, it seems that we may agree that SAAV (DOBV-Aa) and DOBV (DOBV-Af) are distinct entities. We have summarized their differences in Table 1. It is important to make the distinction since the two viruses differ drastically in pathogenicity and circulate in the same geographic regions in central and southeastern Europe.

REFERENCES

TABLE 1. Differences between SAAV and DOBV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SAAV</th>
<th>DOBV</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent carrier</td>
<td>Apodemus agrarius</td>
<td>Apodemus flavicollis</td>
<td>1, 3, 9, 13, 14</td>
</tr>
<tr>
<td>Where first discovered</td>
<td>Estonia (Saaremaa Island)</td>
<td>Slovenia (Dobrava Village)</td>
<td>3, 13</td>
</tr>
<tr>
<td>Other areas where found</td>
<td>European Russia, Slovakia,</td>
<td>Albania, Greece, Serbia,</td>
<td>17 (review)</td>
</tr>
<tr>
<td></td>
<td>Slovenia, Hungary, Germany,</td>
<td>Croatia, Hungary, Slovakia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Denmark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive isolation documented</td>
<td>Slovenia (Prekmurje),</td>
<td>Slovenia (Prekmurje),</td>
<td>1, 14</td>
</tr>
<tr>
<td></td>
<td>Slovakia (Kosice region)</td>
<td>(Kosice region)</td>
<td></td>
</tr>
<tr>
<td>Lethality for humans (%)</td>
<td>None reported</td>
<td>9–12</td>
<td>2, 10</td>
</tr>
<tr>
<td>Lethality for succumbing mice</td>
<td>No</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>Antigenic distinction by rabbit sera</td>
<td>Yes*</td>
<td>Yes*</td>
<td>9</td>
</tr>
<tr>
<td>Antigenic distinction by human sera</td>
<td>Yes*</td>
<td>Yes*</td>
<td>3a</td>
</tr>
</tbody>
</table>

*a A two- to fourfold or greater difference.

*b A greater-than-fourfold difference in the majority of human convalescent-phase sera.

Authors’ Reply

The comment of Plyusnin et al. on our recent paper (10) gives us a welcome opportunity to discuss the current taxonomic situation within the hantavirus species Doebavira-Belgrade virus (4) (usually named Dobrava virus [DOBV] in the scientific literature), as well as the clinical relevance of the representatives of this species. In a reply to a similar letter by the same authors 2 years ago (11) and in various other publications (8–10), we have explained why the Saaremaa virus (SAAV) strain does not meet the criteria of the International Commission on Taxonomy of Viruses (4) to be considered a hantavirus species separate from Doebavira-Belgrade virus (DOBV).

The upper part of Table 1, we have summarized the taxonomically important properties of the different DOBV lineages, including SAAV. Only the different hosts harboring the virus lineages speak for the occurrence of different virus species. However, the very recent discovery of members of DOBV in an additional reservoir host, Apodemus ponticus, demonstrates the complexity of the ecological and phylogenetic situation within the DOBV species (18). Furthermore, all other criteria of species demarcation are not fulfilled when comparing SAAV with the other DOBV lineages (Table 1). For example, species have to exhibit at least a 7% difference in amino acid identity in a comparison of the complete glycoprotein precursor and nucleocapsid protein sequences (4). This is obviously not the case for the different DOBV strains, including SAAV.

Most interestingly, the nucleocapsid protein sequence diversity between Apodemus agrarius-borne DOBV-Aa isolate SK/Aa and the SAAV strain is even higher (3.1%) than the difference between the representatives of DOBV-Aa and Flavivirus-borne DOBV-Af (only 2.4%). In other words, while the data do not confirm a definition of SAAV as a separate species, within the DOBV species the DOBV-Aa lineage and the SAAV lineage can be clearly separated (8, 10; Table 1).

Plyusnin and colleagues correctly state that there are “difficulties in inferring correct S segment-based phylogeny” of the virus lineages. A host-switching event of an ancestral DOBV representative from Flavivirus to A. agrarius, followed by different evolutionary rates for the S and L segments on the one hand and for the M segment on the other hand might be an interesting concept to explain the molecular phylogenetic clustering of the SAAV S and L segments with DOBV-Af rather than DOBV-Aa; however, such a host switch was strongly doubted by others (7). Moreover, this concept cannot explain why only certain strains (those from Saaremaa island in Estonia) behave differently in S, M, and L phylogenies while all other A. agrarius-borne strains behave equally in all three segment-specific phylogenies. Following the host-switching concept, a possible explanation is that these two groups (DOBV-Aa and SAAV) are results of two independent host-switching events. This scenario, however, is not in line with the monophyletic character of all A. agrarius-borne strains in M segment trees. Therefore, we still believe that genetic reassortment is the most plausible explanation for the various S, M, and L segment phylogenies.

Because even extremely modest differences in viral gene sequences can have profound effects upon pathogenesis (for hantaviruses, see references 3 and 6), differences in disease severity associated with different virus lineages are not a useful criterion for classification or taxonomy of viruses. Moreover, we are not aware that infection by the SAAV lineage was conclusively shown in any patient exhibiting hemorrhagic fever with renal syndrome (HFRRS). On the other hand, the clinical importance of DOBV-Aa was undoubtedly demonstrated by detection of a DOBV-Aa sequence in a German HFRRS patient (9). However, these findings should not be exploited as evidence of the putative clinical relevance of SAAV. It is clearly
<table>
<thead>
<tr>
<th>Virus lineage</th>
<th>Representative strain</th>
<th>Host species</th>
<th>Nucleocapsid protein $%$ difference</th>
<th>Glycoprotein precursor $%$ difference</th>
<th>Difference in titer of neutralizing antibody to DOBV-Aa</th>
<th>Genetic reassortment reported</th>
<th>Main geographic distribution</th>
<th>Putative pathogenicity for humans</th>
<th>Cross-reactivity of neutralizing antibodies from human sera to DOBV-Aa</th>
<th>Recovery and characterization of virus RNA from infected patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOBV-Aa</td>
<td>SK/Aa</td>
<td>A. agrarius</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>No</td>
<td>Central Europe</td>
<td>No clinical cases defined</td>
<td>0–40</td>
<td>Yes</td>
</tr>
<tr>
<td>DOBV-Af</td>
<td>Slo/Af</td>
<td>A. flavicollis</td>
<td>2.4</td>
<td>6.6</td>
<td>—</td>
<td>No</td>
<td>Southeastern Europe (Balkans)</td>
<td>Moderate/severe</td>
<td>0–16$m$</td>
<td>Yes</td>
</tr>
<tr>
<td>Saaremaa</td>
<td>Saa/160v</td>
<td>A. agrarius</td>
<td>3.1</td>
<td>4.3</td>
<td>2–4</td>
<td>Yes</td>
<td>Northeastern Europe (Estonia)</td>
<td>No clinical cases defined</td>
<td>0–32$m$</td>
<td>No</td>
</tr>
<tr>
<td>DOBV-Ap</td>
<td>Ap1584/Sochi-01$^{k}$</td>
<td>A. ponticus</td>
<td>2.8</td>
<td>ND</td>
<td>ND</td>
<td>No</td>
<td>Southeastern Europe (Black Sea)</td>
<td>Moderate$^{k}$</td>
<td>0–8$m$</td>
<td>Yes</td>
</tr>
</tbody>
</table>

$^a$ Originally defined in reference 4.

$^b$ Data from references 2, 10, 13, and 18.

$^c$ Data from reference 10 and 18.

$^d$ Data from reference 10.

$^e$ Data from references 13, ND, not done. Values are ranges indicating the fold difference (sera of experimentally infected rabbits).

$^f$ Data from references 8 and 10.

$^g$ Data from references 10 and references therein, as well as reference 18.

$^h$ Data from references 1, 7, 9, 14, and 16.

$^i$ Data from references 5, 10, 12, 15, and 17. Values are ranges indicating the fold difference.

$^j$ Data from references 9, 14, and 18.

$^k$ E. K. Tkachenko, personal communication.

$^l$ No comparison (same virus strain).

$^m$ The human sera have been assigned to different DOBV lineages according to the geographic region of serum sampling. No molecular evidence is available that serum donors were really infected by the respective virus lineage.
the DOBV-Aa, and not the SAAV, lineage which was found in patients in central Europe.

In addition, it has to be stated that all available data supporting the idea of a different pathogenicity of DOBV-Af versus DOBV-Aa are rather limited; no evidence with statistical porting the idea of a different pathogenicity of DOBV-Af versus DOBV-Aa, and not the SAAV, lineage which was found in patients in central Europe.

We cannot follow Plyusnin et al. in their statement that “it obviously makes a major difference for patients to be diagnosed with SAAV and not DOBV infection.” First, no HFRS patients with SAAV infection were unequivocally diagnosed yet (see above). Second, the representatives of the different DOBV lineages, including SAAV, cannot be distinguished by any of the routine serological methods (enzyme-linked immunosorbent assay, indirect fluorescent-antibody assay, immunoblotting) and in a substantial number of cases not even by focus reduction neutralization assays under biosafety level 3 conditions (which can be achieved in only a few laboratories in Europe). Third, since focus reduction neutralization assays are time-consuming and usually conducted with convalescent-phase sera, their outcome would have no diagnostic value for patients during the acute phase of disease. Fourth, virus typing could not influence the patients’ care since there is no hantavirus-specific treatment available so far and the therapy is only symptomatic.

A number of additional molecular epidemiological and seroepidemiological studies of rodents and humans (including patients suffering from hantavirus infection) in Europe are needed to shed more light on the obviously complex situation within the DOBV species and the medical importance of their members. Unfortunately, in recent years it has become more rather than less common to apply new place names to viruses that differ only modestly from known species, a phenomenon that has made it all but impossible for even hantavirus experts to accurately catalogue the plethora of newly proposed hantavirus “species.”

REFERENCES

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