Review

Epidemiology and Epizootiology of Hantavirus Infection in Japan

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SUMMARY: Hantaviruses cause two severe human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Various rodent species act as animal reservoirs for hantavirus. In Japan, urban rat- (Rattus norvegicus) and laboratory rat-derived human infections were reported during the 1960s and 1970S-1984, respectively. Although no human cases of infection have been reported since 1984, infected urban rats have been found throughout Japan, and infected grey red-backed voles (Clethrionomys rufocanus) have been identified in Hokkaido. These carriers can be considered to be potential sources of human infection. This review examines the epidemiology and epizootiology of this important zoonosis in Japan.

Introduction
Hantaviruses are members of the Hantavirus genus of the Bunyaviridae family (1). Hantaviruses are associated with rodents and other small mammals and are the causative agents of hemorrhagic fever with renal syndrome (HFRS) (2) and hantavirus pulmonary syndrome (HPS) (3) in humans. Transmission of the virus to humans occurs either via the inhalation of infectious aerosols generated from hantavirus-containing animal secretions (4-6) or via contaminated saliva in animal bites (7). Thus, HFRS and HPS are regarded as important rodent-borne viral zoonoses (8).

Between 150,000 and 200,000 HFRS cases are reported annually throughout the world (9). The majority of these cases occur in Asia and Europe, particularly in China, where 50,000 to 100,000 cases have been registered annually since 1985 (10). In Japan, there have been two recent outbreaks of HFRS, one in the 1960s and one in the 1970s-1984. The former outbreak was traced to brown rats (Rattus norvegicus) in Osaka City (11,12), and the latter to laboratory rats (13). Although no human HFRS cases have been reported in Japan since 1984, brown rats in several port areas (14-17) and field grey red-backed voles (Clethrionomys rufocanus) in Hokkaido (18,19) have been found to be hantavirus-positive. Therefore, the re-emergence of HFRS as a human infectious disease in Japan remains possible.

HPS was first reported in the Southwestern United States (US) in 1993 as an acute respiratory distress syndrome with greater than 40% mortality (20). The causative virus of HPS is transmitted from Peromyscus maniculatus and several other rodent species, all of which are members of the subfamily Sigmodontinae (21). Since these rodent species inhabit the American continent only, no HPS cases have been reported outside the Americas. However, the introduction of infected rodents into other countries represents a potential public health problem.

In 1998, a new law aimed at controlling infectious diseases was introduced in Japan. Under the new law, HFRS and HPS are graded as group 4 diseases, and afflicted patients must be reported to the government. In spite of these new regulations, information on these diseases is still quite limited.

In this report, we review hantavirus epidemiology and epizootiology in Japan, and emphasize the necessity for caution in dealing with unfamiliar diseases.

Clinical manifestations of HFRS (22,23) and HPS (23,24)

The premonitory symptoms common to both HFRS and HPS, including fever, myalgia, and headache, are seen in more than 90% of infections. However, the later-stage clinical symptoms differ between HFRS and HPS. In HFRS, severe cases usually show four different phases: febrile, hypotensive, oliguric, and diuretic. However, the severity of infection varies, primarily according to the type of infecting virus, but also in
cases involving viruses of the same type. Therefore, disease confirmation is based not only on clinical signs and laboratory findings, but also on the demonstration of specific IgM antibodies by ELISA or a fourfold or greater rise in anti-hantavirus antibody titer in paired sera, as measured by serological testing. Moreover, renal failure, as evidenced by proteinuria, is observed in 100% of cases (Table 1). In addition, mild cases of HFRS occasionally show hepatic dysfunction (25). It is important to differentiate HFRS from other acute infectious diseases such as influenza, tsutsugamushi disease, and leptospirosis. HPS, on the other hand, is characterized by a respiratory syndrome such as tachypnea caused by pulmonary edema and a high mortality rate (30 to 40%) due to pulmonary edema and the rapid onset of shock.

Diagnostic protocols for HFRS and HPS have previously been published (26,27) and released as an information leaflet by the Ministry of Health and Welfare of Japan in 1999 (28).

Viral agents of HFRS and HPS

To date, at least 22 hantaviruses have been identified by based on comparisons of nucleotide-sequence similarity and evolutionary classifications of viral genomes (29). All hantaviruses have specific rodent reservoirs. Phylogenetic

analysis of the rodent-associated hantaviruses has revealed three primary lineages: the Murinae, Arvicolinae, and Sigmodontinae subfamilies (30,31). Since the evolutionary trees of the hantavirus and rodent genomes are superimposable (32), hantavirus-rodent coevolution has been hypothesized. Among the known hantaviruses, the Hantaan, (HTN), Seoul (SEO), Dobrava (DOB), and Puumala (PUU) viruses are known to cause HFRS, and the Sin Nombre (SN), New York (NY), Black Creek Canal (BCC), Bayou (BAY), Lagna Negra (LN), and Andes (AND) viruses cause HPS (22, 29) (Table 2).

Distribution of HFRS and HPS

Due to the close relationship between hantavirus type and rodent species, the distribution of HFRS and HPS cases has been confined to areas inhabited by the specific rodent reservoir (Fig. 1). Since HPS-reservoir rodents live in North and South America, HPS has only been reported in these regions. HFRS cases caused by HTN, PUU, and DOB viruses have been reported primarily in Eastern Asia, Northern and Eastern Europe, and Central Europe, respectively. Since brown rats infected with SEO virus are distributed worldwide, infections with this virus might be expected in many countries. However,

<table>
<thead>
<tr>
<th>Viruses</th>
<th>(Abbreviation)</th>
<th>Principal species</th>
<th>Original isolation in Distribution of reservoir</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murinae subfamily-associated viruses</td>
<td>Hantaan</td>
<td>HTN</td>
<td>Korea</td>
<td>Asia, Europe</td>
</tr>
<tr>
<td></td>
<td>Dobrava-Belgrade</td>
<td>DOB</td>
<td>Apodemus flavicolis</td>
<td>Slovenia</td>
</tr>
<tr>
<td></td>
<td>Seoul</td>
<td>SEO</td>
<td>Rattus norvegicus, R. rattus</td>
<td>Korea</td>
</tr>
<tr>
<td>Arvicolinae subfamily-associated viruses</td>
<td>Puumala</td>
<td>PUU</td>
<td>Clethrionomys glareolus</td>
<td>Finland</td>
</tr>
<tr>
<td>Sigmodontinae subfamily-associated viruses</td>
<td>Sin Nombre</td>
<td>SN</td>
<td>Peromyscus maniculatus</td>
<td>New Mexico</td>
</tr>
<tr>
<td></td>
<td>New York</td>
<td>NY</td>
<td>Peromyscus leucopus</td>
<td>New York</td>
</tr>
<tr>
<td></td>
<td>Black Creek Canal</td>
<td>BCC</td>
<td>Sigmodon hispidus</td>
<td>Florida</td>
</tr>
<tr>
<td></td>
<td>Bayou</td>
<td>BAY</td>
<td>Oryzomys palustris</td>
<td>Louisiana</td>
</tr>
<tr>
<td></td>
<td>Lagna Negra</td>
<td>LN</td>
<td>Calomys laucha</td>
<td>Paraguay</td>
</tr>
<tr>
<td></td>
<td>Andes</td>
<td>AND</td>
<td>Oligoryzomys</td>
<td>Argentina</td>
</tr>
</tbody>
</table>

Table 1. Clinical manifestations of hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS)

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>HFRS</th>
<th>HPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation period (week)</td>
<td>2-3</td>
<td>1-2</td>
</tr>
<tr>
<td>Prodrome</td>
<td>fever, myalgia and headache (common to both HFRS and HPS patients)</td>
<td>cough</td>
</tr>
<tr>
<td>Symptoms</td>
<td>[severe case; caused by HTN, DOB, SEO]</td>
<td>tachypnea, tachycardia, hypotension, pulmonary edema, bilateral interstitial pulmonary infiltrates, shock</td>
</tr>
<tr>
<td></td>
<td>1) febrile phase</td>
<td>2) hypotension phase (hemorrhagic)</td>
</tr>
<tr>
<td></td>
<td>3) oliguria phase (proteinuria)</td>
<td>4) diuretic phase (recovery)</td>
</tr>
<tr>
<td>Target organ</td>
<td>kidney</td>
<td>lung</td>
</tr>
<tr>
<td>Hematology</td>
<td>elevated white-cell count, thrombocytopenia, hemoconcentration (common to both HFRS and HPS)</td>
<td></td>
</tr>
<tr>
<td>Death rate</td>
<td>1-15% (HTN, SEO, DOB)</td>
<td>30-40%</td>
</tr>
<tr>
<td></td>
<td>&lt;1% (PUU)</td>
<td></td>
</tr>
</tbody>
</table>
HFRS cases due to SEO virus have been confined, thus far, to Asian countries.

**Historical background of HFRS in Japan (Table 3)**

Russian and Japanese physicians encountered severe febrile disease among troops in Far Eastern Russia and North Eastern China between 1930 and 1940, respectively. The disease was referred to as "epidemic nephrosonephritis" in Russia and "epidemic hemorrhagic fever" in Japan. These physicians identified the agent of disease as a virus and *Apodemus agrarius* as the rodent reservoir. They also identified the gamasid mite (*Laelaps jettmari*) as an arthropod vector of the hantavirus (33). Recent reports by Chinese researchers have demonstrated hantavirus multiplication in gamasid and chigger mites as well as transovarial and transstadial transmission of the virus (10). However, no reports of hantavirus transmission via arthropods have been forthcoming from other endemic countries.

In Japan, both brown rat- and laboratory rat-associated HFRS cases were reported in the 1960s (11) and 1970s-1984 (13), respectively. SEO-type hantavirus strain SR-11 was first isolated from infected rat-lung tissues in 1982 (34). Subsequently, SEO viruses closely related to strain SR-11 were isolated from both laboratory rats (35) and brown rats in Japan (14, 36). Isolation of the viruses allowed for the establishment of serological diagnostic procedures (37) and facilitated epidemiologic and epizootiologic investigations. Due to extensive surveillance of laboratory rat populations, no laboratory-rat-related HFRS cases have been reported since 1984. However, infected brown rats have been detected throughout Japan (38), and infected grey red-backed voles have been identified in Hokkaido (18,36). SEO and PUU viruses have been isolated from both brown rats and voles, respectively. Therefore, these animals might be considered potential sources of human infection. The results of the epidemiologic and

<table>
<thead>
<tr>
<th>Year</th>
<th>Place</th>
<th>Reservoir rodent</th>
<th>No. patient</th>
<th>No. died</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940th</td>
<td>China (Manchuria)</td>
<td><em>Apodemus agrarius</em></td>
<td>10,000</td>
<td>3,000</td>
</tr>
<tr>
<td>1960th</td>
<td>Umeda (Osaka)</td>
<td><em>Rattus norvegicus</em></td>
<td>119</td>
<td>2</td>
</tr>
<tr>
<td>1970-1984</td>
<td>Laboratory for animal experimentation (21 Institutions throughout Japan)</td>
<td>Laboratory rat (<em>Rattus norvegicus</em>)</td>
<td>126</td>
<td>1</td>
</tr>
<tr>
<td>1984-present</td>
<td>Port arcas (more than 13 port arcas)</td>
<td><em>Rattus norvegicus</em></td>
<td>No patient has been reported</td>
<td></td>
</tr>
<tr>
<td>1992-present</td>
<td>Hokkaido (more than 10 arcas)</td>
<td><em>Clethrionomys rufocanus</em></td>
<td>No patient has been reported</td>
<td></td>
</tr>
</tbody>
</table>
epizootiologic studies are described in the following section.

**Prevalence of anti-hantavirus antibodies in the Japanese population**

The sera of individuals not involved in animal experimentation were examined for hantavirus antibody (Table 4). Thus far, only five positive sera with low antibody titers (1:16, 32 by IFA test) have been identified (39). In contrast, high-risk groups, including workers at dumping grounds inhabited by infected rats (39) and members of the Japanese Self-Defense Forces who trained on lands where infected grey red-backed voles have been detected (40), showed higher antibody titers than the general public. However, no members of these two high-risk groups have shown any of the typical symptoms of HFrs. During a laboratory rat-associated outbreak of HFrs in the 1970s-1984, a total of 126 people were found to be positive for hantavirus antibody. Among these, at least 18 were reported as cases of apparent infection (13). Although only a limited amount of sera was examined, these sero-epidemiologic results indicate that human-pathogenic hantaviruses exist in Japan. A correlation between hepatitis and hantavirus infection has been reported in South Asia (41) and Belgium (42). Antibody-positive cases among hepatitis patients with unknown etiology have also been reported in Japan (43). The anti-hantavirus antibodies were confirmed by several serologic procedures, including IFA, ELISA, Western blotting, and neutralization tests. However, since all of the positive sera were obtained from patients with chronic rather than acute hepatic disease, no virus or viral genomic samples were isolated from the patients. Therefore, further study is needed to elucidate whether hantavirus causes hepatitis in Japan.

**Epizootiologic study of hantavirus infection among rodents in Japan**

Hantavirus-infected brown rats have been reported, primarily in the port areas of Otaru, Kamiiso, Tokyo, Yokohama, Nagoya, Kobe, Tokuyama, Moji, Hakata, Nagasaki, Niigata, Osaka, and Shimizu, and at Kasaai Airport (38). These results suggest that brown-rat-associated SEO virus can be readily distributed worldwide through transportation systems. In Nagoya City, SEO-virus-infected rats were captured in a residential area adjoining a pier in the port (44), indicating that the brown-rat-borne SEO virus is a possible source of human HFrs. Analysis of the nucleotide sequences of the entire coding region of the enveloped glycoprotein of SEO viruses isolated from brown rats from China (strain R22), Seoul (strain R22), Osaka (strain B-1), Sapporo (strain SR-11), and Kamiiso-Hokkaido (strains K-83, 85, and 88) indicated a close relationship between geographic origin and strain phylogeny (45). However, nucleotide-sequence identities among the strains were more than 93%. In addition, phylogenetic analysis based on the partial sequences of 23 SEO viruses from China, Korea, Japan, and the US revealed a single cluster (46) (Fig. 2). Therefore, the relatively low genetic variability might be explained by a recent expansion, via ships arriving at various countries, of infected rat populations from a common source.

The results obtained in Japan for rodents other than brown rats are summarized in Table 5. These results were collected from published papers (18), abstracts (47), and unpublished data. The majority of seropositives was obtained from grey red-backed voles (C. rufocanus) in Hokkaido. Antibody-positive animals were captured at 10 different points around Hokkaido, indicating that hantaviruses are widely distributed among C. rufocanus in Hokkaido. Although attempts at virus isolation from C. rufocanus were unsuccessful, viral sequences amplified from seropositive rodent lung tissues, designated as Tobetsu and Kamiiso, indicated PUU virus infection (18). Since all the PUU viruses identified thus far are harbored by either Clethrionomys spp. or Eothenomys regulus (formerly classified as Clethrionomys regulus) (48), a close relationship between hantavirus type and rodent reservoir was confirmed. Phylogenetic analysis indicated that the Japanese PUU viruses form a lineage distinct from that of the European PUU viruses. Therefore, it is possible that the PUU virus was introduced by infected Clethrionomys spp. brought from the Eurasian continent through Sakhalin during glacial era when Hokkaido was connected with the continent. Two seropositives were detected; one from an Apodemus speciosus in Okushiri Island, Hokkaido, and the other from an A. speciosus in Hirotsuka City in Aomori Prefecture (unpublished observations). However, since no information relating to the virus or viral genomic sequences is available, further study is necessary to confirm the source of infection.

Phylogenetic analysis based on 300 base pairs of a nucleotide sequence from the M genome segments of 38 HTN viruses (34 from China, 4 from Korea) and 24 SEO viruses (19 from China, 2 from Japan, 2 from US, 1 from Korea) isolated from

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**Table 4: Seroepidemiologic study of hantavirus infection among human**

<table>
<thead>
<tr>
<th>Prefecture</th>
<th>Year of collection</th>
<th>Number of sera</th>
<th>Number of positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General public</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tokyo</td>
<td>1983</td>
<td>530</td>
<td>5 (0.94)</td>
</tr>
<tr>
<td>Akita, Iwate, Miyagi, Niigata, Gunma, Nagano, Sataita, Fukuoka</td>
<td>1991</td>
<td>550</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Hokkaido</td>
<td>1993</td>
<td>1,000</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2,080</td>
<td>5 (0.24)</td>
</tr>
<tr>
<td>High risk group (Positive group)</td>
<td>Workers at dumping ground in Tokyo Bay</td>
<td>1983</td>
<td>732</td>
</tr>
<tr>
<td>Members of Japanese Self-Defence Forces (Hokkaido)</td>
<td>1999</td>
<td>207</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Hepatitis patient with unknown etiology (Tokyo)</td>
<td>1990th</td>
<td>105</td>
<td>3 (2.9)</td>
</tr>
<tr>
<td>(Hokkaido)</td>
<td></td>
<td>1990th</td>
<td>60 (1.7)</td>
</tr>
<tr>
<td>Total</td>
<td>1,104</td>
<td></td>
<td>39 (3.5)</td>
</tr>
</tbody>
</table>
Table 5. Seroepizootiologic study of hantavirus infection among rodents in Japan

<table>
<thead>
<tr>
<th>Prefecture*</th>
<th>Rodent species</th>
<th>Number of sera examined</th>
<th>(Number of area surveyed)</th>
<th>Number of positive sera</th>
<th>(Number of area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hokkaido</td>
<td><em>Clethrionomys rafocanis</em></td>
<td>385</td>
<td>(13)</td>
<td>36</td>
<td>(10)</td>
</tr>
<tr>
<td>1985-2000</td>
<td><em>Clethrionomys rutilus</em></td>
<td>5</td>
<td>(2)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td><em>Apodemus speciosus</em></td>
<td>274</td>
<td>(13)</td>
<td>4</td>
<td>(2)</td>
</tr>
<tr>
<td></td>
<td><em>Apodemus argentus</em></td>
<td>223</td>
<td>(12)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td><em>Sorex sp.</em></td>
<td>48</td>
<td>(6)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>935</td>
<td></td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Rest of the</td>
<td><em>Eothenomys smithii</em></td>
<td>35</td>
<td>(4)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>parts of Japan</td>
<td><em>Eothenomys andersoni</em></td>
<td>4</td>
<td>(1)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>1988-2000</td>
<td><em>Apodemus speciosus</em></td>
<td>163</td>
<td>(8)</td>
<td>1</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td><em>Apodemus argentus</em></td>
<td>102</td>
<td>(7)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td><em>Urotrophus talpoides</em></td>
<td>7</td>
<td>(2)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td><em>Crocidura daurica</em></td>
<td>3</td>
<td>(2)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td><em>Microtus montemerianus</em></td>
<td>43</td>
<td>(1)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td><em>Suncus murinus</em></td>
<td>4</td>
<td>(1)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>361</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Phylogenetic trees for hantaviruses based on partial sequences of (a) the M (nucleotides 2001-2301) and (b) S (nucleotides 1211-terminus) segments, excluding the primer sequences. The numbers at the nodes are bootstrap confidence levels for 100 replicates. The numbers on the right side of the figure indicate the subgroup numbers of HTN and SEO viruses. The reference viruses (Sin Nombre, Thailand, and Dobrava/Belgrade) are indicated in boldface type. (ref. 46)

either humans or rodents indicates the existence of 9 HTN and 5 SEO genetic subtypes (46) (Fig. 2). Interestingly, one HTN virus isolated from *Niviventer confucianus* (strain NC167) captured in the Anhui Province of China, and one SEO virus isolated from *Rattus rattus* (strain Gou3) captured in the Zhejiang Province of China showed the highest nucleotide divergence among all the HTN or SEO viruses examined. Moreover, examples of *Microtus fortis* captured in Khabarovsk (49) and Vladivostok (19) bore hantaviruses distinguishable from the PUU virus (Fig. 3). In Korea, Mju virus was identified from *Eothenomys regulus* (47). Phylogenetic analysis of full-length S genome segments suggested that Mju virus was evolutionarily distinct from other *Clethrionomys-* and *Microtus-* borne hantaviruses (48). These results indicate that a variety of rodents carrying different hantaviruses exist in Far-East Asia (Fig. 4).
Fig. 3. Phylogenetic analyses of hantaviruses. Hantavirus sequences were analyzed by neighbor-joining methods using Phylip. Bootstrap confidence limits were calculated from 100 replicates and are shown in parentheses. (A) Phylogenetic dendrogram of the S genome sequences from nucleotides 1 to 1251 in the PUU sequence. (B) Phylogenetic dendrogram of the M genome sequences from nucleotides 2673 to 3652 in the PUU sequence.

Fig. 4. Distribution of HFRS and hantaviruses in Japan and Far-East Asia.
Conclusion

Although no recent cases of HFRS have been reported in Japan, infected brown rats are distributed throughout Japan. In addition, grey red-backed voles in Hokkaido are heavily infected with PUU virus. There may therefore be many cases of undiagnosed HFRS in Japan.

HFRS is still prevalent in Far-East Asia, as are the several rodent varieties carrying different hantaviruses (Fig. 4). Therefore, steps must be taken to prevent the introduction of infected rodents via increased international transport. In addition, the epizootiologic investigations among wild rodents, particularly in Honshu, Shikoku and Kyushu, have been quite limited. A program of serological surveillance should therefore be carried out among wild rodents throughout the country.

In addition to HFRS and HPS, various zoonotic diseases have been registered as category 4 diseases, i.e., disease cases that must be reported to the government by a physician in accordance with the new law on infectious disease control. Nevertheless, since many category 4 diseases are nonexistent or occur rarely in Japan, many physicians are unfamiliar with them. As such zoonotic disease information, including geographical distribution of enzootic foci and reservoir animals, may assist physicians and personnel in related fields in diagnosis and control of these unfamiliar infections.

REFERENCES


