HANTAVIRUS INFECTION AMONG RATTUS NORVEGICUS IN JAPAN

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SUMMARY: Seroepizootiological study of hantavirus infection among 393 urban rats (Rattus norvegicus) captured in six regions in Japan during the period from 1990 to 1994 was carried out by the indirect fluorescent antibody (IFA) test and Western blot (WB). Fifteen out of 393 (3.8%) rat sera were antibody-positive by IFA, i.e., Tokyo Port (12.8%, 6/47), Shimizu Port (5.7%, 2/35), Otaru Port (1.5%, 1/65) and Nagoya City (3.6%, 6/167). In two other regions, i.e., Kasai Seaside Park and Haneda Airport, rat sera were antibody-negative. One serum with a lower IFA titer, 1:64, from Otaru Port was confirmed to be antibody-positive by WB, while two sera from Shimizu Port (IFA titer, 1:32 and 1:64) were not. In Nagoya City, one out of four sera (IFA titer, 1:32) and one of two sera...
Hemorrhagic fever with renal syndrome (HFRS) viruses are widely distributed among various rodent species in Eurasia, Japan, the Americas and Africa. HFRS is a zoonosis transmitted to man by rodents. Urban rats have been shown to carry hantavirus and infection to man is caused by the rodent in China (1) and Korea (2). In Japan, occurrence of HFRS among inhabitants of Osaka City in 1960's was demonstrated retrospectively by the indirect fluorescent antibody (IFA) test with Hantaan virus (3). Furthermore, after 1975 HFRS virus infection among laboratory workers caused by infected laboratory rats were reported. In 1982, the causative virus, SR-11 strain, of which the antigen was closely related to Hantaan virus, was successfully isolated from the infected rat lung tissue in Vero E-6 cell (4). Prevalence of anti-hantavirus antibody in wild rats captured in various ports in the world was reported (5,6). Thus, HFRS is regarded as one of the imported infectious diseases. The prevalence of antibody in rats in Tokyo Port in 1960's (7) and 1983 to 1990 (8), in Kobe Port and Yokohama Port in 1978 and 1979 (9) and in Shimizu Port in 1981 and 1982 (10) were reported. Hantaviruses were also isolated from rats captured in a reclaimed land in Tokyo Bay and Tokyo Port (11,12). Since 1985, however, no incidence of laboratory-type or urban-type HFRS has been reported in Japan. Continuous serosurveillance of wild rats for hantavirus infection in five regions and first examination in Otaru Port were conducted during the period from 1990 to 1994.

Rats were captured in Tomahawk-type traps. The captured rats were anesthetized with chloroform or ether and bled by cardiac puncture. Altogether 393 sera were obtained from 1990 to 1994, i.e., 75 in Kasai Seaside Park, 47 in Tokyo Port, four in Haneda Airport, 35 in Shimizu Port, 65 in Otaru Port and 167 in Nagoya City. Hantavirus, SR-11 strain, which had been isolated from the lung of an infected Wistar rat associated with an outbreak of HFRS at the Animal Experimental Laboratory of Sapporo Medical College, was used. Vero E-6 cells were cultivated in Dulbecco's MEM supplemented with 7 or 2% fetal calf serum (FCS). The IFA test was carried out by the method described elsewhere (4). Briefly, Vero E-6 cells infected with SR-11 strain were spotted on slide glasses. The cells were dried and fixed with cold acetone for 10 min, then, the antigen slides were stored at $-70 \, ^\circ\mathrm{C}$ until used. Fluorescein isothiocyanate conjugates of (IFA titer, 1:64) were also confirmed to be antibody-positive by WB. Continuous hantavirus infection among rats in Tokyo Port, Shimizu Port and Nagoya City and the existence of hantavirus among rats in Otaru Port were demonstrated.
antibodies to rat IgG (Cappel, Cochranville, PA) were used. The sera with titers of 1:32 or higher were regarded as antibody positive. Western blot (WB) was carried out with the antigen prepared with recombinant baculovirus expressing Hantaan N protein (13). S. f.-9 cells inoculated with recombinant virus showed cytopathic effects (CPE) in 2 or 3 days of infection. When CPE were observed, the cells were harvested, disrupted with lysis buffer (2% SDS, 60 mM Tris-HCl, pH 6.8, 5% 2-ME, 10% glycerol) and boiled for 5 min. Then, the antigen was stored at −30 C until used. Five microliters of antigen was loaded in each well and then electrophoresed in a 9% SDS-polyacrylamide gel. Subsequently, the proteins were electrophoretically transferred to Immobilon membrane (Millipore, Bedford, MA) and then cut to strips. Ten microliters of each serum was diluted with 1,000 μl of 5% skim milk in PBS (1:100), followed by overnight incubation at room temperature. The strips were washed three times each with 2 ml of PBS. Peroxidase-labeled goat anti-rat IgG (H+L) (ZYMED, San Francisco, CA) was used as the second antibody in dilution of 1:1,500. Each strip was dipped in 2 ml of peroxidase conjugate solution, which was incubated for an hour at room temperature, and then washed three times with PBS. Finally, 0.05% of diaminobenzidine (DAB) and H2O2 (5 μl of a 31% solution/10 ml of PBS) were added. A serum of a rat immunized against SR-11 and two rat sera collected in Nagoya Port were used as positive control and normal Wistar rat serum was also used as negative control. SDS-7B (Sigma, St. Louis, MO) was used as a molecular weight marker.

The results of antibody detection by IFA in rats sera from six regions are shown in Table I. Fifteen (3.8%) of 393 rat sera were antibody-positive by IFA, i.e., Tokyo Port (12.8%, 6/47), Shimizu Port (5.7%, 2/35), Otaru Port (1.5%, 1/65) and Nagoya City (3.6%, 6/167); however, in other two regions, i.e., Kasai Seaside Park and Haneda Airport, no antibody-positive case was shown. With 15 IFA-positive sera, the following examinations by WB were proceeded. As shown in Fig. 1, positive bands were constantly detected at the position of 50 kDa when sera with IFA titers above 1:128 were applied. Six sera with IFA titers higher than 1:512 collected in Tokyo Port were all WB positive. Four of six sera are shown in lanes Nos. 7 to 10 in Fig. 1. On the other hand, in the serum from Shimizu Port (IFA, 1:32), no positive band was shown (lane No. 5). Of two sera with IFA titers of 1:64, the one from Otaru Port was WB positive (lane No. 11), but the other from Shimizu Port was negative (lane No. 6). In Nagoya City, six sera were IFA positive, i.e., four had titers of 1:32 and the other two of 1:64 (Table I). With one serum collected in 1993 (lane No. 13) of four sera (IFA titer, 1:32)
Table I. Detection of antibody in *Rattus norvegicus* by IFA in six regions in Japan

<table>
<thead>
<tr>
<th>Capture place</th>
<th>Capture date</th>
<th>No. positive /No. tested</th>
<th>(%)</th>
<th>IFA titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kasai Seaside Park</td>
<td>1990 Oct</td>
<td>0/29</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1991 May-Nov</td>
<td>0/46</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>Tokyo Port</td>
<td>1991 Apr, Sep</td>
<td>6/14</td>
<td>(42.9)</td>
<td>512*-4096*</td>
</tr>
<tr>
<td></td>
<td>1992 Jan, Sep</td>
<td>0/3</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1993 Apr-Nov</td>
<td>0/18</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1994 Feb-Jun</td>
<td>0/12</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>Haneda Airport</td>
<td>1993 Nov</td>
<td>0/2</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1994 Jan, May</td>
<td>0/2</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>Shimizu Port</td>
<td>1992 Nov, Dec</td>
<td>1/2</td>
<td>(50)</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>1993 Jan-Sep</td>
<td>1/33</td>
<td>(3.0)</td>
<td>64</td>
</tr>
<tr>
<td>Otaru Port</td>
<td>1992 Nov, Dec</td>
<td>1/39</td>
<td>(2.6)</td>
<td>64*</td>
</tr>
<tr>
<td></td>
<td>1993 Jan-Nov</td>
<td>0/19</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1994 Jan-Jun</td>
<td>0/7</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>Nagoya City</td>
<td>1993 Nov, Dec</td>
<td>4/97</td>
<td>(4.1)</td>
<td>32*,32,64*,64</td>
</tr>
<tr>
<td></td>
<td>1994 Jan-Mar</td>
<td>2/70</td>
<td>(2.9)</td>
<td>32, 32</td>
</tr>
</tbody>
</table>

**Total** 15/393 (3.8)

Fifteen sera of IFA positive were examined by WB. *=WB positive. In Tokyo Port, all six sera were WB positive and four of six sera are shown in lanes Nos. 7 to 10 in Fig. 1. Sera from Shimizu (lanes Nos. 5,6), Otaru (lane No. 11) and Nagoya City (lanes Nos. 12,13; both sera WB positive) are also shown in Fig. 1.

and another collected also in 1993 (lane No. 12) of two sera (IFA titers, 1:64), positive bands were detected but the other four were negative.

We reported that antibody-positive rats were found in Tokyo Port, from 1983 to 1986, while no positive case from 1987 to 1990 (8). In the present study, antibody-positives were shown again in rats captured in 1991 at No. 13 reclaimed land (R.L.), where antibody-positive rats had been found and furthermore hantaviruses were isolated (8). From 1992 to 1994, no antibody-positive rats were
Fig. 1. Detection of hantavirus antibody in rat sera by the Western blot. The wild rat sera shown in Table I (Shimizu Port, Tokyo Port, Otaru Port and Nagoya City) were examined (No. 5 to No. 13). Two rat sera collected in Nagoya Port (No. 3 and No. 4) were used as positive control. Antigen was prepared from S.f. cells infected with recombinant baculovirus expressing Hantaan N protein. Lanes Nos. 5 and 6 sera are WB negative, while lanes Nos. 7 to 13 are WB positive.

No. 1: positive control.
(immune rat serum against hantavirus SR-11 strain, IFA titer 1:8192).
No. 2: negative control (normal Wistar rat serum, IFA titer <1:16).
No. 3: positive control (rat serum from Nagoya Port, IFA titer 1:256).
No. 4: positive control (rat serum from Nagoya Port, IFA titer 1:128).
No. 5: rat serum from Shimizu Port, IFA titer 1:32.
No. 6: rat serum from Shimizu Port, IFA titer 1:64.
No. 7: rat serum from Tokyo Port, IFA titer 1:2048.
No. 8: rat serum from Tokyo Port, IFA titer 1:4096.
No. 9: rat serum from Tokyo Port, IFA titer 1:512.
No. 10: rat serum from Tokyo Port, IFA titer 1:2048.
No. 11: rat serum from Otaru Port, IFA titer 1:64.
No. 12: rat serum from Nagoya City, IFA titer 1:64.
No. 13: rat serum from Nagoya City, IFA titer 1:32.

found; during these years only two rats were obtained from No. 13 R.L. In Shimizu Port, antibody-positive rats were found in 1981 and 1982 (10). Although
two sera showed low IFA titers, they were antibody-negative in WB. In Nagoya City, antibody-positive rats were found in 1988, 1990, 1991 and 1992 (14). In 1993 and 1994, six rats with low IFA titers, i.e., 1:32 or 1:64 were found. Furthermore two of six sera were demonstrated to be antibody-positive by WB. It seems that hantavirus may still be persisting among rats in these regions.

Although antibody-positive rats were found, in Kasai Seaside Park in 1989 and 1990 (8) and in Haneda Airport in 1983, no antibody-positive case was shown in the present study.

Though the sera which showed specific granular staining in the infected cell cytoplasm in dilutions higher than 1:32 are regarded as IFA positive, in sera of low IFA titers, it seems to be quite difficult to exclude completely false positive cases. Therefore, in the determination of hantavirus infection in rat populations or colonies with only low IFA titers, i.e., 1:32 or 1:64, additional tests like WB or others seem necessary. In the present study, there was the discrepancy between IFA and WB results in several sera. In the examination with rat immune serum, the sensitivity of WB was twofold higher than IFA. Further, 20 wild rat sera with IFA titers lower than 1:16 were WB negative. Although the specificity and sensitivity of WB used in this study were not assessed completely, the results of screening in 1:100 dilution by WB seems to be reliable. One serum specimen from Otaru Port was serologically confirmed as positive by both IFA and WB, although its IFA titer was low (1:64). Thus, in Hokkaido, in addition to Kami-iso (15), hantavirus may be prevalent among rats in Otaru Port. Further study for serological confirmation of low titer sera is needed.

In the present study, continuous prevalence of hantavirus among rats in three regions was shown and furthermore the existence of hantavirus in Otaru Port was demonstrated for the first time. Hantavirus infection is still one of the public health problems. Continuous survey of the rat or other rodents and man for hantavirus is needed to clarify the ecology of hantavirus in Japan.

REFERENCES


