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EPIDEMIOLOGICAL STUDY OF HEMORRHAGIC FEVER WITH RENAL SYNDROME RELATED VIRUS INFECTION AMONG URBAN RATS IN TWO ISLANDS IN TOKYO BAY, JAPAN

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Summary. — A seroepidemiological survey of hemorrhagic fever with renal syndrome (HFRS) virus infection in 403 urban rats (*Rattus norvegicus*) captured on two different islands in Tokyo Bay was carried out using the indirect immunofluorescent antibody (IFA) test with the SR-11 strain of HFRS virus. Antibody positive sera were detected in 99 out of 355 (27.9%) rats from the island with reclaimed ground area, but none were detected in 48 rats from the other islands. IFA titers of the positive sera varied from 16 to 4,096 (mostly 128) with a peak at 1 : 128, and antibody positive ratios were significantly higher in the small rats (< 50 g, probably of younger age) than in the large ones. A causative agent antigenically related to HFRS virus was isolated from lung tissue of seropositive rat using Vero-E6 cells. These results revealed the existence of HFRS related virus among urban rats in the coast of Tokyo Bay, Japan.

Key words: hemorrhagic fever; seroepidemiology; immunofluorescence; Bunyavirus

Introduction

HFRS is a predominantly rural disease endemic in Euro-Asia, which is transmitted to humans by wild rodents (Gajdusek, 1962). It has been found that various species of wild rodents play a role as a reservoir of HFRS virus in different countries (Lee *et al.*, 1978; Brummer-Korvenkontio *et al.*, 1980; Gavrilovskaya *et al.*, 1983). In addition to wild rodents, urban rats (*Rattus norvegicus* and *Rattus rattus*) have been shown to carry HFRS virus and to cause human infection in China and Korea (Harng *et al.*, 1982; Lee *et al.*, 1982). In the United States, urban rats also have been found to contain fluorescent antibody to Hantaan virus, which is the causative agent of

Korean hemorrhagic fever (KHF) (Lee *et al.*, 1978; Tsai *et al.*, 1982; LeDuc *et al.*, 1982). In Japan, it was retrospectively demonstrated by a serologic examination with Hantaan virus that HFRS occurred among dwellers of Osaka in the 1960's (Lee *et al.*, 1979). Although an urban rat was suspected as a possible reservoir, no epidemiological survey was carried out at that time.

Since 1978, however, outbreaks of HFRS virus infection among laboratory workers caused by infected laboratory rats have been reported (Umenai *et al.*, 1979; Hayashi *et al.*, 1982). In 1982, we succeeded in isolating the causative virus, which was antigenically closely related to Hantaan virus, from infected rat lung tissue using Vero-E6 cells (Kitamura *et al.*, 1983). This finding made it possible to conduct HFRS survey in suspected areas of its occurrence in Japan. However, very little is known of the epidemiological conditions existing among rats.

We have, therefore, carried out a serological survey among urban rats to elucidate the existence of HFRS virus infection. The investigation was carried out on two different islands in areas where a large rat colony has been maintained for a long time.

Materials and Methods

Collection of rodents sera. Surveys were carried out on two islands, a reclaimed ground area and an abandoned island in Tokyo Bay. The geographical location of these islands are shown in Fig. 1. The reclaimed ground (A), 1 kilometer in width and 3 kilometers in length, was made with rubbish from city of Tokyo. A total of 355 *Rattus norvegicus* were captured in the area by using a live trap or an adhesive mat (Chu-Clean, Ikari Ltd., Japan) in November 13—16 and December 4—5 in 1982. The rats were divided into three groups according to their body mass (large: over 150 g; medium: 50 to 150 g; small: less than 50 g). Blood samples were collected by cardiac puncture under ether anesthesia. The abandoned island (B, 0.15 kilometers in width and 0.4 kilometers in length) was constructed as the fortress about 100 years before. It was destroyed by the big earthquake in 1923 and has been abandoned. In this island, a total of 48 serum specimens were obtained from *Rattus norvegicus* in July 1—4 in 1983.

Virus. HFRS virus, SR-11 strain, which was isolated from the lung of an infected Wistar rat obtained in an outbreak at the Animal Experiment Laboratory of Sapporo Medical College, Sapporo, Japan, was used in this experiment (Hayashi *et al.*, 1982; Kitamura *et al.*, 1983). This strain was antigenically indistinguishable from Hantaan virus by the cross IFA test. The contamination with reovirus in the SR-11 strain stock was denied by the IFA test with specific immune sera to reovirus types I, II and III (Kitamura *et al.*, 1983).

IFA technique. Spot slides of Vero-E6 cells infected with SR-11 strain after the 7th passage were used as antigen (Kitamura *et al.*, 1983). Two-fold serial dilutions of rat sera were mounted on the cells for 30 min at 37 °C, then washed with 0.01 mol/l phosphate buffered saline (PBS) for 5 min and run three times on a vibrator. Anti-rat IgG (Goat, Cappel Laboratories) was labelled with fluorescein isothiocyanate (FITC) by the method of Kawamura (1977). Then eight staining units of FITC-conjugate were mounted. Incubation and washing were performed as described above. IFA titers were expressed as the reciprocals of the highest dilution giving specific granular fluorescence in the cytoplasm. Serum specimens which showed an IFA titer higher than 16 were regarded as positive.

Detection of viral antigen. The lung, spleen and kidney were removed and fixed in 2% paraformaldehyde dissolved in PBS containing 0.075 mol/l of lysine and 0.01 mol/l of sodium meta periodate (PLP fixative, pH 6.2) for 4 days at 4 °C. Detection of viral antigen was performed after trypsin treatment of the PLP-fixed and paraffin-embedded sections according to the method of Hondo *et al.* (1982). Viral antigen was detected by the IFA technique with human convalescent phase serum, which was taken 21 days after the onset of fever and considered to represent pri-

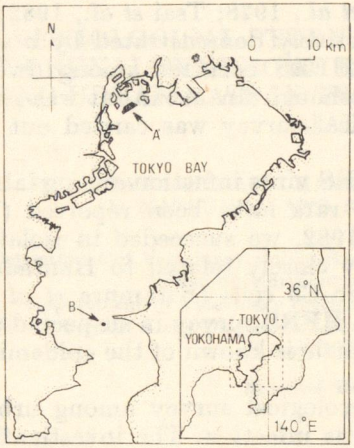

Fig. 1.
The geographical location of two islands where surveys were carried out
Arrow indicates the reclaimed ground area (A), and the abandoned island (B).

mary antibody. The sera with IFA titer of 4 096 to the SR-11 strain were used at 1 : 20 dilution. Anti-human IgG (Goat: Cappel Laboratories) was conjugated as mentioned above and used as a second antibody.

Isolation of the causative agent. Lung tissue was homogenized as 10% suspension (w/vol) in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum, kanamycin (60 μg/ml) and amphotericin B (2 μg/ml). The suspension was maintained for 1 hr at 4 °C without centrifugation, then the supernatant was mixed with the Vero cell suspension in the above medium and incubated at 37 °C. At three-week intervals the monolayer cells were passaged by the ordinary method. At that time, part of the cells were dropped on a slide glass for examination of viral antigen by the IFA technique as described above.

Results

The results of the examination of antibody titers to HFRS virus are shown in Table 1. In the reclaimed ground area, positive sera were obtained from nine different sites with positive ratios ranging from 15.4% to 42.9%. In addition, IFA titers of the rat sera formed a normal distribution pattern varying from 16 to 4 096 with most frequently occurring titer of 128 (Fig. 2). The seroepidemiological study revealed that an agent antigenically related to HFRS virus existed among rats on the island having reclaimed ground area. On the other hand, no positive serum was obtained from rats on the abandoned island.

A total of 197 sera collected in the first series of surveys in the reclaimed ground area, (November 13—16, 1982) were divided into three groups according to the rat body masses as described (Table 2). Positive ratios of large, medium and small rats were 18.9% (17/90), 26.6% (21/79) and 50.0% (14/28), respectively. The overall positive ratio was significantly higher in the small rat group than in the large and medium rat group ($p < 0.01$, chi-square test). The geometric mean titers of positive sera were higher in the large rats' group (401) than in the medium and small rat group (141).

Table 1. Detection of IFA to HFRS virus SR-11 strain, in sera from rats inhabiting a reclaimed ground area and an abandoned island of Tokyo Bay

Reclaimed ground area site	No. tested	*No. positive	(%)
1	14	6	(42.9)
2	37	8	(21.6)
3	16	4	(25.0)
4	81	26	(32.1)
5	26	7	(26.9)
6	65	10	(15.4)
7	44	15	(34.1)
8	61	20	(32.8)
9	11	3	(27.3)
Total	355	99	(27.9)
Abandoned island	48	0	(0.0)

* Reciprocal IFA titer $\geqslant 16$

It was noted that the positive ratios did not increase with the aging of the rats.

Detection of viral antigen in the lung, spleen and kidney was carried out in 11 seropositive rats by IFA procedures as described. HFRS antigen showing granular fluorescence was detected in the cytoplasm of alveolar cells and in the interstitial tissue in the lung of one rat, which possessed the highest serum FA titer (2 048).

Attempts were made to isolate the causative virus from lung tissue of four of these seropositive (IFA titers from 256 to 2 048) rats. After three successive passages of one specimen (with IFA titer of 1 : 256), specific fluorescence was seen in a half of the cultured cells by IFA technique using the immune rat

Table 2. Positive ratios and geometric mean titers to HFRS virus in sera from three groups of rats collected in December 1982

Group*	No. tested	No. positive	Ratio (%)	GMT
large	90	17	(18.9)	401
medium	79	21	(26.6)	141
small	28	14	(50.0)	141
Total	197	52	(26.4)	199

* Rats were divided according to their body mass (< 50 g; 50—150 g; > 150 g)
GMT = geometric mean tifer

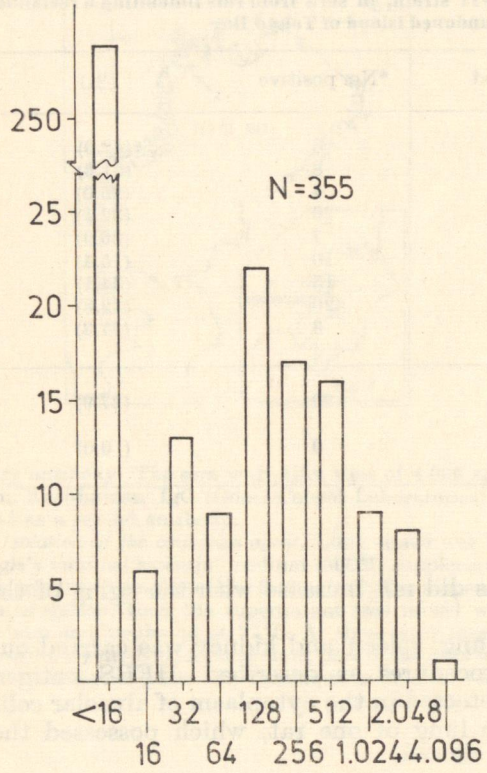

Fig. 2.
Distribution of IFA to HFRS virus SR-11 strain in the rat sera
Titers were expressed as the reciprocals of the highest serum dilution giving specific immunofluorescence in the cytoplasm of infected Vero cells.
Abscissa: serum dilution reciprocals; ordinate: number of serum specimens.

serum to SR-11 strain and human convalescent phase serum. Subsequent passage of this agent led to the 100% infection in the cultured cells. No contamination with reovirus was determined. Antigenicity of the newly isolated virus, named Tokyo Bay-314 strain (TB-314), was compared to SR-11 strain by the IFA test by the use of the immune rat serum to SR-11 strain and the positive serum obtained in this survey. Essentially, the same IFA titers were obtained with both antigens. These results revealed the antigenic relationships between the two strains.

Discussion

Antibodies to HFRS virus were recognized in about 30% of the rats inhabiting an island in Tokyo Bay. Viral antigen was detected in the seropositive rat lung with human convalescent phase serum. A causative virus which was antigenically related to HFRS virus was also isolated from the lung tissue. From these epidemiological surveys follows that an endemic

focus of HFRS virus was maintained in an urban rat colony in Japan. As reported by Morita *et al.* (1983), seropositive cases of HFRS virus were discovered among rat sera collected in 1978 and 1979 at Kobe and Yokohama port areas. However, specific contaminated regions were not observed because the positive ratio was very low (1.9%) and the positive cases were scattered in different places. Moreover, no attempts at virus isolation were carried out.

In the United States, seropositive rats to Hantaan virus were recognized in New Orleans and Houston ports in 1982. It was suspected that these endemic foci were due to the introduction of infected rats from abroad by international shipping, because of the focal distribution of seropositive rats (Tsai *et al.*, 1982; LeDuc *et al.*, 1982). In Tokyo, an endemic focus was also recognized in a reclaimed island near to the port area. Moreover, a non-endemic island was discovered in the same bay. These regional distribution within a bay may also suggest the same introduction route as suspected in the United States. However, an urban type epidemic of HFRS was already reported in Osaka, Japan, in the 1960's (Lee *et al.*, 1979). Moreover, since 1978, outbreaks of HFRS virus infection among laboratory workers caused by infected laboratory rats have been reported (Umenai *et al.*, 1979; Hayashi *et al.*, 1982). It is, therefore, necessary to conduct surveys also among rats in other than port areas to determine whether the HFRS virus infection exists in areas outside this island.

An unexpected finding was that positive sera to SR-11 strain were observed among small rats rather than large ones, and that the positive ratios did not increase with the age of the rats, although the animals lived in an area where the virus was prevalent. Two possibilities which explain this phenomenon may be considered. One is that newborn rats are much more susceptible to fatal HFRS virus infection than are adult ones, and that they are highly infected at the same time via a contaminated nest. Maternally acquired antibody to HFRS virus of newborn rats was also suspected. On the other hand, antibody titers increased with the rat age. This may be due to the continuous stimulation with virus atnigen resulting from persistent infection.

In the study of urban rats in Korea by Lee *et al.* (1982), approximately 50% of seropositive rats revealed viral antigen in the lung cryostat sections stained by IFA method. In the present study, however, the lower antigen detection rate (1/11) may be due to the formalin fixation employed to prevent laboratory infection.

Antigenic characterization of HFRS viruses isolated from urban rats in this island, a laboratory rat (SR-11) and a wild rodent (Hantaan virus) are now in progress. In addition, further seroepidemiological surveys among the workers of the reclaimed ground area are being planned.

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