Isolation and Characterization of a Hantavirus from *Rattus norvegicus* in a Residential Area of Nagoya City, Japan

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ABSTRACT. The prevalence of antibodies to hantavirus among *Rattus norvegicus* in Nagoya city was examined during the period from 1986 to 1993. Eight sera of 675 were antibody positive in an indirect fluorescent antibody test. Antibody positive rats were found in 1988, 1990, 1991 and 1992 and the numbers of rats were 1, 1, 3 and 3, respectively. A hantavirus strain was isolated from a rat captured in 1990 at a residential area adjoining to a pier in Nagoya port. The virus strain, named NR-9, was identified antigenically as the Seoul type of hantavirus in a neutralization test. Suckling rats inoculated with NR-9 strain survived, while those with SR-11 strain succumbed. A difference between the virulence of strains, NR-9 and SR-11 was demonstrated.—KEY WORDS: epizootiology, hantavirus, hemorrhagic fever with renal syndrome, virulence.

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Hemorrhagic fever with renal syndrome (HFRS) viruses are globally distributed among various rodents species. China, Korea, Russia, Scandinavia and the Balkans are endemic areas of clinical human HFRS. An acute respiratory illness with high mortality was recently reported in the southwestern United States. Genetic identification of a hantavirus associated with an outbreak of this illness was reported [7]. The virus causing Korean hemorrhagic fever (KHF), Hantaan virus 76–118 strain was isolated from a wild striped mouse, *Apodemus agrarius coreae*, in 1978 [6]. Hantaan virus has been classified as a prototype virus of the fifth genera of the Bunyaviridae family, i.e., genus Hantavirus. Hantavirus could be divided into at least four serotypes specific to the species of rodent hosts, i.e., *Apodemus, Rattus, Clethrionomys* and *Microtus* species [8, 11].

In Japan, human HFRS cases had occurred in Osaka city in the 1960s [13]. Since 1970 there have been a number of incidences of HFRS associated with laboratory rats in medical institutions. In 1982, the causative viruses, named SR-11 and SR-14, were isolated at Sapporo Medical College [5] from rat lung specimens associated with HFRS incidence. Since 1985 no laboratory type HFRS incidence has occurred, but antibody positive cases among rats and humans were reported at Kyoto University in 1992 and 1993 [9]. On the other hand, a seroepizootiological survey among wild rats demonstrated the prevalences of antibodies in rats in Tokyo port [10], the Tama area in the western suburbs of Tokyo in the 1960s, and in other port areas, e.g., Kobe, Yokohama and Shimizu. Hantavirus strains were also isolated from *Rattus norvegicus* captured in nonresidential areas, i.e., reclaimed land near Tokyo port [12] and a dumping ground in Kami-iso town near Hakodate, Hokkaido [1]. Normally, the general public could not enter port areas where the rats were captured, and rat populations seemed higher than in ordinary residential areas. In Nagoya city, investigations of rat infestations and rat exterminations have been carried out to control rodent-borne diseases. In the present study, an epizootiological survey of hantavirus infection in rats was conducted in residential areas of Nagoya city from 1986 to 1993 to measure the public health involvement of hantavirus infection in rats.

MATERIALS AND METHODS

Sera of wild rats: Rats in the public sewers in sixteen wards of Nagoya city were captured with Tomahawk-type traps from 1986 to 1993. Manhole covers were opened and the traps were set inside the sewers. The captured rats were anesthetized with chloroform or ether and bled by cardiac puncture. Altogether 675 sera were obtained.

Viruses and cells: SR-11 strain isolated from a laboratory rat associated with the incidence of laboratory type HFRS [5], TR-352 strain isolated from a *Rattus norvegicus* captured on reclaimed land in Tokyo Bay [12] and

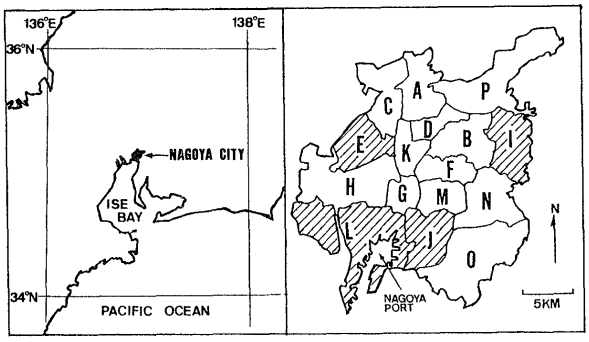

Fig. 1. Map of Nagoya city: A to P corresponds with names of wards which are shown in Table 1. Sero-positive rats were found in the four wards (Minato, Minami, Meito and Nakamura) indicated by diagonal lines.

Hantaan virus 76–118 strain [6] were used. Vero-E6 cells were cultivated in Dulbecco's MEM supplemented with 7 or 2% fetal calf serum (FCS).

Immune rat sera: Eight-week-old female Wistar rats, specific pathogen free (SPF), (Japan SLC, Inc., Shizuoka) were immunized against SR-11 or 76–118 strain and three-week-old female Wistar rats were immunized against TR-352 strain.

Serological tests: Indirect fluorescent antibody (IFA) test with SR-11 antigen slides was carried out [5]. Fluorescein isothiocyanate conjugates of antibodies to rat IgG (Cappel, Cochranville, Pa) were used. The sera which showed 1:32 or more were regarded as antibody positive. Serological cross-reactions of NR-9 strain to Seoul type strains and 76–118 strain were tested by IFA and neutralization test (NT). NT with peroxidase-antiperoxidase (PAP) was carried out by the method described elsewhere [14]. Serial twofold dilutions of sera were mixed with an equal amount of virus solution containing 200 focus forming units (ffu). Vero E-6 cells were inoculated with mixtures containing 100 ffu of the virus. Neutralizing antibody titers were expressed as the reciprocal of the highest dilution of the serum which showed over 50% PAP focus reduction.

Virus isolation: Virus isolation was carried out [5] on lung specimens from *Rattus norvegicus* captured in Minato ward, Nagoya in 1990 and 1991. Vero E-6 cells were inoculated with a 10% lung homogenate and then the cells were maintained in Dulbecco's MEM supplemented with 2% FCS at 37°C. The cells were subcultured after three weeks' incubation. The virus isolation was confirmed by an IFA test with anti SR-11 rat immune serum.

Animal and virus inoculation: The pathogenesis of NR-9 or SR-11 strain in newborn rats was examined. Pregnant Wistar rats (SPF) were obtained from Japan SLC, Inc., Shizuoka. A virus suspension (0.05 m*l*) containing 10^3 ffu of NR-9 or SR-11 strain was used to intraperitoneally inoculate a litter of suckling rats within 24 hr after delivery.

RESULTS

Detection of antibody: The prevalence of antibodies to SR-11 strain among *Rattus norvegicus* captured in sixteen wards in Nagoya city is shown in Fig. 1 and Table 1. From 1987 to 1992, 8 seropositive rats were found in Meito, Minato, Nakamura and Minami wards. In Minato ward, seropositive rats were found consecutively during the three years from 1990 to 1992 in the same site adjoining the pier in Nagoya port.

Virus isolation: At the second passage of a cell culture inoculated with a lung specimen from a *Rattus norvegicus* captured in a residential area in Minato ward, Nagoya in 1990 (IFA titer was 1:2048), the cells showed specific fluorescence stain in cytoplasm and the newly isolated virus strain was named NR-9.

Antigenic characteristics of NR-9 strain: Antigenic relationships of NR-9 strain to other hantavirus strains are shown in Table 2. NR-9 strain cross-reacted with the rat strains isolated in Japan and 76–118 strain by IFA but could be differentiated from 76–118 strain by NT. NR-9 strain was also shown to be a Seoul type virus as well as other rat isolates in Japan.

Virulence of NR-9 strain: The results of the inoculation of newborn rats with NR-9 strain are shown in Table 3. Seven rats out of nine inoculated with SR-11 strain had

Table 1. Detection of antibody to SR-11 in rats captured in Nagoya city by indirect fluorescent antibody test

Ward	1986	1987	1988	1989	1990	1991	1992	1993	Total
A Kita	8*	13	12						33
B Chikusa	19		8	13		5			45
C Nishi	14	10	15			6			45
D Higashi	16		19						35
E Nakamura	12	11	10			2**/22	1/9	14	3/78
F Showa	9		5	7		3			24
G Atsuta	11					9			20
H Nakagawa	6					5			11
I Meito	4	18	1/12			15	21	3	1/73
J Minami		8			10	7	1/42	33	1/100
K Naka		19		6		6			31
L Minato		14			1#/42	1/12	1/28	19	3/115
M Mizuho		12	7						19
N Tenpaku		13	4						17
O Midori		2	10						12
P Moriyama		3	14						17
Total	99	123	1/116	26	1/52	3/90	3/100	69	8/675 (1.2%)

IFA titers in positive cases were as follows:
Meito ('88; 1: 1024), Minato ('90; 1: 2048, '91; 1: 2048, '92; 1: 1024), Nakamura ('91; 1: 128, 1: 2048, '92; 1: 128), Minami ('92; 1: 32)
*: Numbers tested. **: Numbers positive. #: NR-9 strain was isolated.

Table 2. Cross IFA test and NT with rat immune sera

Antisera	IFA				NT			
	Virus				Virus			
	NR-9	SR-11	TR-352	76-118	NR-9	SR-11	TR-352	76-118
anti NR-9 rat	16384	16384	16384	8192	5120	10240	10240	20
anti SR-11 rat	8192	8192	8192	4096	640	640	1280	<20
anti TR-352 rat	8192	8192	8192	2048	2560	1280	2560	40
anti 76-118 rat	1024	512	1024	2048	<40	<40	<40	320

Table 3. Mortality of new born rats inoculated with SR-11 or NR-9

Inoculated with SR-11		Inoculated with NR-9
Mortality[a] (%)	Mean time to death	Mortality
7/9(77.8)	30.9[b]	0/10(0.0)

a) Mortality at 6 weeks after virus inoculation.
b) Mean time in days.

ruffled fur, paralysis of limbs and hypertension, and most of them died within 35 days, the mean survival time being 30.9 days. On the other hand, ten suckling rats inoculated with NR-9 strain survived for the 6 weeks' observation period without any manifestations except the loss of body weight.

DISCUSSION

The evidence of hantavirus infection among rats in Nagoya city was demonstrated by a seroepizootiological survey. A hantavirus strain named NR-9 was isolated by means of Vero E-6 cells from a rat captured in the proximity of the pier in Nagoya port where hantavirus was prevalent among *Rattus norvegicus* living around warehouses from 1987 to 1993 (unpublished data). So far hantavirus had been isolated from wild rats captured in the port areas or a dumping ground which the general public could not normally enter. This is therefore the first isolation of a hantavirus from *Rattus norvegicus* captured in a general residential area in Japan. It seemed that rodents infected with hantavirus might be introduced into the port by vessels which came from HFRS endemic areas, and infected rodents have been prevalent in the port area. It may easily be presumed that rats infected with hantavirus moved to neighboring areas from the pier. As seropositive rats were also found in Nakamura and Meito wards which do not adjoin Minato ward, it seems that rats infected with hantavirus had already gradually spread into the inner part of the city through rivers or sewers.

No HFRS patient has yet been reported in Nagoya city except human infections which might be associated with laboratory animals. It is still not known whether viruses in rats in this area are pathogenic in man or not. A seroepidemiological survey of hantavirus infection in human subjects conducted by Dr. Komatsu *et al.* demonstrated that the antibody positive rate for sanitation workers on reclaimed land in Tokyo Bay where a hantavirus, TR-352, was isolated was 4.5% (33 out of 732) and that of people in Metropolitan Tokyo was 0.9% (5 out of 530), but no clinical signs of HFRS were shown in any of the antibody positive cases (unpublished data). In a survey in Baltimore, U.S.A., patients with proteinuria were more commonly seropositive (1.46%) than the reference group (0.25%). The antibody positive rate among dialysis patients with end-stage renal disease was 2.76%. This indicated that hantavirus infection could be associated with hypertensive renal disease in the U.S.A. [2].

Newborn laboratory mice have been shown to be susceptible to, and able to be killed by infection with Hantaan virus [4]. It was reported, however, that Hantaan virus showed lower virulence in newborn rats than in newborn mice, and B-1 strain isolated from laboratory rat also showed higher virulence in newborn rats than in newborn mice [15]. This indicated that higher virulence was seen when the virus was used in inoculating homologous species in which virus was isolated. It was also reported that KI-262 and TB-314 strains isolated from *Rattus norvegicus* captured in a dumping ground in Kami-iso, Hokkaido and on reclaimed land in Tokyo Bay, respectively, did not cause any clinical signs, indicating less pathogenicity in newborn rats [3]. In the present study, in addition to KI-262 and TB-314 strains, NR-9 strain isolated from a wild rat showed low virulence in newborn rats, while SR-11 and B-1 strains isolated from experimental rats showed high virulence in newborn rats. It seemed that the virulence in newborne rats of Seoul type virus strains may vary. Further studies on the virulence of other strains should be conducted to determine the character of the Seoul virus.

The first incidence of HFRS in Japan occurred in Osaka during the 1960s [13]. It had ceased because of improvements in residential buildings and the living environment. In Nagoya city, extermination of rats has been vigorously continued and chances of contact with contaminated feces and urine seem to be few because of improvements in the living environment. On the other hand, in the Tokyo Bay area, the prevalence of hantavirus among *Rattus norvegicus* at least until 1990 has been demonstrated [10]. In the present study, the prevalence of hantavirus among *Rattus*

norvegicus in ordinary residential area has also been demonstrated. Hantavirus infection in Japan is still a public health problem, and continuous surveillance of hantavirus infection among rats, other rodents and people is needed to understand the hantavirus infection situation in Japan.

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