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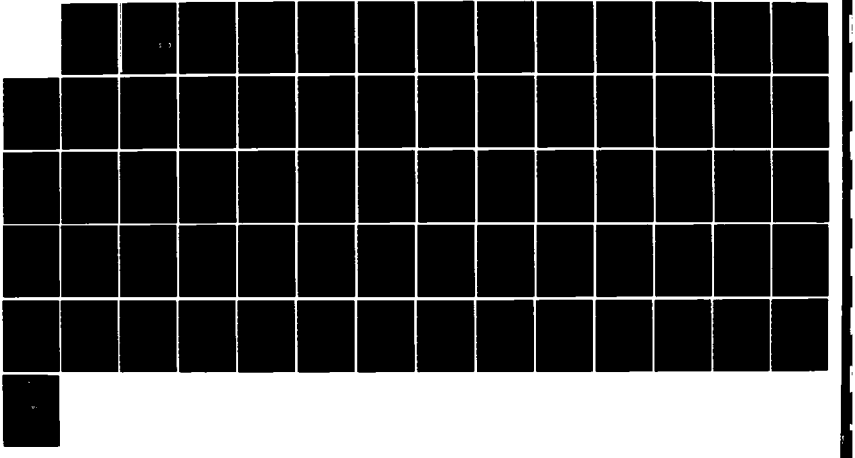

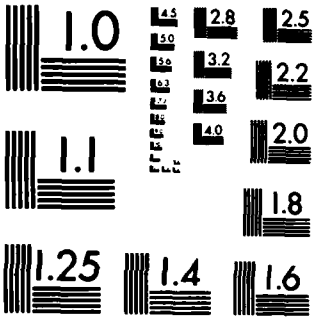

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KOREAN HEMORRHAGIC FEVER
(HEMORRHAGIC FEVER WITH RENAL SYNDROME(HFRS))

Final Report

HO WANG LEE, M.D.

August 1983

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Supported by

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In 1982 WHO adapted to call diseases clinically compatible to Korean hemorrhagic fever as "Hemorrhagic fever with renal syndrome (HFRS)". HFRS was an important military disease transmitted to man by field mice in past wars. Although predominantly associated with

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Hemorrhagic Fever With Renal Syndr...

rural areas, it is now being recognized as an urban problem in some countries and a particular hazard to laboratory staff using rodent for biomedical research.

This report presents the results of seroepidemiologic studies of laboratory infections with Hantaan virus in animal rooms of institutes in Korea and Japan during last 5 years, and isolation of Hantaan virus from urban rats caught in Incheon harbor and Tokyo harbor and, isolation of 7 strains of Hantaan virus from lung tissues of rodent in tissue culture cells.

From 1975 to 1981, 126 cases of (HFRS), of which one was fatal, had occurred in 22 animal rooms of research laboratories in Korea and Japan. Among colonized laboratory rats of the animal rooms, 71% (Korea) and 40% (Japan) had antibodies to Hantaan virus. In Korea, 23% of those 71% were proven to have pulmonary viral antigen and 7 strains of Hantaan virus were isolated from those rats. 203 urban rats caught in Tokyo harbor yielded 5 isolates of Hantaan virus of which one strain was propagated in Vero E-6 cells. 215 urban rats caught in Incheon harbor yielded 14 isolates of Hantaan virus of which one strain was propagated in Vero E-6 cells and found to have both of the antigenic characteristics as that of Korean agent and NE agent.

Five strains of Hantaan virus were isolated and propagated in Vero E-6 cells; three strains are from blood of HFRS patients and two strains from wild Apodemus mice. Originator ... (Oct. 19)

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SUMMARY

From 1975 to 1981, 126 cases of HFRS, of which one was fatal, had occurred in 22 animal rooms of research laboratories in Korea and Japan. Among colonized laboratory rats of the animal rooms, 71% (Korea) and 40% (Japan) had antibodies to Hantaan virus. In Korea, 23% of those 71% were proven to have pulmonary viral antigen and 7 strains of Hantaan related virus were isolated from those rats. 203 urban rats caught in Tokyo harbor yielded 5 isolates of Hantaan related virus of which one strain was propagated in Vero E-6 cells. 215 urban rats caught in Incheon harbor yielded 14 isolates of Hantaan related virus of which one strain was propagated in Vero E-6 cells and found to have both of the antigenic characteristics as that of Hantaan virus and NE virus.

Five strains of Hantaan virus were isolated and propagated in Vero E-6 cells; three strains are from blood of HFRS patients and two strains from wild Apodemus mice.

FOREWORD

In conducting the research described in this report, the investigators(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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A. Seroepidemiologic and Virologic Studies of Laboratory Infections with Hantaan related Virus, Etiologic Agent of Hemorrhagic Fever with Renal Syndrome, in Korea and Japan.

INTRODUCTION

Seroepidemiologic studies have shown that Hantaan virus, the etiologic agent of hemorrhagic fever with renal syndrome (HFRS) and related agent are ubiquitous in the World (1-12), of which the impetus for the study was the discovery of Hantaan virus for the first time in Korea (4,13). WHO has recently adapted to call Korean hemorrhagic fever and clinically similar diseases with a different name, HFRS (1). In addition, Lee et al (14,15,16) has very recently reported the finding of the Hantaan and related virus not only in wild rural mice, but also in urban rats as well as laboratory rats. Interestingly, many cases of animal room personnels who were afflicted with HFRS have been reported in the 1970's (17,18), at which time studies of HFRS had not yet even begun in these animal rooms. We report here 126 cases of HFRS recognized during 1975 to 1981. All of these cases, of which one was fatal, were of scientists and animal room caretakers who had worked in animal rooms of Korea and Japan. This report provides the evidence that various kinds of colonized laboratory rats are the carriers of Hantaan and related virus, thus, significantly expanding the epidemiologic horizon for the transmission of the virus to humans.

MATERIALS AND METHODS

1. Sera of Patients

781 human sera from suspected HFRS patients, animal caretakers and personnels of 17 laboratories in Japan where HFRS cases had occurred was sent to Seoul, Korea. Sera of animal caretakers of six laboratories in Seoul was also used. None of 23 laboratories in Japan and Korea had ever conducted Hantaan virus experiments. All of the sera were stored in -60°C refrigerator until needed for the antibody test.

2. Sera of Colonized Rodent

Sera of 474 rats and of 108 mice obtained from seven laboratories of the aforementioned 17 laboratories in Japan was sent to Korea. Sera of 196 rats was also obtained from the aforementioned six laboratories in Seoul. All of the sera were stored in -60°C refrigerator until needed for the antibody test.

3. Measurement of Immunofluorescent Antibodies to Hantaan Virus

Spot slides of A549 cells infected with the 76/118 strain of Hantaan virus (19) were used for antigen and the antigen preparations were free of immunofluorescent reoviral antigens when tested with polyvalent antiserum to reovirus. The indirect immunofluorescent method (4) was used for detection and titration of antibodies to Hantaan virus.

4. Detection, Isolation and Identification of Viral Strains from Lung Tissue of Rats

Of the 196 rats from the six laboratories in Seoul, 178 rats were autopsied, and portions of their excised lung tissue were sent to the immunofluorescent room for demonstration of Hantaan viral antigen and Hantaan viral isolation. All of the lung tissues were stored in -60°C refrigerator until needed. Isolation of Hantaan virus from laboratory rats and the method of identification have been previously described (14).

5. Hantaan Virus

For this experiment, 76-118 strain of Hantaan virus (4) was used. A549 cells infected with 76-118 strain was used as antigen for the antibody test.

RESULTS

1. Number of HFRS Cases by Annual and Seasonal Groups

The number of HFRS cases occuring in animals rooms in Korea and Japan (1975-1981) are correlated with regional groups as shown in Figure 1; in Japan, the Hantaan viral infection in animal rooms spreads throughout the entire region of Japan, whereas in Korea, the region surveyed was only that of Seoul. Table 1 shows the annual number of HFRS patients (1975-1981) and its corresponding number of research institutes having those patients.

Statistical data shows that there was only one patient in one institute in 1975, while there was 34 cases in five institutes in 1978, and 30 cases in six institutes in 1981 in Japan. A total of 111 patients in 17 institutes in Japan had been ill with HFRS, whereas in Korea, a total of 15 patients in six institutes had been ill during 1975 to 1981. Figure 2 illustrates the monthly number of confirmed HFRS cases in Japan; most of the cases in Japan occurred in Spring and Winter, the seasons in which the animal rooms are dried by heating. It was impossible to figure out the monthly number of confirmed HFRS cases in Korea because the patients could not remember exactly the month of their illness when we drew their blood in 1981 but most of them claimed to have been sick in Spring and Winter.

2. Condition of Hantaan Viral Infection of Rats in Animal Rooms where HFRS Cases had Occurred

The conditions of Hantaan viral infection of rats and of personnels in seven research institutes in Seoul, Korea, are shown in Table 2. The results of antibody test showed that 140 rats (75%) out of 196 rats from six institutes had antibodies to Hantaan virus. Lung tissue test showed that 41 rats (23%) out of 178 rats had Hantaan viral antigen. Also, 15 (31%) out of 48 animal caretakers of animal rooms in six institutes had antibodies to Hantaan virus and all of them claimed to have had moderate to mild forms of HFRS.

Table 3 shows the infection rate of rats (Charles river) with Hantaan virus and antibody titers of sera in a small animal room of an institute in Seoul as an example. About 90% of rats were antibody positive out of 36 rats examined regardless of sex, and two strains of Hantaan related virus were isolated from nine antigen positive rats. All of four different strains of colonized laboratory rats were equally infected with Hantaan related virus as shown in Table 4.

Table 5 shows the data of all laboratories and their corresponding number of HFRS patients in Japan from 1975 to 1981. Infected rats in animal rooms of medical centers were the source of infection for 111 patients. 474 rodents from seven out of these 17 laboratories were examined, and 191 rodents (40%) proved to have antibodies to Hantaan virus. Infection pattern of laboratory rats with Hantaan virus in an animal room at J-C Medical School in Japan where five cases of HFRS had occurred is shown in Table 6 as an example. 33 out of 38 rats from this small animal room were infected and antibody titers to Hantaan virus were almost as high as the infected laboratory rats in Seoul. Virus isolation was not made from these infected rats since lungs of these rats were not available.

Table 7 shows, by strain groups, the condition of Hantaan related viral infection of rats in seven laboratories in Japan; results of the antibody test of the various rats from these animal rooms where HFRS cases had occurred show that 12 strains of colonized rats and four out of 108 albino mice were positive for the antibody. Antibody titers of four positive sera from albino mice were low (1:15-1:64)。

Data indicates that all kinds of colonized laboratory rodents are infected with Hantaan virus, and these rodents were the source of Hantaan virus infection for the personnels who had worked in the animal rooms.

3. Clinical Diagnosis for HFRS Cases Occurring in Animal Room

The clinical diagnosis for HFRS cases that had occurred in animal rooms of 17 laboratories in Japan and seven laboratories in Korea is shown in Table 8. 156 out of 781 human sera were positive for the antibodies to Hantaan virus and 126 persons exhibited clinical symptoms whereas 30 persons were asymptomatic. 96 out of 126 seropositive patients were hospitalized with severe clinical symptoms.

The 126 symptomatic patients showed the following diagnosis; about 1/3 of these 126 patients were suspected to have HFRS, and the rest were diagnosed as having various viral diseases. The suspected HFRS patients all showed strong clinical symptoms. In Korea, even though 15 patients who were personnels of animal rooms suffered from a high fever, lumbago and other clinical symptoms of HFRS, they were not diagnosed as having HFRS, but as having an influenza-like illness and none of them were hospitalized.

DISCUSSION

The occurrences of HFRS cases in laboratories can be classified into two categories; one is comprised of all the cases reported when the known source of infection is from a laboratory where Hantaan virus experiment is in progress and it contains Apodemus mice and urban rats that were infected with Hantaan virus, and the other, from a laboratory where its tissue culture cells and colonized laboratory rats were never subjected to the Hantaan virus experiment.

In 1976, an animal room personnel was afflicted with a disease of an unknown etiology in Sendai, Japan. It was at first suspected to be Marburg disease because he was working with monkeys in an experiment prior to his illness. But his serum test was negative for it. This disease, accordingly named as "Sendai fever", was further studied by Ishida et al. of the Medical College of Tohoku University. In 1978, the serum of this patient was sent to Seoul, tested with Hantaan virus, and thereupon discovered to be the first positive serum for antibodies to Hantaan viral agent that had come from an animal room in Japan. Soon after, it was evident that there were many other HFRS cases occurring in animal rooms of Medical Centers in Japan. This incident rapidly became a sensation in Japan for HFRS had been thought to be a virtually non-existing disease in Japan and the source of viral infection leading to HFRS in the 1960's in Osaka was thought to have been brought into Japan from abroad.

Until quite recently, HFRS had been thought to be a regional disease occurring mainly in Korea, China, Mongolia and Russia, and other diseases similar HFRS occurring in Europe had been called by other names.

In 1976, however, Lee et al (13) discovered the antigen of etiologic agent of HFRS in lung tissues of Apodemus mice and the serologic diagnosis for HFRS. Consequently, sera positive for antibodies to Hantaan virus from human and rodent have proven to be everywhere in USA (12), Africa (10), South America (11), South East Asia and Pacific island (20) as well as in Euro-Asia (1,2). Lee et al (15) also recently reported after isolating Hantaan related virus from urban rats that HFRS cases could occur even in areas such as center city (16). Furthermore, it was proven for the first time that HFRS cases could occur not only in the fields of rural and urban areas but also in animal rooms of laboratories via colonized laboratory rats infected with Hantaan related virus, i.e., the reservoirs of Hantaan related virus are all different kinds of rats infected with Hantaan related virus, as revealed in this report. It is important to note that most of all the laboratory rats used in Korea are imported from Japan and some from U.S.A. and England. Most of all the rats in Japanese laboratories where HFRS cases had occurred were provided by companise that breed animals and these rats were used for clinical research purposes, such as in studying hypertension,

diabetes, cerebral diseases and cancer etc. This report shows that there were 111 cases of HFRS that had occurred in the animal rooms in Japan. It is thought, however, that the actual number of cases of HFRS may be more than 111 cases, the most likely reason being that since the 17 laboratories described in this report had not revealed the occurrences of HFRS cases at first, but only eventually, it can be assumed that other laboratories could have just as well not reported any such occurrences either, thereby giving a false low- ount.

In Korea, we confirmed 15 patients to be infected with the virus ever since we started to survey rats infected with Hantaan virus in several animal rooms of Institutes in Seoul in 1981, but they were diagnosed clinically not as HFRS but as influenza. It was reported that most of the cases had occurred in spring, but the authenticity of the reported data could not be verified. Most of HFRS cases had occurred in animal rooms in spring and winter, at which time the animal rooms in temperate zones like Korea are dry and rather stuffy, due to the heating system. In winter, therefore, Hantaan virus in excrements are dried indoors, suggesting that the air might be the medium of transmission of aerosols of Hantaan virus.

Survey of the animal rooms where HFRS cases had occurred revealed that most of the rats were bred in ill-ventilated rooms, and several animal rooms were heated using oil burners during winter; HFRS cases had occurred only in such rooms having infected rats, thus, providing further evidence that these animals were capable of producing aerosols of highly infectious virus.

In Korea, among 48 animal caretakers, only 15 persons were infected, indicating that the rate of infection is not as high as expected, considering the fact that many examined rats of various kinds were infected.

Due to lack of sufficient evidence no significant conclusion can be derived as to when and how the laboratory rats were infected with Hantaan virus, but, this presents a matter of great importance.

In 1977, Charles river rats were sent by air flight from London, England to an Institute in Seoul, Korea where only one laboratory had used them. Since those rats were not examined for infection at that time, it is not known whether they were, in fact, infected with the virus or not. On the other hand, if those rats had been proven to be non-infected, then we could obviously conclude that they had been infected after their arrival in Seoul in 1977. As reported recently, urban rats infected with Hantaan viurs in Seoul could have been the source of infection for the laboratory rats. The urban rats may have been infected with the virus from infected wild rural Apodemus mice. The existence of Hantaan virus even in Japan is not of a recent finding and had been documented in 1960's. Since the symptoms

of Japanese patients with HFRS were not quite clinically compatible with classic HFRS patients described in 1940-1950s in Korea, China, and Russia, it is likely that those Japanese patients had been diagnosed for a long time as having diseases other than HFRS.

HFRS cases occurring in laboratories have been also documented in Belgium, Europe. Whether or not those infected special strain of colonized rats with Hantaan virus were imported from Japan is uncertain. Regardless of any evidence showing the original reservoir of Hantaan virus these reports of sporadic HFRS cases occurring in different countries provide strong possibility of further global infection via the dangerous transmission of sending rats to different laboratories in different countries.

Antibodies of laboratory rats in Korea and Japan react strongly to 76-118 strain of Hantaan virus, and the virus strains from laboratory rats are like 76-118 strain antigenically by indirect IFAT. The observation that the rats from both Korea and Japan are probably the same strain correlates well with laboratory findings showing that the Japanese and Korean patients' sera are similar in titers and both Japanese and Korean rats'sera are of also compatible titers.

Although the greatest amount of antigen was usually observed in lungs of laboratory rats, some were also detected in the liver and spleen of a majority of the animals. The duration of existence of the viral antigen in the lung tissues was found to be at least two months, whereas the duration of excretion of the viral content from the kidney through the urinary tract is yet unknown. As described in the dissemination trace which shows that a large amount of virus is excreted through urine in the Apodemus mice, rats probably excrete the virus through urine and saliva, contaminate the laboratory when the urine and saliva dries by releasing aerosols of highly infectious virus, and infect the laboratory personnels when they become subjected to breathing such contaminated air.

CONCLUSION

1. From 1975 to 1981, 126 cases of HFRS had occurred in 22 animal rooms of laboratories in Korea and Japan. Most of them occurred in winter and spring.
2. Among colonized laboratory rats of animal rooms where HFRS cases had occurred, 71% (Korea) and 40% (Japan) had IF antibodies against Hantaan virus. In Korea, 23% of those 71% were proven to have pulmonary viral antigen, and seven strains of Hantaan related virus were isolated from these rats. 16 strains of laboratory rats and albino mice were proven to have IF antibodies to Hantaan virus. The titers of antibody to Hantaan virus were in the range of 1:32 to 1:8,192, with the mean of 1:512.
3. Clinical diagnosis of HFRS cases that had occurred in laboratories in Korea and Japan were incorrect. Of 126 persons who were believed to have HFRS according to serologic test, only 1/3 were suspected to actually have UFRS and the rest were diagnosed of not having HFRS. This shows that without an accurate serologic test the diagnosis for the Hantaan viral infection is quite difficult. It was revealed for the first time that 30 out of 156 antibody-positive persons had inapparent infection with Hantaan related virus.

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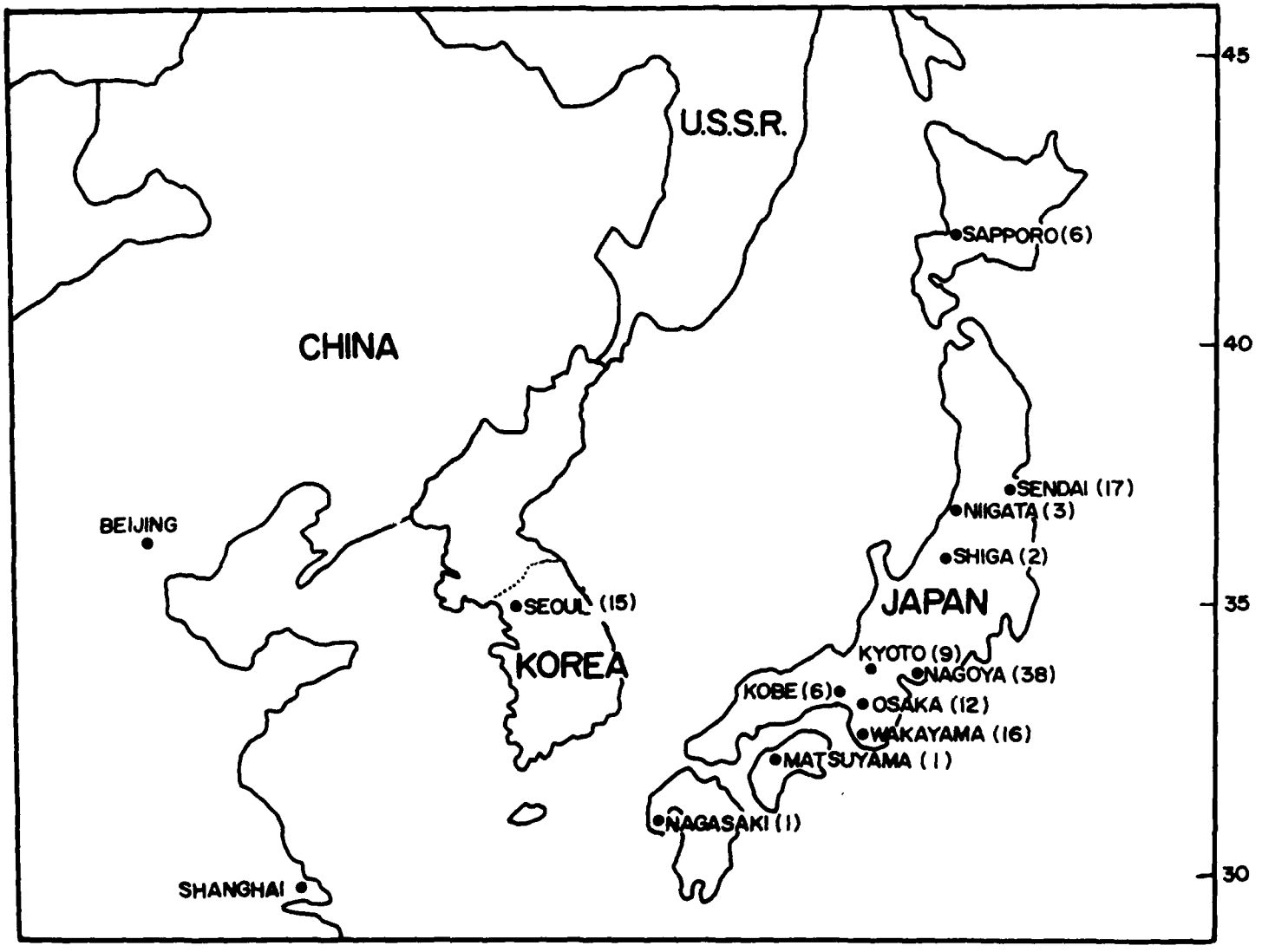

FIGURE 1.
MAP SHOWING NO. OF HEMORRHAGIC FEVER WITH RENAL SYNDROME PATIENTS WHO WORKED IN ANIMAL ROOMS OF MEDICAL CENTERS IN KOREA AND IN JAPAN FROM 1975 TO 1981

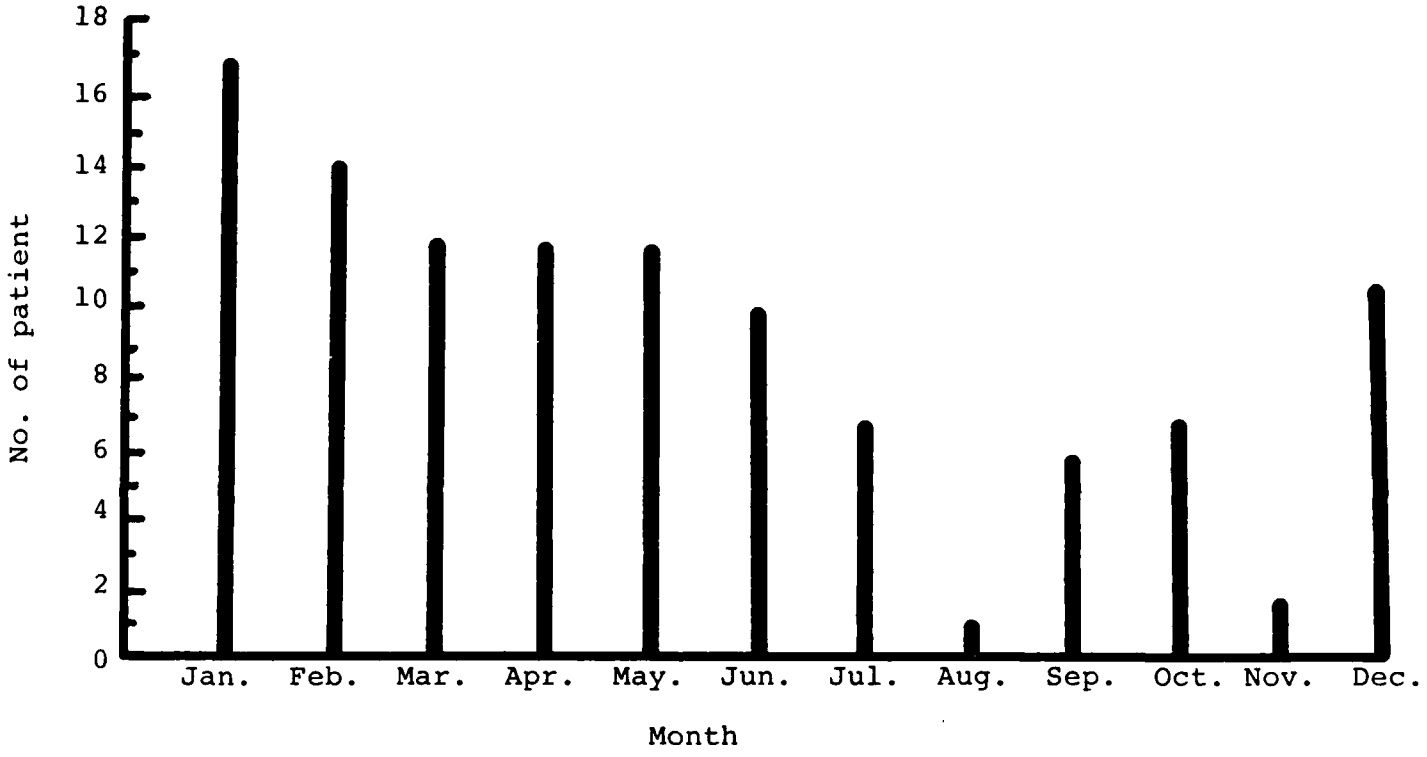

Figure 2.
Number of HFRS patients who worked in animal rooms of research institutes in Japan from 1975 to 1981.

Table 1
Number of HFRS patients who worked in animal rooms of Research Institutes in Japan and Korea

Year	Japan		Korea	
	No. of patient	No. of Institute	No. of patient	No. of Institute
1975	1	1		
1976	11	3		
1977	11	5		
1978	34	5	1	1
1979	13	6	3	2
1980	11	6	3	2
1981	30	6	5	3
Total	111	17	15	6

Table 2.
Infection of rat (Wistar, S.D. and C.R.) and animal caretaker with Hantaan related virus, etiologic agent of HFRS, in animal rooms of Research Institutes in Korea, 1981.

Institution	Hantaan viral IF antibodies and pulmonary antigen in laboratory rat		Animal caretaker
	antigen positive/ no. tested	antibody positive/ no. tested	antibody positive/ no. tested
K-A	8[1]/52 (12.3%)	47/55 (75.4%)	2/13
K-B	24[2]/48 (50.0%)	43/49 (91.7%)	3/5
K-C	9[3]/36 (25.0%)	32/36 (88.9%)	3/17
K-D	0/20	9/20 (60.0%)	0/2
K-E	0/16	5/16 (31.3%)	4/8
K-F	0/6	4/20 (20.0%)	n.d.
K-G	n.d.[4]	n.d.	3/3
Total:	41/178 (23.0%)	140/196(71.4%)	15/48(31.3%)

[1] : Two strain of Hantaan related virus was isolated from 8 antigen positive rats in Wistar rats.

[2] : Three strain of Hantaan related virus was isolated from 24 antigen positive rats in Wistar rats.

[3] : Two strain of Hantaan related virus was isolated from 9 antigen positive rats in Wistar rats.

[4] : not done

Table 3.
IF antibody titers against Hantaan virus of sera from rats of K-C Institute, Seoul, Korea, 1981.

No. of serum	Strain/ sex	Antibody titer	Antigen in lungs	No. of serum	Strain/ sex	Antibody titer	Antigen in lungs
1	C.R./M	-	-	19	C.R./F	1,024	-
2	" /M	64	-	20	" /F	1,024	-
3	" /M	512	++	21	" /F	128	-
4	" /M	4,096	-	22	" /F	64	-
5	" /M	-	-	23	" /F	1,024	-
6	" /F	8,192	+	24	" /M	4,096	-
7	" /F	8,192	-	25	" /M	4,096	-
8	" /F	256	++ Ψ	26	" /F	1,024	-
9	" /M	-	-	27	" /M	4,096	-
10	" /M	512	+	28	" /F	1,024	-
11	" /M	4,096	-	29	" /F	4,096	++
12	" /F	1,024	+	30	" /F	128	-
13	" /M	1,024	-	31	" /F	128	+
14	" /F	128	-	32	" /M	8,192	-
15	" /M	128	-	33	" /F	512	-
16	" /F	1,024	+ Ψ	34	" /F	1,024	-
17	" /F	4,096	-	35	" /F	1,024	+
18	" /F	-	-	36	" /F	512	-
	No. positive/no. tested					32/36	9/36
		male				12/15	2/15
		female				20/21	7/21

Ψ : Hantaan virus was isolated from lungs in Wistar rat.

Table 4
Strain of infected colonized laboratory rats with Hantaan related virus in animal rooms of Institutes in Korea, 1981.

Strain of rat	Hantaan viral IF antibodies and pulmonary antigen in laboratory rat	
	antigen positive/ no. tested	antibody positive/ no. tested
Wistar rat	32/115	101/133
Charles River rat	9/36	32/36
S. D. rat	0/22	6/22
Fatty rat	0/5	1/5

↓ : IF antibody titers against Hantaan virus of positive sera from rats ranged between 1:32 to 1:8,192 (Mean 1:512).

Table 5.
Laboratory infection of Hantaan and related virus, etiologic agent of HFRS, in animal rooms of medical centers in Japan from 1975 to 1981.

Institute	No. HFRS patient	No. antibody positive rat/no. tested	No. antibody positive mouse/no. tested
J-A	18	11/20	
J-B	17	21/63	4/108
J-C	16	104/132	
J-D	15	11/72	
J-E	11		
J-F	8		
J-G	6		
J-H	4	30/65	
J-I	3	4/78	
J-J	3		
J-K	2		
J-L	2		
J-M	2		
J-N	1		
J-O	1		
J-P	1		
J-Q	1	10/44	
17 Institute	111	191/474(40.3%)	4/108(3.7%)

Table 6.
IF antibody titers of rat sera against Hantaan virus from an animal room of J-C Medical School, Japan

Code No. of serum	Strain/ sex	Antibody titer	Code No. of serum	Strain/ sex	Antibody titer
WR79-1	Wister/M	2,048	WR79-20	SHR X ch/F	2,048
WR79-2	Wister/M	32	WR79-21	SHR X ch/M	128
WR79-3	Wister/M	2,048	WR79-22	Wister/F	-
WR79-4	Wister/M	256	WR79-23	Wister/M	32
WR79-5	Wister/M	1,024	WR79-24	Wister/M	64
WR79-6	Wister/M	32	WR79-25	Wister/M	2,048
WR79-7	Wister/M	1,024	WR79-26	Wister/M	2,048
WR79-8	Wister/M	256	WR79-27	Wister/F	512
WR79-9	Wister/M	256	WR79-28	Wister/F	32
WR79-10	CRJ:CD/M	1,024	WR79/29	Wister/M	64
WR79-11	Wister/M	32	WR79-30	Wister/M	256
WR79-12	SHR/M	512	WR79-31	Wister/F	32
WR79-13	SHR/M	-	WR79-32	Wister/M	512
WR79-14	SHR/F	-	WR79-33	Wister/M	-
WR79-15	SHR X HR/F	32	WR79-34	Fatty/F	1,024
WR79-16	SHR X HR/F	32	WR79-35	Hr-1/F	256
WR79-17	SHR X HR/M	256	WR79-36	SHR X HR/M	32
WR79-18	SHR X ch/F	64	WR79-37	Wister/F	1,024
WR79-19	SHR X HR/F	-	WR79-38	Wister/F	512

$$\frac{\text{No. of serum positive}}{\text{No. of serum tested}} = \frac{33}{38}$$

Table 7.
Species and strain of infected experimental rodent with Hantaan related virus in animal rooms of Medical Centers where HFRS cases had occurred in Japan from 1975 to 1981.

Species and strain of rodent	No. of antibody positive$^{\Psi}$ against Hantaan virus/no. tested
Wistar rat	130/309
SHR rat	22/57
S.D. rat	9/32
Fisher rat	9/26
Gunn's 191-1 rat	9/17
WKA rat	1/14
Long Evans rat	4/11
Fatty/F rat	2/2
190 CXXIV rat	2/2
Lewis rat	1/2
CRJ:CD rat	1/1
Hr-1 rat	1/1
Albino mice	4/108

Ψ :IF antibody titers against Hantaan virus of positive sera from rats ranged between 1:16 to 1:2,048 (Mean 1:256).

Table 8
Clinical diagnosis of HFRS patients who worked in animal rooms of research institutes in Japan and Korea from 1975 to 1981

Clinical diagnosis	No. of seropositive to Hantaan virus	No. of hospitalized patient
Suspected HFRS	47	47
Viral infection	25	25
Viral hepatitis	15	15
Influenza-like illness	15	0
Common cold or influenza	11	0
Unknown fever	6	4
Nephritis	2	2
Acute tonsillitis	2	0
Weil's disease	2	2
Pneumonitis	1	1
Subtotal	126	96
Inapparent infection	30	
Total	156	

IF antibody titers against Hantaan virus of positive sera from HFRS patients and from inapparent infection had mean and range of 64 - 16,384 and 16 - 256, respectively.

B. Isolation of Hantaan Related Virus from Urban Rats in Incheon Harbor, Korea.

INTRODUCTION

Hantaan virus (1), the etiologic agent of Hemorrhagic fever with renal syndrome (HFRS) was found for the first time in Korea, 1976, by Lee et al (2), isolated from Apodemus mice and patients in 1978 (3) and subsequently recovered from not only Apodemus (2,3) but wild urban rats (4) as well as colonized laboratory rats (5). Further studies revealed that urban rats from different parts of the world had antibodies to Hatnaan virus (6,7) and thus, strongly suggesting the possibility of unexpected occurrences of sporadic HFRS cases in large port cities of the world. It could become a matter of serious global concern should the infected urban rats from infected areas of Euro-Asia where HFRS cases already occur annually aboard ships and upon landing at foreign non-infected port cities transmit the Hantaan virus to that particular region of the world.

We report here the results of our investigation on isolation of Hantaan related virus and distribution of antibodies in wild urban rats captured in the second largest port city of Korea, Incheon Harbor, where no HFRS cases have been previously reported.

MATERIALS AND METHODS

Survey areas

Survey areas for collection of urban rats as seen in Fig. 1 were primarily the Yonan Pier, Walmi Island and Buksong-dong district of Incheon city.

Capture and processing of rodents.

Rats were purchased from householders who used live traps supplied by us. Live animals were identified upon receipt at the laboratory. They were bled by cardiac puncture while anesthetized with chloroform; samples centrifuged at 1,500 g for 10 min to obtain sera were stored at -20°C for antibody studies. The rats were then killed to provide lung, liver, spleen, kedney, and parotid gland tissues. Portions of lung tissue were saved for immediate cryostat sectioning to search for Hatnaan viral antigens, and the remaining samples were stored at -70°C.

Measurement of levels of immunofluorescent antibodies to Hantaan virus.

Two preparations of Hantaan viral antigen were used: (a) frozen sections of Apodemus lung infected with the 76/118 strain in its 12th passage in Apodemus lung (7) and (b) spot slides of human adenocarcinoma A549 cells infected with the 76/118 infected with the 76/118 strain after three passages in Apodemus lung and seven passages in A549 cells. Both of these preparations were free of immunofluorescent reoviral antigens when tested with polyvalent antiserum to reovirus (Reference Reagents Branch, Centers for Disease Control, Atlanta). The indirect immunofluores-

cent method (3) was used for detection and titration of antibodies to Hantaan virus. Fluorescein isothiocyanate-conjugated goat antibodies to rat IgG and mouse IgG were obtained from Cappel Laboratories (Cochranville, Pa.); slides were examined with an epi-illumination UV microscope (model SM-Lux;E. Leitz, Rockleigh, N.J.)

Detection of viral antigens.

Frozen cryostat cut sections (6 μ) of lung and other tissues were examined for viral antigens by indirect immunofluorescence with use of serum from a patient convalescent from KHF.

Isolation and identification of viral strains.

Details of this technique have been described (4).

RESULTS

The city of Incheon is located within 50 km of Seoul. Even though considerable number of HFRS cases with classic HFRS clinical symptoms have been recently reported in metropolitan area of Seoul (7), not one such HFRS case had been documented in Incheon prior to our investigation.

1. Detection of immunofluorescent antibodies to Hantaan virus and of viral antigen in wild urban rats.

215 R. norvegicus and six R. rattus rats from three different locations of Incheon city as shown in Fig. 3 were captured and examined.

The number of rats tested and those positive for antigen and antibody are shown in Table 9. We detected antigen in rats that also had antibodies to Hantaan virus. Only three out of 221 rats had antigen but no antibody and of these three rats only one yielded a viral isolate. About 52% of rats out of 215 R. norvegicus were antibody positive and the infected rats had been distributed equally in the three localities of Incheon.

2. Isolation of viral strains from wild urban rats.

Lung suspensions from ten rats with antibodies to Hantaan virus and undetectable pulmonary Hantaan viral antigen and 50 rats with both detectable antibodies and Hantaan viral antigen were inoculated into Wistar rats. Lungs of these animals were in turn examined 30 days later for immunofluorescent antigen as an index for viral replication. As shown in Table 10. Fourteen isolates were obtained. It is noteworthy that among the ten rats with only antibodies to Hantaan virus and undetectable immunofluorescent antigen one isolate was obtained, an indication that IFA technique was not a sensitive measure of presence of minute amount of antigen in these rats.

In general, the intensity of immunofluorescent antigen in lungs was directly proportional to the subsequent recovery of virus. Sera from all Wistar rats inoculated with antigen positive lung suspensions were examined 30 days later for immunofluorescent antibodies to Hantaan virus. All antigen negative rodents had

no antibodies, an indication that antibody was a reliable index of viral infection.

Table 11 shows the distribution of viral antigen as determined in tissues of six urban rats, all of which yielded viral isolates. The greatest amount of antigen was alsays detected in lungs with lesser amount in spleen, liver and kidneys.

Passages of lung suspensions from 14 isolates of Incheon urban rats as shown in Table 10 were made in Vero E-6 cells, and of the passaged virus only one strain, KIRN/82/3, successfully replicated in the tissue culture cells. By the third passage, the presence of viral strain was detected by immunofluorescence.

3. Biological characteristics of Hantaan virus isolated from urban rats, Incheon.

The strain KIRN/82/3 from an Incheon rat, and strain 76/118 from Apodemus mice were both propagated in the tissue culture cells to yield antigens for antibody test in which the two strains induced compatible antibody titers in the sera of KHF patients, but strain KIRN/82/3 induced high antibody titer whereas strain 76/118 induced low titer in sera of Nephropathia epidemica patients of Scendinavia (Tables 12 and 13). Also virus isolated from Incheon rats induced no antibody response, in all but one, in positive sera of Americans and antibodies in sera of both Korean and Japanese urban rats exhibited compatible titers. Sera of Hawaiian urban rats had antibodies only to strain 76/118.

Antisera obtained from rats inoculated with strain 82/3 yielded hightiter antibodies against homologous virus, but low titer to heterologous strain 76/118.

Table 13 shows detailed data of similar antibody titers detected in the sera of Nephropathia epidemica patients of Finland and Norway, a strong evidence that isolate 82/3 has both of the antigenic characteristics of Korean and Scandinavian agent of HFRS.

4. Distribution of IF antibodies to Hantaan virus in sera of residents, Incheon.

As shown in Table 14, 67 out of 658 residents (10%) of Incheon had antibodies to Hantaan virus regardless of age and sex and levels of antibodies were low, ranging from 1:16 to 1:256. This percentage is much higher than 1% of Seoul residents.

DISCUSSION

This study to isolate Hantaan or related virus from wild urban rats captured in Incheon harbor was started in spring of 1982 soon after our isolation of Hantaan virus from wild urban rats caught in metropolitan area of Seoul and in adjacent cities of Seoul in 1980 and aims to correlate the infectivity of Incheon urban rats with other ubiquitous urban rats of the world where urban rats are transported by ships to all different countries from port to port; should we find that a large portion of urban

rats caught in Incheon harbor are infected with Hantaan virus, we could speculate that urban rats in many port cities of the world are already contaminated with Hantaan virus.

It was unbelievable that no HFRS cases had ever been reported in Incheon city populated with more than a million people even though Incheon is only 50 km away from Seoul where many cases of HFRS had occurred already and 50% of urban rats living in Incheon harbor are infected. Therefore, we surveyed the distribution of IF antibodies to Hantaan virus in sera of residents of Incheon in summer of 1982 to find out human infection with the virus. To our surprise, 67 out of 658 residents of Incheon (10.2%) who were hospitalized with various diseases other than HFRS were antibody positive against Hantaan virus, showing a much higher percentage than the 1% antibody positive residents of Seoul.

As a result of successful isolation and propagation of KIRN/82/3 strain from an Incheon urban rat in Vero E-6 cells, it was possible to compare Incheon KIRN/82/3 strain with prototype strain 76/118 of Hantaan virus serologically and found that Incheon strain has antigenic characteristics as that of causative agents of both Asian type of HFRS and European type of HFRS by indirect IFAT. The pathogenicity and virulence of Incheon strain of Hantaan virus to man still remains to be studied, and should this strain be avirulent and have good antigenicity in man, this virus could be a strong candidate for vaccine which is in urgent need.

Urban rats caught in many port-cities of the world proved to have antibodies to Hantaan virus and data indicates that Hantaan or related virus exists in many parts of the world where HFRS is not yet known to exist. It is too early to speculate, however, that people are ill with HFRS in these areas of the world because no classic type of severe HFRS patient was reported yet in these areas. Danger of spreading Hantaan virus to big cities of the world is great but more knowledge on virulence of viruses in new group of Hantaan virus family and global surveillance of occurrences of HFRS-like illness are required to prevent spreading this new plaque.

Studies of morphology and molecular biology of virus strains isolated from urban rats remain although it was reported that Hantaan virus has a Byunyaviridae morphology (8,9).

CONCLUSION

1. 112 out of 215 rats collected from Incheon Harbor, Korea, had antibodies to Hantaan virus and 51 out of 215 rats were antigen positive.
2. Pulmonary tissues of 60 wild urban rats yielded 14 strains of Hantaan like viral isolates of which one was from ten lungs of rats which had antibodies but no pulmonary antigen. Greatest amount of viral antigen was observed in lungs with lesser amount in spleen, liver and kidneys.
3. The isolated strain 82/3 was propagated in Vero E-6 cells and found to have both of the antigenic characteristics as that of the causative agents of Korean and Scandinavia N.E.
4. About 10% of residents of Incheon City had IF antibodies to Hantaan virus regardless of age and sex. However, so far there had been no HFRS patients reported in this city. The prevalent virus in Incheon rats might be a non-virulent strain of Hantaan or Hantaan related virus to man.

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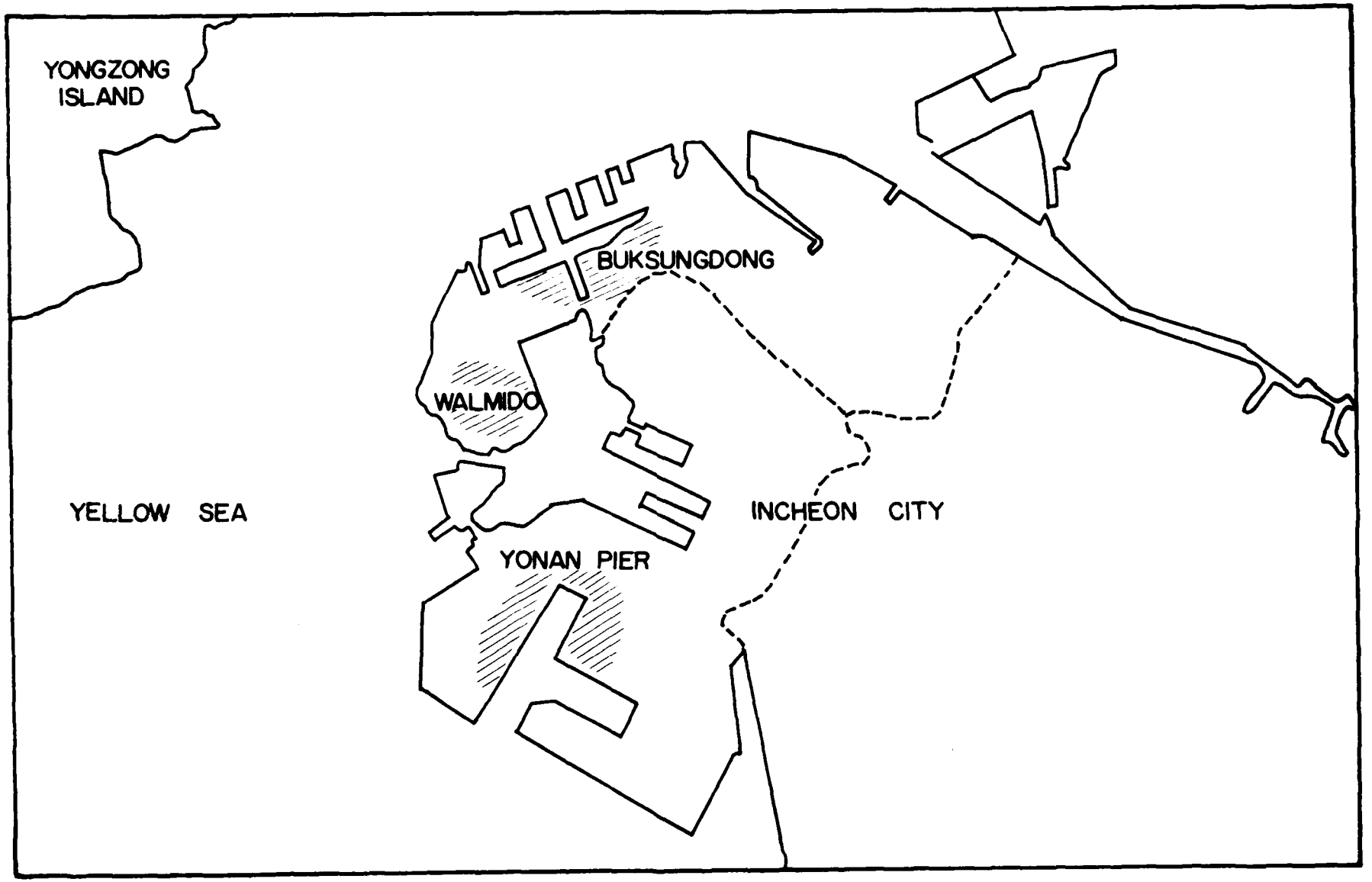

FIGURE 3. MAP OF INCHEON HARBOR, KOREA, SHOWING (▨) LOCATIONS OF COLLECTION OF URBAN RATS

Table 9
Hantaan viral IF antibodies and pulmonary antigen in wild Rattus, Incheon, Korea, March 12 - May 20, 1982.

Locality	IF antibody and pulmonary antigen in R. norvegicus			% positive	
	No.	antibody	antigen	antibody	antigen
Yonan Pier	106	66	31	62	29
Wolmido	41	35	16	85	39
Buksungdong	68	11	4	16	6
Total	215	112	51	52	24
% positive		52	24		

Table 10
Isolation of Hantaan-like virus from wild urban rats positive for viral antigen by immunofluorescence in Incheon, Korea in 1982.

Immunofluorescence intensity	No.yielding isolate/ no. tested (%)	Mean and range of antibody
-	1/10 (10)	3,058 (16 - 8,192)
+	3/23 (13)	3,696 (16 - 8,192)
++	9/23 (39)	3,139 (16 - 8,192)
+++	1/4 (25)	644 (16 - 2,048)
Total	14/60 (23)	

Table 11
Distribution of Hantaan-like viral IF antigen in the organs of wild R. norvegicus in which Hantaan-like virus was isolated, Incheon harbor, 1982.

Code no. of R. norvegicus	Weight (gm)	Sex	IF antibody titer of serum against Hantaan virus	Presence of Hantaan viral antigen			
				Lung	Kidney	Liver	Spleen
82/I/HR/3	150	M	16	++	+	+	+
82/I/HR/25	175	F	1,024	+++	-	-	+
82/I/HR/29	218	F	256	+++	-	++	++
82/I/HR/66	206	M	2,048	++	+	+	+
82/I/HR/165	160	F	8,192	++	+	+	+
82/I/HR/167	135	M	4,096	+	-	+	+

Table 12
Comparative titration of IF antibodies against two strains of Hantaan and related virus, of sera from human and rats in Korea, Japan, Finland and U.S.A.

	Tested sera	Hantaan virus antigen and antibody titer of sera	
		AP/76-118 A549 cells	KIRN/82-3 Vero-E6
Control	KHF-81-605-3	16,384	8,192
	B-Normal-39	-	-
	Reo-polyvalent	-	-
Human	KHF-81-308-1	4,096	4,096
	KHF-81-308-2	16,384	8,192
	Incheon Hu-17	32	128
	Japan-S-1	4,096	4,096
	Finland-277	256	512
	Finland-317	512	2,048
	Finland-323	512	4,096
	NE-79-276	128	128
	US-Aug-82-64	128	-
Rat	SRN-80-39-WR	8,192	4,096
	SNUIH-Fr-81-24	512	1,024
	Jap-WR-14	2,048	1,024
	Hawaii-Rr-6191	32	-
	Hawaii-Rr-6387	16	-
	Anti-KIRN/82-3 rat immune serum	1,024	4,096

Table 13
Comparative titration of IF antibodies against two strain of Hantaan and related virus antigen, of human sera from Korea, Finland, Norwary and U.S.A.

Code no. of serum	Hantaan viral antigen and antibody titer	
	AP/76-118 A549 cells	KIRN/82-3 Vero E6 cells
KHF-81-308-1	4,096	4,096
KHF-81-308-2	16,384	8,192
KHF-81-605-3	16,384	8,192
. . . . .		
Finland-643	16	256
Finland-645	16	2,048
Finland-646	64	1,024
Finland-647	64	1,024
Finland-648	16	256
Finland-649	32	256
Finland-650	256	4,096
Finland-651	32	1,024
Finland-652	64	1,024
Finland-653	32	2,048
. . . . .		
Norway-597	16	128
Norway-598	32	256
Norway-599	128	256
Norway-600	128	512
Norway-602	64	128
Norway-603	64	64
Norway-604	16	64
Norway-605	32	256
Norway-607	32	64
Norway-608	32	64
Norway-609	32	128
Norway-610	64	512
. . . . .		
US-J-82-16	160	-
US-J-82-35	40	-
US-J-82-38	80	-
US-J-82-48	40	-
US-J-82-127	40	-
US-J-82-145	80	-
US-J-82-191	80	-
US-J-82-191	80	-
US-J-82-252	160	20
US-J-82-285	80	-

Table 14
Occurrence of IF antibodies against Hantaan virus, etiologic agent of Korean hemorrhagic fever, among resident of Incheon city, 1982.

Age group	Male		Female		Total	
	No. positive /no. tested	Incidence of antibody positive	No. positive /no. tested	Incidence of antibody positive	No. positive /no. tested	Incidence of antibody positive
0-10	8/26	38.8%	2/19	10.5%	10/45	22.2%
11-20	1/8	12.5%	4/16	25.0%	5/24	20.8%
21-30	3/35	8.6%	7/179	3.9%	10/214	4.7%
31-40	17/90	18.9%	3/99	3.0%	20/189	10.6%
41-50	8/58	13.8%	8/45	17.8%	16/103	15.5%
Over 51	2/38	5.3%	4/45	8.9%	6/83	7.2%
Total	39/255	15.3%	28/403	7.0%	67/658	10.2%

C. Isolation of Hantaan and Related Virus from Lung Tissues of Rodent in Tissues Culture Cells.

INTRODUCTION

Hantaan virus (1), the etiologic agent of Hemorrhagic fever with renal syndrome (HFRS), was isolated by Lee et al (2,3). Thereafter, the method of virus isolation was by inoculating early phase blood of HFRS patients and lung tissues of infected rodent into striped field mice and laboratory rats using the indirect immunofluorescent antibody technique (4,5). Animals exhibited no clinical symptoms even when injected with Hantaan virus (6).

Until now, only 76/118 strain of Hantaan virus isolated from lung tissues of striped field mice was cultured in tissue culture cells without exhibiting CPE(7,8,9) and any findings of direct isolation of Hantaan virus from experimental tissues in tissue culture cells has not been yet reported.

We report here for the first time the results of seven strains of Hantaan and related virus isolation obtained by inoculating lung suspension of rodent positive for Hantaan viral antigen into tissue culture cells.

MATERIALS AND METHODS

1. Tissues of infected rodent: Seven infected lung tissue of striped field mice caught in endemic areas of HFRS in Tongducheon, Kyungido (76/309, 78/197, 79/89, 79/90, 79/237, 79/242, 79/246), 20 positive lungs of urban rats infected with Hantaan virus caught in Incheon (82/3, 82/29, 82/30, 82/52, 82/66, 82/153, 82/157, 82/158, 82/160, 82/161, 82/163, 82/164, 82/165, 82/167, 82/177, 82/182, 82/199, 82/200, 82/215, 82/216), 14 positive lungs of urban rats caught in urban areas of Seoul (80/4, 80/11, 80/12, 80/21, 80/23, 80/24, 80/39, 80/115, 80/200, 80/337, 80/404, 80/482, 80/502, 80/516) and eight positive lungs of urban rats caught in Tokyo harbor, (82/11, 82/16, 82/17, 82/22, 82/24, 82/25, 82/27, 82/28) were used.
2. Tissue culture cells: Vero E-6 and A549 cells were used for the isolation and propagation of Hantaan virus and the method of tissue culture has been previously described (7,8,9).
3. Propagation of Hantaan virus in Vero E-6 and A549 cells: 0.1 ml of supernatant of 20% lung suspension obtained after centrifugation at 5,000 rpm, 4°C for 30 min., was inoculated into a tissue culture tube. Three tubes of cells were used for one specimen and inoculated cells were kept at 36°C CO_2 incubator. The supernatant of 20% lung suspension was filtered through 0.45 u millipore filter before inoculating into tissue culture cells. The fluid of inoculated tissue culture cells with suspension of positive lung tissues was passaged at 12 day intervals for three times and then cells were examined for growth of virus in the cells by indirect

immunofluorescent technique (3).

RESULTS

Isolation and propagation of Hantaan and related virus in Vero E-6 cells:

Table 15 shows that strains of Hantaan virus isolated from lung tissues of rodent are comprised of five striped field mice strains (AP/76/309, AP/78/197, kHF/79/89, KHF/79/90, KHF/79/237) and two urban rat strains (JTRN/82/17, KIRN/82/3). AP/76/309 and AP/78/197 strains are from infected pulmonary tissues of Apodemus mice caught in the endemic areas of HFRS. KHF/79/89, KHF/79/90 and KHF/79/237 strains are from positive pulmonary tissues of Apodemus mice inoculated with acute stage blood from HFRS patients into normal Apodemus mice. JTRN/82/17 strain is from positive pulmonary tissues of Japanese urban rat and KIRN/82/3 strain is from positive pulmonary tissues of Incheon urban rat, Korea. Five strains out of seven Apodemus lungs and two strains out of 28 urban rat lung were isolated in Vero E-6 cells but no strain was isolated in A549 cells even though 14 suspensions of urban rat lung tissues were inoculated. AP/76/118 strain of Hantaan virus is an adapted strain of virus in A549 cells described previously (7). The results of comparative titration of antibodies of sera from HFRS patients and urban rats against eight antigens of Hantaan virus strains grown in tissue culture cells are shown in Table 15. It was noteworthy that antibody titers of sera from HFRS patients in Korea, Japan and Finland are equally high against KIRN/82/3 strain of Hantaan virus isolated from urban rats caught in Incheon harbor. It suggests that this strain might have common antigenic properties as that of causative agents of both Asian HFRS and Scandinavian NE.

DISCUSSION

Until recently, only one strain of Hantaan virus, 76/118 (3) was successfully propagated in A549 cells (7) and Vero E-6 cells (8,9) since the isolation of the virus from pulmonary tissues of infected Apodemus mice in 1978. Thereafter, it was possible to study biochmical and morphological characteristics of Hantaan virus which was grown in tissue culture cells.

It was necessary to isolate and propagate other strains of Hantaan and related virus from tissues of rodent in tissue culture cells so that a comparison of virus properties with prototype strain of Hantaan virus, 76/118, could be further made.

Results clearly show Vero E-6 cells were a better detector system than A549 cells for isolation and propagation of Hantaan virus from rodent tissues. Since Hantaan virus from sera of HFRS patients has not yet been isolated in tissue culture cells despite tremendous efforts made to isolate the virus in A549 and other tissue culture cells, the practical approach to replicate

the Hantaan virus as candidate for vaccine would be to directly inoculate positive pulmonary tissues of Apodemus mice rather than positive pulmonary tissues of urban rats into and isolate the specimen in Vero E-6 cells.

Results of simultaneous titration of antibodies of sera from HFRS patients by IFA technique strongly suggest the possibility that antigenically different strains of Hantaan virus do indeed exist, but, it is yet too early to predict as such.

These new findings reemphasize the need for further work to devise more sensitive and simpler host system for the isolation and production of Hantaan and related virus from experimental animal specimens in tissue culture laboratories where specially facilitated animal rooms are not available.

CONCLUSION

1. Seven strains of Hantaan and related virus were isolated; three strains are from early phase blood of HFRS patients, two strains are from wild Apodemus mice and two strains are from wild urban rats.
2. All seven strains of the virus could be used as antigens of Hantaan virus for titration of IF antibodies. KIRN/82/3 strain isolated from urban rat in Incheon had different antigenic properties from other strains and had common antigenic properties as that of causative agents of both Asian type of HFRS and Europian type of HFRS.
3. Antigenic differences between strains of viruses isolated from man, mice and rats by plaque neutralization test remains to be studied.

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Table 15
Comparative titration of IF antibodies against different strains of Hantaan virus of sera from human and rats in Korea, Japan. Finland and U.S.A.

	Tested sera	Hantaan virus antigens and antibody titers of sera							
		AP/76-118 A549	AP/76-309 Vero E6	AP/78-197 Vero E6	JTRN/82-17 Vero E6	KIRN/82-3 Vero E6	KHF/79-89 Vero E6	KHF/79-90 Vero E6	KHF/79-237 Vero E6
Control	KHF-81-605-3	16,384	512	512	512	8,192	1,024	512	1,024
	B-Normal-39	-	-	-	-	-	-	-	-
	Reo-polyvalent	-	-	-	-	-	-	-	-
Human	KHF-81-308-1	4,096	4,096	8,192	1,024	4,096	1,024	4,096	8,192
	KHF-81-308-2	16,384	8,192	8,192	4,096	8,192	2,048	8,192	8,192
	Sappro MS-K	1,024	512	512	4,096	4,096	1,024	1,024	512
	Osaka S-1	4,096	256	1,024	4,096	4,096	1,024	1,024	2,048
	Wakayama-35	64	64	64	256	256	64	256	128
	Finland-277	256	64	64	128	512	128	64	32
	Finland-317	512	128	256	128	2,048	128	128	256
	Finland-323	512	512	512	256	4,096	512	128	128
	NE-79-276	128	64	32	64	128	64	64	64
	US-Jul-82-252	128	16	-	-	16	-	128	32
	US-Aug-82-64	128	16	-	64	-	16	16	64
Rat	SRN-80-39-WR	8,192	2,048	4,096	4,096	4,096	2,048	4,096	4,096
	SNUIH-Fr-81-24	512	128	256	4,096	1,024	128	1,024	256
	Jap-WR-14	2,048	512	512	512	1,024	128	1,024	256
	JTRN-82-4	1,024	512	512	2,048	1,024	512	128	128
	Hawaii-Rr-6191	32	-	-	-	-	-	32	32
	Hawaii-Rr-6387	16	-	-	-	-	-	32	32

D. Isolation of Hantaan Related Virus from Urban Rats in Tokyo Harbor, Japan.

INTRODUCTION

The etiologic agent of Korean hemorrhagic fever (KHF) was found for the first time in Korea, 1976 (1) and isolated in 1978 (2) and then registered as Hantaan virus (3). WHO has recently adapted to call clinically compatible KHF-like illness in Euro-Asia Continent as Hemorrhagic fever with renal syndrome (HFRS) (4). The epidemic cycle of Hantaan virus has very recently been found after Lee et al. isolated Hantaan virus has very recently been found after Lee et al. isolated Hantaan virus from urban rats (5) and laboratory rats (6), not to mention field mice (1,2,7).

Patients of Hemorrhagic fever with renal syndrome (8) was reported in Osaka in the 1960's and some 116 patients of hemorrhagic fever were found in animal rooms of 17 medical centers in the 1970's in Japan (6). The etiologic agent of hemorrhagic fever with renal syndrome is not reported to have been isolated yet in Japan.

We report here the initial results of a search for Hantaan virus infection among urban wild rats in Japan and together with data concerning isolation of the agent in tissue culture.

MATERIALS AND METHODS

Urban rat:

203 urban rats were captured in Osaka, Nagoya and Tokyo, Japan. Among them, 89 urban rats were caught in an area of Tokyo harbor; 50 caught in December 1981, and 39 in February 1982. Others were caught in summer of 1982 in Osaka and Nagoya. They weighed 85-300 gm each and were classified by genders before autopsy.

Isolation of Hantaan virus-like antigen and demonstration of antibodies from urban rats.

Methods used for virus isolation and demonstration of immunofluorescent and neutralizing antibodies to Hantaan virus were as described previously (2,5).

Isolation of virus from lungs of rats in tissue culture cells.

0.1 ml of filtered 20% suspension of Hantaan virus antigen positive lungs with 0.45 μ millipore filter was added into a tube of Vero E-6 (9) cells and kept in CO_2 incubator at 36°C. Three tubes of Vero E-6 cells were used for a specimen of antigen positive lungs. We checked the proliferation of virus in cells by IF staining after three blind passages at intervals of 12 days.

RESULTS

1. Demonstration of IF antibodies to Hantaan virus and of viral antigen in wild urban rats.

It was proven that 36 (40.5%) out of 89 rats captured in U-harbour area of Tokyo had IF antibodies to Hantaan virus, and eight rats had Hantaan viral antigen in their pulmonary tissues as shown in Table 16.

Of 109 urban rats captured in Osaka city, 33% of urban rats near Senpoku-Wharf had antibodies. Urban rats in some parts of Tennoji-ku also proved to have antibodies whereas 13 urban rats of Nishi and Kita-ku, Osaka were negative. Only 5 rats of Nagoya city were examined, all proving to be antibody negative.

2. Isolation of Hantaan-like virus strains from wild rats.

The details of eight urban rats, i.e., weights, genders, antibody titers of sera, amount of viral antigen in various tissues that were proven to have Hantaan viral antigen in pulmonary tissues, are shown in Table 17.

Urban rats infected with Hantaan virus had high titers of immunofluorescent antibodies regardless of genders, and viral antigen was found mainly in lungs. We isolated virus by inoculating 10% suspension of pulmonary tissues of eight rats into S.D. rats and Apodemus mice intramuscularly, resulting in the isolation of 5 strains from S.D. rats and one strain from Apodemus mice. We have identified 3 strains of virus isolated from urban rats in Japan with a prototype strain of Hantaan virus, 76-118, previously reported (2) and the result is shown in Table 18. Against such antigens the antibody titers of serum from a patient convalescent increased 100-1,000 times in comparison with the titer of an early stage serum. A positive serum of Japanese urban rat (82/11) also showed the same reactions to Hantaan virus of many strains.

The results of neutralization test of 76/118 strain and JTRN/82/22 strain with convalescent serum of Hemorrhagic fever patient in Apodemus mice showed neutralizing index of $10^{4.3}$ and $10^{3.9}$, respectively. Virus antigen of strain JTRN/82/11 was found mainly in lungs and spleen tissues of S.D. rats after inoculation of the viurs, as shown in Table 19.

3. Isolation of Hantaan-like virus from lungs of wild urban rats in Vero E-6 cells.

We tried to isolate virus by injecting 20% suspension of eight lung tissues positive for Hantaan viral antigen into A549 cells and Vero-E6 cells, but only JTRN/82/17 strain proliferated in Vero-E6 cells. CPE was not observed and immunofluorescent antigen in cells was detected after three passages.

After the 4th passage, 80% of cells were infected on the 7th day after being inoculated with virus, enabling the

infected cells to be used as antigen for antibody test. Comparative titration of antibodies of sera from HFRS patients and positive rats against A549 cells antigens infected with 76/118 strain and Vero-E6 cell antigens infected with JTRN/82/17 are in Table 20. It is noteworthy that antibody titers of sera from Japanese HFRS patients were high against Japanese rat strain JTRN/82/17 but low to Korean strain 76/118.

DISCUSSION

In 1980, Hantaan-like virus was isolated from urban rats of Seoul city and in 1981, the virus was also isolated from urban rats captured in Incheon harbour, 50 miles away from Seoul (5,10). These findings suggest that urban rats may have carried Hantaan-like virus to other harbours of the world, thus, carrying the virus far beyond the presumptive Euro-Asian origins of the virus. These findings were reported to WHO in 1981, and as a result, urban rats in America, especially in the most logical targets such as port cities, have begun to be surveyed (11).

In order to investigate additional epidemiologic feature of Hantaan virus, we began to examine in specific detail the excrement of virus, antibody formation, and dissemination trace of virus, etc., of urban rats. Since the Korean urban rats, however, were already infected with the virus, they were unsuitable for our laboratory work.
This prompted Prof. Tanaka of Osaka City University of assist us by sending 50 R. norvegicus Japanese urban rats, thought to be free of Hantaan virus. Upon examination of the sera from the obtained 50 Japanese rats, 40% surprisingly showed antibodies to Hantaan viurs, thus, rendering them unsuitable for our work as well. Prof. Tanaka sent us another 40 of the same strain, of which we once again found 40% to have antibodies to Hantaan virus and eight rats of those 40% to have Hantaan viral antigen in lungs.

Eight lung suspensions from rats positive for antigen and antibodies when inoculated into various rodent and tissue culture cells yielded five strains of Hantaan virus. The five isolates were identified physiognomically with 76/118 strain recovered from Apodemus mice. Inoculating eight lung suspensions of urban rats (82/17) into Vero E-6 cells directly made it possible for isolation of only one strain of virus (82/17) which was discovered to have almost same antigenic properties with 76/118 strain by IFAT, and thus, enabling it to be adaquately used as viral antigen for antibody test. Hantaan-like virus in various tissues of Japanese urban rats shared biologic characteristics with virus recovered from urban rats captured in Seoul.

Sera of urban rats captured in the city of Osaka have begun to be examined from the summer of 1982. Results showed that

most of rats near Senpoku-Wharf were infected, while only a few rats in Tennoji-ku were infected. From 1976 to 1982, 111 cases of HFRS had been reported, all of which had their origin of infection in animal rooms of 17 medical centers in Japan. One case was fatal. Although it is known that the laboratory rat was the reservoir of virus, its original mode of infection is yet unknown. Logically, however, we could assume that the viral content of wild rural mice, host of KHF in Korea, contaminated urban rats, which in turn infected laboratory rats. This assumption is further supported by the finding that noninfected laboratory white rats when left for three months in an infected urban area of Seoul were found to be later infected with the virus.

Among all of urban rats with high titers of immunofluorescent antibodies, only a few of them proved to have virus antigen. We could isolate five strains from S.D. rats, and only one strain from Apodemus mice. The strain from Apodemus mice resembled the virus isolated by inoculating the strain from Vero E-6 cells into S.D. rats. In order to further study the epidemiology of HFRS, we will investigate the optimal conditions necessary for viral proliferation in tissue-cultivating cells.

Our recent findings show that urban rats of all harbours we have investigated in the world, Incheon, Manila, Hongkong, Suva, Honolulu, Bangkok and Alexandria, have antibodies to Hantaan virus, thus, indicating that further surveys of urban rats, especially in port-cities worldwide, are urgently needed.

CONCLUSION

1. Wild urban rats caught in Tokyo harbour and in Osaka city had IF antibodies to Hantaan virus.
2. Five strains of Hantaan-like virus were isolated from Wistar rats which had been inoculated with positive pulmonary tissues of wild urban rats caught in Tokyo. One strain of Hantaan-like virus was isolated and propagated in Vero E-6 cells which had been inoculated with positive pulmonary tissues of wild urban rats.
3. Results of comparative titration of antibodies from sera of HFRS patients of different parts of the World showed that antibody titers of sera from Japanese HFRS patients were high against Japanese strain JTRN/82/17 but low to Korean strain 76/118, thus, suggesting that JTRN/82/17 strain of Hantaan virus isolated from Japanese urban rats may have different antigenic properties from that of 76/118 strain.

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Table 16.
Distribution of Hantaan viral IF antibodies and pulmonary antigen in wild rats caught in Japan, 1981-1982.

Location	Name of species	No. antibody positive[1] no. serum tested	No. antigen positive[2] no. lung tested
Tokyo city			
U-harbour	R. norvegicus	36/89(40.5%)	8/89(9.0%)
Osaka city			
Senpoku-wharf	R. norvegicus	18/54(33.3%)	0/29
Tennoji-ku	R. norvegicus	4/42(9.5%)	n.t.[3]
Nishi & Kita-ku	R. rattus	0/13	n.t.
Nagoya city	R. rattus	0/5	n.t.

[1] IF antibody titers against Hantaan virus of positive sera from rats ranged between 1:16 to 1:4,096 (mean 1:128).

[2] IF Hantaan virus antigens against convalescent serum from HFRS patient in the lung tissues of rats.

[3] n.t. : not tested.

Table 17.
Isolation of Hantaan-like virus from Hantaan viral IF antigen and antibody positive wild *R. norvegicus* caught in Tokyo harbour in 1982.

Code no. of *R. norvegicus*	Weight (g)	Sex	IF antibody titer against Hantaan virus	Presence of Hantaan virus antigen in tissue of rats				Hantaan virus isolated in		
				lung	liver	spleen	kidney	S. D. rats	*Apodemus* mice	Vero E6 cells
JTRN/82/11	176	F	4,096	+++	++	-	+	4/4[1]	0/4	0/3
JTRN/82/16	235	M	1,024	++	-	+	-	0/4	0/4	0/3
JTRN/82/17	158	F	2,048	+++	++	++	+	3/8 (1/4, 2/4)[2]	0/4	3/3[3]
JTRN/82/22	232	F	1,024	++	-	-	-	0/4	3/8 (1/4, 2/4)[2]	0/3
JTRN/82/24	285	F	2,048	+	-	-	-	2/8 (1/4, 1/4)[2]	0/4	0/3
JTRN/82/25	290	M	512	++	+	-	-	2/4	0/4	0/3
JTRN/82/27	225	F	1,024	++	-	-	-	0/4	0/4	0/3
JTRN/82/28	232	F	1,024	+++	++	++	+	3/4	0/4	0/3

[1] No. positive/no. inoculated.
[2] No. positive/no. tested on primary and re-isolation attempt.
[3] Infected cells with Hantaan virus were demonstrated by IFA. technique after three blind passages of cells.

Table 18.
Identity of strains of Hantaan and related virus isolated from wild R. norvegicus caught in Tokyo.

Virus	Reciprocal IF and N antibody titer of indicated serum			
	HFRS patient 81/745		Immune rabbit serum	
	Acute (day3)	Convalescent (day38)	IF antibody	LNI[1]
76/118 A549 cells (passage 16)	16	16,384	4,096	
JTRN/82/17 Vero cells (passage 12)	16	8,192	2,048	
76/118 Apodemus lung (passage 12)	16	8,192	2,048	4.3
JTRN/82/22 Apodemus lung (passage 4)	-	4,096	1,024	3.9
JTRN/82/17 Rat (Original)	16	8,192	2,048	
JTRN/82/28 Rat (Original)	-	8,192	2,048	

[1] LNI = log of the neutralizing index with indicated serum vs. homologous normal serum.

Table 19.
Growth and distribution of viral antigen by immunofluorescence in S.D. rats inoculated with strain of Hantaan-like virus, JTRN/82/11. isolated from wild R. norvegicus in Japan.

No. of rat	Presence of viral antigen in tissues at 30 days			
	lung	spleen	kidneys	liver
1	+++	-	-	-
2	++++	+	-	-
3	+++	+	-	-
4	+++	+	-	-
5	+++	+	-	-

Note: All rats were inoculated I.M. with 0.5 ml of 10% lung suspension.

The distribution of Hantaan viral antigen was graded on a scale of - to ++++.

Table 20.
Comparative titration of IF antibodies of sera from HFRS patients against strains of Hantaan and related virus isolated in Korea and in Japan.

Sera tested	Virus antigen grown in tissue culture cells and antibody titers of sera	
	AP/76-118 A549 cells	JTRN/82-17 Vero E6 cells
B-Normal-39	-	-
Reo-polyvalent	-	-
KHF-81-605-3	16,384	512
KHF-81-308-1	4,096	1,024
KHF-81-308-2	16,384	4,096
KHF-76-21-3	4,096	1,024
Sapporo MS-K	1,024	4,096
Sapporo MS-H	1,024	1,024
Niigata-JH3	4,096	4,096
Niigata-IH3	1,024	4,096
Kyoto MS-K2	1,024	4,096
Kyoto MS-K5	64	256
Kobe MS-1	64	256
Nagoya-CM1	64	256
Osaka-S1	1,024	4,096
Finland-277	256	128
Finland-317	512	128
Finland-323	512	256
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