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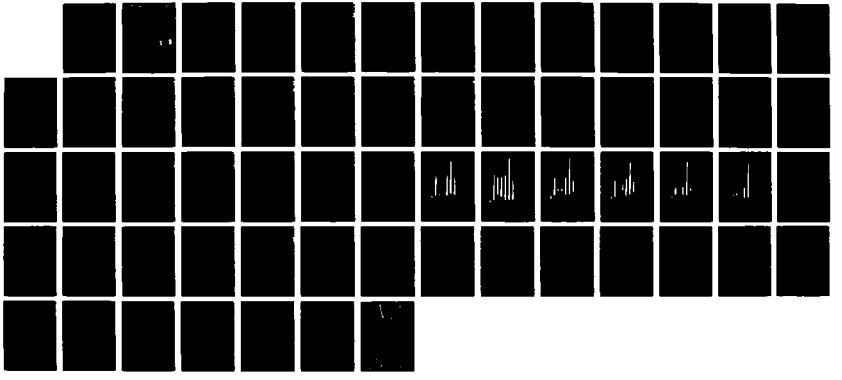

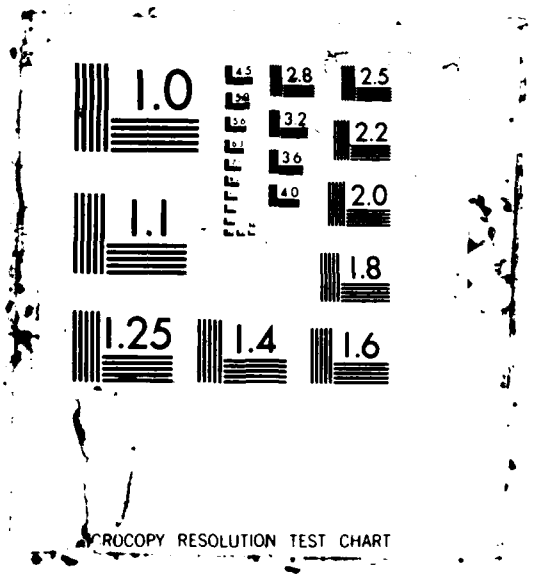
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HEMORRHAGIC FEVER WITH RENAL SYNDROME (KOREAN HEMORRHAGIC FEVER)

ANNUAL AND FINAL REPORT

HO WANG LEE, M.D.

23 July 1986

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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 problem since large epidemics of HFRS occurred among soldiers in the past wars. Although predominantly associated with field mice in rural areas, it is now being recognized that urban rats and laboratory rats are also reservoirs of Hantaan & Seoul viruses the etiologic agents of HFRS, in many parts of the world.

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Therefore, seroepidemiologic survey of distribution of Hantaviruses and surveillance of occurrences of HFRS in the world are urgently needed to prevent the further spreading of this highly fatal disease. It is also important to investigate antigenic differences of strains of Hantaviruses isolated from rats caught in different parts of the world because HFRS cases had never been documented in many areas despite our finding of positive rats there. Study to explore the mechanism of transmission of Seoul virus among rats was started already but consistant results are not obtained as of yet. Our preliminary experimental findings showed that HI test is a reliable serologic test for diagnosis of HFRS.

The methods for diagnosis of HFRS, isolation of Hantaviruses from man and rodents, intraspecific transmission of Hantaan virus in field mice and HI test are described previously.

There were about 700 cases of HFRS in Korea in 1984 and 1985, respectively and recently no. of HFRS patients are increasing in Seoul, and large epidemics of leptospirosis and Scrub typhus were occured during epidemic seasons of HFRS. Studies have demonstrated a near global distribution of Seoul virus among urban rats. We have isolated 8 and 13 strains of Hantaan virus from HFRS patients and Apodemus mice, respectively, 12 strains of Seoul virus from laboratory and urban rats and 1 strain of Seoul-like virus from a hamster in Vero E6 cells. A total of 41 strains of Hantavirus is presently on hand and preliminary results indicate that there are 4 distinct serotypes and may be more. Subclinical chronic infections characterized by transient viremia, prolonged virus shedding in saliva and virus persistence in tissues developed in laboratory and urban rats inoculated intramuscularly with Seoul virus. Horizontal transmission coincided with virus shedding in saliva and infectious virus was found in the lungs from 14 to 90 days and saliva collected 14-60 days postinoculation. A small amount of infectious virus was found in urine and feces 20-60 days postinoculation. HI test was a useful serologic diagnostic test to differentiate Hantaan and Seoul virus infection in man and rats. HFRS patients with clinical diagnosis of hepatitis, dengue and leptospirosis-like illness were confirmed in Tropic areas for the first time.

Hantaviruses are ubiquitous in the world and HFRS with diverse clinical symptoms will be a major public health problem through-out the world.

SUMMARY

In 1984 and 1985, there were 730 and 697 cases of hospitalized HFRS patients in Korea, respectively and large epidemics of leptospirosis and Scrub typhus were occured during epidemic seasons of HFRS. No. of HFRS patient in urban areas of Seoul is increasing every year. A near global distribution of Hantavirus infection in man and urban rats was demonstrated.

We have isolated 8 and 13 strains of Hantaan virus from bloods of HFRS patients and Apodemus mice, respectively, 12 strains of Seoul virus from laboratory and urban rats, and 1 strain of Seoul-like virus from a Syrian hamster in Vero E6 cell cultures. A total of 41 strains of Hantavirus is presently on hand and preliminary results indicate that there are 4 serotypes and may be more.

Infection and transmission of Seoul virus among rats resemble findings in Apodemus agrarius experimentally infected with Hantaan virus. Subclinical chronic infections characterized by transient viremia, prolonged virus shedding in saliva and virus persistence in tissues developed in laboratory and urban rats inoculated intramuscularly with Seoul virus. Horizontal transmission coincided with virus shedding in saliva and infectious virus was found in the lungs from 14 to 90 days and saliva collected 14-60 days postinoculation. A small amount of infectious virus was found in urine and feces 20-60 days postinoculation.

HI test with mouse brain hemagglutinins of Hantaan and Seoul virus could differentiate Hantaan and Seoul virus infection of HFRS patients and rats.

HFRS patients were confirmed serologically in Singapore, Malaysia and Hong Kong for the first time.

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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INTRODUCTION

During the Korean War more than 3,000 United Nations troops in Korea developed a rare hemorrhagic fever which attracted worldwide attention (1). Since then it has been known as Korean hemorrhagic fever (KHF). This disease is an important military problem because large epidemics have occurred among soldiers during several wars. More than 12,600 cases of epidemic hemorrhagic fever (EHF) occurred among Japanese soldiers in Manchuria (2) and several hundred cases occurred among Russian soldiers in the Far East (3) during World War II. Several thousand cases of war nephritis, clinically similar to Nephropathia epidemica (NE), were reported among British soldiers stationed in Flanders during World War I (4), and about 16,000 cases occurred among German soldiers in Lapland and prisoners in Yugoslavia during World War II (5). About 14,000 cases of war nephritis clinically similar to NE were described among Northern Armies in the American Civil War (6). In South Korea, 500 to 900 persons are hospitalized annually with this disease and about half of them are soldiers. There were 124,000 cases of HFRS in China in 1983 with 7% mortality, and several hundred cases of HFRS occurred in other countries of Asia and Europe (7). The causative agent was first discovered in 1976 from Apodemus mice (8) and isolated from patients in 1978 (9). The etiologic agent of KHF has been propagated in a human cell culture line (10), and it was named Hantaan virus after the Hantaan river which runs along the 38th Parallel between South and North Korea (11). Antigenic, genetic properties and EM findings indicated that Hantavirus is a new genus of Bunyaviridae (12,13,14,15). A close etiological relationship was established between KHF and HFRS in USSR, NE in Scandinavia and EHF in Japan and in China (9,16,17,18). The working group on HFRS at a WHO meeting in Tokyo, 1982 recommended that the above mentioned diseases with different names should be referred to as "Hemorrhagic Fever with Renal Syndrome (HFRS)" (7). Recent seroepidemiologic surveys showed that Hantaviruses are widely distributed throughout much of the world. Antibody against Hantaan virus in human sera were demonstrated in India, Thailand, Iran, Greece, U.S., Canada, Bolivia, Brazil, Gobaon and Republic of Central Africa (19,20, 21,22,23) and recently in Taiwan, Philippine, Malaysia, Singapore, Hong Kong, Fiji and Hawaii (24). Intraspecific transmission of Hantaan virus in Apodemus mice (25) was shown and infection occurred among cage-mates up to 360 days after infection, while large amounts of virus were excreted in urine, and no evidence for the participation of ectoparasites in virus transmission was obtained. Infection with Hantaan virus is thought to be silent in animals (26), but is associated with diverse clinical symptoms in man (27). A severe form is common in East Asia, while most European cases are mild. It usually produces sporadic disease, but under special circumstances epidemics occur. Although predominantly associated with rural areas, it is now being recognized as an urban problem in some countries (28,29)

and a particular hazard to laboratory staff using rodents for biomedical research (30,31,32). From 1975 to 1982, 126 cases of HFRS, of which one was fatal, 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 seven strains of Hantavirus were isolated from those rats. 203 urban rats caught in Japan yielded five isolates of Seoul virus, of which two strains were propagated in Vero E-6 cells (33). 215 urban rats caught in Incheon harbor yielded 14 isolates of Hantavirus, in which one strain was propagated in Vero E6 cells (34). Nineteen strains of Hantaan virus from blood of HFRS patients were isolated in Apodemus mice and 8 strains of Hantaan virus in Vero E6 cells cultures (35). Commercial rabbits bought from breeding firms in Korea and Japan were seropositive to Hantaan virus. Serum antibodies were found in 3.5% of 792 New Zealand rabbits (36). We have registered a Hantaan related virus isolated from an urban rat caught in Seoul in 1980 as Seoul virus in 1985 (37). This report describes a) new epidemiological features of HFRS in Korea b) serologic surveys for the presence of Hantavirus infection throughout the world c) the dynamics of infection and intraspecific transmission of Seoul virus in rats experimentally d) hemagglutination inhibition test for a differential diagnostic method and e) HFRS patients in tropic areas.

MATERIALS AND METHODS

Survey areas

Survey areas for reservoir of HFRS and isolation of Hantaan and related agents from field mice and urban rats were Manila harbor, Singapore, Hong Kong, urban areas of Kuala Lumpur and Cairo. Frozen lungs of rodents from above mentioned areas were shipped in dry ice to Seoul by Air Flight.

Collection of field and urban rodents

Field and house rodents were captured by means of baited live traps and normal Apodemus mice were captured on Jeju island as described (9,29). Seronegative Apodemus mice and Wistar rats were used as sensitive detectors for Hantavirus.

Processing rodents

Living rodents were identified and bled by cardiac puncture under chloroform anesthesia. Serum was separated for antibody titration. Necropsy tissues include lungs, liver, spleen, kidneys, and parotid glands. A portion of each organ was examined immediately by FA for Hantavirus antigen and the remaining portion were frozen at -70°C until processing for virus isolation.

Specimens from patients

Blood and urine were obtained from acute phase patients for virus isolation and sera collected from suspected HFRS patients were used for serodiagnosis. Larger amounts of hyperimmune conva-

lescent serum was collected from HFRS patients for experimental use.

Hantaviruses

All experimental and diagnostic work were done with Vero E-6 and A549 cells infected with Hantaan virus, strains 76/118, Lee and Hub/9/80 isolated from patient blood and adapted in Vero E-6 cells and Seoul virus, strains 80/39 and 82/3 isolated from Seoul and Incheon urban rats in Vero E-6 cells and JTRN82/17 isolated from a Japanese urban rat in Wistar rats and adapted in Vero E-6 cells. To titrate the virus from rat lungs, 10% lung suspensions are prepared with BSS containing 0.2 % bovine albumin clarified at 5,000 G for 20 min. at 4°C and supernatants are used as inoculum. The ID_{50} of strains 76/118 and Lee in Apodemus mice is $10^{6.3}$, $10^{7.2}$ and ID_{50} of strains 80/39 and JTRN 82/17 in Wistar rat is $10^{7.3}$ and $10^{6.8}$/1.0 ml, respectively. All strains of Hantaan and Seoul viruses are free from reovirus. It was proved by FA staining with polyvalent anti-reovirus immune sera and by antibody responses in rabbits and rats after inoculation of Hantaan and Seoul virus I.M.

Preparation of antisera

In addition to convalescent sera obtained from HFRS patients and antisera from naturally infected rats and mice, laboratory animals were used as a source of antibody. Sera from immunized rabbits and rats as well as hyperimmune mouse acutic fluids were employed.

Tissue culture cells

A549 (10) and Vero E6 cells (12) were grown as described previously and used for virus isolation, preparation of FA antigen and virus plaque assay.

Virus isolation

The details of techniques used for demonstration of Hantavirus antigen by IFAT and virus isolation from HFRS patients and animals in Vero E6 cell cultures and in animals have been described previously (9.31,33,35).

Demonstration of antigen and antibodies of HFRS by use of immunofluorescent antibody techniques (IFAT)

The techniques employed for demonstration of antibodies and antigens of Hantavirus in specimens from patients, rodents and other animals have been described in detail (9,29,35).

Plaque reduction neutralization test (PRNT)

Neutralizing antibody titers were determined by plaque reduction methods employing immunoperoxidase staining (38). Hantaan and Seoul virus plaques developed readily in 5 to 7 days under 0.5% methylcellulose. PRNT titers are expressed as the reciprocal of the highest dilution of serum resulting in 80% or greater reduction in the number of virus plaques.

Hemagglutination inhibition (HI) test

A modification of the Clarke-Casals method for the HI test (39) was used. Hantavirus hemagglutinating antigens were made in suckling ICR albino mice brain, less than 48 hrs old, by inoculation of 0.01 ml of virus previously adapted to mice by intracerebral passage. Infected mouse brains were harvested

about 14 days after inoculation of virus when about 20% of the mice have died. Infected suckling mouse brain suspensions were acetone treated and sonicated at 300W for 15'. The final pH of the antigen-serum-RBC mixtures for Hantaan virus 76/118 and Seoul virus 80/39 was 5.8 and 6.4, respectively.

RESULTS

A. New epidemiological features of HFRS and outbreaks of leptospirosis and rickettsiosis during epidemic seasons of HFRS in Korea.

1. New epidemiologic features of HFRS

There were 730 and 697 hospitalized cases of HFRS confirmed serologically at our Institute in 1984 and 1985, respectively and 13 of them were US Army soldiers as shown in Table 1. One of the new epidemiologic features of HFRS in Korea is increasing number of HFRS patients in urban areas of Seoul as shown in Table 2. There were about 100 cases of HFRS in Seoul city in 1984 and in 1985, respectively. These patients were only hospitalized severe cases but usually moderate and mild cases are not included because they were diagnosed clinically as influenza. Patients occur throughout the year but peak is in fall in urban areas of Seoul (Table 3). HFRS cases occur in all district of Seoul as shown in Table 4. Recent findings show that there is only one epidemic peak of HFRS in late fall in Korea as shown in Table 5, and there are an increasing no. of cases of HFRS among children (Table 6). Male patients are dominant group of HFRS as shown in Table 7 eventhough 159 male soldier patients were not included in the no. of male cases of HFRS.

2. Epidemic outbreaks of leptospirosis and rickettsiosis during epidemic season of HFRS

As shown in Table 8, total no. of confirmed cases of HFRS in 1985 is 690 among 2,114 HFRS suspected sera were tested. These suspected sera were sent to our laboratory from hospitals in and nearby cities of Seoul for serologic diagnosis of HFRS but HFRS was only 33% of total sera tested. During epidemic season of HFRS, we have tested 1,547 sera from suspected HFRS patients for leptospirosis and confirmed 435 cases (28%) of leptospirosis serologically and monthly incidence of leptospirosis was shown in Table 9. No. of leptospirosis patients among civilians were only patients diagnosed serologically from September to December 1985 but sera from soldier patients were tested throughout the year. There were 39 mixed infection patients with Hantaan virus and leptospira according to serologic tests. Furthermore, we have sent 324 non-HFRS and non-leptospirosis sera to NIH, Japan for serologic survey against rickettsia and 131 sera (40%) among these unknown patients were seropositive against R. tsutsugamushi. There were 7 cases of HFRS patients among 21 HFRS suspected sera from US soldiers hospitalized in U.S. Army hospital in Seoul but we did not tested these non-HFRS sera against leptospira and rickettsia. Monthly no. of confirmed cases of HFRS, leptospirosis and Scrub typhus are shown in Table 10.

Table 1.
Hospitalized cases of Hemorrhagic fever with renal syndrome patients in the Republic of Korea

Year	US forces	Korean soldiers	Korean civilians	Total
1951	827	...	...	827
1952	833	...	...	833
1953	455	...	...	455
1954	307	...	19	326
1955	20	...	...	20
1956	28	26	...	54
1957	13	21	...	34
1958	15	20	...	35
1959	79	47	...	126
1960	10	185	...	195
1961	27	341	...	368
1962	29	311	...	340
1963	11	257	...	268
1964	22	205	18	245
1965	99	110	2	211
1966	36	82	11	129
1967	31	86	13	130
1968	28	102	26	156
1969	9	134	48	191
1970	13	221	131	365
1971	2	358	391	751
1972	0	203	186	389
1973	0	237	241	478
1974	0	251	176	427
1975	1	370	466	837
1976	4	304	585	893
1977	7	241	288	536
1978	10	168	207	385
1979	1	122	241	364
1980	1	72	185	258
1981	2	164	377	543
1982	3	123	378	504
1983	3	98	402	503
1984	6	156	568	730
1985	7	159	531	697
Total	2,939	5,174	5,490	13,603

Nos. of patients since 1977 are serologically confirmed cases at The Institute of Viral Diseases, Korea University.

Table 2.
Number of serologically confirmed hospitalized Hemorrhagic fever with renal syndrome patients in Provinces of the Republic of Korea from 1980 to 1985

Province	Number of patients					
	1980	1981	1982	1983	1984	1985
Seoul City	18	65	73	46	91	70
Kyungkido	82	143	146	145	240	240
Chungcheongdo	44	89	101	44	125	109
Kangwondo	18	67	37	128	67	62
Kyungsangdo	17	6	14	23	21	20
Chulrado	6	7	7	16	24	30
Total	185	377	378	402	568	531

Table 3.
Monthly incidence of serologically confirmed Hemorrhagic fever with renal syndrome patients in metropolitan areas of Seoul from 1980 to 1985

Year	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
1980	2	0	0	1	0	1	0	1	0	1	8	4	18
1981	3	1	0	1	0	1	1	0	0	14	29	15	65
1982	6	0	4	4	1	0	2	3	5	10	22	16	73
1983	12	1	0	1	4	0	0	0	0	4	16	8	46
1984	4	1	4	6	0	3	4	1	3	15	34	15	91
1985	6	1	4	0	5	2	3	4	3	4	22	16	70
Total	33	4	12	13	10	7	10	9	11	48	131	75	363

Table 4.
Number of Hemorrhagic fever with renal syndrome patients in district of Seoul, 1981 - 1985

Name of district	1981	1982	1983	1984	1985
Sungbuk-ku	5	5	2	8	3
Tobong-ku	4	6	6	7	8
Tongdaemun-ku	5	8	5	5	2
Chongro-ku	1	3	4	2	4
Chung-ku	3	2	0	3	4
Yongsan-ku	2	2	0	4	3
Mapo-ku	0	2	1	3	3
Seungdong-ku	6	12	7	6	5
Seodaemun-ku	3	1	3	3	2
Eunpyung-ku	3	3	4	4	2
Kuro-ku	3	0	4	8	9
Yungdungpo-ku	9	4	0	4	2
Kwanak-ku	6	5	2	4	5
Kangnam-ku	6	14	3	10	5
Kangdong-ku	4	5	4	6	7
Tongzak-ku	3	0	1	6	1
Kangseo-ku	2	1	0	8	5
Total	65	73	46	91	70

Table 5.
Monthly incidence of Hemorrhagic fever with renal syndrome patients in the Republic of Korea, 1966 - 1985

Year	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
1966	2	3	3	1	4	9	6	2	1	16	56	26	129
1967	2	1	0	1	4	10	2	4	8	29	50	19	130
1968	3	1	0	4	7	9	7	6	8	40	50	21	156
1969	4	0	4	1	8	12	7	8	5	41	66	35	191
1970	1	0	0	1	6	9	8	1	15	58	154	112	365
1971	13	1	2	7	14	23	13	19	33	140	348	148	761
1972	15	5	5	12	17	27	16	10	18	80	142	42	389
1973	12	3	3	4	6	10	11	13	19	117	211	69	478
1974	11	0	1	7	17	13	13	10	19	113	151	72	427
1975	25	5	3	3	8	32	22	22	27	177	360	153	837
1976	40	12	5	11	12	36	46	33	111	156	319	112	893
1977	7	0	0	2	8	57	21	19	29	93	226	74	536
1978	17	8	2	2	11	10	11	9	9	78	156	93	406
1979	12	4	6	7	21	16	21	12	9	79	124	53	364
1980	19	6	4	8	14	11	5	5	6	40	74	66	258
1981	12	7	1	4	4	17	21	6	15	80	233	143	543
1982	44	11	10	9	15	13	16	15	15	79	178	99	504
1983	34	7	2	5	9	16	16	3	13	60	186	152	503
1984	35	7	8	10	13	24	12	10	13	125	304	169	730
1985	45	18	12	8	21	32	21	21	12	74	254	181	699
Total	353	99	71	107	219	386	295	228	385	1,675	3,642	1,839	9,299

Table 6.
Incidence of serologically confirmed Hemorrhagic fever with renal syndrome by age group in the Republic of Korea, 1979 - 1985

Age group	Period						
	1979	1980	1981	1982	1983	1984	1985
1 - 4	0	0	0	0	0	0	0
5 - 9	2	0	1	3	1	1	1
10 - 14	2	1	2	4	2	13	9
15 - 24	198	103	197	145	134	354	217
25 - 44	121	86	180	201	187	193	239
45 - 64	41	62	143	137	159	148	191
65 +	6	5	18	11	20	21	33
Total	370	257	541	501	503	730	690

Table 7.
Number of Hemorrhagic fever with renal syndrome patients by sex and month in 1985

Sex	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Female	10	6	1	1	6	10	6	5	3	17	64	42	171
Male	35	12	11	7	15	22	15	16	9	57	190	130	519

Table 8.
Number of Hemorrhagic fever with renal syndrome, leptospirosis and rickettsiosis confirmed serologically at The Institute of Viral Diseases, Korea University in 1985

Korean

$$\frac{\text{Total no. of HFRS patient}}{\text{Total no. of HFRS suspected serum tested}} = \frac{690}{2,114} \ (33\%)$$

$$\frac{\text{Total no. of leptospirosis patient}}{\text{Total no. HFRS suspected serum tested}} = \frac{435}{1,547} \ (28\%)$$

$$\frac{\text{No. of rickettsiosis patient}}{\text{Total no. of non-HFRS and non leptospirosis serum tested}} = \frac{131}{324} \ (40\%)$$

$$\frac{\text{Total no. of un-identified patient}}{\text{Total no. of HFRS suspected serum tested}} = \frac{858}{2,114} \ (41\%)$$

US soldier

$$\frac{\text{Total no. of HFRS}}{\text{Total no. of HFRS suspected serum tested}} = \frac{7}{21} \ (33\%)$$

All of the patients are hospitalized cases at the hospitals in and nearby cities of Seoul.

Table 9.
Number of serologically confirmed hospitalized HFRS, leptospirosis and rickettsiosis patients at Institute of Viral Diseases, Korea University in Korea in 1985

Month	No. of serologically diagnosed HFRS / No. of tested		No. of serologically diagnosed leptospirosis / No. of tested		No. of serologically diagnosed rickettsiosis / No. of tested	
	Civilian	Soldier	Civilian	Soldier	Civilian	Soldier
1	45/107	0/1	n.t.	0/1	n.t.	0/1
2	15/61	3/20	n.t.	0/20	n.t.	0/20
3	10/32	2/3	n.t.	0/3	n.t.	0/3
4	6/45	2/7	n.t.	0/7	n.t.	0/7
5	18/65	3/10	n.t.	0/10	n.t.	0/10
6	23/61	9/15	n.t.	1/15	n.t.	0/15
7	17/52	4/8	n.t.	0/8	n.t.	0/8
8	17/75	4/5	n.t.	0/5	n.t.	0/5
9	10/83	2/4	42/83	2/4	n.t.	0/4
10	55/293	19/82	75/293	41/82	37/293	3/82
11	178/593	76/140	161/593	36/140	72/593	17/140
12	137/303	35/49	73/303	5/49	n.t.	0/49
Total	531/1770	159/344	351/1272	84/344	109/886	20/344

Table 10.
Number of confirmed hospitalized HFRS, leptospirosis and mixed infection patients serologically at The Institute for Viral Diseases, Korea University in Korea in 1985

Month	No. of serologically diagnosed HFRS		No. of serologically diagnosed leptospirosis		No. of mixed infection with HFRS & leptospirosis	
	No. of tested		No. of tested		No. of tested	
	Civilian	Soldier	Civilian	Soldier	Civilian	Soldier
1	45/107	0/1	n.t.	0/1	n.t.	0/1
2	15/61	3/20	n.t.	0/20	n.t.	0/20
3	10/32	2/3	n.t.	0/3	n.t.	0/3
4	6/45	2/7	n.t.	0/7	n.t.	0/7
5	18/65	3/10	n.t.	0/10	n.t.	0/10
6	23/61	9/15	n.t.	1/15	n.t.	0/15
7	17/52	4/8	n.t.	0/8	n.t.	0/8
8	17/75	4/5	n.t.	0/5	n.t.	0/5
9	10/83	2/4	42/83	2/4	3/83	0/4
10	55/293	19/82	75/293	41/82	5/293	1/82
11	178/593	76/140	161/593	36/140	21/593	5/140
12	137/303	35/49	73/303	5/49	3/303	1/49
Total	531/1770	159/344	351/1272	84/344	32/1272	7/275

B. Seroepidemiologic survey of distribution of Hantaviruses and isolation of Hantaviruses from urban rats in the world.

1. Collaborations with laboratories of the world

From 1980 to 1985, we have supplied Hantaan and Seoul virus that were isolated from HFRS patients, urban and laboratory rats to Drs. J. Dalrymple and C. Gajdusek in U.S.A., Dr. C. Kang in Canada, Drs. T. Yamanouchi, T. Kitamura, K. Yamanishi, T. Tamura and Dr. A. Tanaka in Japan, Dr. F. Lian in China, Dr. S. Antoniades in Greece, Dr. V. Dhanda in India, Dr. K. Chang in Hong Kong, Dr. T. Wong in Singapore, Dr. S. Ambu in Malaysia, Dr. L. Zoller in W. Germany and Dr. T. Chang in Taiwan. We have also supplied several hundreds killed Hantaan virus antigen spot slides to Drs. T. Tamura, A. Tanaka, J. Kawamata, A. Oya, A. Tomiyama, V. Dhanda and F. Lian. We have received some strains of Hantavirus from Drs. T. Kitamura, J. Dalrymple, J. LeDuc, P.W. Lee, G. van der Groen, S. Drozdov and C. Kang and monoclonal antibodies made from Hantaan virus 76/118 from Drs. J. Dalrymple, J. McCormick and K. Yamanishi. We have been collaborating with Drs. N.Y. Agustino, S.B. Villarubio and J. Cross in Manila, Drs. T.W. Wong and C.Y. Cheong in Singapore, Drs. S. Ambu, T.W. Lim and S.K. Lam in Malaysia, Drs. W.K. Chang and K.F. Shortridge in Hong Kong, Dr. J.U. Mataika in Fiji, Drs. T. Tamura, J. Kawamata, T. Yamanouchi, H. Takada and T. Tanaka in Japan, Dr. H. Hoogstraal in Cairo, Dr. A. Diwan in Hawaii, Drs. M. Kalunda and I. Chu in Uganda, Dr. C. Kang in Canada and Dr. Pavri in India, Drs. P. Pilaski, L. Zoller in Germany Dr. C. Charlo in France and Dr. M. Weissenbacker in Argentina, to investigate the distribution of Hantavirus in the world. In July 1985, we have distributed the following reagents and protocols of techniques for standardization of techniques to be used for serologic diagnosis of HFRS to the participating laboratories according to the recommendation of the WHO Ad Hoc meeting in Antewerp, 1983 and are expecting to receive the results for evaluation of data.

1) Virus:
   a) Hantaan virus, 76/118 b) Puumala virus, NE/Fin
   c) USSR/CLS1/452 d) Seoul virus, 80/39.
2) Serum:
   a) ROK81-499-5, convalescent serum from Korean hemorrhagic fever patient b) Fin-79-278, convalescent serum from Nephropathia epidemica patient c) USNS/80H, negative human serum.
3) Standard protocol:
   a) IFA technique b) Plaque reduction neutralization test c) ELISA technique d) IAHA test e) HI test.

2. Global center for HFRS serology and virus isolation

As WHO Collaborating Centre for Research on Hemorrhagic

Table 11.
Seroepidemiologic survey of Hantavirus among human and rodent in some parts of the world where HFRS is not known to exist from 1981 to 1985 at WHO Collaborating Centre for Virus Reference and Research (HFRS), Seoul

Country	No. of IF antibody positive to Hantaan virus/No. tested			
	Human	Urban rats	Laboratory rats	Mice
Hong Kong	16/322[1] (5.0%)	26/140[5] (18.6%)	3/62 (4.8%)	0/40
Philippines	20/400 (5.0%)	86/167 (51.5%)		
Malaysia	3/329[2] (1.0%)	10/204 (4.9%)	42/154 (27.3%)	
Singapore	2/21[3] (9.5%)	6/52[6] (11.5%)	5/38 (13.2%)	
Taiwan	31/240[4] (13.0%)			
India	1/89 (1.1%)			
Fiji	8/145 (5.5%)	6/98 (6.1%)		0/3
Hawaii	15/252 (6.9%)	131/1,482 (8.8%)		0/22
Egypt	6/458 (1.3%)	499/2,499[7] (20.0%)		5/71 (7.0%)
Sudan		28/352 (8.0%)		
Uganda	15/355 (4.3%)	3/64 (4.7%)		
Brazil	37/500 (7.4%)			
Canada	29/2,063 (1.4%)			

[1] : Two HFRS patients were confirmed serologically.
[2] : Several HFRS patients were confirmed serologically.
[3] : One HFRS patient was confirmed serologically.
[4] : Several HFRS patients were confirmed serologically.
[5] : Four strains of Hantavirus were isolates in Vero E6 cells.
[6] : One strain of Hantavirus was isolated in Vero E6 cells.
[7] : Two strains of Hantavirus were isolated in Vero E6 cells.

fever with renal syndrome (HFRS), we have provided serological diagnosis for suspect HFRS in sera from throughout the world, but especially from the Asian region. In addition, we have collaborated with a number of investigators conducting small mammal surveys for evidence of Hantavirus infection and isolation of strains from host animal tissues. Results of these preliminary studies indicate that human disease due to Hantavirus infection is present in several areas where HFRS had not been previously diagnosed. The results of the serosurvey of Hantaviruses among rats and human populations in many parts of the world where HFRS patients are not known to exist are shown in Table 11.

Human sera from 12 countries; 7 countries in Pacific Ocean, 1 country in North America, 1 country in South America and 3 countries in Africa were found to have IF antibodies to Hantaan virus as shown in the table. The prevalence rate of antibodies to Hantaan virus was between 1.1% - 9.5%, data much higher than those of residents of Seoul, the endemic area of HFRS. Very recently, we have confirmed HFRS patients serologically among hospitalized patients in Hong Kong, Taiwan, Singapore and Malaysia.

Urban rat sera from the Philippines, Hong Kong, Malaysia, India, Singapore, Fiji, Hawaii, Egypt, Sudan and Uganda were also found to have IF antibodies to Hantaan virus with a high prevalence rate of 51.5% among Philippine rats and 20.0% in Egypt rats.

Forty two out of 154 laboratory-bread white rats from two institutions in Malaysia and several Wistar rats from Hong Kong and Singapore were sero-positive against Hantaan virus. Five out of 71 house mice from Egypt were also positive to Hantaan virus. Clearly the genus Hantavirus is a near global distribution and maintained in a variety of different ecological settings. The degree to which Hantaviruses cause human disease, especially in areas where HFRS has not been traditionally recognized, is presently unknown.

3. Antigenic comparison of Hantaviruses by monoclonal antibodies.

HFRS is caused by a number of serologically related viruses. From a recent biochemical analysis the viruses contain genomes consisting of three segments of single stranded RNA and have an RNA-protein structure consistent with classification in the family Bunyaviridae (13). Numerous viruses have been described but not all have been associated with the disease. Studies using monoclonal antibodies have revealed significant antigenic variation within the group of viruses as shown in Table 12. Hantaan and Seoul viruses are pathogenic to suckling mice when the virus is inoculated intracerebrally. There

Table 12.
Comparative FA titers of monoclonal antibodies against different strains of Hantaan and related virus

No.	Virus strain	Monoclonal antibodies									KHF Pt's serum 81-605
		BD01-BB08	HC02-BE08	HC02-BD05	FD03-AF03	MCAb-33-B	MCAb-40-A	FD03-AA11	EC02-BE04	MCAb-80-A	
1	KHF83-61	8,192	256	2,048	16	64	32	256	256	8,192	4,096
2	Lee #188604	8,192	1,024	1,024	128	-	-	128	1,024	8,192	2,048
3	76-118#050323	8,192	256	1,024	128	-	-	128	4,096	4,096	2,048
4	Apo. 76-118	8,192	256	256	-	64	32	128	4,096	8,192	8,192
5	Apo. 79-89	8,192	64	256	-	64	-	128	2,048	8,192	8,192
6	Apo. 83-14	4,096	64	64	-	32	-	128	512	8,192	4,096
7	Apo. 83-138	4,096	64	-	-	64	32	128	2,048	8,192	8,192
8	KHF 83-109	-	512	2,048	-	-	-	256	2,048	8,192	4,096
9	CHV-78	-	-	-	128	-	-	128	4,096	8,192	4,096
10	S/RN 80-39	-	1,024	1,024	-	-	-	32	4,096	8,192	2,048
11	SR-11	-	1,024	1,024	32	-	-	128	4,096	8,192	4,096
12	B-1	-	1,024	1,024	32	-	-	128	4,096	8,192	2,048
13	Girard pt	-	1,024	1,024	32	-	-	128	1,024	8,192	4,096
14	Tchoupitoulas	-	1,024	1,024	32	-	-	32	64	8,192	2,048
15	I/RN 82-3	-	-	-	-	-	-	-	2,048	8,192	1,024
16	JTRN 82-17	-	-	-	-	-	-	256	2,048	8,192	1,024
17	TR-352	-	-	-	32	-	-	128	4,096	8,192	4,096
18	NE-Finn	128	-	-	-	256	-	128	512	4,096	8,192
19	Prospect Hill	-	-	-	-	-	-	-	-	256	256

Table 13.
List of stock Hantaviruses adapted in Vero E-6 cells at WHO Collaborating Centre for Virus Reference and Research (HFRS), The Institute for Viral Diseases, Korea University, Seoul by February 1986

A. Human strain:

1. ROK79/89	(blood)	Korea	7. US84/2	(serum)	Korea
2. ROK79/90	(blood)	Korea	8. ROK84/105	(serum)	Korea
3. ROK79/237	(blood)	Korea	9. Hubei/1	(serum)	China
4. LEE#188604	(blood)	Korea	10. Hubei/2	(serum)	China
5. ROK83/61	(blood)	Korea	11. Hubei/3	(serum)	China
6. ROK83/109	(serum)	Korea			

B. Apodemus mice strain:

1. 76/118	Korea	8. 83/15	Korea
2. 76/309	Korea	9. 83/18	Korea
3. 78/197	Korea	10. 83/23	Korea
4. 83/7	Korea	11. 83/27	Korea
5. 83/10	Korea	12. 83/125	Korea
6. 83/11	Korea	13. 83/138	Korea
7. 83/14	Korea	14. TCM/2508/84	Yugoslavia

C. Urban rat strain:

1. 80/39(#211808)	Korea	8. Egypt R/13120	Egypt
2. I/RN/82/3	Korea	9. Thailand #605	Thailand
3. Girard Pt. #820132	USA	10. Brazil 2-4	Brazil
4. Tchoupitoulas #401613	USA	11. Hong Kong R/14	Hong Kong
5. JTRN/82/17	Japan	12. Hong Kong R/19	Hong Kong
6. TR-352 VE8	Japan	13. Hong Kong R/35	Hong Kong
7. Egypt R/12915	Egypt	14. Hong Kong R/40	Hong Kong
		15. Singapore R/36	Singapore

D. Laboratory rat strain:

1. KSNUSD 84/30	Korea	3. SR-11 #191811	Japan
2. KSNUSD 84/34	Korea	4. B-1 strain	Japan

E. Clethrionomys mice strain:

1. NE-Finn	Finland	3. RUV/38-83	USSR
2. USSR/CLS1/452	USSR	4. CG/18-20	USSR

F. Microtus mice strain:

1. Prospect Hill USA

G. Hamster strain:

1. SNUS/Hamster 85/4 Korea

H. Bandicota indica strain:

1. Thailand #749 Thailand

is no animal model yet that produces clinical symptoms similar to those of HFRS patients.

Preliminary results indicate that 4 distinct viruses compose the genus Hantavirus (15): Hantaan, Puumala, Seoul and Prospect Hill viruses. It appears that several subtypes of strains exist within each virus type and epidemiological evidence suggests that other Hantaviruses still exist unrecognized in nature. Our preliminary experiments suggest that there are two serotypes of Hantaan virus in Korea: human isolates from HFRS patients and isolates from naturally infected Apodemus mice. There are also three serotypes of Seoul virus in Asia: the first represented by isolates from HFRS patients and urban rats in Seoul, the second from HFRS in China, and the third from Incheon and Tokyo rats. Our recent experience with virus isolates from Egypt, Singapore, and Hong Kong suggests that multiple antigenic serotypes exist. A serologic classification is thus needed to clearly differentiate specific Hantaviruses and to aid in the recognition of new viruses.

4. Stock of Hantaviruses isolated from man and rodents in the world at WHO Collaborating Centre.

A total of 41 strains of Hantaviruses is presently on hand in our laboratory as shown in Table 13 and available for serological characterization. We have isolated 8 strains from HFRS patients, 13 strains from Apodemus mice, 10 strains from urban rats, 2 strains from laboratory rats and 1 strain from golden hamster in Vero E6 cells until 1985. All other strains of Hantaviruses in Table 13 were obtained from scientists from other countries as described in section 1.

C. Infection and intraspecific transmission of Seoul virus in laboratory and urban rats.

Experimenatal parometers of infection and intraspecific transmission of several strains of Seoul virus adapted to grow well in rats were determined. Subclinical chronic infection characterized by transient viremia, prolonged virus shedding in saliva and short period virus excretions in urine and feces, and virus persistence in tissues, particularly lungs, developed in rats inoculated intramuscularly with Seoul virus, the etiologic agent of HFRS.

1. Antibody responses to Seoul virus after inoculation into and S.D. rats and R. norvegicus

IF antibody response curves against Seoul virus strain (80/39) isolated from R. norvegicus in Seoul after inoculation into R. norvegicus by the intramuscular route are shown in Fig. 1. Antibodies were produced about two weeks after inoculation of the virus and reached maximum on 35-45 days and then decreased slowly but still demonstrable on 90 days after inoculation. IF antibody response curves against Seoul virus strain (80/39) after inoculation of the virus into S.D. rats by the intramuscular route are shown in Fig. 2. Antibody response patterns

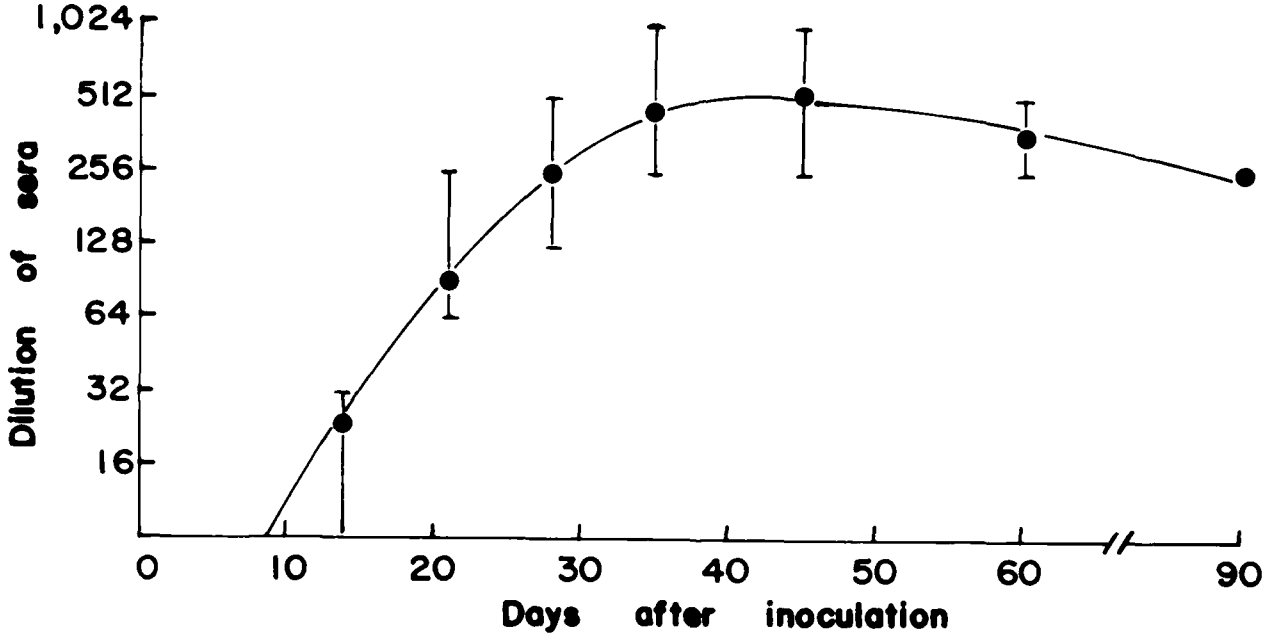

Fig. 1. Immunofluorescent antibody responses against Seoul virus (HR 80-39 WR_{p6}) after inoculation into Rattus norvegicus.
●: mean value —: range of antibody titer

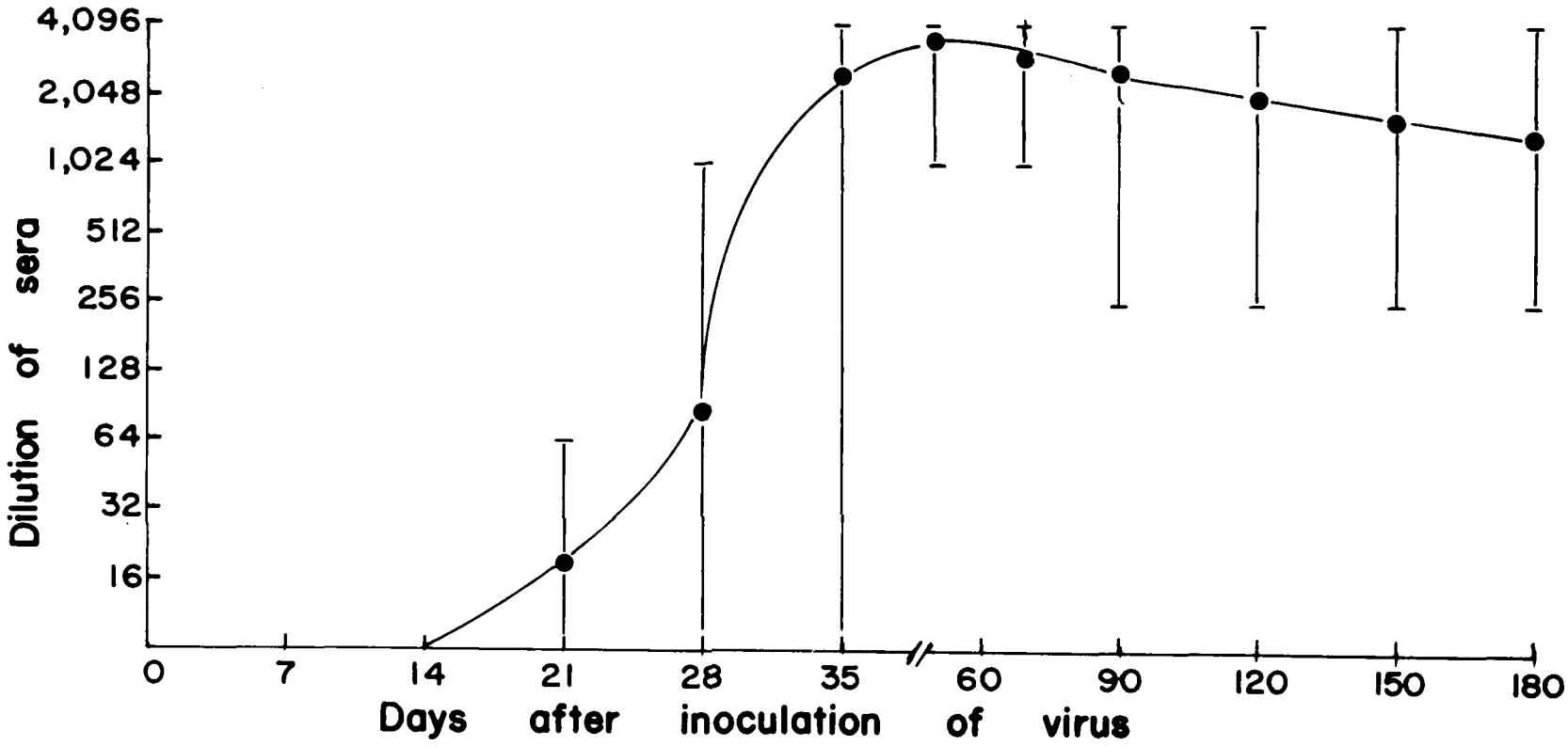

Fig. 2. Immunofluorescent antibody responses to Seoul virus (HR80-39/WRP6 + SDP3) after inoculation into S.D. rats.
●: mean value —: range of antibody titer

are almost same as in R. norvegicus but more amounts of antibodies were produced, reached peak on about 40 days and then slowly declined and demonstrable untill on 180 days after inoculation of the virus.

2. Growth and distribution of Seoul virus antigen in the tissues of R. norvegicus

As shown in table 14, Seoul viral antigens as evidence by granular fluorescence were demonstrated in various tissues of rats after inoculation of the virus strain 80/39 intramuscularly. Large quantities of virus antigens were persisted for 90 days until examined in lung tissues and for about 30 days in liver, kidney spleen and parotid gland. Some amounts of viral antigens were also detected in lacrimal glands, urinary blader and small intestine. There are always some rats that did not show viral antigen in the tissues after inoculation of the virus at the same time with same dose of virus though antibody responses of the rats were very good.

3. Infectivity of excreta from infected S.D. rats with Seoul virus

Tables 15 and 16 show quantitative infectivity of excreta from S.D. rats inoculated with Seoul virus, strain 80/39 isolated from Seoul urban rat and strain JTRN82/11 isolated from Tokyo urban rat. Large quantities of infectious virus was excreted in saliva for about two months from 18 to 100 days and from 18 to 70 days postinoalation with two different strains of virus, respectively but less amounts of infectious virus was found in urine and feces for a month or less.

4. Infection and intraspecific transmission of Seoul virus in rats

Horizontal transmission coincided with virus shedding in oropharyngeal secretions the period of transmission of Seoul virus from infected rats to normal rats is about one to two months depend on virus strains and rats while large quantities of virus is excreted in saliva. This result is in contrast with Apodemus mice that excrete Hantaan virus in urine for an year post-inoculation.

The course of infection and infectivity of Seoul virus strains, 80/39 isolated from Seoul urban rat, JTRN82/11 isolated from Tokyo bay rat and I/HR 82/216 isolated from Incheon harbor rat, in S.D. rats and R. rattus are shown in Figs. 3,4,5,6,7 and 8. There are strain differences in terms of infectivity of saliva, urine and feces in S.D. rats and R. rattus.

D. Hemagglutination inhibition test for a differential diagnostic method of Hantaan and Seoul virus infection.

Tsai et al's (39) a modification of the Clarke-Casals method for HI test was used. The hemagglutinins were pre-

Table 14.
Demonstration and distribution of immunofluorescent antigen of Seoul virus in the tissues of Rattus norvegicus after experimental inoculation

Virus strain, dose & route of inoculation	Tissue	Presence of Seoul virus antigen in various tissues on days after inoculation.							
		7	14	21	28	36	48	60	90
Seoul virus, HR80/39 WR-P6, 10% Lung susp. 0.5ml/IM	Lungs	-* -- 0/4** -	++ ++ 3/4 +++	- +++ 2/3	- - 2/4 -	++ ++++ 3/4 ++++	- +++ 2/4 ++++	++ + 3/4 +	+++ - 1/4 -
	Kidneys	- - 0/4 -	- - 0/4 -	- - 1/3	- - 0/4 -	+ + 3/4 ++	- ++ 2/4 +	- - 0/4 -	+ - 1/4 -
	Liver	- - 0/4 -	+ - 2/4 +	- ++ 2/3	- - 0/4 -	+ ++ 3/4 ++	- ++ 2/4 ++	- - 0/4 -	- - 0/4 -
	Spleen	- - 0/4 -	+ - 1/4 -	- + 2/3 +++	- - 0/4 -	- - 2/4 ++ 3/4 +++	- ++ 2/4 +++	- - 0/4 -	++ - 1/4 -
	Parotid glands	- - 0/4 -	- - 0/4 -	- + 2/3	- - 0/4 -	- - 1/4 +	- ++ 2/4 +	- - 0/4 -	- - 0/4 -
	Lacrimal glands	- - 0/4 -	- - 0/4 -	- - 2/3	- - 0/4 -	- - 0/4 -	- - 0/4 -	- - 0/4 -	- - 0/4 -
	Urinary blader	- - 0/4 -	- - 0/4 -	- ++ 1/3	- - 0/4 -	- - 1/4 +	- - 0/4 -	- - 0/4 -	- - 0/4 -
	Intestine	- - 0/4 -	- - 0/4 -	- ++ 1/3	- - 0/4 -	- - 0/4 -	- - 0/4 -	- - 0/4 -	- - 0/4 -

* : Distribution of immunofluorescent antigen of Seoul virus in the tissues from Rattus norvegicus was graded as - or + (from + to ++++).

** : No. of viral antigen positive/no. of inoculated.

Table 15.
Quantitative infectivity of excreta from S.D. rats inoculated with Seoul virus, HR80-39/WRP6-SDP3 (Seoul strain)

Excreta	S.D. rat $-\log_{10}$ ID_{50}/0.5 ml on days after virus inoculation										
	14	18	21	28	35	50	70	90	100	120	150 day
Saliva	0	1.0	3.0	1.5	≥4.0	≥4.0	2.0	2.4	1.2	0	0
Urine	0	0	0	0	≥2.0	≥2.0	1.0	1.0	0	0	
Feces	0	0	0	≥2.0	1.4	1.0	1.0	0	0	0	

Table 16.
Quantitative infectivity of excreta from S.D. rats inoculated with Seoul virus, JTRN82-11/SDP4 (Tokyo strain)

Excreta	S.D. rat $-\log_{10}$ ID_{50}/0.5 ml on days after virus inoculation									
	14	18	21	28	35	50	70	90	120	150 day
Saliva	0	2.5	3.0	2.0	3.0	3.0	1.5	0	0	0
Urine	0	0	0	0	1.0	0	0			
Feces	0	0	0	0	2.0	0	0			

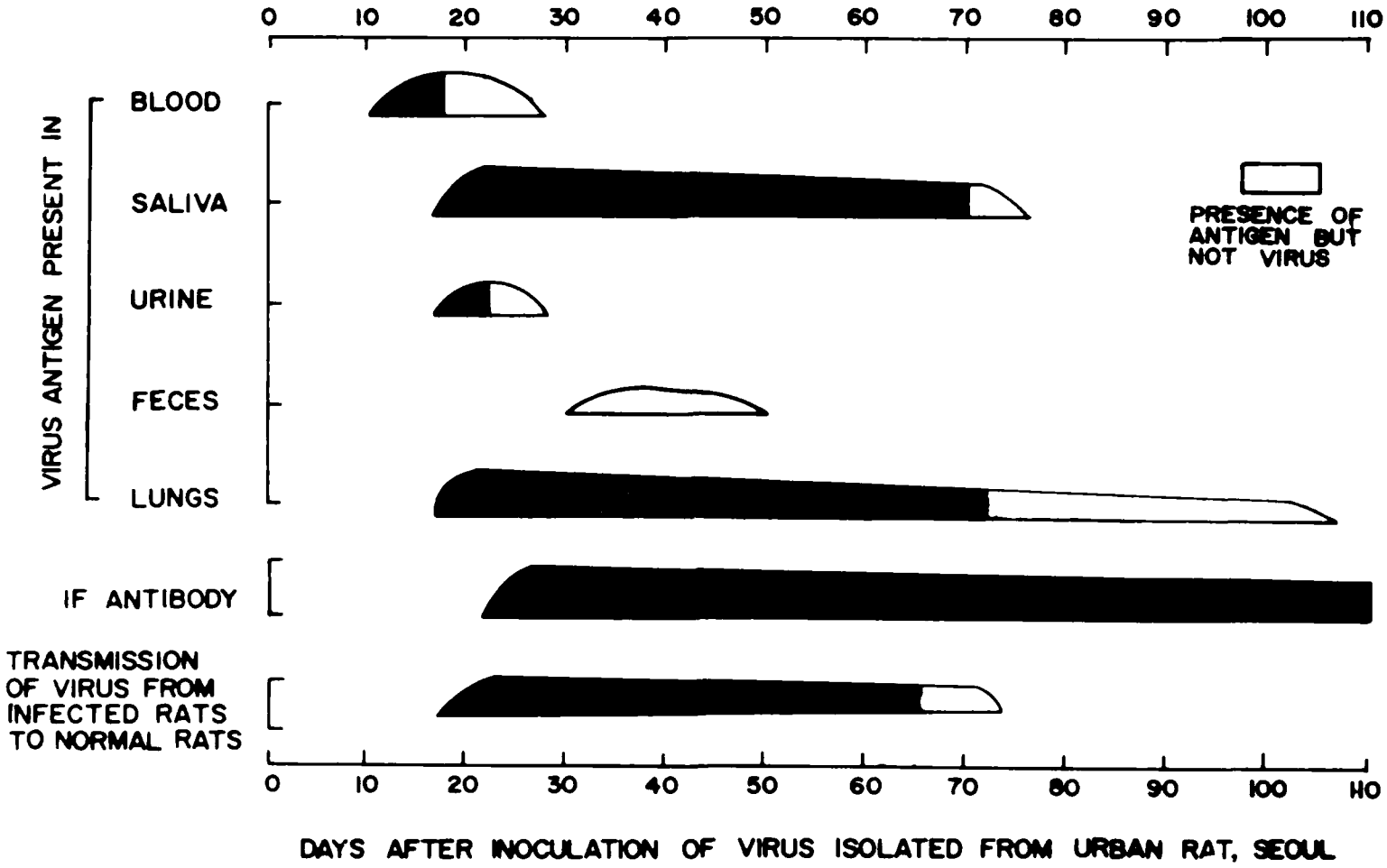

FIG. 3. THE COURSE OF INFECTION AND INFECTIVITY OF SEOUL VIRUS (HR 80-39/WR P6) IN S.D. RAT.

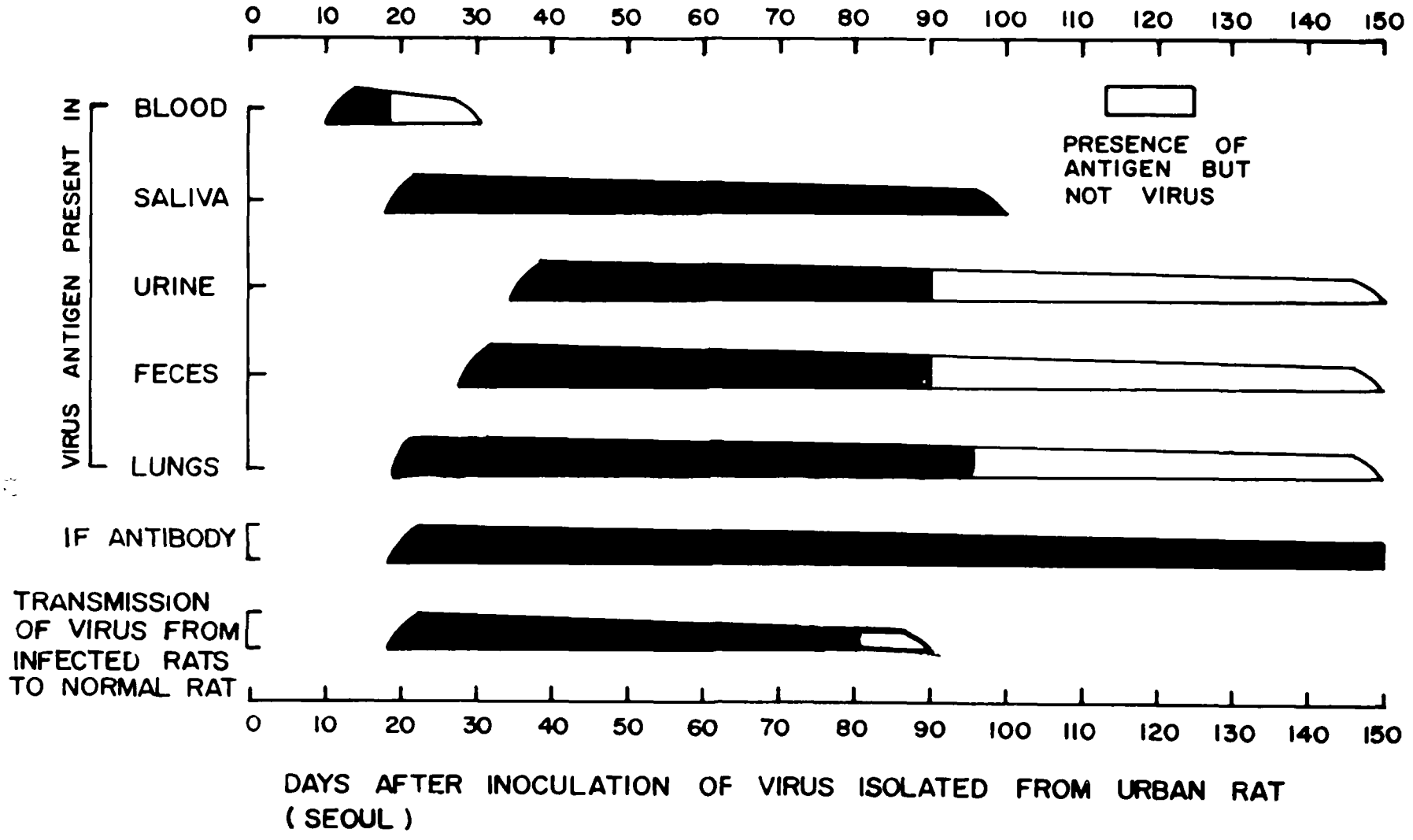

FIG. 4. THE COURSE OF INFECTION AND INFECTIVITY OF SEOUL VIRUS (HR 80-39/WRp6-SDp3) IN S.D. RAT.

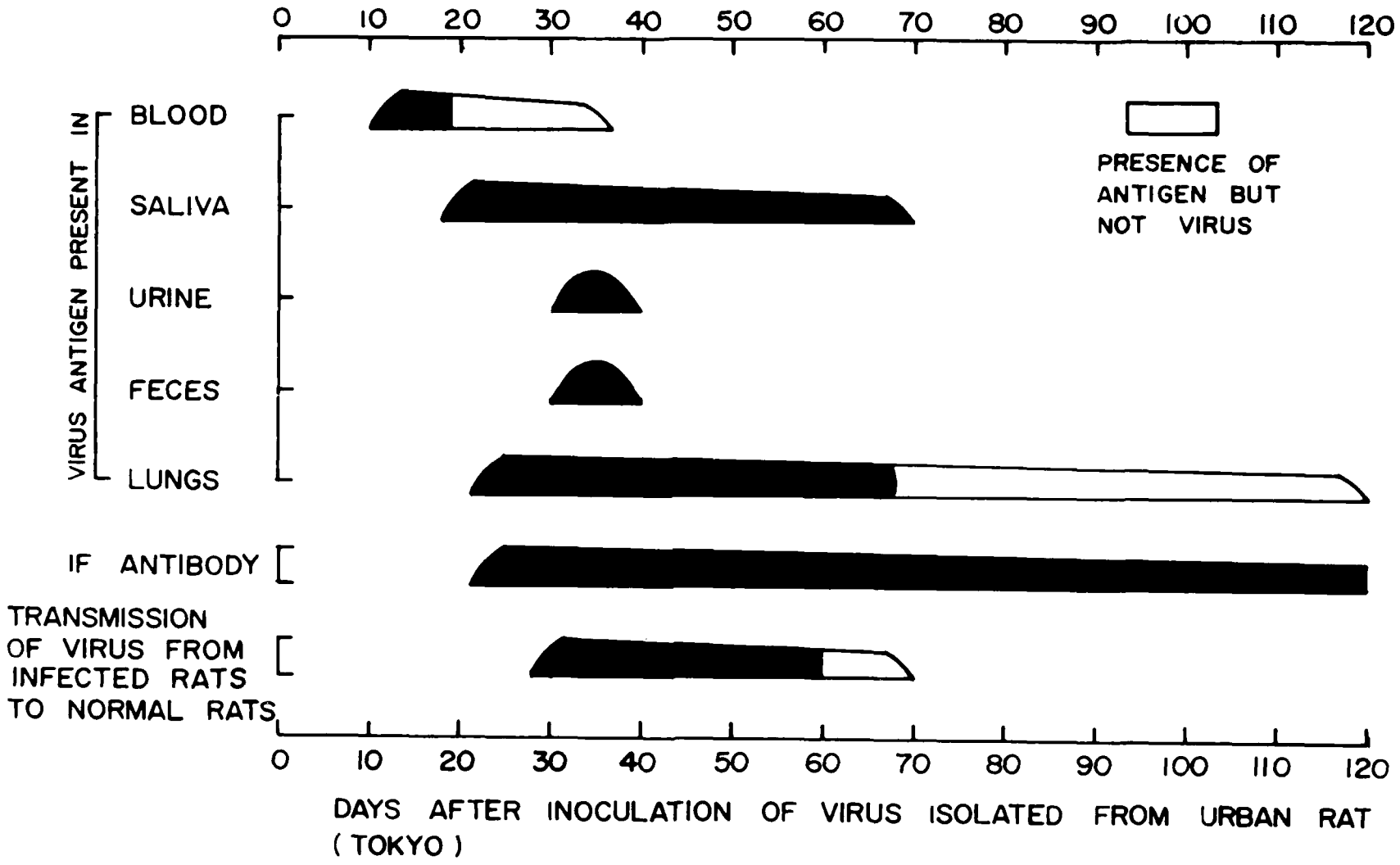

FIG. 5. THE COURSE OF INFECTION AND INFECTIVITY OF SEOUL VIRUS (JTRN 82-11/SDp4) IN S.D. RAT.

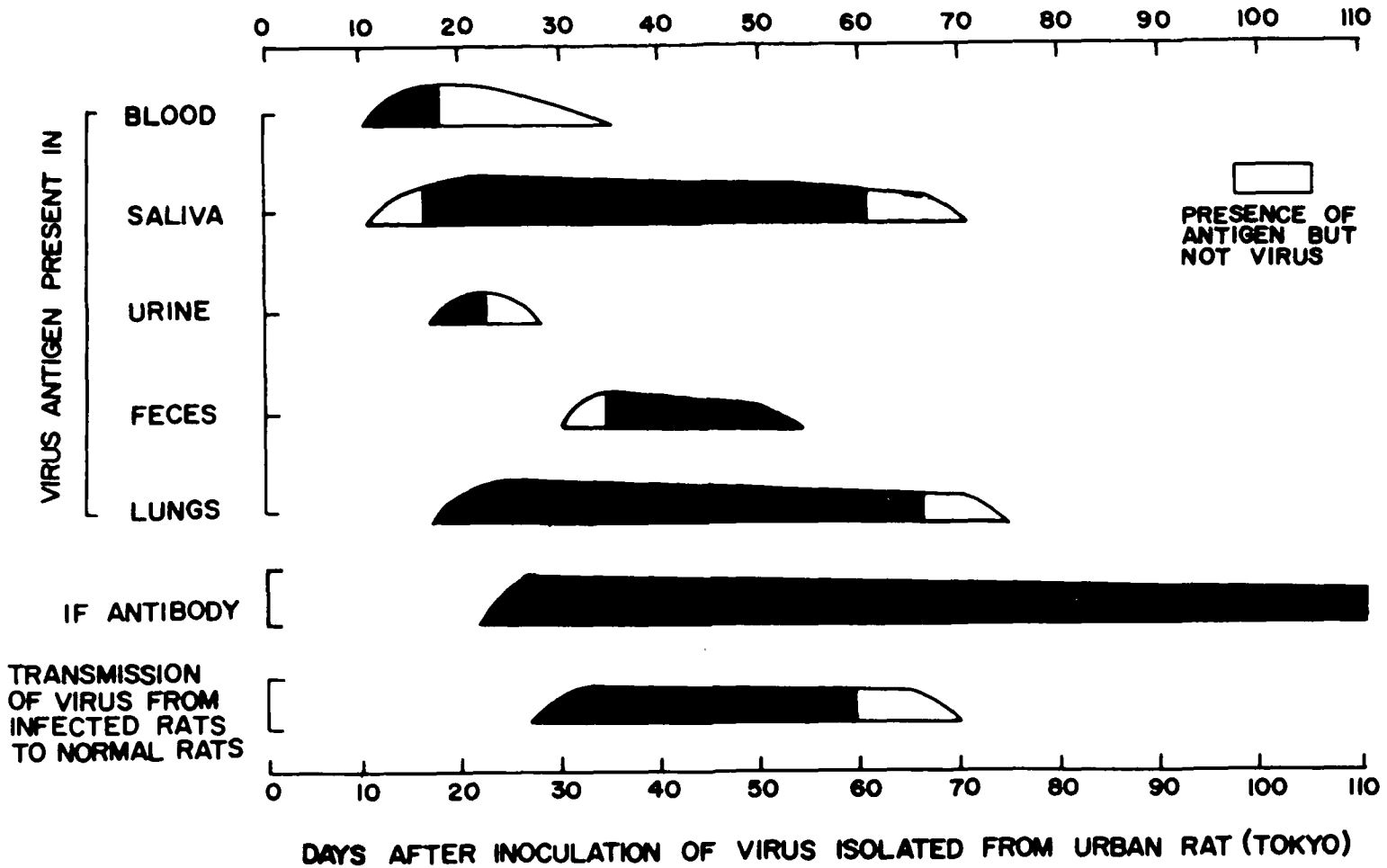

FIG. 6. THE COURSE OF INFECTION AND INFECTIVITY OF SEOUL VIRUS (JTRN 82-11/SD P1/GR P1/SD P1) IN S.D. RAT.

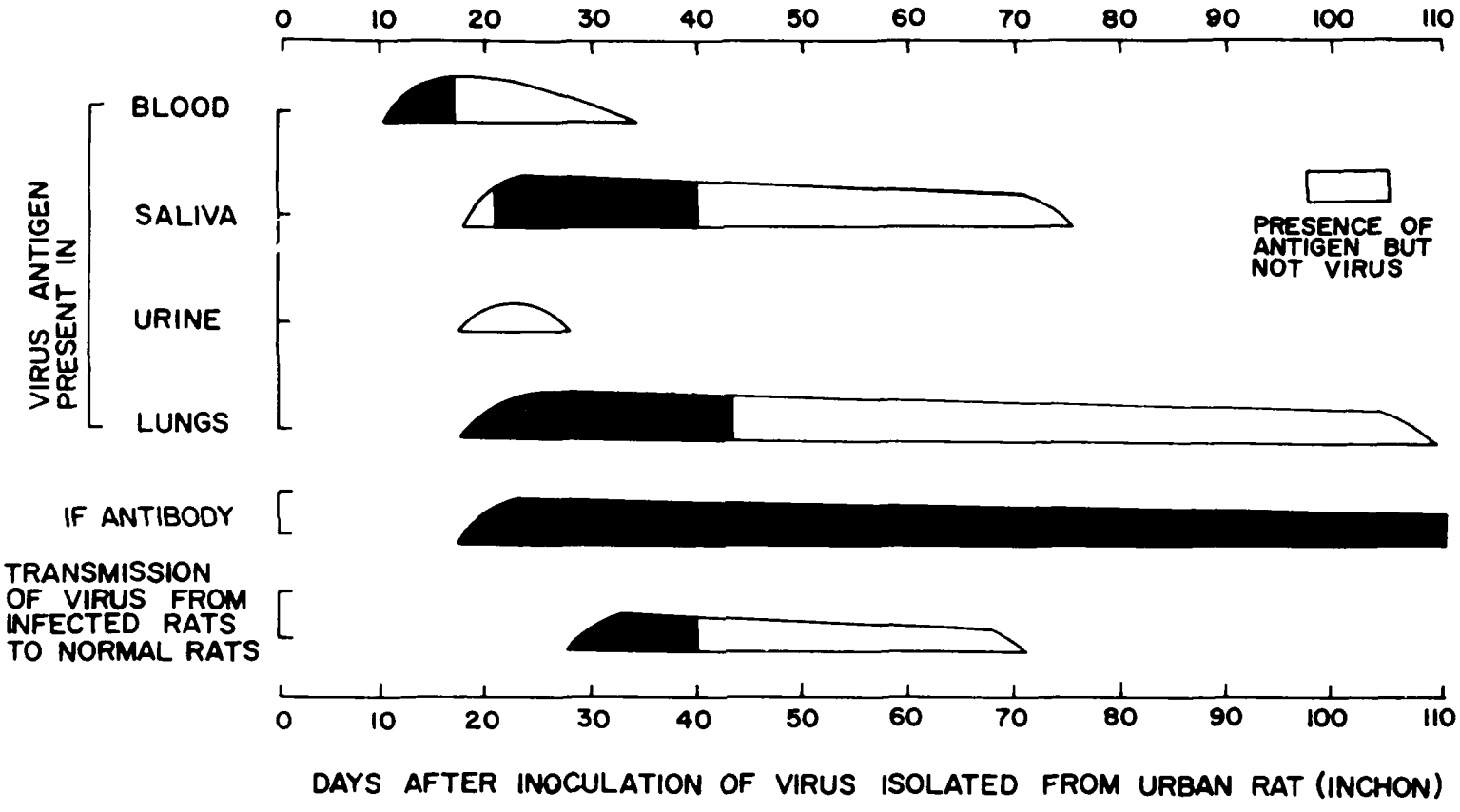

FIG. 7. THE COURSE OF INFECTION AND INFECTIVITY OF SEOUL VIRUS (1/HR 82-216/WRP1) IN S.D. RAT.

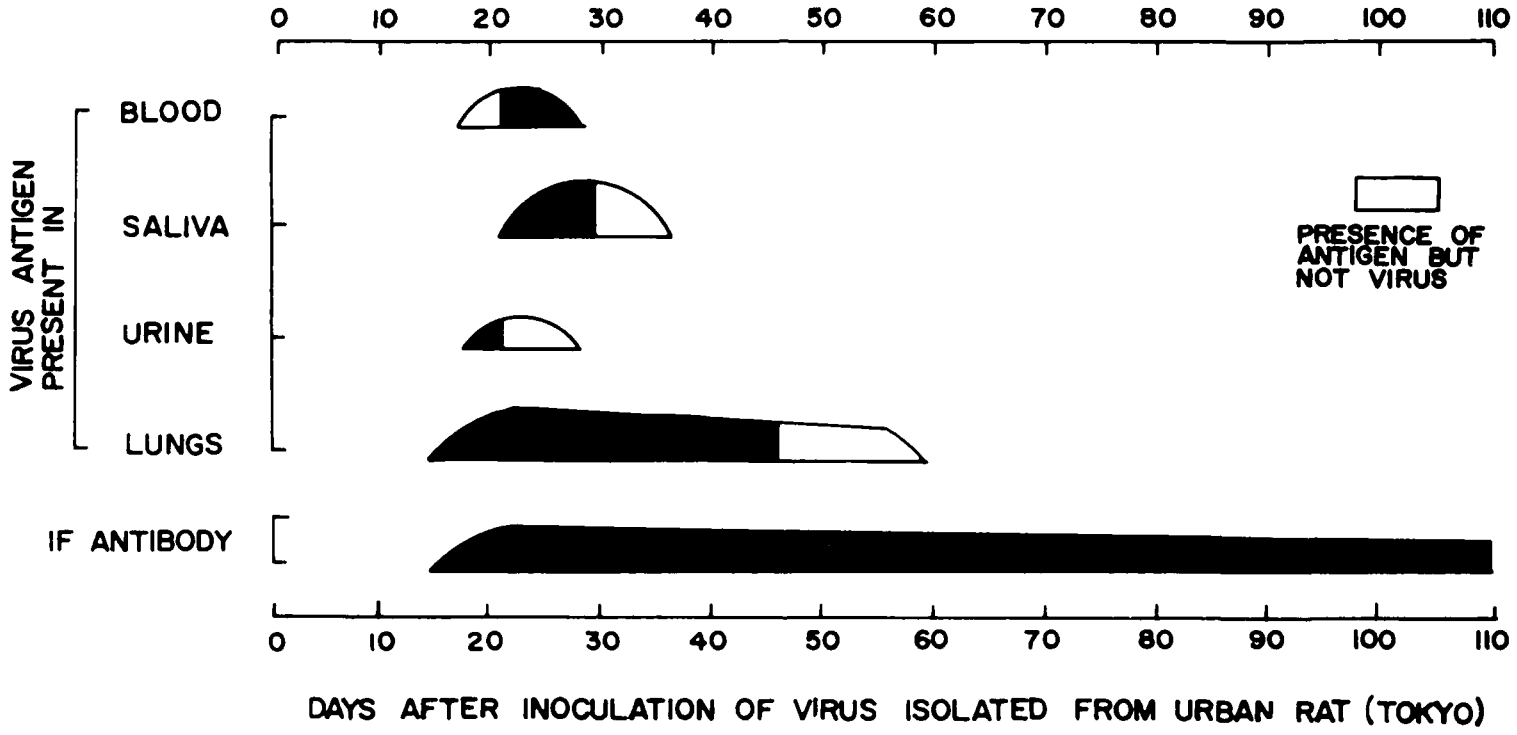

FIG. 8. THE COURSE OF INFECTION AND INFECTIVITY OF SEOUL VIRUS (JTRN 82-11/SD P1/GR P1/SD P1) IN RATTUS RATTUS.

pared by sucrose-acetone extraction and sonication with suckling mouse brains infected with Hantaan and Seoul viruses.

Hemagglutination of goose erythrocytes by the viral hemagglutinins was pH dependent. pH range of Hantaan virus (76/118) hemagglutination was 5.75-6.4 and the optimal pH was 5.75 and Seoul virus (80/39) hemagglutination pH range was 6.2-6.5 and the optimal pH was 6.4. High titers of hemagglutinins of Hantaan and Seoul viruses were obtained after several passages of the viruses in less than 48 hours old suckling mouse brain and titers of hemagglutinins were 1,024 and 512, respectively. HI test with Hantaan and Seoul virus hemagglutinins with sera from HFRS patients was performed to differentiate antibodies against the two viruses.

1. HI antibody response curves against Hantaan and Seoul virus of sera from HFRS patients.
   As shown in Fig. 9. HI antibody titers of six HFRS patients infected with Hantaan virus for two months after onset of illness could be differentiated but it was difficult after 65 days. Similar results were also obtained in rat sera immunized with Hantaan virus as shown in Fig. 10. There are some differences in HI antibody titers against two viruses and antibody titer difference was between two to four folds after 14 day from illness. Therefore, it will be significant if there are four-fold or more differences in antibody titers in sera of HFRS patient against two viruses
2. HI antibody response curves against Hantaan and Seoul virus of sera from rats immunized with Hantaan and Seoul viruses
   However, there are always significant HI antibody titer differences for two months after inoculation of the virus into rats intramuscularly in sera from five rats immunized with Seoul virus. It was impossible to test serially bled human sera from HFRS patients infected with Seoul virus in this experiment since we did not have such sera. But it can be concluded that Seoul virus infection could be differentiated by HI test using Hantaan and Seoul virus hemagglutinins.

E. Hemorrhagic fever with renal syndrome patients in Tropic areas.
   1. HFRS patients in Singapore
      Table 17 shows data of antibody positive sera against Hantaan virus among 437 suspected leptospirosis and acute nephritis patients in Singapore in 1985. There were 12 seropositives against Hantaan virus, 3 positives to Seoul virus and 1 positive to Puumala virus. Their IF antibody titers to the viral antigens were low but one patinet L85/697. IF antibody titers of this patient were very high against both to Hantaan and Seoul viruses and PRN

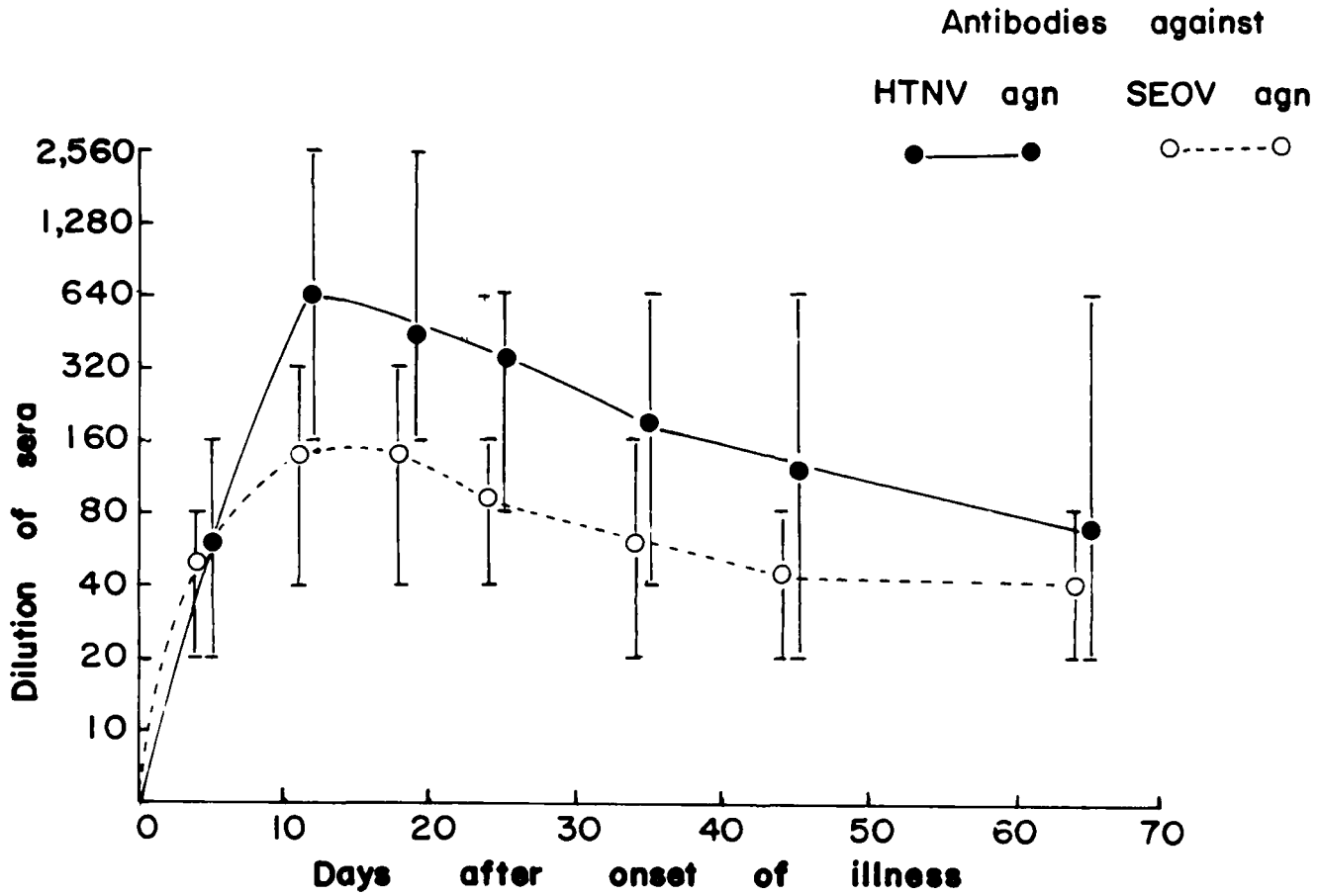

Fig. 9. HI antibody response against Hantaan and Seoul virus of sera from HFRS patients infected with Hantaan virus.
●, ○ : mean value - : range of antibody titer

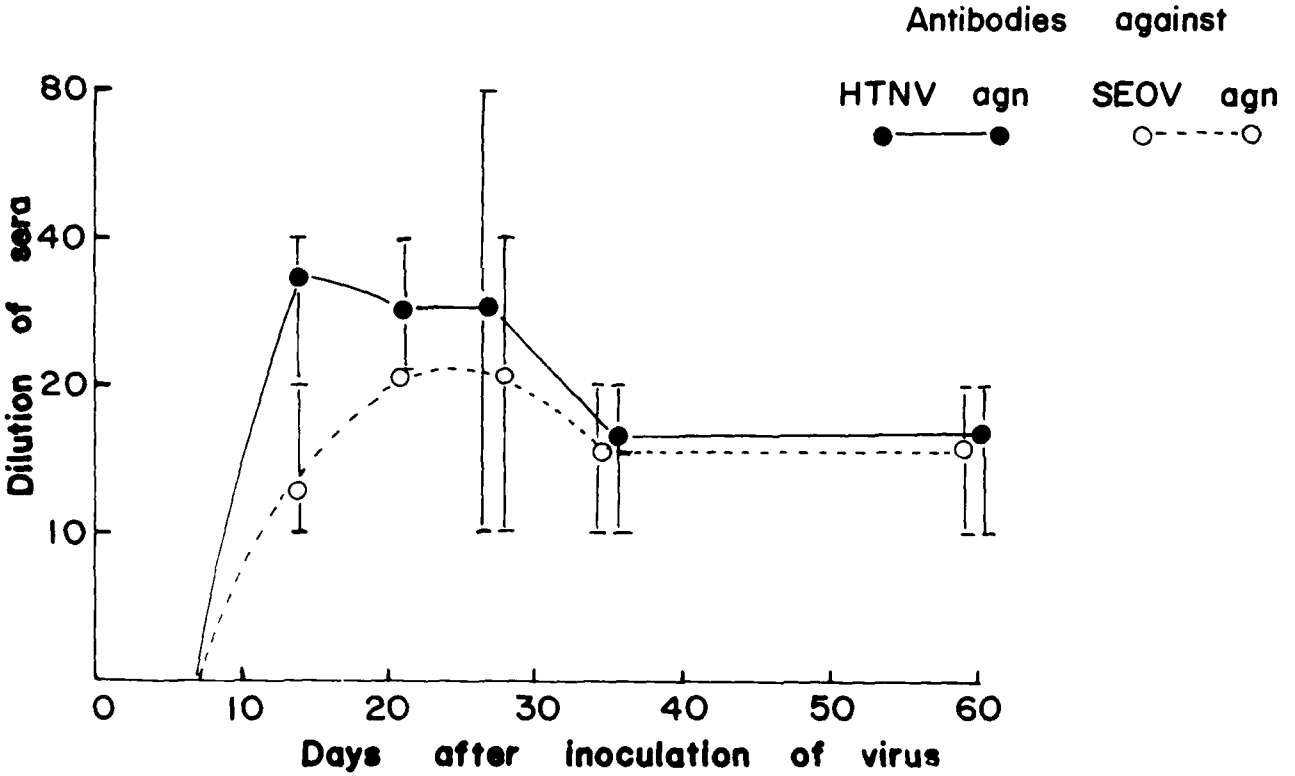

Fig. 10. HI antibody response against Hantaan and Seoul virus of sera from S.D. rats inoculated Hantaan virus(76/118) intramuscularly.
●, ○: mean value —: range of antibody titer

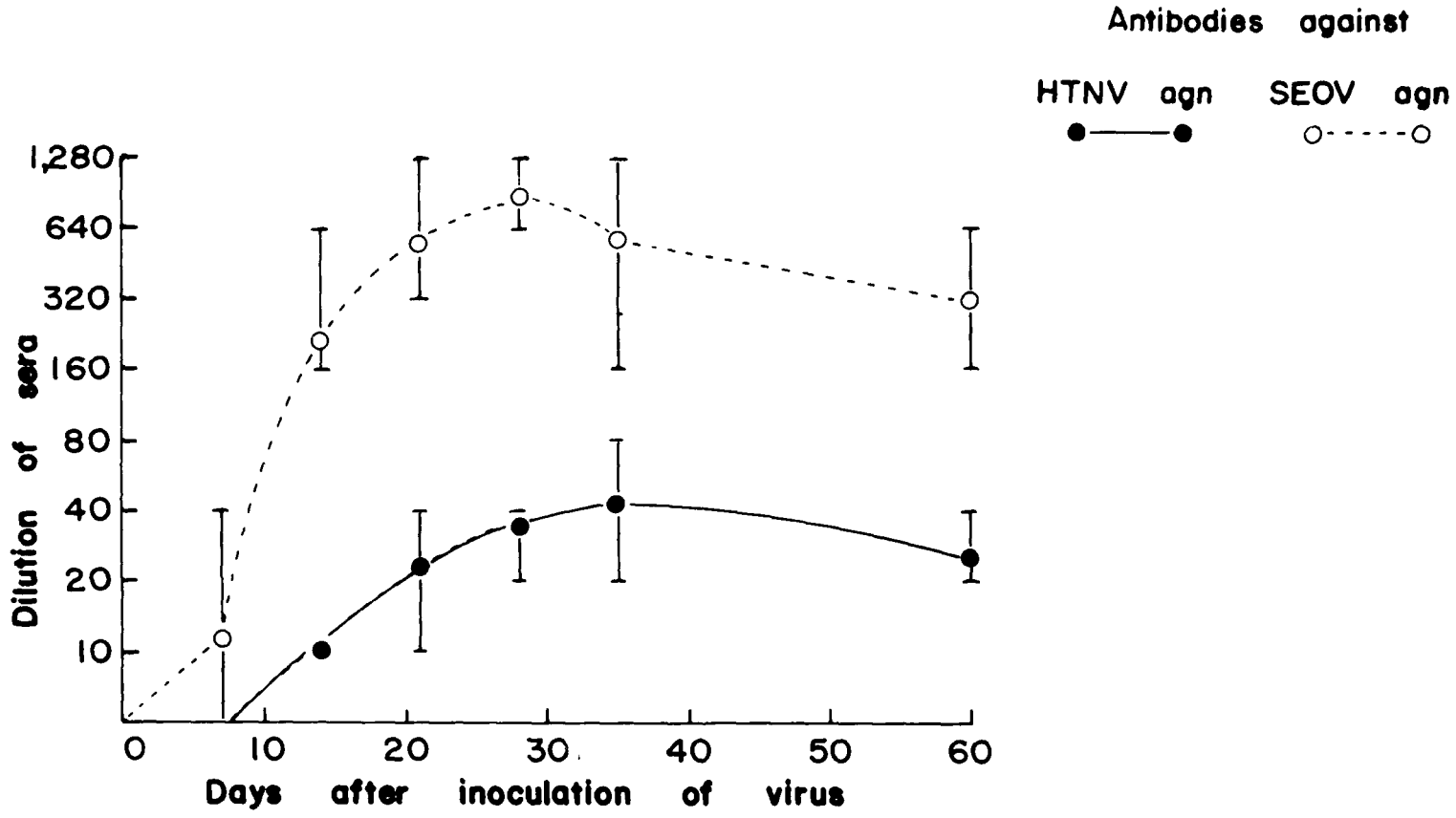

Fig. 11. HI antibody response against Hantaan and Seoul virus of sera from S.D. rats inoculated Seoul virus (80/39) intramuscularly.
●,○: mean value –: range of antibody titer

Table 17.
Results of the antibody positive sera against Hantaan virus among suspected leptospirosis and acute nephritis patients in Singapore, 1985

Code no. of serum	Virus name and antibody titers					
	Hantaan (76/118)		Seoul (80/39)		Puumala (NE #2)	
	IF	PRN	IF	PRN	IF	PRN
L85/48	16		-		-	
L85/564	32		-		-	
L85/588	32		-		-	
L85/697	1,024	2,000	4,096	20	64	<20
L85/867	32		-		-	
L85/884	64		32		-	
L85/913	64		-		-	
L85/928	32		-		-	
N/577	64		32		-	
N/880	64		-		-	
N/1328	32		-		-	
N/1431	32		-		-	

$$\frac{\text{No. of positive sera}}{\text{No. of suspected leptospirosis sera tested}} = \frac{8}{261} \quad (3.1\ \%)$$

$$\frac{\text{No. of positive sera}}{\text{No. of nephritis sera tested}} = \frac{4}{176} \quad (2.3\ \%)$$

antibody test confirmed that the patient were HFRS infected with Hantaan virus because PRN antibody titers to Hantaan and Seoul viruses were 2,000 and 20, respectively. Retrospective study showed that the patient was a 39 year-old American Vietnamese male and diagnosed clinically as Dengue haemorrhagic fever. He worked in Malaysia and admitted to hospital in Singapore and clinical details are as follows. He was admitted to hospital with the complains of fever, chills, nausea, headache and muscle ache in July 1985. One day after admission he developed petechial haemorrhage over his body and limbs and in the mucous membrane of the palate. There was marked thrombocytopenia (platelet count of $7000/mm^3$ on the third day). The total white cell count was normal but atypical mononuclears were observed. There was evidence of disseminated intravascular coagulation with prolonged prothrombin time and partial thromboplastin time and an increase in fibrin degradation products. Blood urea and electrolytes were normal. There was a transient mild proteinuria and a diminished urine output but no overt manifestation of acute renal failure. The serum bilirubin and alkaline phosphatase were mildly raised. SGOT and SGPT transaminases and lactic dehydrogenase (LDH) were markedly raised. Creatine phosphokinase was normal. The patient's illness was clinically diagnosed as Dengue haemorrhagic fever (DHF) but could not be confirmed in the laboratory by HI test. Acute and convalescent phase sera (taken two weeks apart) had HI antibody titres of 1:20 to both dengue types 1 and 2 virus antigens. Leptospirosis and Scrub typhus were excluded as the sensitized erythrocyte lysis test for leptospira and Weil-Felix test, respectively were negative. His acute serum, however, was strongly positive for Hantaan virus (1:1024) and Seoul virus (1: 4096) by the immunofluorescent antibody test, suggesting that his illness could be due to a Hantavirus infection. Additional plaque reduction neutralization test in Vero E6 cell culture confirmed that he was a HFRS patient infected with Hantaan virus because his PRN antibody titer to Hantaan virus was 2,000. The patient's illness improved with supportive treatment and he made an uneventful recovery.

The case reported here appears to be a variant of classical Hemorrhagic fever with renal syndrome. There was little renal involvement but the liver was affected. The clinical presentation is consistent with that of DHF. In the past Malaysian doctors have encountered a number of cases of clinically diagnosed DHF which did not show the expected dengue antibody rise. The findings of this case show that some of these unconfirmed cases could

be due to infection by Hantavirus.

2. HFRS patients in Malaysia

Fifty paired sera samples from hospitalized suspected leptospirosis in Kuala Lumpur were investigated against Hantaviruses and the results are shown in Table 18. It is surprising that IgG antibody titers of seropositive patients to Hantavirus antigens are quite low but there were antibody incerease from acute stage to convalescent stage and importantly, IgM antibodies which are very important indication of recent infection of Hantaviruses were demonstrated in five out of six patients. These serologic findings are a little different from HFRS patients that occuring in Euro-Asia continent. It could be interpreted that the local strain of causative agent of HFRS in Malaysia is different antigenically from known Hantaviruses because titers of IgG antibodies are low but IgM antibodies were demonstrated.

Six patients were found positive representing the first time HFRS is recorded in Malaysia. From the clinical data HFRS in the tropics would appear to be a severe disease more closely related to classical KHF than NE. The main clinical features of these cases were fever, shock, bleeding manifestations and liver dysfuction. However, there was only minimal or no renal impairment in these patients.

In would appear that this disease is common in this country as these six cases were actually detected from cases which were originally investigated and found to be sero-negative for leptospirosis. We are expecting to receive more clinical details of these patients soon. We have also tested 65 urban rats caught in Penang, Malaysia and found six antibody positive rats against Seoul virus but failed to demonstrate presence of viral antigen in lung tissues of rats as shown in table 19.

3. HFRS patients in Hong Kong

In Collaboration with Hong Kong Government and our WHO Collaborating Centre, HFRS survey was started from February 1984 and the program is in still progress. As shown in Table 20, 53 sera from patients with unknown fever were screened against Hantaan virus and one patient was positive and IF antibody titer was 64. 210 sera from urban rats caught in Hong Kong island were also tested against Hantaan and Seoul viruses and 24 (13.3%) sera were positive and antibody titers of positive sera were ranged between 32-2,048. Various laboratory animals from animal rooms in Hong Kong Hospital were also tested antibodies against Hantaan virus, only 3 rats out of 10 rats tested were antibody positive and all rats in that animal room were destroyed immediately as soon as we reported the results to Hong Kong

Table 18.
IF antibody titers of sero-positive sera from suspect leptospirosis against Hantaviruses in Malaysia in 1985

No. & code of serum	IF antibody titers against different virus						
	Hantaan (76/118)		Seoul (80/39)		Singapore (R/36)		Puumala (NE#2)
	IgG	IgM	IgG	IgM	IgG	IgM	IgG
1-1 L-4621	-	-	-	-	-	-	-
1-2 L-4657	32	16	32	16	32	16	-
2-1 L-4592	-	-	-	-	-	-	-
2-2 L-4641	32	-	32	-	32	-	-
3-1 L-4520	-	16	16	16	16	64	-
3-2 L-4596	-	-	32	16	32	16	-
4-1 L-4324	-	-	-	-	-	-	-
4-2 L-4407	32	16	32	16	32	16	-
5-1 L-3874	-	-	-	-	-	-	-
5-2 L-3949	32	16	32	16	32	16	-
6-1 L-3551	-	64	-	64	-	64	-
6-2 L-3661	64	32	64	64	128	64	-

$$\frac{\text{Total no. positive patient}}{\text{Total no. patient tested}} = \frac{6}{50}$$

Table 19.
IF antibody titers of sero-positive house rats sera against Hantaviruses in Malaysia in 1985

Code no. of serum	IF antibody titers against different viruses			
	Hantaan (76/118)	Seoul (80/39)	Singapore (R/36)	Puumala (NE#2)
	IgG	IgG	IgG	IgG
304	128	64	64	-
306	-	32	32	-
307	32	32	32	-
312	32	32	32	-
317	-	32	32	-
320	64	64	64	-

$$\frac{\text{Total no. of positive serum}}{\text{Total no. serum tested}} = \frac{6}{65}$$

$$\frac{\text{Total no. antigen positive}}{\text{Total no. lungs tested}} = \frac{0}{65}$$

Table 20.
Occurrence of IF antibody to Hantaan virus in human and rat sera in Hong Kong

Type of serum	No. positive / No. tested	
	1984	1985
Human	1/53 (1.9%)	7/163 (4.3%)
Urban rat	28/210 (13.3%)	67*/344 (19.5%)
Mice		0/10
ICR mice		0/10
CBA/N mice		0/4
Balb/c An mice		0/4
C57BL/6 N mice		0/4
C57BL/6 N mice		0/4
Rats		3/10
SD rats		0/10
F344 rats		0/10
Hamster		0/10
Rabbit		0/10
Guinea pig		0/19

* Four strains of Seoul-like virus were isolated from sero-positive urban rats both in S.D. rats and Vero E6 cells.

Table 21.
Serologic diagnosis of HFRS patient in Hong Kong in 1985

Code no.	Age/ sex & accu- pation	Clinical diagnosis	Date of illness	Days after onset	Antibody titer against Hantavirus									
					Hantaan[1]			Seoul[2]			Hong Kong[3]		Puumala[4]	
					IgG[5]	IgM[5]	PRN	IgG	IgM	PRN	IgG	IgM	IgG	IgM
H9-A		Hepatitis	3/19/85	3	256	32		256	64		n.t.		16	-
H9-B				19	4096	256	200	4096	256	20			64	-
H27-A	F/73	Urinary	4/12/85	4	-			-			n.t.		-	
H27-B	House wife	tract infection		10	32			32					-	
H71-A	M/19	Fever	8/14/85		64						128	-		
H71-B			8/22/85		64						128	-		
H75-A	M/50	Transient	?		256						2048	128		
H75-B		thrombocyto-	9/9/85		512						8192	-		
H75-C		penia due to viral infection	7/11/85		1024			4096			4096		64	
H132A	F/4	Nephritis	11/11/85		32			16			32		-	
H132B			11/20/85		32			32			32		-	
H143A	M/8	Acute	12/10/85		16			16			32		-	
H143B		nephritis	12/18/85		64			32			32		-	
H157A	M/10	Acute	1/3/85		-			-			-		-	
H157B		nephritis	1/9/85		32			32			32		-	

[1] : Hantaan virus strain 76/118.
[2] : Seoul virus strain 80/39.
[3] : Hong Kong virus strain 85/19.
[4] : Puumala virus strain NE.
[5] : IgG and IgM antibodies are IF antibodies.

Government. In 1985, 163 paired sera from hospitalized patients in Queen Elizabeth Hospital were tested against Hantavirus antigens and 7 patients were antibody positive against Hantaan virus as shown in Table 21. Four patients among 7 seropositives showed significant increase of antibodies against Hantaan virus during course of illness. The clinical diagnosis of patients were hepatitis, urinary tract infection, transient thrombocytopenia and acute nephritis, code nos. H9, H27, H75 and H157. IgM antibodies to Hantaan virus were demonstrated in sera from H9 patient and PRN antibody titer was 200 against Hantaan virus but unfortunately the other IF antibody positive sera were not tested for IgM and PRN antibodies due to loss of sera. It was confirmed for the first time that HFRS patients occur in Hong Kong and causative agents are related to Seoul virus antigenically according to antibody titers of sera from H75 patient. It was unfortunate that sera from H9 patient were not tested against Hong Kong virus since the virus was not isolated at that time. Sera and lungs from 344 urban rats caught in Hong Kong island in 1985 in where positive urban rats were caught in 1984 survey were tested for antibodies against Hantavirus antigens. As shown in Table 20, 67 rats were seropositive and four strains of Seoul-like virus were isolated in Vero E6 cell cultures from antigen positive lungs and characterization of these virus strains by monoclonal antibodies and PRNT is in progress. Further studies for demonstration of HFRS patients among non-A, non-B hepatitis, nephritis, thrombocytopenia and UFO in Hong Kong are in progress. Preliminary results of HFRS survey suggest that there are Hantaan and Seoul virus like viruses in Hong Kong according to the serologic findings of H9 and H75 patients.

DISCUSSION

Numbers of 730 and 697 severe hospitalized cases of HFRS patients in 1984 and 1985 in Korea are only no. of serologically confirmed patients at our Institute. Sera from suspected HFRS patients were came from limitted no. of hospitals in Seoul and nearby cities, therefore, it can be estimated that real total no. of HFRS patients in entire South Korea would be at least three times more than no. of patients in table 1 because I think we might have examined only less than one third of severe HFRS cases occured in Korea during last 2 years. Therefore, I estimate that there are at least over 2,000 cases of HFRS patients in S. Korea every year. It is surprising to learn that no. of HFRS patients in metropolitan areas of Seoul is increasing since 1980 and many cases occur in late fall from October to November, same epidemic season of HFRS in rural areas of Korea. More than half of total patients were occured in two Provinces, Kyunggi

and Chungcheong, as shown in table 2 but it does not mean that the two Provinces are more heavily infected foci of HFRS than other Provinces since other provinces are far from Seoul and it is difficult to send sera from the patients to our Institute for serologic diagnosis of the disease. All of the hospitals in endemic rural areas of Korea have incapability to make serologic diagnosis of HFRS although patients occur all over the South Korea except Jeju island. Distribution of occurence of HFRS patient in Seoul is in all districts and every district had several cases of HFRS every year. It remains to be studied why only a few patients are occuring in every district of Seoul where over 8 million people live and more than 10% of urban rats population in Seoul area are infected with Seoul virus (29).

There were several epidemic outbreaks of unknown fever patients with similar clinical symptoms of HFRS and pulmonary hemorrhages in several parts of South Korea since 1982 and almost all of the patients were farmers and soldiers, and fatality rate was about 20-40%.

Fortunately, it became clear that many of the unknown fever patients were leptospirosis caused by Leptospira icterohemorrhagica in 1984 after isolation of the leptospira from the patients and from field mice, Apodemus agrarius, and using serologic diagnostic method of leptospirosis. However, still many unknown fever patients were remained undiagnosed until 1984 and it became possible to make serologic diagnosis of these unknown patients with Scrub typhus antigen by IFAT in 1985 after collaboration with Dr. Oya A. NIH, Japan. Korean doctors never paid attention to leptospirosis and rickettsiosis because they believed the research data of infectious disease in South Korea that were done by American scientists during Korean War and in 1960s. Many eminent scientists including famous microbiologists from U.S. have done extensive microbe hunting and survey of infectious diseases to discover the causative agent of Korean hemorrhagic fever in Korea for 18 years since 1951 but they did not mentioned about a possibility of outbreak of these diseases. It is surprising to know that about one third of total sera from suspected HFRS patients were HFRS, leptospirosis and Scrub typhus, respectively. We do not know how many cases of leptospirosis and Scurb typhus have occured among US soldiers stationed in Korea because there have been no such diagnostic serologic tests were requested by American doctors from US Army Hospital in Seoul. It is interesting and surprising to know that epidemics of leptospirosis and Scrub typhus occured during epidemic seasons of HFRS in Korea for several years although limitted sero-epidemiologic studies were carried out by us recently. Further studies on sero-epidemiology, reservoir hosts of the diseases and vectors of Scrub typhus in Korea are urgently needed for better understanding and prevention of the diseases. Leptospirosis and rickettsiosis are very important military diseases for both Korean and US soldiers as well as for farmers.

Recent studies have demonstrated a near global distribution of Seoul virus among urban rats and the presence of this or other Hantaviruses among several different species and genera of small mammals (16-20,23). Clearly the genus Hantavirus (15) is widely distributed and maintained in a variety of different ecological settings. The degree to which Hantaviruses cause human disease, especially in areas where HFRS has not been traditionally recognized, is presently unknown. As WHO Collaborating Centre for Research on HFRS, we provided serological diagnosis for suspect HFRS in sera from throughout the world. In addition, we have collaborated with a number of investigators conducting small mammal surveys for evidence of Hantavirus infection and isolation of strains from urban rats. Results of these preliminary studies indicate that human disease due to Hantavirus is present in several areas where HFRS had not been previously diagnosed. One patient with clinical diagnosis of Dengue hemorrhagic fever was confirmed serologically as HFRS in Singapore, six patients with suspect Leptospirosis in Malaysia are certain HFRS patients and IgG antibody responses to local strain of the virus remain to be analyzed further, and there is no question about the existance of HFRS in Hong Kong and their clinical diagnosis of Hepatitis is very interesting. These preliminary data on existence of HFRS patients with different variety of clinical diagnosis would be a clue to find HFRS patients in tropic areas and other areas of the world where HFRS was not known to exist. There are also accumulating evidences about existence of infected laboratory small animals with Hantaviruses in many different research Institutes of the world (30-32).

Preliminary results indicate that 4 distinct viruses compose the genus Hantavirus, Hantaan; Puumala; Seoul; and Prospect Hill viruses. It appeare that several subtypes of strains exist within each virus type by monoclonal antibodies and molecular biologic studies and epidemiological evidence suggests that other Hantaviruses still exist unrecognized in nature. A further serological classification is thus needed to clearly differentiate specific Hantaviruses and to aid in the recognition of new viruses. A total of 41 strains of Hantavirus is presently on hand in our laboratory and available for serological characterization. Using these viruses and those isolated in future studies, we will identify serological groupings of viruses generating a formal classification scheme using standard serological tests such as IFA, HAI, PRNT and EIA. This information will be useful in establishing which viruses are responsible for human disease and will insure that diagnostic tests and developed vaccines are reactive with all Hantaviruses.

Recently, we are analyzing antibodies of convalescent sera from HFRS patients occurred at different areas of Korea against Hantaan, Seoul and Puumala virus antigens by PRNT because we have found some convalescent sera from HFRS patients

and a few positive rats sera to Hantaan by IFAT contained equal titers of neutralizing antibodies against both Hantaan and Seoul viruses simultaneously. We have proved the convalescent serum from a HFRS patient which was used for neutralization test in rats for the first virus isolated from urban rat in Seoul in 1980 contained equal titers of neutralizing antibodies against both Hantaan and Seoul viruses (29). We are now attempting to isolate a new virus from patients and rodents caught in such areas which has antigenicities of both Hantaan and Seoul viruses for additional classification of Hantaviruses and a possible use of a vaccine candidate virus.

Since we discoverd Seoul virus from an urban rat in an apartment building in Seoul in 1980 and from HFRS patients, and from laboratory rats, we have tried to demonstrate dynamics of infection and intraspecific transmission of Seoul virus in urban rats and laboratory colonized rats but it took several years to obtain convincing constant results using Seoul virus strains that were adapted well in laboratory rats. Antibody responses against Seoul virus strains in urban rats gave relatively low antibody response curves in compare with in S.D. rats and viral antigen was demonstrated in various tissues of rats after inoculation of the virus intramuscularly. Seoul virus isolated from Seoul rats gave better results than the virus isolated from Tokyo urban rats and Incheon urban rats. There were some differences on the results in different experiment at different time with same strains of Seoul viruses which have different passage histories in laboratory rats as observed in Fig. 3-6. The pattern of infectivity and mode of transmission of Seoul virus among rats were almost same as Apodemus agrarius (25) but large amounts of virus were excreted in saliva than urine and feces and, average duration of excretion of the virus in saliva was about one to two months. These results were after inoculation of seoul virus strains in rats that were adapted well in laboratory rats, therefore, infection pattern and intraspecific transmission of street Seoul virus in nature might be a little different from the results described in the report.

Infected suckling mouse brain suspensions are currently the preferred source of virus hemagglutinating antigen but are only available for Seoul and Hantaan viruses. It was demonstrated that HI test can be used for a differential diagnosis of Hantaan and Seoul virus infection in man and rats. This is a simple, cheap and reliable test which can be used in developing countries where expensive equipments and reagents are not available. Further experiments to make very high titers of pure hemagglutinins with different serotypes of Hantaviruses from suckling mice brains are needed for a sensitive simple serologic diagnostic test for HFRS and for a classification of Hantaviruses as well.

As I have been expected that we could discover HFRS patients in tropic areas as described in this report. All

of the HFRS patients were diagnosed clinically Dengue hemorrhagic fever, hepatitis and leptospirosis-like illness because HFRS is not known to exist in tropic areas, Singapore, Malaysia and Hong Kong. This is the first evidences of existence of HFRS patients in tropic areas and we expect to find more HFRS patients in other areas of the world if HFRS surveillance program countinues. HFRS will be a major public health problem through-out the world but there are still many problems to be solved for better understanding of mechanisms of pathogenesis of the disease in man, finding anti-Hantavirus drugs for better treatment of the patient, and to develope an effective vaccine to prevent outbreaks of HFRS in animal rooms and for the people in the endemic areas of the disease.

CONCLUSION

1. There were 730 and 697 hospitalized cases of confirmed HFRS patients serologically at our laboratory in 1984 and 1985, respectively and 13 of HFRS patients were US Army soldiers in Korea. No. of HFRS patient is increasing in urban areas of Seoul and nearby cities every year. Epidemics of leptospirosis and Scrub typhus had occured during epidemic seasons of HFRS in 1985 among soldiers and farmers in Korea.
2. Eight and 13 strains of Hantaan virus from bloods of HFRS patients and Apodemus mice, respectively, 12 strains of Seoul virus from laboratory and urban rats, and 1 strain of Seoul-like virus from a Syrian hamster were isolated directly in Vero E6 cell cultures. A total of 41 strains of Hantavirus is presently on hand and preliminary results indicate that there are 4 serotypes and may be more.
3. Infection and transmission of Seoul virus among rats resemble findings in Apodemus agrarius experimentally infected with Hantaan virus. Subclinical chronic infections characterized by transient viremia, prolonged virus shedding in saliva and virus persistence in tissues developed in laboratory and urban rats inoculated intramuscularly with Seoul virus. Horizontal transmission coincided with virus shedding in saliva and infectious virus was found in the lungs from 14 to 90 days and saliva collected 14-60 days postinoculation. A small amount of infectious virus was found in urine and feces 20-60 days postinoculation.
4. HI test with mouse brain Hantaan and Seoul virus hemagglutinins with sera from HFRS patients and immunized rats could differentiate antibodies against the two viruses and it is a useful simple serologic diagnostic method for differentiation of Hantaan and Seoul virus infection.
5. HFRS patients with clinical diagnosis of hepatitis, dengue and leptospirosis-like illness were confirmed in tropic areas for the first time.

   Hantaviruses are ubiquitous in the world and HFRS with diverse clinical symptoms will be a major public health problem through-out the world.

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