Committee 3
The Threat of Epidemics

Draft – February 1, 2000
For Conference Distribution Only

The Threat of Hantaviruses and Vaccine

Ho Wang Lee
Director
Asian Institute for Life Sciences
Seoul, Korea

The Twenty-second International Conference on the Unity of the Sciences
Seoul, Korea February 9-13, 2000

Com 3

THE THREAT OF HANTAVIRUSES AND VACCINE

H.W.Lee
WHO Collaborating Centre for Virus Reference and Research (Hantaviruses)
Asan Institute for Life Sciences, Seoul, Korea

Hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in Americas are re-emerging and emerging viral diseases and causative agents are hantaviruses. There are about 100,000~150,000 patients with 1-60% fatality in the world each year.

The outbreak of deadly HPS in the U.S.A. in 1993 changed the concept of hantavirus infection because the clinical manifestations of this newly described hantavirus disease are quite different from the known typical forms of HFRS caused by Hantaan, Seoul, Belgrade and Puumala viruses and because it occurred in America, where hantavirus disease had not been known to exist previously.

A severe form of HFRS caused by Hantaan and Belgrade virus occurs in Asia and Balcan countries. A new clinical form with 70% fatality, HPS, caused by novel hantaviruses occurs in Americas. A moderate form of HFRS caused by Seoul virus occurs in Asia and a mild form of HFRS caused by Puumala virus occurs in Europe. Recently, Puumala virus infection in man and mice were found in Siberia, Korea and Japan. The reservoirs of hantaviruses are rodents and other small mammals including bats and birds. The mode of transmission of hantaviruses is aerosol. There are some evidences for existence of Sin Nombre-like virus in Asia. Recently we isolated hantaviruses from rats in Thailand and Indonesia. Three different serotype hantaviruses are circulating in Asia. An inactivated Hantaan virus vaccine against HFRS is available in Korea and recently we developed a Hantaan-Puumala combination vaccine for prevention of HFRS in Eurasia.

Recently, we developed simple diagnostic kits for Puumala and Sin Nombre virus infections by suing HDPA. The results show comparative sensitivity and specificity of HDPA test and FA test for diagnosis of HFRS and HPS. HTNV HDPA test is specific for diagnosis of HFRS in Korea, China and Japan, and PUUV HDPA test is specific for diagnosis of HFRS in Yugoslavia. Russia and Finland and SNV HDPA test is specific for diagnosis of HPS in the U.S.A.

In the 21 century, it is highly possible to identity new hantavirus illnesses in parts of the world where the disease is not known because of the availability of diagnostic tools.

The Threat of Hantaviruses and Vaccine

H. W. Lee M.D., Ph.D.

WHO Collaborating Centre for Virus Reference and Research (Hantaviruses)
Asan Institute for Life Sciences
388-1 Poongnap-dong, Songpa-ku,
Seoul 138-736, Korea.

Introduction

Hemorrhagic fever with renal syndrome (HFRS) cuased by hantaviruses is a good example of an important re-emerging viral disease with 1~15% fatality because it is an old-disease existed for more than 100 years in Eurasia (1). Hantavirus pulmonary syndrome (HPS) with 60% fatality caused by novel hantaviruses is an emerging viral disease in Americas. However, these diseases are quite new to Western Medicine because HFRS attracted the attention of the world during Korean War and HPS in 1993 when a deadly mysterious lung-swelling illness occurred in the U.S.A.

The outbreak of deadly HPS in the United States in 1993 (2) changed the concept of hantavirus infection because the clinical manifestations of this newly described hantavirus infection are quite different from the known typical forms of HFRS caused by Hantaan, Seoul, Belgrade and Puumala viruses and because it occurred in the United States, where hantavirus disease had not been known to exist previously (3). Recent data on hantavirus disease show that HFRS occurs in the old world and HPS occurs in the new world (4). The emerging diseases caused by hantaviruses threatens the world in the 21st century.

History

The history of diseases caused by hantaviruses is very interesting and useful to understand rodent-borne hantavirus infections because it has many episodes among soldiers in the past wars with different names in

different parts of the world as shown in Table 1. Large outbreaks of epidemic nephritis and HFRS were occurred among soldiers during the American Civil War, the First World War, the Second World War, the Korean War and War in Bosnia and number of the victims was more than several thousands respectively (3). However, the deadly HPS with fever and pulmonary syndrome is quite different clinically from classic HFRS occurred in the U.S. in 1993 and same diseases were identified subsequently in Canada and in S. America (4).

The discovery of the new virus causing Korean hemorrhagic fever (KHF) by Lee et al (5), now called Hantaan virus, launched a new era in the study of HFRS throughout the world. Various clinical forms of HFRS occur not only in Eurasia but also in Southeast Asia and in Africa. About 150,000 people are hospitalized each year with HFRS and 3-15% of them die (4). A new clinical form of hantavirus infection, HPS, with 60% fatality was identified in the U.S.A. in 1993 for the first time (2, 6~8).

Since the isolation of Hantaan virus and improvements in sero-diagnostic tests for infection with Hantaan virus, numerous Hantaan-related viruses have been isolated from man and rodents in different parts of the world. In addition, it has been confirmed serologically that KHF-like illnesses in various parts of the world are cuased by Hantaan or Hantaan-related viruses (1,3,7). In 1982, the WHO recommended naming KHF-like diseases caused by Hantaan and Hantaan-related viruses "Hemorrhagic fever with renal syndrome (HFRS)" (9).

Hantaviruses

Recently, Hantaan group viruses have been classified as members of a newly established genus Hantavirus in the family Bunyaviridae (10). Of these, Hantaan (HTN), Seoul (SEO), Belgrade (BGD), Puumala (PUU), Sin Nombre (SN) and Sin Nombre-like virus are known to cause illnesses in humans (3). However, the pathogenicities of Prospect Hill, Thottapalayam and isolates of Seoul-like viruses from non-endemic areas of HFRS transmitted from rodents to humans are unknown.

Figure 1 is the classification of registered hantaviruses in the International Catalogue of Arboviruses. The new genus name Hantavirus comes from Hantaan virus and Hantaan is after Hantaan river that runs

between South Korea and North Korea where we discovered the virus from lungs of mice caught near Hantaan river in 1976.

Hantavirus is spherical RNA virus with envelope, 80-120 nm diameter, grows well in Vero cells and A549 cells but do not produce CPE. Table 2 shows the list of hantaviruses at my WHO Collaborating Center for HFRS and HPS and serotyping of hantaviruses isolated from 5 continents by plaque reduction neutralization test in Vero E6 cells and by PCR technique. The results of PCR were 100% agreeable with that of serological typing (11).

Geographical distribution of humans and rodents infected with hantaviruses

Sero-epidemiological surveys show that hantavirus infections are distributed throughout much of the world, as demonstrated by the presence of antibodies against hantaviruses in sera from humans and rodents as shown in Fig. 2.

Hantaan virus is found in Korea, China, Thailand, Mongolia, Yugoslavia, Greece and the Far-East of the Russia. Seoul and related viruses exist essentially world-wide: 14 Asian countries (Japan, Korea, China, Hong Kong, Philippines, Malaysia, Indonesia, Singapore, India, Sri Lanka, Fiji, Thailand, Vietnam, Taiwan), 4 North American countries (Canada, U.S.A., Mexico, Panama), 6 South American countries (Brazil, Bolivia, Colombia, Argentina, Uruguay, Paraguay), and 13 African countries (Egypt, Sudan, Uganda, Kenya, Benin, Cameroun, Mauritania, Senegal, Tchad, Central African Republic, Gabon, Madagascar, Nigeria), and 4 countries in Europe (Belgium, Netherlands, Germany, Italy). Puumala virus is in Europe (Russia, Scandinavian countries, Finland, Belgium, Germany, Yugoslavia, France and England) and there are evidences of existence of Puumala virus in Asia. Sin Nombre virus, New York virus, Prospect Hill and Seoul viruses have been found in the U.S.A. and Sin Nombre-related viruses have been found in S. America.

HFRS patients have been documented clinically and serologically throughout Eurasia and, recently, in Africa. Fifteen countries in Asia are focally enzootic for hantaviruses : Japan, South Korea, North Korea, China, Mongolia, Russia, Hong Kong, Malaysia, Thailand, Indonesia, Vietnam,

Cambodia, Myanmar, India and Sri Lanka; 17 countries in Europe : Russia, Finland, Sweden, Norway, Denmark, Bulgaria, Hungary, Albania, Germany, France, Portugal, Belgium, Netherlands, Switzerland, England, Yugoslavia and Greece : and 1 country in Africa : Central African Republic (1,3). More than 700 cases of HPS have been reported throughout North and South America; The U.S.A., Canada, Mexico, Argentina, Brazil, Peru, Paraguay, Uruguay, Bolivia and Chile (4, 6,7,8,14,15)

Field rodents infected with hantaviruses were demonstrated in Asia, Europe, Africa and the Americas (12~17), specifically : Korea, China, Russia, Sweden, Findlan, Norway, Yugoslavia, Greece, Egypt, U.S.A., Canada, Mexico, Brazil, Paraguay, Costa Rica, Beolivia and Argentina. Urban rats infected with hantaviruses are in 10 Asian countries : Japan, Korea, China, Hong Kong, Malaysia, Sri Lanka, India, Singapore, Fijim Philippines; in 3 European countries : Belgium, Federal Republic of Germany, Italy; and in 3 countries in Americas : Canada, U.S.A., Brazil.

Laboratory rats infected with hantaviruses have been determined in 11 countries of the world : Japan, Korea, China, Russia, Belgium, England, Malaysia, Hong Kong, Singapore, Hawaii, Argentina (3). Infections of laboratory workers with Hantaan, Puumala and Seoul viruses have been reported from Russia, Korea, Japan, China, Belgium and England. One patient died out of 149 laboratory-acquired HFRS in Japan as shown in Table 3 (1,3). Laboratory infection with hantaviruses is very dangerous and it is strongly recommended to screen laboratory animals against Hantaan, Seoul and Puumala viruses before animal experiment. Importataion and exportation of hantavirus free small laboratory animals from one country to another country should be controlled under international quarantine rule.

Clinical manifestations

Clinical symptoms of hantavirus infections are diverse and very difficult to make clinical diagnosis because the early symptoms and mild form of HFRS are flu-like upper respiratory illness. And only 1/3 of HFRS patients show hemorrhages and nephritis. There are 4 different clinical forms of diseases caused by different serotypes of hantaviruses : 1. HFRS by Hantaan virus (fever, hemorrhage and renal syndrome), 2. HFRS by Seoul virus (fever, hemorrhage, renal and hepatic syndrome) 3. NE (fever

and renal syndrome) and 4. HPS (fever and pulmonary syndrome).

Table 4 shows the comparative features of 4 different clinical forms of diseases caused by different serotypes of hantaviruses with fatality and their geographical distribution.

Total number of hospitalized hantavirus patients in the world is about 100,000~150,000 each year as shown in Figure 2. Severe and moderate clinical forms of HFRS occur in Asia and Balkan countries with 3-15% fatality. HPS caused by novel hantaviruses occurs in U.S.A., Canada and S. America with 60% fatality. Annually, between 60,000 and 150,000 people are hospitalized with HFRS in China, about 500~2,000 cases in South Korea, several hundreds cases in North Korea, and several hundreds cases in Russia and Vietnam. Recently, several cases of HFRS were documented in Malaysia and Sri Lanka. Most HFRS patients in Asia live in rural areas but there have been many infections acquired in urban areas of Japan, Korea, China and Hong Kong. In Europe, several hundreds cases of HFRS are reported annually and the disease is usually mild illness, known as Nephropathia Epidemica, in which renal involvement dominated and hemorrhagic features are less prominent; the fatality rate is about 0.2%. However, the severe type occurs in parts of Yugoslavia and Greece.

The discovery of new HPS in the U.S.A. in 1993 opened a new era for hantavirus research because HPS is completely different clinical disease from known typical forms of HFRS. Since the initial outbreak in the Four Corners region in 1993, 217 cases of HPS were reported in U.S. as of May 28, 1999 (14) and from 1996 to January 1999, 210 cases of HPS were confirmed in Argentina (15). The impact of discovery of HPS patients and new hantaviruses is great because the new findings and new knowledges found in the U.S.A. would be implemented to other parts of the world where pneumonia-like dseases with unknown etiology exist for a long time by use of the available serologic diagnostic tests and PCR.

Epidemiology

It was long believed that HFRS only occurs in endemic rural areas of Asia, with farmers and soldiers being the most likely victims. Areas infected with Hantaan and Puumala viruses were also thought to be limited to certain locations of Eurasia, infecting only their inhabitants and travelers.

Recent findings (3), however, have shown that HFRS patients can be found not only in rural areas and also in urban and animal rooms. There are three epidemiologic patterns of HFRS according to the location of outbreak and the reservoirs host of the disease.

A. Rural type

The reservoir of HFRS in the rural endemic areas is the field mice. These rodents live only in fields but will invade houses during the snow season. Rural type cases occur all year around in the endemic rural areas. There are two seasonal peaks in late spring and fall when incidences of the infected Apodemus mice are high in Asia. Epidemic season of NE in Scandinavia is fall-winter and epidemic season of HFRS in Bashkiria is summer. Victims are primarily farmers, soldiers and construction employees, ranging in age from 20 to 50, working or stationed in the field. There is increasing evidence that many species of wild rodent and insectivora are also reservoirs of hantaviruses in Russia and China. Outbreaks of HPS in Americas usually occur in summer. Recently, Tkachenko et al (3), Lee et al (16) and Kim et al (17) demonstrated that wild birds and bats are possible reservoirs of Hantaan virus in Russia and Korea.

B. Urban type

The reservoir for the urban type of HFRS is house rats. This type threatens to endanger the world as never known before. The 130 cases of HFRS were reported among residents of urban areas of Osaka, Japan, during the 1960s. Recently, over 100 cases of HFRS were reported in metropolitan areas of Seoul and other large cities in Korea annually where the patients had never been outside the city limits, but who had histories of contact with house rats (3). Cases of the urban type occur throughout the year, but tend to be more frequent in fall and winter seasons.

C. Animal room type

The reservoir for animal room HFRS is colonized experimental rats and hamsters. This was proven by demonstration of antibody and isolation of the

virus from experimental rats and hamsters (1,3). There were 33 outbreaks of HFRS from 1976 to 1985 among personnels in the medical centers of animal rooms in Korea and Japan where Hantaan or Seoul virus experiments had not been conducted. The victims in Japan was 149 and one of them died. Animal room HFRS may occur at any time of the year, but a series of outbreaks occurred during the winter season in non-ventilated animal rooms when the air in the rooms was dry. Laboratory infection with hantavirus where hantavirus experiments have never been conducted have occurred not only in Asia but also in Europe. The 116 cases of HFRS occurred in an institute in Moscow after introduction of wild mice caught in the endemic areas of HFRS into animal rooms of an institute in 1965 and a few cases occurred in animal rooms of research institutes in Belgium and Great Britain. These incidents demonstrate that exports, imports, and exchanges of special animal models of infected rats among research institutes are very dangerous.

Table 5 shows infection rate of people with hantaviruses in South Korea and telephone line construction employees and Golf course employees are high risk group. Man is susceptible and show clinical symptoms but no animals are susceptible after inoculation of hantaviruses though small animals are reservoir host of the viruses.

Model of Transmission

Mode of transmission of hantaviruses is air-borne infection via respiratory tract from excreta of infected rodents to rodents (18) and to humans (1) but no direct patient to human transmission of the virus in HFRS was documented. However, direct patient to human infection in HPS was suggested in Argentina, recently (19).

Hemorrhagic fever with renal syndrome in Korea

HFRS is an important public health problem in Korea since Korean War and Table 6 shows no. of hospitalized patient from 1951 to 1998. Serologic diagnosis of HFRS patient was started from 1977 and HTNV vaccine was available since 1991 in Korea. However, actual total no. of the patient in Korea is not clear since no. of the patient in this table is no. of the patient diagnosed serologically at 5 laboratories only in Seoul. Vaccination

campaign was started from 1991 and no. of the patient was decreased significantly from 1995, 3 years after the vaccination started. Insecticide (Benzyl-benzoid) was used to prevent HFRS in the US Army from 1951 to 1977 and Korean Army used it from 1956 to 1978. As shown in Table 6, benzyl-benzoid had no effect to decrease no. of patient in Korean Army and it means that mites are not important vector to transmit the disease from rodents to man. Table 7 shows no. of HFRS patient and no. of vaccinees in the Korean Army from 1981 to 1998 and no. of patient was decreased significantly since 1991 after vaccination campaign was started. According to the Korean Army, the efficacy of Hantavax in soldiers from 1994 to 1998 is 86%.

Hantaan virus vaccine

In 1989, Lee et al. (20~21) developed a formalin inactivated suckling mouse brain HTNV vaccine and this vaccine (Hantavax^{TM}) is available commercially since 1991 in Korea. HTNV 84-105 strain isolated from blood of a HFRS patient in Vero-E6 cells was used as the vaccine seed virus because it showed the best immunogenicity in animals among 8 human isolates in Korea. The comparative immunogenicity of HTNV vaccines made with 84/105 human isolate and with 76/118 Apodemus isolate is shown in Table 8. HTNV 84/105 showed better immunogenicity in rats by immunofluorescent (IF) and neutralizing (N) antibody test than HTNV 76/118. As shown in Table 9, HTNV antigen harvested from suckling mouse brain had the best antigenicity compared with other virus antigens from Vero cells, Baculovirus and Adenovirus vectors in guinea pigs. Hantavax was made according to the modified method of Japanese encephalitis (JE) vaccine with HTNV 84/105 by inoculation into 1 day old ICR mouse brain and alum gel was used as adjuvant. Total amount of protein and myelin basic protein of Hantavax, JE vaccine and PUUV vaccine are shown in Table 10. Total amount of protein and myelin basic protein of Hantavax is 10 ug/ml and <0.01 ng/ml, respectively. These proteins are far less than the amount of WHO minimum requirement. One dose of Hantavax for adult contains 5,120 unit/ELISA of virus antigen in 0.5 ml and 2,560 unit/ELISA for children in 0.5 ml. Immune response and antibody persistence against HTNV of 61 vaccinees after vaccination twice subcutaneously (SC) at one month interval

according to the recommended vaccination schedule were measured at 1-4 months and 1 year after primary basic vaccination, and one month and 2-4 years after booster vaccination at one year by IFA, high density particle agglutination (HDPA) and plaque reduction neutralization test (PRNT). As shown in Fig. 3, seroconversion of 61 vaccinees on 1-4 months after primary vaccination were 20/21 (95.2%), 19/21 (90.5%) 14/21 (66.7%) whose geometric mean antibody titers were 262, 248, 120; 40 vaccinees on 1 year after primary vaccination were 25/40 (62.5%), 18/40 (45.0%), 9/40 (22.5%) whose geometric mean antibody titers were 90, 56, 24 by IFA, HDPA and PRNT, respectively. Seroconversion of 12 vaccinees on 20 months after booster vaccination was 11/12 (91.7%), 9/12 (75.0%) whose mean antibody titers were 296, 33 and 7 vaccinees on 3 months after second booster vaccination on 2 year were 7/7 (100%), 6/7 (85.7%) whose mean antibody titers were 549, 46 by IFA and PRNT, respectively. It was concluded that the booster vaccination is necessary at 1 year after primary basic vaccination for maintaining high level of antibodies, and antibodies after boost shot at 1 year persist for two years at least. Since Hantavax was available in 1991, total no. of hospitalized HFRS patient in South Korea decreased significantly from 1,234 cases in 1991 to 415 cases in 1997 as shown in Table 6.

Field efficacy trial of Hantavax in Yugoslavia

Recently, the efficacy trial of Hantavax in the endemic areas of HFRS was carried out under a randomized, placebo-controlled field study with vaccinees who received Hantavax twice in 1996 and a booster vaccine in 1997 in Yugoslavia. Twenty HFRS patients were documented among control group of 2,000 non-vaccinees but none were reported among 2,000 vaccinees as shown in Table 11. Data on neutralizing antibody responses in Hantavax vaccinees are limited and some investigators have been presented 60~80% seroconversion rate by PRNT (22~23).

It was shown that heat inactivation of sera from vaccinees reduce N antibodies significantly against HTNV, therefore, it is recommended to use non-inactivated sera from vaccinees for evaluation of protective N antibodies as shown in Table 12.

HTNV-PUUV combination vaccine

PUUV exist not only in Europe and also in Asia, such as in eastern parts of Russia, Japan and Korea (24~25). Recently, we confirmed HFRS patients infected with PUUV in Korea. Based on these results, for complete protection of HFRS occurring in Eurasia, an effective combination vaccine against HTNV and PUUV infection is urgently needed.

Recently, we have developed a formalin inactivated suckling hamster brain-derived PUUV vaccine to prevent PUUV infection and HTNV-PUUV combination vaccine to prevent HFRS caused by HTNV and PUUV infection in the world simultaneously.

PUUV K27 strain isolated from a HFRS patient in Ufa, Bashikiria was used as seed virus and the total protein and myelin basic protein content of PUUV vaccine are 12 ug/ml and <0.01 ng/ml, respectively as shown in Table 10. One dose of PUUV vaccine contains 5,120 unit/ELISA of virus antigen in 0.5 ml. Hamsters after inoculation of inactivated Hantavax and PUUV vaccine produced high titers of IF and N antibodies against HTNV and PUUV.

One dose of HTN-PUU combination vaccine contains both 5,120 unit/ELISA of HTNV and PUUV antigen in 1.0 ml. Antibody responses of hamsters after inoculation of 0.1 ml of combined HTN-PUU vaccine showed good IF and N antibody production against 2 viruses as shown in Fig. 4. Actually, the combined vaccine produced higher N antibodies against PUUV than monovalent vaccine.

To examine the potential usefulness of HTN-PUU combination vaccine against HFRS and HPS caused by several hantavirus serotypes, vaccinated hamsters with HTN-PUU combination vaccine twice at one month interval were challenged with 5 different serotype hantaviruses and tested immune response, incidence of viremia, and presence of virus in the target organs. Ten volunteers were also vaccinated with HTN-PUU combination vaccine and immune responses were analyzed.

Inactivation of HTNV and PUUV in the vaccines with 0.4% formalin were confirmed by passing inactivated virus three times in Vero E6 cells and in suckling mouse and in suckling hamster brains. There was no evidence of virus replication in the inoculated cells, brains and lungs of vaccinated suckling animals by IFA and nested RT-PCR. Three viral structural proteins in vaccines were detected by western blot with HFRS

convalescent sera.

Hamsters were given 0.1 ml of vaccine twice intramuscularly at one month interval and bled from retro-orbital sinus on 30 days after each vaccination. Antibody titers were measured by IFA and PRNT against 5 serotype hantaviruses; HTNV strain Howang, SEOV strain 80/39, Belgrade/Dobrava virus (BEL/DOBV) strain Belgrade 1, PUUV strain K27 and New York virus (NYV). Mean IF antibody titers on 30 days after 1st shot were 78.4, 68.8, 68.8, 37.9 and 15.6; mean N antibody titers were 65.4, 12, 6.1, 65.6 and 0.5 against HTNV, SEOV, BEL/DOBV, PUUV and NYV, respectively. Mean IF antibody titers on 30 days after 2nd shot were 686.9, 567.5, 550.4, 516.3 and 430.9; mean N antibody titers were 710.8, 41.9, 24.3, 409.9 and 1.6 against HTNV, SEOV, BEL/DOBV, PUUV and NYV, respectively as shown Table 13.

It was reported that HTNV, SEOV and PUUV RNA could be detected in infected hamsters' lungs, kidneys and blood by nested RT-PCR (23). Hamsters were infected with 1,000 pfu of each serotype of hantaviruses and then bled on reglular intervals and lungs were excised on 30 days after inoculation of viruses. Sera from all infected hamsters have shown high antibody titers to homologous viruses by IFA and PRNT. Viremia and virus RNA in lungs could be detected from all of the infected hamsters by nested RT-PCR using serotype-specific primers as shwon Table 14 and Table 15.

Vaccinated hamsters with HTN-PUU combination vaccine twice at 30 day intervals were challenged on 30 day after 2 dose of vaccination with 6 different serotype hantaviruses; HTNV, SEOV, BEL/DOBV, PUUV, SNV and NYV. None of the vaccinated hamsters challenged with live HTNV, SEOV, BEL/DOBV, PUUV showed viremia nor virus in lung tissues. In contrast, vaccinated hamsters challenged with live SNV and NYV showed viremia and virus in lung tissues as shown Table 18 and Table 19. In immune response, the vaccinated hamsters challenged with HTNV, SEOV, BEL/DOBV, PUUV did not show significant increase of IF and N antibodies but vaccinated hamsters challenged with SNV and NYV showed dramatic increase of N antibodies against NYV.

In our limited study, 10 volunteers who are working with hantaviruses at our institute were vaccinated with HTN-PUU combination vaccine 3 times subcutaneously at 1 month interval with various dose of the vaccine. All vaccinees produced relatively high IF antibodies (128~2,048)

and medium level of N antibodies (10~640) against homologous hantaviruses after second and third vaccination as shown in Table 16. There was no side effect of the combined vaccine among vaccinees who were vaccinated and not vaccinated with Hantavax before. HTN-PUU combination vaccine could be used after safety tests and a field efficacy trial in the endemic areas of HFRS where these 2 viruses co-exist in the world.

Differential diagnosis

In the individual case with mild to moderate clinical findings, it is impossible to diagnose hantavirus infection on clinical grounds alone. A serologic diagnosis of hantavirus infection can be made by demonstrating a rise of antibody titer to hantaviruses. We developed a simple rapid diagnostic test for Hantaan virus infection by using high density particle agglutination (HDPA) that can be used in the field. There are about five serologic tests against hantavirus infections but IFAT, HDPA and ELISA are simple, rapid and sensitivie tests.

High density particle agglutination test

Recently, we developed simple diagnostic kits for Puumala virus and Sin Nombre virus infections by using HDP. Table 17 shows comparative sensitivity and sepcificity of HDPA test and FA test for diagnosis of HFRS and HPS patients. HTNV HDPA test is sensitive and specific for diagnosis of HFRS patient in Korea, China and Japan and PUUV HDPA test is sensitive and specific for diagnosis of HFRS patient in Yugoslavia, Russia and Finland and SNV HDPA test is sensitive and specific for diagnosis of HPS patient in the U.S.A. However, IFA test with HTNV, PUUV and SNV is group specific against many hantaviruses.

References

1. Lee HW, van der Groen G. Hemorrhagic fever with renal syndrome. *Prog Med Virol.* 1989;36:62~102 .
2. Duchin JS, Koster FT, Peters CS, Simpson GL, Tempest B, Zaki SR, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients

with a newly recognized disease. *N Engl J Med*. 1994;330:949~55.
3. Lee HW. Epidemiology and pathogenesis of hemorrhagic fever with renal syndrome. In: Elliott RM, eds. *The Bunyaviridae*. New York: Plenium press, 1996;253~267.
4. Schamljohn C, Hjelle B. Hantaviruses: A global disease problem. *Emer Infect Dis*. 1997;3:95~104.
5. Lee HW, Lee PW, Johnson K. Isolation of the etiologic agent of Korean hemorrhagic fever. *J Infect Dis*. 1978;137:298~308.
6. Nichol ST, Siropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science*. 1993;262:914~917.
7. Hjelle B, Jenison S, Torrez-Martinez N, Yamada T, Notte K, Zumwalt R, Macinnes K and Meyers G. A novel hantavirus associated with an outbreak of fatal respiratory disease in the southwestern United States; Evolutionary relationships to known hantaviruses. *J Virol*. 1994;68:592~596.
8. Ksiazek TG, Peters CJ, Rollin PE, Zaki S, Nichol S, Spiropoulou C, et al. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. *Am J Trop Med Hyg*. 1995;52:117~123.
9. World Health Organization Regional Office for the Western Pacific : Memorendum from a working group on development of vaccines for arthropod and rodent-borne viruses. *Manila, Phillipines*, 1984.
10. Schamljohn C, Hasty S, Dalrymple J, LeDuc J, Lee HW, von Bonsdorff C-H, et al. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. *Science*. 1985;227:1041~1044.
11. Puthavathana P, Lee HW, Kang CY. Typing of hantaviruses from five continents by polymerase chain reaction. *Virus Res*. 1982;26:1~14.
12. Mills JN, Yates TL, Ksiazek TG, Peters CJ, James EC. Long-term studies of Hantavirus reservoir pupolations in the Southwestern United States: Rationale, Potential, and Methods. *Emerging Infect Dis*. 1999;5:95~101.
13. Abbott KD, Ksiazek TG, Mills JN. Long-term Hantavirus persistence in rodent populations in Central Arizona. *Emerging Infect Dis*. 1999;5:101~112.
14. Kitsutani PT, Denton RW, Fritz CL, Murray RA, Todd RL, Pape WJ, Frampton JW, Young JC, Khan AS, Peters CJ, Ksiazek TG. Acute Sin Nombre Hantavirus infection without pulmonary syndrome, United States.

Emerging Infect Dis. 1999;5:701~705.

15. Calderon G, Pini N, Bolpe J, Levis S, Mills J, Segura E, Guthmann N. et al. Hantavirus reservoir hosts associated with peridomestic habitats in Argentina. *Emerging Infect Dis*. 1999;5:792~797.
16. Lee HW, Baek LJ, Lee YT. Seroepidemiologic study of hantavirus infection of wild birds and bats in Korea. *J Kor Soc. Virol*. 1991;21:127-134.
17. Kim GR, Lee YT, Park CH. A new natural reservoir of hantvirus; isolation of hantaviruses from lung tissues of bats. *Arch Virol*. 1994;134:85~95.
18. Nuzum E, Rossi C, Stephenson E, LeDuc J. Aerosol transmission of Hantaan and related viruses to laboratory rats. *Am J Trop Med Hyg*. 1988:38;636~640.
19. Enria D, Padula P, Segura EL, Pini N, Edelstein A, Riva PC. et al. Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission. *Medicina (B Aires)*. 1995;58:709~711.
20. Lee HW, Ahn CN. Development of a vaccine against hemorrhagic fever with renal syndrome. *J Kor Soc Virol*. 1988;18:143-148
21. Lee HW, Ahn CN, Song JW, Baek LJ, Seo TJ, Park SC. Field efficacy trial of an inactivated vaccine against hemorrhagic fever with renal syndrome in humans. *Arch Virol*. 1990;[Suppl 1]:35-47
22. Cho HW, Howard CR. Antibody responses in humans to an inactivated hantavirus vaccine (Hantavax). *Vaccine*. 1999;17:2569-2575.
23. Chu YK, Woo YD, Lee HW. Immune response and antibody persistence against hantaan virus of vaccinees with Hantavax. *J Kor Soc Infect Dis*. 1998;30:317-324
24. Horling J, Chizhikov V, Lundkvist A, Jonsson M, Ivanov L, Dekonenko A, Niklasson B, Dzagurova T, Peters CJ, Tkachenko E, Nichol S. Khabarovsk virus: a phylogenetically and serologically distinct hantavirus isolated from Microtus fortis trapped in far-east Russia. *J Gen Virol*. 1996;77: 687-94
25. Arikawa H, Yoshizumi S, Arikawa J, Yoshimatsu K, Takahashi K, Takashima I, Hashimoto N. Evidence for the existence of Puumala-related virus among Clethrionomys rufocanus in Hokkaido, Japan. *Am J Trop Med Hyg*. 1995;53:222-227.

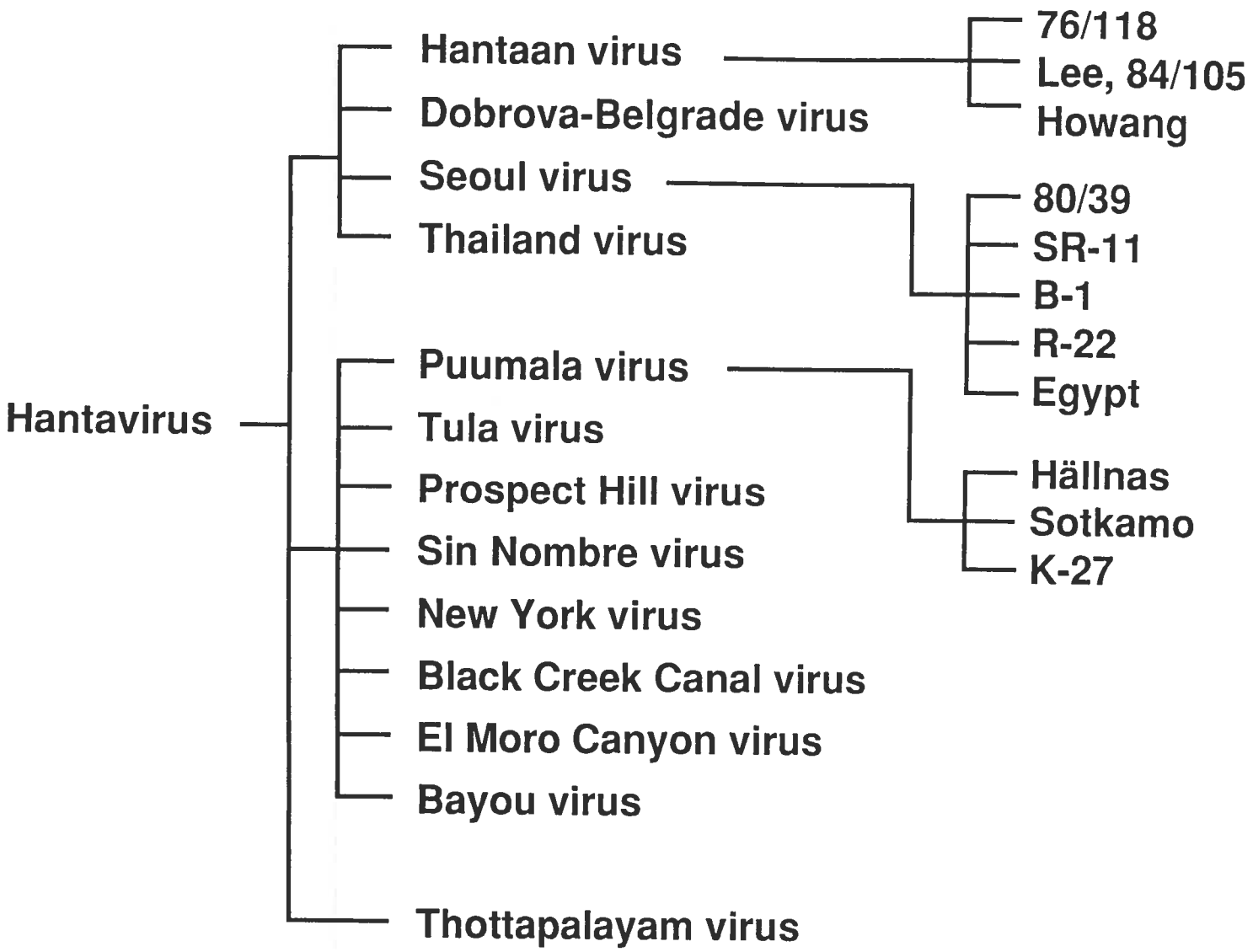

Figure 1. Classification of hantaviruses

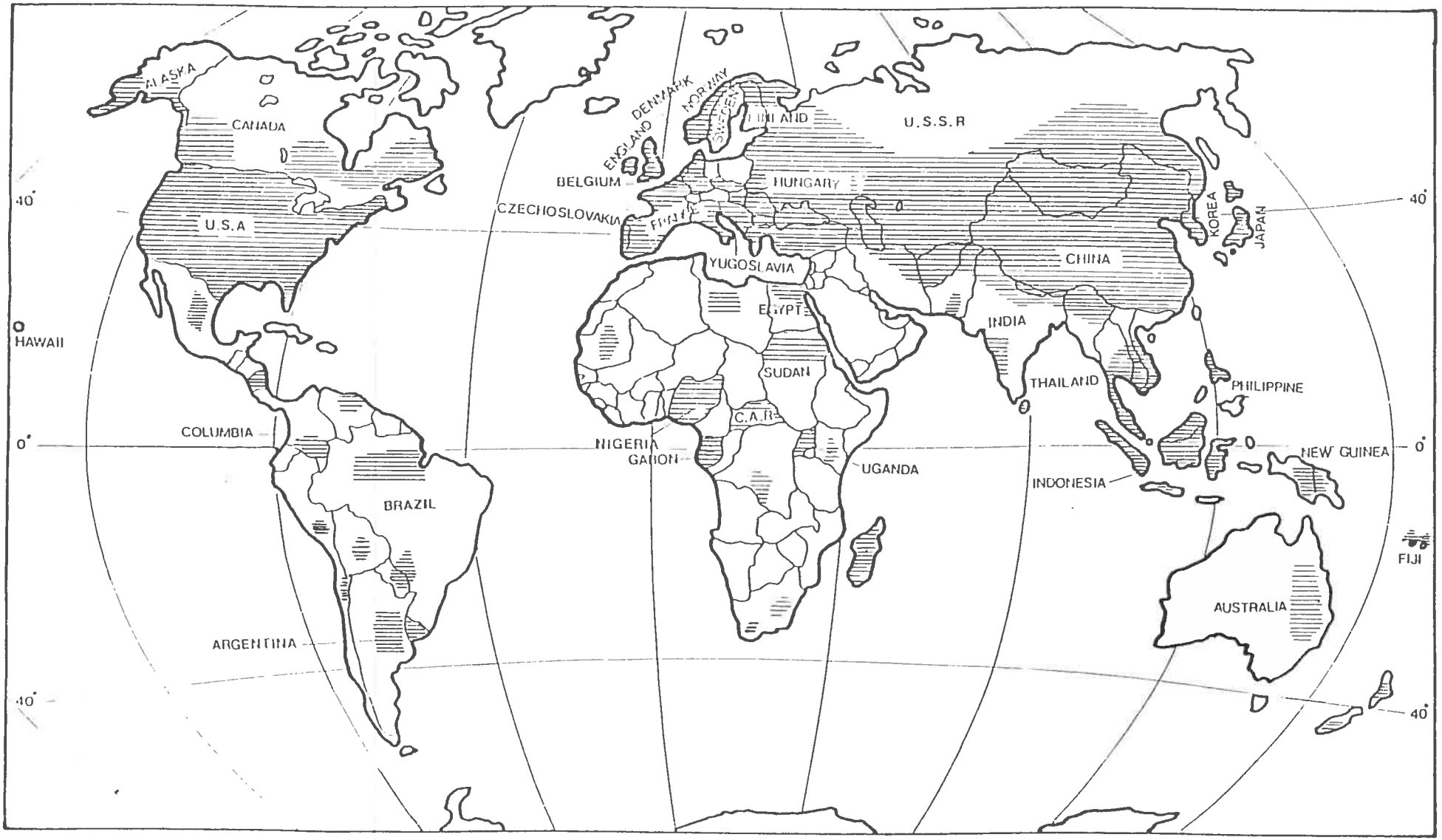

Figure 2. Global distribution of hantaviruses

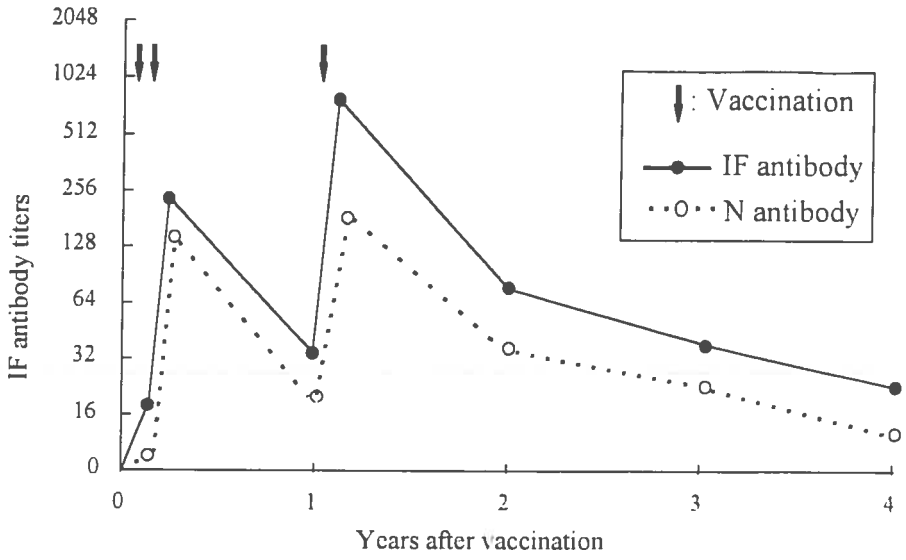

Figure 3. Persistence of IF and N antibodies against Hantaan virus in the Hantavax™ vaccinees

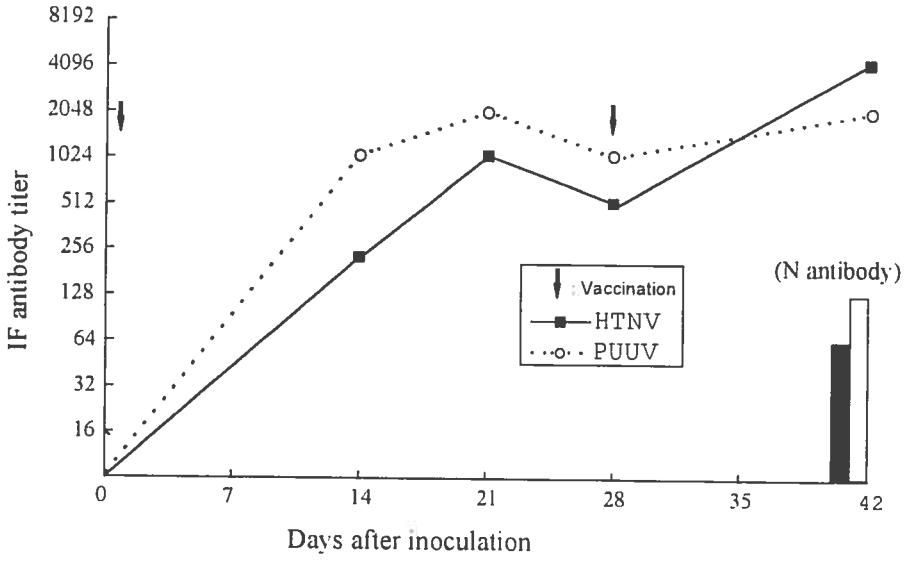

Figure 4. Antibody response of hamsters after inoculation of Hantaan-Puumala virus combination vaccine

Table 1. War and Hemorrhagic fever with renal syndrome

Year	Name of war	Name of disease	Number of patient (fatality)	Local hantavirus
1861-1866	The American Civil War	Epidemic nephritis	14187	Sin Nombre Seoul Prospect Hill
1914-1918	The First World War British troops	War nephritis, Trench nephritis, Epidemic nephritis	12000	Puumala Seoul
1939-1945	The Second World War			
	1. Japanese troops in China	Epidemic haemorrhagic fever	12500 (15-30%)	Hantaan Seoul
	2. Soviet troops in Far East	Hemorrhagic nephrosonephritis	8000 (10-20%)	Hantaan Puumala
	3. German and Finland troops in Lapland	Nephropathia epidemica, Field fever	10000	Puumala Seoul
	4. German War Prisoners in Yugoslavia	Epidemic nephritis, Virus glomerulonephritis	6000	Puumala Hantaan Belgrade
1951-1954	The Korean War UN troops	Korean hemorrhagic fever	3256 (10-20%)	Hantaan Seoul
1993-1995	Bosnia War in Yugoslavia	Hemorrhagic fever with renal syndrome	700	Belgrade Puumala

Table 2. Hantaviruses and their serotypes

Virus isolate	Country	Host of origin	Serotype PRNT	PCR Typing
ASIA				
76/118	Korea	Apodemus agrarius	HTN	HTN
A9	China	Apodemus agrarius	HTN	HTN
Jinhae 87/526	Korea	Apodemus agrarius	HTN	HTN
Maaji	Korea	Apodemus agrarius	HTN	HTN
ROK 79/90	Korea	Human	HTN	HTN
US 84/2	Korea	Human	HTN	HTN
83/14	Korea	Apodemus agrarius	HTN	HTN
83/138	Korea	Apodemus agrarius	HTN	HTN
Thailand #605	Thailand	Rattus norvegicus	SEO	SEO
Thailand #749	Thailand	Bandicota indica	SEO	SEO
80/39	Korea	Rattus norvegicus	SEO	SEO
Hamster 85/4	Korea	Syrian hamster	SEO	SEO
JTRN/82/17	Japan	Rattus norvegicus	SEO	SEO
I/RN/82/3	Korea	Rattus norvegicus	SEO	SEO
EUROPE				
Cg 3883	Russia	Clethrionomys glareolus	HTN	HTN
Fojnica	Yugoslavia	Apodemus flavicollis	HTN	HTN
NE 672	Finland	Clethrionomys glareolus	PUU	PUU
Hallnas B1	Sweden	Clethrionomys glareolus	PUU	PUU
Yanagihara	Finland	Clethrionomys glareolus	PUU	PUU
GBB	England	Laboratory rat	SEO	SEO
NORTH AMERICA				
Baltimore rat	USA	Rattus norvegicus	SEO	SEO
Houston rat #4	USA	Rattus norvegicus	SEO	SEO
Tchoupitouas	USA	Rattus norvegicus	SEO	SEO
Prospect Hill Virus I	USA	Microtus pennsylvanicus	PH	PH
Sin Nombre	USA	Peromyscus maniculatus	SN	SN
SOUTH AMERICA				
Brazil 2/4	Brazil	Rattus norvegicus	SEO	SEO
AFRICA				
Egypt R/12915	Egypt	Rattus norvegicus	SEO	SEO

Table 3. Laboratory-acquired infection with hantaviruses

Country	No. of patient
Russia	113 (1962)
Japan	149 (1 death) since 1964 at 24 Institutes
Korea	9 since 1980 at 8 Institutes
China	Many
Belgium	4
France	2
Netherland	2
England	1

Table 4. Clinical manifestations of hantavirus disease

Geographical distribution	Virus	Disease	Severity	Fatality (%)	Chief clinical manifestations
Americas	Sin Nomber	HPS	Severe	60	Fever, pulmonary syndrome
Balkan	Belgrade	HFRS	Severe	15	Fever, hemorrhagic, renal syndrome
Eurasia	Hantaan	HFRS	Severe	3~7	Fever, hemorrhagic, renal syndrome
Worldwide	Seoul	HFRS	Moderate	1~2	Fever, hemorrhagic, renal and hepatic syndrome
Europe	Puumala	HFRS (NE)	Mild	0.2~1	Fever, renal syndrome

HPS, hantavirus pulmonary syndrome; HFRS, haemorrhagic fever with renal syndrome;

NE, nephropathia epidemica

Table 5. Infection rate of peoples with hantaviruses

Group by profession or locality	IF antibody positive rate to Hantaan virus (n=150 – 950)
Resident of cities (Seoul, Pusan, Incheon)	1.0%
Blood donors (Seoul)	1.2%
Soldiers (ROK & U.S. Army)	1.1%
Resident of the rural endemic areas of HFRS (Tongducheon)	3.8%
Golf course employees (caddies, workers)	4.2%
Telephone line construction employees	9.0%

Table 6. Hemorrhagic fever with renal syndrome in Korea

Year		Korean	ROK Army		US Army		Total
1951		...	26		827	Benzyl-B	853
1952		...	18		833	started	851
1953		...	...		455		455
1954		19	...		307		326
1955		...	...		20		20
1956		...	26	Benzyl-B	28		54
1957		...	21	started	13		34
1958		...	20		15		35
1959		...	47		79		126
1960		...	185		10		195
1961		...	341		27		368
1962		...	311		29		340
1963		...	257		11		268
1964		18	205		22		245
1965		2	110		99		211
1966		11	82		36		129
1967		13	86		31		130
1968		26	102		28		156
1969		48	134		9		191
1970		131	221		13		365
1971		391	358		2		751
1972		186	203		0		389
1973		241	237		0		478
1974		176	251		0		427
1975		466	370		1		837
1976		858	304		4		893
1977	Serodiagnosis	288	241		7	Benzyl-B	536
1978	started	207	168	Benzyl-B	10	stopped	385
1979		241	122	stopped	1		364
1980		185	72		1		258
1981		377	164		2		543
1982		378	123		3		504
1983		402	98		3		503
1984		568	156		6		730
1985		531	159		7		697
1986		530	166		14		710
1987		533	163		5		701
1988		264	97		6		367
1989		306	104		6		416
1990		964	73		6		1,043
1991	Vaccination	1,190	44		0		1,234
1992	started	1,116	49		2		1,167
1993		1,230	62		1		1,293
1994		1,006	34		1		1,041
1995		752	29		1		782
1996		662	23		1		687
1997		390	23		2		415
1998		709	37		4		750
Total		15,142	6,123		2,988		24,253

Table 7. Number of HFRS patient before and after vaccination program in ROK Army

Year	No. of hospitalized patient	No. of vaccinated soldier
1981	164	0
1982	123	0
1983	98	0
1984	156	0
1985	159	0
1986	166	0
1987	163	0
1988	97	0
1989	104	0
1990	73	0
1991	44	16,500
1992	49	72,500
1993	62	66,500
1994	34	71,000
1995	29	116,000
1996	23	77,500
1997	23	81,000
1998	37	80,000

Table 8. Comparison of immunogenicity of inactivated suckling mouse brain Hantaan virus vaccines with different strains

Vaccination schedule	Vaccine (virus)	Dose & route	Rat no.	IFA titers against HTNV on days after vaccination				PRN antibody mean titer
				D-0	D-10	D-20	D-30	
1 time	HTNV[1] 76/118	512 unit/IM	1	<16	64	256	256	<10
			2	<16	128	512	256	
			3	<16	128	128	64	
	HTNV[2] 84/105	512 unit/IM	1	<16	512	2048	2048	11
			2	<16	2048	4096	2048	
			3	<16	2048	4096	2048	
3 times (10 day interval)	HTNV 76/118	512 unit/IM	1	<16	512	4096	8192	7
			2	<16	256	4096	4096	
			3	<16	128	4096	4096	
			4	<16	256	8192	8192	
	HTNV 84/105	512 unit/IM	1	<16	1024	16384	16384	47
			2	<16	1024	16384	16384	
			3	<16	1024	8192	8192	
			4	<16	512	8192	8192	

[1]Hantaan virus 76/118 isolated from an *Apodemus* mice.

[2]Hantaan virus 84/105 isolated from a HFRS patient.

Table 9. Comparison of antigenicity of Hantaan virus vaccines harvested from different source

Source of virus	ELISA antigen unit/0.1 ml	High density particle agglutinin/0.1 ml	Antibody titers in guinea-pigs	
			IF	PRN
Vero cells	3,200	1,600	1,600	10
Baculo virus expressed nucleocapsid protein	3,200	<40	800	<10
Adenovirus expressed G1, G2 glycoproteins	6,400	<40	160	80
Suckling mouse brain	12,800	6,400	6,400	160

Table 10. Myelin basic protein and total protein content of JE and HFRS vaccines

Vaccine	Old JE vaccine 25 day old mouse	New JE vaccine 10 day old mouse	Hantavax™ 7 day old mouse	PUU vaccine 14 day old hamster
Brain weight (g)	0.3	0.2	0.1	0.4
ELISA antigen unit/0.1 ml	1,200	1,800	1,024	1,024
Protein content (ug/ml)[1]	22	17	10	12
Myelin basic protein (ng/ml)[2]	0.8	0.08	<0.01	<0.01

[1]WHO minimum requirement is ≤80 ug/ml [2]WHO minimum requirement is ≤2.0 ng/ml

Table 11. Field efficacy trial of Hantavax™ and HFRS incidence among vaccinated and non-vaccinated control groups in Yugoslavia, 1997-1998

Group	Vaccinated group (n=2,000)	Control group (n=2,000)
HFRS case informed	0	20

Table 12. Comparison of neutralizing antibody titers of Hantavax vaccinees between results of heat-inactivated and non-inactivated sera

Vaccination	After 1 dose			After 2 dose		
Treatment	56°C 30'	56°C 30'	not inactivated	56°C 30'	56°C 30'	Not inactivated
No. positive /no. tested	1/30 (3.3%)	4/30 (13.3%)	6/30 (20%)	5/30 (16.7%)	11/30 (36.6%)	20/30 (66.7%)

Table 13. Detection of virus RNA from blood of 5 infected hamsters with 6 different hantaviruses by nested RT-PCR

Virus	Virus detection on days after infection					
	D-4	D-8	D-12	D-16	D-23	D-30
Hantaan virus HW	4/5	1/5	0/5	0/5	2/5	1/5
Seoul virus 80/39	0/5	3/5	2/5	0/5	0/5	1/5
Belgrade virus	0/5	3/5	2/5	0/5	1/5	2/5
Puumala virus K27	3/5	2/5	0/5	1/5	0/5	2/5
Sin Nombre virus CC107	0/5	3/5	3/5	1/5	1/5	2/5
New York virus	0/5	4/5	3/5	0/5	2/5	2/5
Uninfected	0/5	0/5	0/5	0/5	0/5	0/5

Table 14. Detection of virus RNA from the lung and kidney tissues of 5 infected hamsters with 6 different hantavirus by nested RT-PCR

Virus	Virus antigen detection in			
	Lungs		Kidneys	
	IFA	Nested RT-PCR	IFA	nested RT-PCR
Hantaan virus HW	5/5	5/5	5/5	5/5
Seoul virus 80/39	0/5	5/5	0/5	5/5
Belgrade virus	0/5	5/5	0/5	5/5
Puumala virus K27	0/5	5/5	0/5	5/5
Sin Nombre virus CC107	0/5	5/5	0/5	5/5
New York virus	0/5	5/5	0/5	5/5
Uninfected	0/5	0/5	0/5	0/5

Table 15. Detection of viremia and virus RNA in the lungs of 6 Vaccinated hamsters after challenge with 6 different hantaviruses by nested RT-PCR

Challenge virus	Viremia	Virus in lung
HTNV Howang	0/6	0/6
SEOV 80/39	0/6	0/6
BELV Belgrade 1	0/6	0/6
PUUV K27	0/6	0/6
SNV CC107	6/6	6/6
New York virus	6/6	6/6
Mock infected	0/6	0/6

Table 16. IF and N antibody titers of Hantaan-Puumala virus combination vaccine vaccinees

No.	Age & Sex	History	Vaccine & dose	IF and PRN antibody titers to Hantaan, Belgrade and Puumala viruses																	
				Before vaccination				After first shot (1 M[d])					After second shot (2 M)					After third shot (13 M)				
				IFA		PRNT		IFA		PRNT			IFA		PRNT			IFA		PRNT		
				HTN	PUU	HTN	PUU	HTN	PUU	HTN	BEL	PUU	HTN	PUU	HTN	BEL	PUU	HTN	PUU	HTN	BEL	PUU
1	38/M	Hantavax	HTN-PUU 0.6 ml[a]	256	-	40	-	2048	512	160	20	40	4096	128	160	40	320	4096	512	160	40	320
2	66/M	Hantavax		32	-	-	-	256	-	20	-	10	256	32	80	-	40	512	32	20	-	40
3	27/M	none		-	-	-	-	2048	128	640	-	160	4096	128	1280	10	160	4096	512	1280	10	640
4	33/M	none		-	-	-	-	128	-	10	-	-	1024	128	20	10	20	2048	256	20	10	40
5	24/M	none		-	-	-	-	128	32	-	-	-	128	64	-	-	10	512	128	20	-	20
6	33/F	none	HTN-PUU 0.8 ml[b]	-	-	-	-	32	-	40	80	-	64	32	160	80	40	64	64	80	10	80
7	26/F	none		-	-	-	-	64	32	20	-	-	128	32	40	10	20	1024	128	80	10	40
8	27/M	none		-	-	-	-	128	32	40	-	10	256	64	40	-	40	256	64	40	-	160
9	28/M	none	HTN-PUU 1.0 ml[c]	-	-	-	-	512	128	20	-	10	512	64	320	20	320	1024	128	640	40	320
10	53/F	none		-	-	-	-	1024	128	20	-	40	1024	64	80	10	160	2048	256	80	10	160
11	25/F	none	PUU vaccine 0.5 ml	-	-	-	-	-	128	-	10	80	-	128	-	20	320	-	256	-	10	640
12	22/F	none		-	-	-	-	-	64	-	-	10	-	128	-	-	80	-	128	-	-	160
13	54/F	none	Hantavax 0.5 ml	-	-	-	-	128	-	40	-	-	1024	-	160	-	-	2048	-	160	20	-
14	26/F	none		-	-	-	-	1024	-	20	-	-	512	-	80	-	-	512	-	80	-	-

a: HTN vaccine 0.3 ml + PUU vaccine 0.3 ml

b: HTN vaccine 0.4 ml + PUU vaccine 0.4 ml

c: HTN vaccine 0.5 ml + PUU vaccine 0.5 ml

d: M : Month after vaccination

Table 17. Comparative titration of HFRS and HPS patients sera by IFAT and high density particle agglutination test

Name of Disease	Country	Code no. of serum	IFA Test			HDPA Test		
			HTNV	PUUV	SNV	HTNV	PUUV	SNV
HFRS	Korea	ROK 88-110-3	10240	80	20	5120	80	80
		ROK 88-119-3	10240	40	20	5120	<40	<40
		ROK 88-123-1	10240	80	80	10240	320	<40
		KHF 81-498-6	20480	80	40	10240	80	<40
		KHF 81-499-6	40960	80	20	20480	20	<40
		KHF 81-566-7	40960	320	80	20480	160	80
		KHF 81-745-4	20480	160	40	20480	160	<40
		KHF 81-790-7	5120	20	20	5120	<20	<40
	China	C-X-15	10240	2560	20	5120	160	<40
		C-X-27-1	20480	2560	20	20480	40	<40
		C-X-46	20480	80	40	20480	80	<40
		C-X-28	20480	<20	<20	10240	<20	<40
		C-X-24	20480	20	40	20480	320	<40
	Japan	Jap 13-3	10240	640	80	5120	<40	<40
		Jap 13-5	640	80	<20	10240	<40	<40
		Jap HM-9	320	20	40	1280	<40	<40
	Yogoslavia	Yugo 4601	20	20480	320	320	10240	640
		Yugo 4965	10240	320	160	2560	<40	<40
		Yugo 5455	80	1280	80	160	2560	<40
	Russia	USSR 1848a	320	5120	1280	1280	5120	<40
		USSR 488	80	5120	1280	160	5120	1280
		USSR 1883a	640	20480	2560	640	10240	<40
	Finland	Fin 85-816	80	5120	320	160	5120	<40
		Fin 85-907	<20	640	20	40	2560	40
		Fin 85-849	640	5120	320	160	5120	<40
		Fin 85-869	80	1280	160	40	1280	<40
		Fin 85-860	80	5120	1280	60	5120	40
		Fin 85-786	320	5120	1280	160	5120	80
HPS	USA	USA RBi	80	320	1280	80	160	2560
		USA GMc	40	5120	5120	80	<40	10240
		USA AH	20	1280	5120	<40	40	640
		USA RGH	320	640	5120	80	<40	40960
Negative control		Control 96-110	<20	<20	<20	<40	<40	<40
		Control 96-111	<20	<20	<20	<40	<40	<40