SEROLOGICAL EVIDENCE OF HANTAVIRUS INFECTIONS IN MALAYSIA

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Abstract. Hantaviruses are primarily rodent-borne and transmission is by inhalation of virus-contaminated aerosols of rodent excreta, especially urine and saliva. The genus *Hantavirus*, family Bunyaviridae, comprises at least 14 serotypes and the symptoms of clinical illness range from mild fever to severe hemorrhagic manifestations with renal complications. Many countries in Southeast Asia are unaware of the importance of hantavirus infections and give them low priority. Malaysia, like other countries in the region, has conducted very few studies on the epidemiology of hantaviruses – and even these were conducted in the 1980s. Using a more extensive range of hantavirus antigens, we conducted a seroprevalence study of rodents and humans and found further evidence of hantavirus infections. Moreover, the data from the antibody profiles strongly suggest the presence of different hantaviruses at the study sites.

INTRODUCTION

The genus Hantavirus, family Bunyaviridae, is made up of enveloped RNA viruses and comprises at least 14 serotypes, including those that cause Hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). The first documented account of a hantavirus outbreak was published during the Korean War, when over 3,250 United Nations troops were affected.

Hantaviruses are primarily rodent-borne and transmission is through inhalation of virus-contaminated aerosols of rodent excreta, especially urine and saliva. The clinical characteristics of HFRS are fever, hemorrhagic manifestations, and varying degrees of renal and hepatic dysfunction; mortality ranges from 5-60%. The illness runs through 5 recognizable phases: febrile, hypotensive, oliguric, diuretic and convalescent. After several days of fever, patients may have an abrupt fall in blood pressure, sometimes to shock levels. Bleeding tendency is most pronounced during the oliguric phase. Among several the members of the hantavirus family that give rise to HFRS, Hantaan virus is likely to be associated with severe HFRS – the rodent involed is *Apodemus agrarius*. Most cases are reported from Korea, China, Japan and USSR. Puumala virus tends to produce a much milder illness (nephropathia epidemica) in Europe, Scandinavia, Commonwealth of Independent States and the Balkans, and produces persistent infection in the bank vole (*Clethrionomys glareolus*). Seoul virus is also associated with mild HFRS syndrome and the reservoir rodent host is principally *Rattus norvegicus*.

In addition to the five original serotypes (Hantaan, Puumala, Seoul, Prospect Hill, Belgrade), there has been a great increase in the isolation of 'new' related hantaviruses: these include the Sin Nombre virus which is responsible for HPS and was first described in 1993 in the Four Corners region of the United States (New Mexico, Arizona, Colorado and Utah). More than 350 cases have been reported throughout North and South America. There has been no report of this virus being active outside of the Americas.

Throughout Southeast Asia, hantavirus infection is not given the priority that it deserves. In Malaysia, there have been only three reports of hantavirus infections in humans. The first case of HFRS in Singapore was reported in 1985 when it was suggested that the patient could have been infected in Singapore or Malaysia (Wong *et al*, 1985): the clinical presentation was more benign and somewhat different from that of the classic form. A second human case was reported in 1987 (France and Burns, 1987); the third patient was a man who had returned to Scotland after working in Malaysia for 6 months. The diagnosis of hantavirus infection was made by demonstrating rising titers of hantavirus IgG in the patient's serum samples by IF. The patient, a man of Vietnamese origin, had been living in Malaysia for 10 months and had been admitted to a private hospital in Singapore; he recovered without serious complications. (Chan *et al*, 1987). His clinical symptoms were not typical of HFRS as renal involvement was minimal and liver dysfunction was prominent.

Since these earlier studies were conducted at least two decades ago, it was felt that a new study should be undertaken using different hantavirus antigens, including Sin Nombre virus.

MATERIALS AND METHODS

Source of serum samples

Kelantan: One hundred and nineteen serum samples from a group of renal patients of the Hospital Universiti Sains Malaysia (HUSM), Kelantan, were tested in Seoul, Korea, for antibodies to hantaan virus (HTNV), Seoul virus (SEOV), Puumala virus (PUUV) and Sin Nombre virus (SNV) by indirect immunofluorescence.

Forty-four rodent (*Rattus norvegicus*) samples were also collected in Kelantan for seroprevalence study.

Port Klang: Eighty-seven rodent (*R. norvegicus*) serum samples were collected from Bagan Hailam fishing village in the South Port vicinity of Klang: this area is a predominantly Chinese settlement where wooden houses stand on stilts in a heavily polluted mangrove swamp.

Rodent samples collection: Tomahawk traps were used to trap rodents at the two study sites. The baited traps were set at 7.00 pm and inspected at midnight and at 7 am the following morning. Trapped rodents were brought back to the laboratory and chloroformed; blood was collected by cardiac puncture using a 5 ml syringe connected to a 21-G needle. Serum samples were kept at -20°C until tested serologically.

Hantavirus specific antibody assay

Monolayer Vero E6 cells in a 25 cm^2 culture flask infected with the respective serotype hantavirus was trypsinized after removal of the supernatant. The infected cells were resuspended in 15 ml of Dulbecco modified minimal essential maintenance medium supplemented with 10% fetal calf serum. Thirty microliters of the cell suspension were seeded into each of 10 wells of a teflon coated slide and incubated at 37°C in a sterile moist chamber. After overnight culture, the medium on the slides was carefully rinsed off using sterile phosphate buffered saline, air-dried and UV-irradiated in a biosafety cabinet class II/B2. The infected cells on the teflon slides were then fixed for 10 minutes in cold acetone and subsequently used as the antigen for serological testing for the presence of hantavirus-specific antibodies by indirect immunofluorescence. The human and rodent sera were screened for the presence of hantavirus IgG to the respective serotype at 1:16 dilution by an indirect immunofluorescent test using the prepared antigens. Two-fold serial dilutions were made on positive sera detected at the initial screen and the titers of the hantavirus specific antibody were similarly assayed by the same test.

RESULTS

Kelantan results

Of the 119 human serum samples tested, three samples (2.52%) were positive; one sample

was positive for HTNV at 1:512 dilution and for SEOV at 1:32 dilution (Table 1). Two samples were positive for only SNV, one at 1:128 and the other at 1:32 dilution. Of the 44 rodent samples tested, 2 were positive for HTNV (1:512 and 1:256) and SEOV(1:256 and 1:128) and two were positive to only SEOV at 1:64 dilution (Table 2).

Port Klang results

Of the 87 rodent samples from Port Klang, 14 (15.91%) were positive (Table 3). There were four serological patterns seen. Nine of the 14 samples gave the highest antibody titer to HTNV. One sample had antibody to only PUUV, two had antibody to only SNV and one to only SEOV. One sample had a higher titer to SEOV than HTNV.

DISCUSSION

Hantavirus infection is a zoonosis and different hantaviruses have been detected in various rodent populations throughout the world. Clinical disease in man due to hantaviruses has been noted wherever the virus has been studied. Most infections occur when man comes into contact with the excreta of persistently infected rodents where the infection in the reservoir host is not usually apparent. Virus may persist after infection and can sometimes be detected for 270 days despite the production of IgM and IgG-hantavirus antibodies. The serum prevalence of hantavirus infection among rodent populations varies considerably, and may reflect seasonal factors and host population size and structure. The dynamics of infection in rodent hosts, combined with animal and human behavior, affect the incidence in humans. In a recent study in Thailand, 2 (3.8%) out of 53 *Rattus norvegicus* trapped in Nakhon Pathom Province were seropositive for hantavirus antibody (Nitapattana *et al*, 2000). In Korea, 14% of 520 *A. agrarius* was found to be seropositive for Hantaan virus.

In this study, patients from Kelantan and rodents from Klang as well as Kelantan were surveyed for evidence of hantavirus infection. Patients from HUSM, Kelantan, with chronic renal failure were selected because an earlier study by Zainal *et al.* (1995) showed that this condition is not uncommon in Kelantan. In their study, it was found that the mortality rate for 60 patients seen between January 1991 and June 1993 was 21.7%. The majority of the deaths occurred in patients with end-stage renal failure. The cause of the chronic renal failure

Table 1
Titration of human sera from Kelantan against hantaviruses.

Sample No.	HTNV 76/118	SEOV 80-93	PUUV K-27	SNV CC-107
B014188	512	32	<16	<16
930181	<16	<16	<16	128
015633	<16	<16	<16	32

Table 2
Titration of rodent sera from Kelantan against hantaviruses.

Sample No.	HTNV 76/118	SEOV 80-93	PUUV K-27	SNV CC-107
KB2	512	256	<16	<16
KB3	256	128	<16	<16
KB81	<16	64	<16	<16
KB145	<16	64	<16	<16

Table 3
Titration of rodent sera from Port Klang against hantaviruses.

Sample No.	HTNV 76/118	SEOV 80-93	PUUV K-27	SNV CC-107
NS12	512	64	<16	<16
NS24	2,048	512	64	<16
NS32	64	16	<16	<16
NS37	4,096	1,024	512	256
NS44	2,048	512	64	64
NS46	4,096	1,024	256	128
NS49	512	2,048	<16	<16
NS62	<16	<16	256	<16
NS69	<16	<16	<16	<16
NS71	16,384	4,096	4,096	4,096
NS73	<16	<16	<16	128
NS74	4,096	2,048	1,024	1,024
NS78	<16	16	<16	<16
NS88	512	256	64	64

in the majority of the patients was unknown; for a minority, death was due to diabetic nephropathy.

Port Klang was chosen for the rodent survey becouse it is easily accessible and is known to have a large rodent population. In an earlier study of rodents–in another port area of Penang and a paddy in Perlis – 17 of 252 (6.7%) animals were positive for HTNV by immunofluorescence (Lim *et al*, 1985). None of the lung tissues was positive by virus isolation. It was speculated that the virus in Malaysian rodents was likely to be related antigenically to Seoul virus. In the same year, a report was published based on 50 paired serum samples from suspected clinical cases of leptospirosis collected between 1984 and 1985 (Lim *et al*, 1987). Of the 50 paired sera examined, only one showed low titres of antibody to Hantaan and Seoul viruses. Using the IgM ELISA test, high titred antibody to Hantaan and Seoul viruses was detected, indicating recent infection clinically, this case of HFRS is similar to classic HFRS and in addition, the patient developed jaundice and severe liver dysfunction.

The present study confirms the previous findings of hantavirus infections in humans and rodents in Malaysia. Since serological diagnosis of hantaviruses in human infections is not routinely offered in this country, it would not be surprising if hantavirus infections are very much underestimated. It is important therefore to include hantavirus serology in the differential diagnosis of similar clinical syndromes such as leptospirosis, renal failure and severe respiratory illnesses.

The hantavirus activity among rodents appears to be higher in Port Klang than in Kelantan. This is not surprising since it is well established that rodents in port areas are prone to hantavirus infections. The data from the antibody profiles strongly suggest the presence of different hantaviruses in that area. This will require confirmation by virus isolation from infected animals. This study should be extended to other parts of the country and serum samples should be collected from a larger variety of rodents.

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