SEROLOGIC EVIDENCE OF HANTAAN-LIKE VIRUS IN RODENTS AND MAN IN THAILAND

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INTRODUCTION

Hantaan virus, the cause of hemorrhagic fever with renal syndrome (HFRS) in man is a rodent borne infection enzootic in *Apodemus sp.* in Korea (Lee *et al.*, 1978), China (Song *et al.*, 1983), and Japan (Umenai *et al.*, 1981). Antigenically closely related but distinct viruses have been isolated from other rodent species, including *Rattus sp.* in several countries. Both serologic evidence of rodent infection and Hantaan-like virus isolations have been made from Brazil (LeDuc *et al.*, 1985), and the United States (LeDuc *et al.*, 1984). In Belgium, a hemorrhagic fever with renal syndrome has been associated with a Hantaan-like virus infection in man (Desmyter *et al.*, 1983). Other isolates from rats such as Girard Point (LeDuc *et al.*, 1984) and Prospect Hill (Yanagihana *et al.*, 1984) viruses are antigenically related to the prototype Hantaan virus but have not been associated with human illness although specific antibody to Prospect Hill virus has been found in man (Yanagihana *et al.*, 1984). There have been no reports of Hantaan virus in Thailand. The presence of *Hantavirus* antibody in rodents and serologic evidence of human infection in Thailand is reported herein.

MATERIALS AND METHODS

Collection sites and sampling procedure: In 1982 and 1983, three rodent species (*Rattus rattus*, *Rattus norvegicus*, and *Bandicota indica*) were trapped alive at the international shipping port (Klong Toey) in Bangkok and at the grain shipping ports of Bang Pakong and Sriracha on the eastern coast of the Gulf of Thailand. Sera of 20 *B. indica* trapped in 3 provinces in 1981 were also tested for *Hantavirus* antibody. Additional specimens of the *B. indica* were trapped around a small farm village in the western inland province of Kanchanaburi. All rodents were anesthetized and exsanguinated by cardiac puncture. Serum was separated and frozen at -70°C. For virus isolation attempts, lung, kidney, and spleen were aseptically removed, sealed in plastic tubes and frozen at -70°C

At the Klong Toey port area, serum samples were also collected from workers at a restaurant adjacent to a 4 acre grassy field where the *B indica* were trapped Serum was also collected from a group of people living on the perimeter of the same field Some of these people trapped, cooked, and ate the *B. indica* At the Kanchanaburi trapping site, serum was collected from local residents. More than half of these people had trapped, cooked, or eaten *B. indica* on occassion. Serum samples from laboratory workers at our laboratory (AFRIMS) in Bangkok and a random group of 30 sera from patients at the port hospital (Klong Toey) were also frozen at -70°C for determination of antibody titer to Hantaan virus.

Immunofluorescent antibody assay: Sera were examined for antibody to Hantaan and related viruses by immunofluorescent antibody (IFA) assays following standard pro-

cedures described previously and using Vero E-6 cells (American Type Culture Collection number CRL 1586) infected with the 76-118 strain of Hantaan virus. Spot slides were prepared by Dr. George French, Salk Institute, Swiftwater, PA, USA and were stored at -20°C prior to use. Sera were examined at dilutions of 1 : 8 through 1 : 2048 or greater in fourfold increments; sera were considered positive if characteristic cytoplasmic fluorescence was present at ≥1 : 32 dilutions. The IFA test has previously been shown to be broadly crossreactive among hantaviruses and thus is known to serve as a good screening assay.

Plaque reduction neutralization tests: Selected sera from *Bandicota indica* with IFA antibody titers to Hantaan virus were further tested by plaque reduction neutralization (PRN) tests. Sera were tested with prototype Hantaan virus, strain 76-118, at dilutions of 1 : 10 and 1 : 40 only. Positive and negative serum controls were included in all IFA and PRN tests.

Virus isolation attempts: Virus isolation procedures were as described previously (LeDuc *et al.*, 1984). Briefly, lung tissues from captured rodents which possessed antibody to Hantaan virus by IFA were dissociated in a mechanical blender (Stomacher Bleuder Model 80, Tekmar Company, Cincinnati, Ohio, USA) as a 10% wt/vol suspension in Hank's balanced salt solution with antibiotics. Each tissue suspension was immediately inoculated intramuscularly (1.0 ml/rat) into 5 seronegative Wistar rats. Inoculated rats were held for 32 days in a containment laboratory with special precautions taken to prevent aerosol transmission. At day 32 post-inoculation, rats were bled and their sera examined for anti-Hantaan virus antibody by IFA.

RESULTS

Serological results of the rodent surveys for *Hantavirus* antibody by the IFA test are shown in Table 1. No positive titers (≥ 32) were detected in any *Rattus sp.* trapped at the Bangkok port (Klong Toey) but nearly 20% of the *Bandicota indica* trapped at that location had positive antibody titers (range 32-512). At the grain ports of Sriracha and Bang Pakong no *B. indica* were trapped but 4 of 16 *R. norvegicus* had titers from 32-512 and 6 *R. rattus* had titers of 32. After serological results were obtained from these trappings, sera were tested from 20 *B. indica* trapped previously (data not shown). The 17

Table 1

Antibody to *Hantavirus* in rodents in Thailand.

Location	*B. indica*	*R. rattus*	*R. norvegicus*
Bangkok (Klong Toey)	6/29a	0/55	0/54
Sriracha	0/0	0/24	4/16
Bangpakong	0/0	6/86	0/0
Kanchanaburi	6/21	0/3	0/0
Total	12/50 (24%)	6/168 (3.6%)	4/70 (5.7%)

A number with positive titers (≥1 : 32) over total tested as determined by immunofluorescent antibody assay using Vero E-6 cells infected with Hantaan virus, strain 76-118.

Table 2

Comparison of immunofluorescent antibody (IFA) and plaque reduction neutralization (PRN) test result on *Bandicota indica* sera from Thailand when tested with Hantaan virus, 76-118.

Serum number	Location	Titer	
		IFA	PRN*
055	Kanchanaburi	512	>40
056	Kanchanaburi	512	>40
058	Kanchanaburi	> 2048	>40
392	Bangkok Port, Klong Toey	32	<10
462	Bangkok Port, Klong Toey	512	>40
505	Bangkok Port, Klong Toey	128	10

Sera tested at 1 : 10 and 1 : 40 dilutions only; results expressed as $\geq 80\%$ reduction points.

samples from the provinces of Korat (8) and Nakhon Pathom (9) were negative but 3 of 3 from Kanchanaburi province had titers of 512, 512 and > 2048. A subsequent trapping of 21 *B. indica* at Kanchanaburi in February, 1983, resulted in positive titers (32-2048) in 6 of these animals. Sera from 6 *Bandicota indica* with positive IFA titers were tested for neutralizing antibody to prototype Hantaan virus (Table 2). All sera with IFA titres> 512 also had neutralizing antibody > 40 as measured by PRN.

Virus isolation attempts were made using lung tissues of 3 *Rattus norvegicus* captured at Sriracha Port and 2 *Bandicota indica* captured at Kanchanaburi. Tissue suspensions from each animal were inoculated intramuscularly (1.0ml/rat) into groups of 5 seronegative Wistar rats. At 32 days post-inoculation, all inoculated rats were bled and their sera examined by IFA for development of anti-Hantaan virus antibody. As shown in Table 3, most inoculated Wistar rats had developed high titered antibody by that time. Inoculated animals were then sacrificed and attempts made to adapt the isolated viruses to growth in cell culture. These studies are still in progress and will be reported subsequently. While characterization of isolated viruses is as yet incomplete, we can conclude from the preliminary results that Hantaan or closely related viruses are indeed present in at least two different rodent species in Thailand.

Positive titers were found in 2 of 5 groups of human serum samples screened for *Hantavirus* antibody by IFA test (Table 2). More than 30% of the people (11/35 and 10/30) living around the fields in the two locations where *B. indica* were trapped had positive titers. Other groups that did not reside near or have known contact with *B. indica* had only 5-7% positive titers.

DISCUSSION

This is the first report of the presence of a *Hantavirus* in Thailand. Based upon findings of Hantaan or a Hantaan-like virus in rodents in other port cities throughout the world (LeDuc *et al.*, 1984; LeDuc *et al.*, 1985), positive antibody titers in rodents in international shipping ports in Thailand were not unexpected. Surprisingly, titers were detected

Table 3

Development of anti-Hantaan virus antibody among Wistar rats inoculated with lung tissue suspensions from rodents captured in Thailand.

Number	Species	IFA Titer	No. Iinoc. Wistar rat	IFA Titer at day 32
605	*Rattus norvegicus*	128	1	2048
			2	512
			3	2048
			4	≥2048
			5	32
610	*R. norvegicus*	512	1	128
			2	512
			3	2048
			4	≥2048
			5	32
624	*R. norvegicus*	512	1	512
			2	512
			3	2048
			4	512
			5	512
728	*Bandicota indica*	2048	1	128
			2	512
			3	128
			4	128
			5	128
749	*B. indica*	128	1	8
			2	128
			3	8
			4	32
			5	Negative

IFA tests on Vero E-6 cells infected with prototype Hantaan virus, strain 76-118; titer reciprocal of highest serum dilution producing characteristic cytoplasmic fluorescence.

in 24% of *B. indica*, a species not previously reported to have *Hantavirus* infection. The PRN is the test of choice for differentiating Hantaan-like viruses (LeDuc *et al.*, 1984). In this study, the *B. indica* with high IFA titers also had antibody by the PRN test to the prototype Hantaan virus. However, these sera were not tested against other Hantaan-like viruses or the agent in the *B. indica* lung suspensions to further differentiate the virus.

The fact that no positive titers were found in rats at the Klong Toey port in Bangkok but an antibody positive focus of *B. indica* there and at the inland site at Kanchanaburi suggests the titers in these native field rodents are the result of a local *Hantavirus* infection rather than one introduced by rodents

Table 4

Antibody to *Hantavirus* in man in Thailand.

Location	Pos/Tested	% Positive
Bangkok port restaurant workers	2/38[a]	5.3
Bangkok port hospital patients	2/30	7 3
AFRIMS lab personnel	1/14	7 1
Bangkok port residents[b]	11/35	31.4
Rural Kanchanaburi residents[c]	10/30	33.3

[a] Number with characteristic cytoplasmic fluorescence at $\geq$ 1 : 32 serum dilution when tested by immunofluorescent antibody assay with Vero E-6 cells infected with Hantaan virus, strain 76-110, over total tested.

[b] Living around field where antibody positive Bandicoots trapped.

[c] Living in rural farming areas where antibody positive Bandicoots trapped.

through international shipping. It is not known how widespread this Hantavirus infection is in rodents in Thailand. Results from the screening of serum samples taken in 2 other provinces had no positive titers in 17 *B. indica*.

It is interesting that people living in the two areas where *B. indica* with antibody were trapped had a high incidence of antibody to a *Hantavirus*. The clustering of infected rodents and human illness from a Hantaan infection has been reported (Desmyter *et al.*, 1983). In our study, people not living in close proximity to the areas containing infected *B. indica* had a lower incidence of positive titers (5-7%) compared to the 31-33% incidence in the two groups living in the immediate area with the *B. indica* and having potential for close contact with these rodents. We have yet to determine if HFRS or another disease caused by a *Hantavirus* is present in Thailand. Although some Hantaan-like viruses have been associated with illness in man (Desmyter *et al.*, 1983), at least one, the Prospect Hill virus is not known to cause human disease (Yanaginana *et al.*, 1984). It was not determined in this study if *B. indica* is the reservoir for *Hantavirus* and a source of human infection in Thailand or if a yet unidentified species is the primary reservoir for the virus.

SUMMARY

A serologic survey was conducted to determine the presence of antibody to *Hantavirus* in rodents in Thailand. Sera from over 300 rodents were tested by an immunofluorescent antibody method. *Bandicota indica*, a field rodent, was found to have a high incidence of infection (20-24%) in 2 locations. A *Hantavirus* was isolated from lung samples of *B. indica*. When sera were tested from humans living in Kanchanaburi and several locations in Bangkok, those people living in close proximity to the infected *B. indica* had greater than 30% prevalence of positive antibody titers.

REFERENCES

DESMYTER, J., JOHNSON, K.M., DECKERS, C., LEDUC, J.W., BRASSEUR, F. and VAN YPERSELE DE STRIHOU, C., (1983). Laboratory rat associated outbreak of HFRS due to Hantaan-like virus in Belgium. *Lancet, 2* : 1445.

LEDUC, J.W , SMITH, G A. and JOHNSON, K.M., (1984). Hantaan-like viruses from domestic rats captured in the United States. *Amer. J. Trop. Med. Hyg., 33* : 992.

LEDUC, J.W., SMITH, G.A., PINHEIRO, F.P., VASCONCELOS, P.F., ROSA, E.S.T. and

MAIZTEGUI, J.I., (1985). Isolation of a Hantaan-related virus from Brazilian rats and serologic evidence of its widespread distribution in South America. *Amer. J. Trop. Med. Hyg., 34* : 810.

LEE, H.W., FRENCH, G.R., LEE, P.W., BAEK, L.J., TSUCHIYA, K. and FOULKE, R.S., R.S., (1981) Observations on natural and laboratory infection of rodents with etiologic agent of Korean Hemorrhagic Fever. *Amer. J. Trop. Med. Hyg., 30* : 477.

SONG, G., CHANG-SHOU, H. and XUE-ZHAO, Q., (1983). Etiologic studies of epidemic hemorrhagic fever (Hemorrhagic fever with renal syndrome). *J. Infect. Dis., 147* : 654

UMENAI, T., WATANABE, M., SEKINO, H., YOKOYAMA, S., KAGURAGI, T., TAKAHASHI, K., LEE, H.W. and ISHIDA, N., (1981). Korean hemorrhagie fever among rural residents in Japan. *J. Infect. Dis., 144* : 460

YANIGIHANA, R., GAJDUSEK, D.C., GIBBS, C.J. and TRAUB, R., (1984). Prospect Hill virus: Serologic evidence for infection in mammologists. *New Eng. J. Med., 310* : 1325.