HANTAANVIRUS AMONG URBAN RATS FROM A SLUM AREA IN BANGKOK

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Abstract. A total of 106 rodents sera from slum Wat Phai Ton and slum Klong Toey were examined by immunofluorescent antibody assay during May to August 1990. The positive sera were further tested by plaque reduction neutralization test with the prototype hantaanvirus and the rat-associated hantaan like virus. Isolation attempts were also performed from their tissues. Antibody-positive rats were found in both slum areas, 32.7% in slum Wat Phai Ton and 5.6% in slum Klong Toey. *Rattus norvegicus* was the major species found positive. Positive plaque reduction neutralization results indicated that the infecting virus was antigenically similar to the strain of rat-associated hantaanvirus. The presence of low titer antibodies (IFA titer 32 to 128) may be an obstacle to isolation of associated virus using tissue culture.

INTRODUCTION

Hantaanviruses have been widely distributed in various rodents, insectivores and humans (LeDuc *et al,* 1986). Several rodent species play an important role as reservoirs of hantaanviruses, transmission to humans occurring by contact with rodent urine, feces and saliva (Tsai, 1987). The agents are capable of producing severe, moderate or mild form of hemorrhagic fever with renal syndrome (HFRS), depending upon the virus strain (Quimby, 1987).

In Thailand, there have been a few studies of hantaanviruses. In 1986, LeDuc *et al* found that the prevalence of antibody to hantaanvirus among rodents caught in Thailand was 7%. Later, Tantivinich *et al* (1988) reported the prevalence rate of hantaanvirus antibody among *Rattus norvegicus* and *Rattus exulans* from slum areas in Bangkok as 9%. Subsequently, in 1989, Swasdikosol *et al* also reported that the antibody positive rates of *Rattus norvegicus* and *Rattus rattus* from some provinces in the northern part of Thailand were 4.0% and 13.0%, repectively.

Even though HFRS has not been reported yet in Thailand, urban rats suggested to be the vehicle for hantaanvirus infection in some areas were detected by the presence of the antibody to hantaanvirus (Tantivanich *et al,* 1988; Swasdikosal *et al,* 1989). Nevertheless, the results from those previous studies were achieved without determination of the possible infecting virus in order to ensure the specificity of those antibodies. Thus this study sought to ascertain the presence of hantaanviruses in certain slum areas of Bangkok.

MATERIALS AND METHODS

One hundred and six urban rats and insectivores were live-trapped randomly from slum Klong Toey and slum Wat Phai Ton in Bangkok during May to August 1990. The captured animals were identified by genus and species as described by Chenchittikul (1984), and sacrified to collect blood, lungs, spleen, and pancreas. The blood was centrifuged at 1,500 rpm for 10 minutes to separate the sera, while the lungs, spleen and pancreas were kept separately in sterile containers and stored at -85°C for virus isotation.

The collected sera were examined for the presence of antibody against the 76-118 strain and the B-1 strain of hantaanvirus by using immunofluorescent antibody assay (IFA). The sera were considered as positive if the characteristic fluorescence was present at 1 : 32 dilution to only the infected spot slides but not to the uninfected cells. Their titers were recorded as the reciprocal of the highest serum dilution yielding specific hantaanvirus fluorescence staining at 2^+ grade. The positive sera were further tested for virus neutralizing antibodies by using

plaque reduction neutralization test (PRNT) (LeDuc *et al,* 1984). The viruses used in this test were hantaanvirus strain 76-118 and B-1 strain. The test was performed by mixing 0.1 ml of each serum dilution with 0.1 ml of the tested virus (100 pfu). After incubation for 1 hour at 37°C, the serum-virus mixture was added onto a confluent monolayer of Vero E6 cells grown in 60 mm dishes and incubated in a CO_2 incubator for 10 days, then a second overlay medium was added and counted for the presence of plaques after 48 hours incubation.

Virus isolation : The isolation of virus from lung, spleen, and pancreas was attempted by using the procedures of Kitamura *et al* (1983) and LeDuc *et al* (1986). Briefly, each tissue was prepared as a 10% (w/v) suspension and minced by hand-blending, then centrifuged at 2,000 rpm for 15 minutes. The supernatants were filtered through a millipore membrane (ACRODISC, Gelman Sciences) and cocultivated with Vero E6 cells in 25 ml flasks for 1 day at 37°C. The cultured fluids were discarded and 5 ml of growth medium were replaced, the cultures were kept at 37°C for 50 days. During this time, subcultures were made 4 times at 10-14 day intervals. The infectious culture fluids of isolates were identified by IFA by using the 76-118 strain, the B-1 strain and viral isolates to prepare the antigen on spot slides. Each antigen was tested with a panel of monoclonal antibodies (provided by Dr Koichi, Yamanighi, Department of Virology, Research Institute for Microbial Diseases, Osaka University, Japan) which reacted specifically against hantaanvirus strains (Dantas *et al,* 1986) and reference polyvalent reovirus antiserum by an indirect IFA technique.

RESULTS

Among one hundred and six captured animals from slum Klong Toey and slum Wat Phai Ton, 2 species of common urban rats, *Rattus norvegicus* and *Rattus exulans,* and an insectivore, *Suncus murinus* comprised 61 (57.6%), 17 (16.0%), and 28 (26.4%) respectively. These animals are illustrated in Fig 1.

The numbers of individual animals captured from each slum area are given in Table 1. Prevalence of positive IFA antibody to hantaanvirus strain 76-118 and strain B-1 from urban rats and insectivores is shown in Table 2. Antibody positive rats from the two slums together represented 18.9%, while the insectivore *S. marinus* was negative for IFA antibody. Rats from Slum Wat Phai Ton and slum Klong Toey had positive IFA antibody rates of 32.7% and 5.6%, respectively with a significant difference ($x^2 = 12.71$, $p < 0.001$).

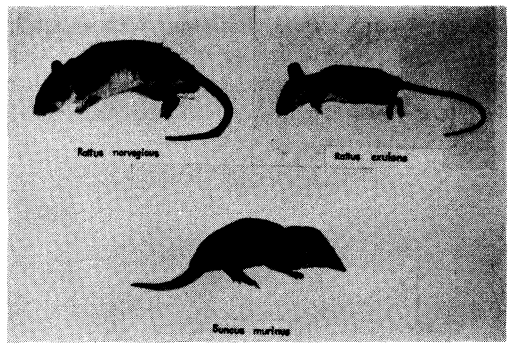

Fig 1—Picture of animals captured *Rattus norvegicus* (A) *Rattus exulans* (B) and *Suncus murinus* (C).

Table 1

Number of individual animals captured from slum areas in Bangkok, Thailand (May to August 1990).

Slum	No. of animals	Species		
		R. norvegicus	*R. exulans*	*S. murinus*
Wat Phai Ton	52	33	5	14
Klong Toey	54	28	12	14
Total	106	61 (57.6%)	17 (16.0%)	28 (26.4%)

Table 2

Prevalence of Positive IFA antibody to hantaanvirus strain 76-118 and rat associated hantaanvirus strain B-1 in urban rats and insertivores captured from slum areas in Bangkok.

Slum	No. of positive sample/No. tested (%)			
	R. norvegicus	*R. exulans*	*S. murinus*	Total
Wat Phai Ton	16/33	1/5	0/14	17/52 (32.7%)
Klong Toey	3/28	0/12	0/14	3/54 (5.6%)
Total	19/61 (31.1%)	1/17 (5.8%)	0/28 (0%)	20/106 (19.9%)

The distributions of reciprocal immunofluorescent antibody titers to hantaanvirus strain 76-118 and rat-associated hantaanvirus strain B-1 from urban rats and insectivores determined by weight are illustrated in Fig 2. If was found that 6/20 (30%) of animals with body weight less than 300 g had positive IFA antibody while 14/20 or 70% of animals with body weight more than 300 g had positive IFA antibody. Determining the correlation between the chance of getting infection by hantaanvirus and the weight of the urban rats, lower than 300 g and over 300 g, it was found that there was a significant difference ($p = 0.001$).

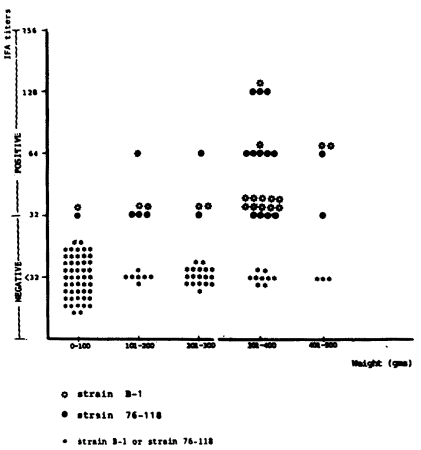

fig 2—Distribution of reciprocal immunofluorescent antibody titers to Hantaan virus strain 76-118 and rat-associated Hantaanvirus strain B-1 from urban rats and insectivores determined by weight ($n = 106$).

Table 3 demonstrates the distribution of IFA titers to strain 76-118 antigen and strain B-1 antigen in urban rats from slum Wat Phai Ton and slum Klong Toey. It was found that 20 of them had positive IFA antibody against HV 76-118 strain and 18 of them had positive IFA antibody against B-1 strain with a range of 1 : 32 to 1 : 128.

Nineteen of twenty IFA seropositive rats were tested by PRNT to evaluate which hantaanviruses likely caused infection. By comparison of IFA titer and PRNT in Table 4, it was found that 10 of 19 (specimens 1-10) of the rat sera which tested positive by IFA to both strains of hantaanviruses gave 4-fold higher NT titers to B-1 strain than to 76-118 strain by PRNT. Six serum samples (11-16) yielded similar NT titers against both types of hantaanvirus (range 8-16) Three serum samples (17-19) had no neutralizing activity against either strain. Overall, a concordance of 16 out of 19 (84.2%) was found between sera positive by IFA and neutralizing ability against two strains of hantaanviruses. Therefore, the prevalence of seropositive rats from slum Wat Phai Ton and slum Klong Toey was 15.1% (16/106).

DISCUSSION

This study showed that the prevalence of antibody to hantaanvirus among urban rats from slum areas in Bangkok was 18.9% which is higher than the previous study by Tantivanich *et al* (1988). This finding may indicate that the hantaanviruses are widely disseminated in the free living rat population in slum Wat Phai Ton and slum Klong Toey areas. The higher prevalence IFA titers in *R.*

Table 3

Distribution of immunofluorescent antibody (IFA) titers to strain 76-118 antigen and strain B-1 antigen in urban rat sera from slum areas in Bangkok, 1990.

Source of sera	Total	Reciprocal IFA titer							
		HV (76-118)				B-11			
		<16	32	64	128	<16	32	64	128
Slum Wat Phai Ton	52	35	10	5	2	35	12	4	1
Slum Klong Toey	54	51	0	2	1	51	3	0	0
Total	106	86	10	1	3	86	15	4	1

Table 4

Comparison of immunofluorescent antibody (IFA) assay and plaque reduction neutralization (PRNT) test : from the IFA seropositive of *R. morvegicus* and *R. exulans.*

Specimen No.	IFA[a]		PRNT	
	Hantaan virus	B-1 virus	Hantaan virus	B-1 virus
1. PT 3	128	32	0	32
2. PT-14	64	32	0	4
3. PT-25	64	128	2	16
4. PT-29	128	32	0	16
5. PT-41	32	32	4	16
6. PT-44	32	32	0	8
7. PT-45	64	32	0	8
8. KT-20	64	32	0	8
9. KT-35	128	32	2	16
10. KT-47	64	32	2	8
11. PT-12	32	32	8	8
12. PT-20	64	64	8	8
13. PT-42	32	32	8	8
14. PT-43	32	64	8	8
15. PT-47	64	64	8	8
16. PT-48	32	64	16	16
17. PT-27	32	32	0	0
18. PT-50	32	32	0	0
19. PT-52	32	32	0	0
20. PT-2	32	32	NT	NT

a = reciprocal of highest serum dilution showing characteristic cytoplasmic fluorescence.
b = reciprocal of highest serum dilution reducing 50% of plaque dose.

norvegicus than *R. exulans* may due to the following reasons : (1) the susceptibility of the infecting agents to these different hosts; (2) the involvement of unresemble infecting viruses or their complexity; (3) differences in the environments in these two slums.

The finding that rats of greater body weight had a greater chance of infection in the case of *R. norvegicus* suggested that the risk of rodent infection is related to the age of the animals.

The result of the PRNT and IFA suggested that hantaanvirus is currently circulating among urban rats in the slum areas in Bangkok. The higher PRN titer to strain B-1 in some serum samples compared with PRNT titer to the prototype hantaanvirus (>4 fold), also suggested that the infecting virus was closely related to the rat associated hantaanviruses.

Even though the PRN antibody assay was used to evaluate which hantaanvirus is likely to cause infection in these urban rats (LeDuc *et al,* 1984), the presence of antibody titers against both strains of virus by PRNT may indicate dual infection or cross reactivity between the prototype hantaanvirus and the rat-associated hantaan-like virus. The observation that no neutralizing antibody occurred in some IFA positive sera may due to infection with an unrecognized hantaanvirus such as reovirus, the natural infectious viruses of respiratory and digestive organ of rats (Yamanishi *et al,* 1983; Song *et al,* 1983) or to non-specific reactions.

Failure to isolate the infecting virus from these animals by tissue culture methods paralled their low antibody titers (1 : 32 to 1 : 128), since the isolation of hantaanvirus from peridomestic rat is usually correlated with high IFA titers of > 1 : 512 (LeDuc *et al,* 1984; Sugiyama *et al,* 1984; LeDuc *et al,* 1985). Rats with low level antibody titers may reflect a low number of infective viruses or past infection. Inactive viruses may need to be propagated in a more susceptible host such as wistar rats (Lee *et al,* 1982; LeDuc *et al,* 1985). Although hantaanvirus is shed for extended periods in urine and feces of infected rodents (Lee *et al,* 1981), the isolation of infective virus from body fluids is rather limited since captured animals were unlikely to be taken care for a long period of time: they died soon after capture.

The results of this study indicated that the urban rats from slum Wat Phai Ton and slum Klong Toey were infected with agents similar antigenically to the rat associated hantaanvirus. These animals are likely to shed the virus in urine, feces and saliva for their life-times (Lee *et al,* 1981; LeDuc, 1987). Therefore, the risk of the people living in these slum areas becoming infected is relatively high. Moreover, *R. norvegicus* has proved to be the main reservoir of the causative agent of the mild type of HFRS (Song *et al,* 1983). This evidence suggests that mild type of hemorrhagic fever due to hantaanvirus infection may occur unnoticed in Thailand. Clinical presentation as a febrile illness with mild renal involvement and few hemorrhagic manifestations should be recognized as a mild type of HFRS (Pon *et al,* 1990). Thus further studies on the role of hantaan or related viruses among Thai people in slum areas should be carried in association with clinical epidemiologic investigations.

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