SEROLOGICAL STUDY OF HANTAVIRUS IN THE RODENT POPULATION OF NAKHON PATHOM AND NAKHON RATCHASIMA PROVINCES THAILAND

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Abstract. A serological survey has been carried out to detect evidence of hantavirus infection in rodents from two provinces of Thailand. This study aimed to examine virus antibody in 354 rodents trapped among 6 different villages of Nakhon Pathom Province (February-March, 1998) and in 326 rodents trapped among 14 villages of Nakhon Ratchasima Province (August-October, 1998). Seroprevalence among rodents from Nakhon Pathom Province (2.3%), was mostly find in *Rattus norvegicus* (3.8%) and *Bandicota indica* (2.6%). In Nakhon Ratchasima Province seroprevalence (4.0%) was mostly in *Bandicota indica* (19.1%) and *Rattus exulans* (3.5%).

INTRODUCTION

Hantaviruses are single-stranded, negative-sense RNA viruses, and members of the Bunyaviridae family. They cause zoonotic diseases which are transmitted to humans by contact with infected rodents. Hantaviruses are associated to rodents of the Muroides superfamily. Muroids are vectors and reservoirs of virus, with one species as a primary reservoir for one virus type. Rodent infection occurs through inhalation of infectious aerosols generated from rodent secretions and excretions, leading to a short, transient viremia (Nuzum *et al*, 1988). Virus persists in rodents despite high-titer antibodies, and disseminates to various tissues, particularly lung, salivary gland and kidney, resulting in a lifelong but asymptomatic infection (Lee *et al*, 1981; Lee, 1982; Yanagihara *et al*, 1985). Horizontal spread of virus occurs through exposure to contaminated nesting material, grooming behavior; interspecies wounding and biting are also important factors in virus maintenance within the enzootic cycle (Glass *et al*, 1988; Yanagihara and Gadjusek, 1988). Vertical transmission probably does not occur (Yanagihara, 1990).The human disease presents two major clinical pictures of severe hantavirus infections: a hemorrhagic fever with renal syndrome (HFRS), which is typically defined by fever, acute interstitial nephropathy with transient renal dysfunction and less commonly hemorrhage (Chun *et al*, 1989) and the more recently described hantavirus group which is responsible of an acute pulmonary syndrome (HPS) (Dohmae *et al*, 1993).

Hantavirus antibodies were previously found in 33% of the population living in slum areas of Bangkok (Elwell *et al*, 1985). Some studies have been conducted in Thailand to determine the presence of hantavirus antibodies in various rodent species. High antibody prevalence by IFA and plaque reduction neutralization test (PRNT) was found in *Bandicota indica* (24.0%) and *Rattus norvegicus* (5.7%) (Elwell *et al*, 1985).Those studies did not clearly identify the risk of infection by hantavirus regarding specific potential vector and environment. In the present paper a preliminary approach to identify risk factors associated with rodent species and environmental issues is presented.

MATERIALS AND METHODS

Study site

Nakhon Pathom Province (2,168.327 km^2) is located in the western part of Bangkok metropolitan area, (13º 49´ 43´´N; 100º 04´ 35´´E) (Fig 1) in a paddy field area. A rainy season lasts 6 to 7 months when the temperature increases to a maximum of 40ºC and a dry season lasts for the other remaining 5 to 6 months. Land use characteristics

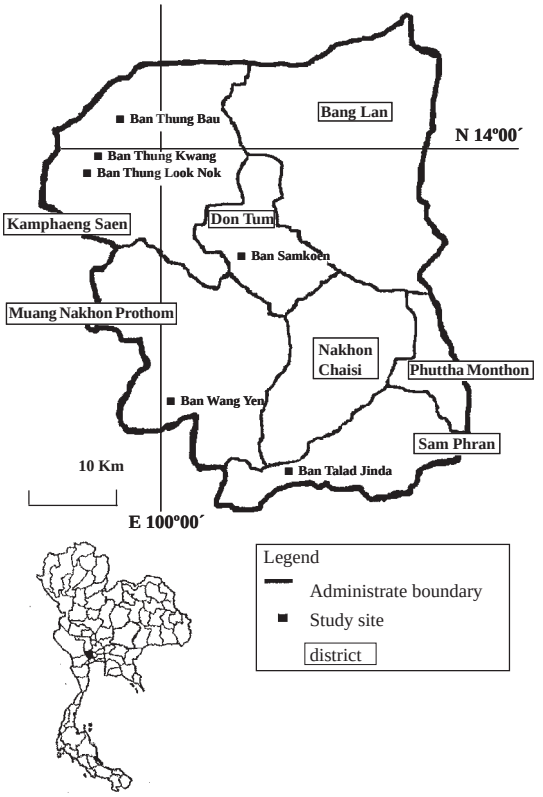

Fig 1–Study sites of Nakhon Pathom Province.

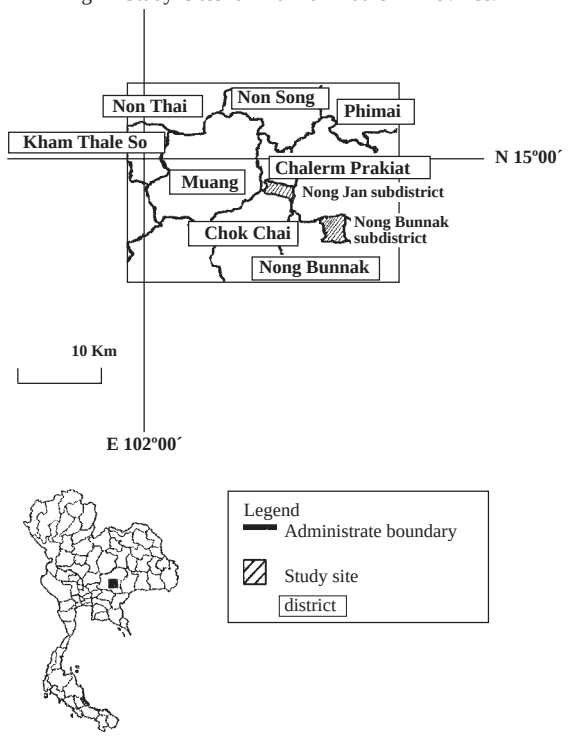

Fig 2–Study sites of Nakhon Ratchasima Province.

are classified as urban area, municipal area, agricultural area (rice and sugar cane fields) and animal farms (especially pigs). The population size of Nakhon Pathom Province in 1997 was 753,599 persons.

Nakhon Ratchasima Province with population of 2,510,839 as of 1997 (20,493.964 km^2) is located in the north eastern part of Bangkok metropolitan area (14º 38´N; 1002º 26´E) (Fig 2). The geographical characteristics are classified as urban area, municipal area, agricultural area (mostly rice, corn fields and sugar cane fields).

Rodent sampling

Rodents from Nakhon Pathom Province were trapped in six villages (February-March, 1998). After carbon dioxide anesthesia, whole blood and organs (liver, kidney, spleen and heart) were collected. Specimens were labeled, kept in cryotube and stored at -70ºC. The study sample were classified as follows by their different geographical origin:

- Maung district: Ban Wang Yen village where most of the land occupation is residential and rice fields.
- Sam Phran district: Ban Talad Jinda village where most of the areas are gardens.
- Don Tum district: Ban Samkoen village, mostly an area covered by rice fields.
- Kamphaeng Saen District: Ban Thung Look Nok, Ban Thung Kwang and Ban Thung Bau villages in a sugar cane field.

Rodents from Nakhon Ratchasima Province were trapped in two villages (August-October, 1998) and sampled as described above. The study areas were classified as follows:

- Nong Bunnak district: villages including moo bahn 1, 4, 5, 6, 7, 9, 10, 12, 15 in Nong Bunnak subdistrict where most of the areas are rice fields.
- Chalerm Prakiat district: villages including moo bahn 1, 5, 6, 7, 9 in Nong Jan subdistrict where most of the areas are rice fields, and gardens.

Serological tests

The ELISA plates (MaxiSorp; Nunc, Roskilde, Denmark) were coated with either hantavirus antigen (Hantaan ROK 84/105 SMRV kindly provided by Professor Ho Wang Lee) dilution 1:1,000 in sterile PBS 7.2 overnight at 4ºC. The next morning the plates were washed with PBS/tween 0.5%. Then 100 µl of rodent serum diluted 1:100 in PBS/ tween 0.5%/ skim milk 3% was added and incubated for 1 hour at 37ºC after three times washing with PBS/

tween 0.5%, 100 µl of HRP/Anti-Rodent IgG (HRP Conjugate Goat Anti-Rodent IgG, ZYMED) was added and incubated for 1 hour at 37ºC. After three times washing, the reaction was stopped with 50 µl 4M H_2So_4 and the plates were read in a Metertech Σ490 spectrophotometer at 490 nm.The cutoff value for presence of antibody was defined as 3 times of the negative control absorbance.

RESULTS

Rodent characteristics and seroprevalence

354 rodents were trapped from different geographical areas of Nakhon Pathom Province. Specimens were identified as *Bandicota indica*, *Bandicota savilei*, *Mus caroli*, *Mus castaneus*, *Rattus argentiventer*, *Rattus exulans*, *Rattus losea*, *Rattus norvegicus* and *Rattus rattus*. The overall seroprevalence against hataanvirus antigen was 2.3%. Mostly it was found among paddy field dike rodents, especially *Bandicota indica* (2.6%), while among the indoor-dwelling rodents, the higher seroprevalence was found in *Rattus norvegicus* (3.8%), *Rattus exulans* (2.2%) and *Rattus rattus* (1.5%) (Table 1). Among the different geographical areas of the province, the highest seroprevalence against hantavirus was found in the rodent population collected from Don Tum district (4.2%), among *Bandicota indica* (2/65), *Rattus norvegicus* (1/7) and *Rattus exulans* (1/11) (Table 2).

In Nakhon Ratchasima Province, we trapped 326 rodents from different geographical areas. Only six species were present: *Bandicota indica*, *Mus caroli*, *Bandicota savilei*, *Bandicota* sp, *Rattus exulans* and *Rattus rattus*. The overall seroprevalence against hantaanvirus antigen was 4.0%. Most of the seroprevalence was found in *Bandicota indica* 19.1% and *Rattus exulans* 3.5% (Table 1).

DISCUSSION

All rodents collected in Nakhon Pathom and Nakhon Ratchasima provinces, were members of the Muridae family. *Bandicoda indica* (44.4%)was the the most frequent species trapped from Nakhon Pathom Province, while in Nakhon Ratchasima Province most of the rodent specimens caught were *Rattus exulans* (78.8%).

The highest hantavirus seroprevalence in Nakhon Pathom was found essentially among *Rattus norvegicus* (3.8%) and *Bandicota indica* (2.6%). In Nakhon Ratchasima only *Bandicota indica* (19.0%) and *Rattus exulans* (3.5%) had hantavirus reacting

Table 1
Hantavirus antibody reacting sera from rodent species trapped in Nakhon Pathom and Nakhon Ratchasima Province, Thailand, 1998.

Species	Nakhon Pathom	Nakhon Ratchasima	Total
Paddy field dike rodents			
Bandicota indica	4/157 (2.6)[a]	4/21 (19.1)	8/178 (4.5)
Bandicota savilei	0/14 (0.0)	0/6 (0.0)	0/20 (0.0)
Bandicota sp	-	0/5 (0.0)	0/5 (0.0)
Mus caroli	0/3 (0.0)	0/1 (0.0)	0/4 (0.0)
Rattus argentiventer	0/9 (0.0)	-	0/9 (0.0)
Rattus losea	0/2 (0.0)	-	0/2 (0.0)
Total	4/185 (2.2)	4/33 (12.1)	8/218 (3.7)
Indoor rodents			
Mus castaneus	0/3 (0.0)	-	0/3 (0.0)
Rattus exulans	1/45 (2.2)	9/257 (3.5)	10/302 (3.3)
Rattus norvegicus	2/53 (3.8)	-	2/53 (3.8)
Rattus rattus	1/68 (1.5)	0/36 (0.0)	1/104 (1.0)
Total	4/169 (2.4)	9/293 (3.1)	13/462 (2.8)
Total	**8/354 (2.3)**	**13/326 (4.0)**	**21/680 (3.1)**

[a]Number of positive/Total tested (percentage)

Table 2
Hantaanvirus antigen antibody reacting sera from rodent species of Nakhon Pathom Province by district of origin.

Species	District				
	Muang	Sam Phran	Don Tum	Kamphaeng Saen	Total
Bandicota indica	2/41 (4.9)[a]	0/8 (0.0)	2/65 (3.1)	0/43 (0.0)	4/157 (2.5)
Bandicota savilei	0/11 (0.0)	0/1 (0.0)	0/2 (0.0)	-	0/14 (0.0)
Mus caroli	-	-	-	0/3 (0.0)	0/3 (0.0)
Rattus argentiventer	0/2 (0.0)	0/4 (0.0)	0/1 (0.0)	0/2 (0.0)	0/9 (0.0)
Rattus losea	0/1 (0.0)	0/1 (0.0)	-	-	0/2 (0.0)
Mus castaneus	-	0/3 (0.0)	-	-	0/3 (0.0)
Rattus exulans	0/4 (0.0)	0/22 (0.0)	1/11 (9.1)	0/8 (0.0)	1/45 (2.2)
Rattus norvegicus	1/32 (3.1)	0/7 (0.0)	1/7 (14.3)	0/7 (0.0)	2/46 (4.3)
Rattus rattus	0/7 (0.0)	1/47 (2.1)	0/10 (0.0)	0/4 (0.0)	1/68 (1.5)
Total	3/98 (3.1)	1/93 (1.1)	4/96 (4.2)	0/67 (0.0)	8/354 (2.3)

[a]positive/total tested

Table 3
Hantavirus antigen antibody reacting sera from rodent species of Nakhon Ratchasima Province by district of origin.

Species	District		
	Nong Bunnak	Chalerm Prakiet	Total
Bandicota indica	3/18[a] (16.7)	1/3 (33.3)	4/21 (19.0)
Bandicota savilei	0/3 (0.0)	0/3 (0.0)	0/6 (0.0)
Bandicota sp	0/4 (0.0)	0/1 (0.0)	0/5 (0.0)
Mus caroli	0/1 (0.0)	0/0 (0.0)	0/1 (0.0)
Rattus exulans	5/156 (3.2)	4/101 (4.0)	9/257 (3.5)
Rattus rattus	0/18 (0.0)	0/18 (0.0)	0/36 (0.0)
Total	8/200 (4.0)	5/126 (4.0)	13/326 (4.0)

[a]positive/total tested

antibodies. These findings show an active hantavirus circulation in the provinces with *Bandicota indica* as a primary virus reservoir candidate as previously reported by others (Elwell *et al*, 1985; Sawasdikol *et al*, 1989; Tantivanich *et al*, 1988, 1992). In some instances farmers and other low-income groups eat rodent meat trapped from rice fields or sugar cane areas. Some rodents like *Bandicota indica* can be found also at the local market. This is a risk factor which needs to be evaluated.

Rattus exulans can be another potential hantavirus vector as well as *Rattus norvegicus* and *Rattus rattus*. *Rattus exulans* have been reported as infected for the first time in those areas. Indoor rodents which have positive hantavirus serology such as *Rattus exulans* can potentially transmit virus to human because of their domestic habits.

Outdoor rodents appear to be more infected, as judged by the rate of antibody prevalence, than indoor rodents. When we compared hantavirus circulation in both provinces we found more paddy field dike rodents with higher hantavirus seropositivity than indoor rodents in Nakhon Ratchasima but no difference in Nakhon Pathom. However the hantavirus prevalence by district of Nakhon Pathom showed higher positivity in Muang and Don Tum areas which are more included in the rice field environment than the two other sites.

From previous studies and our observation (Elwell *et al*, 1985) *Bandicoda indica* and *Rattus exulans* appear as a primary potential reservoir/ vector of hantavirus in the central plain of Thailand. *Bandicoda indica* lives outside the housing and its domain overlaps with that of *Rattus exulans* which is the most abundant domestic rodent in Thailand living in close contact with humans. *Rattus exulans* is the smallest rat in Thailand and originally comes from the Pacific Islands where it is known to be an outdoor rodent. From our study we can hypothesize that in Thailand *Bandicota indica* can play a role as a wild reservoir of virus and *Rattus exulans* will be more likely a vector of hantavirus to humans.

It appears from a previous study done in the human population that 10 years ago 1.2% of the population from the Bangkok area had hantavirus antibody (Sawasdikol *et al*, 1989). We found similar seroprevalence in humans from Nakhon Pathom Province (1.9%) however, a high density population living in slum areas of Bangkok showed an unexplained, much higher, prevalence of 31.4% (Elwell *et al*, 1985).

Those findings of seroprevalence of antibodies against hantavirus among rodents will lead to other serological surveys among human populations who have experienced contact with rodents. Also it is important to address a warning to physicians to emphasize laboratory investigations and differentiate the causes of acute respiratory syndrome (Kiatboonsri *et al*, 1995), renal failure and leptospirosis cases in Thailand (Hindrichsen *et al*, 1993).

ACKNOWLEDGEMENTS

This study was supported by the Department of Technical and Economic Cooperation (DTEC) Thailand; the Center for Vaccine Development. (CVD) Thailand; CDC/MOPH Thailand and the Institut de Recherche pour le Developpement (IRD), France.

We would like to thank all staff in Environmental and Sanitation Section, Nakhon Pathom Provincial Health Office, Dr Darika Kingnate Department of Communicable Diseases Control, Ministry of Public Health, Nonthaburi, Thailand. Miss Yuvaluk Khoprasert Agricultural Zoology Research Group, Division of Entomology and Zoology, Department of Agriculture. Mr Greangsak Hamarit, Agricultural Zoology Research Group, Division of Entomology and Zoology, Department of Agriculture. Mr Noppadol Sanngjun, Armed Forces Research Institute of Medical Sciences. Dr Yupha Rongsriyam, Department of Medical Entamology, Faculty Tropical Medicine, Mahidol University, University and Office for their Cooperation, CVD-RCEVD in the program of emerging viral diseases.

We are also deeply indebted to Professor Natth Bhamarapravati for his constant support to the project.

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