

PRELIMINARY STUDY ON POTENTIAL CIRCULATION OF ARENAVIRUSES IN THE RODENT POPULATION OF NAKHON PATHOM PROVINCE, THAILAND AND THEIR MEDICAL IMPORTANCE IN AN EVOLUTING ENVIRONMENT

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Abstract. Preliminary serological investigations were preferred to detect evidence of arenavirus infection in rodents. The study examined virus antibody in 367 rodents trapped in 6 different geographical areas of Nakhon Pathom Province, Thailand from February-March, 1998. The overall seroprevalence among rodents was 13.3%, mostly in *Bandicota savilei* (35.7%) and *Rattus norvegicus* (31.5%). Between ecology, behavior and sex of the rodents, seroprevalence was not significantly different ($p > 0.05$), however the seroprevalence found among different geographical areas of Nakhon Pathom Province were significantly different ($p < 0.0001$).

INTRODUCTION

Arenavirus infections are zoonotic diseases transmitted to human by contact with rodents arenavirus (Howard, 1986; Salvato, 1993). The viruses of the Arenaviridae family are associated with rodents, especially those belonging to the Muridae family. These rodents can be chronically infected. Rodents can maintain or sustain the virus cycle in nature without a secondary host, then transmit the virus by direct contact through blood or biological effluents or by aerosols to other vertebrates including humans. The rodents experience a silent virus reservoir but persistent and lifelong infection, the viruses being adapted to this highly successful ecological niche by asymptomatic horizontal or vertical transmission at or near birth. Arenaviruses comprise 22 recognized virus types, six of which are known as human pathogens and found in both the new and the old world. In humans, arenavirus infections take an altogether different course which is relatively common in some areas of the world and can cause severe illness. Infections range from a febrile disease with aseptic meningitis caused by lymphocytic choriomeningitis virus (LCMV), to total collapse and death with circulatory and respiratory failure caused by Lassa fever, Argentine hemorrhagic fever or Bolivian hemorrhagic fever. Arenavirus infection in humans is incidental to the natural cycle of the virus and occurs when an individual ingests contaminated food or the skin comes into direct con-

tact with rodent excrement. LCMV is responsible for outbreaks of febrile illness and neurological syndromes in Europe, North and South America while Lassa fever is responsible for severe epidemics in West Africa and viruses of the Tacaribe complex from the new world are responsible for human infection in the Americas. In terms of epidemiology and risk of infection we have to consider that Southeast Asian countries are located at similar latitudes as countries of Africa or South America enzootic for most of the known arenaviruses.

The ecology of old world arenaviruses is still emerging. The unique human reservoir hosts are rodents, especially the Muridae family which are found in Thailand. In this study, we identify serological prevalence of arenavirus infections among rodents in order to evaluate the risk for the human infection by a human pathogenic arenavirus.

MATERIALS AND METHODS

Study site

Nakhon Pathom Province (2,168,327 km²) is located in the western part of the Bangkok metropolitan area, (13° 49' N ; 100° 04' E) (Fig 1). It is a paddy field area with a rainfall season of 6 to 7 months, the hot temperature increases to a maximum of 40°C and a dry season lasts for the remaining months. The geographical characteristics are

classified as urban area, municipal area, agricultural area (mostly rice fields and sugar cane) and animal farms (especially pigs). The population of Nakhon Pathom Province in 1997 was 753,599. The population density is high in the urban and municipal areas.

Rodent sampling

All of the rodents were trapped in six villages (February-March, 1998). After chloroform anesthesia, whole blood and organs (liver, kidney, spleen and heart) were collected. Specimens were labeled, kept in cryotubes and stored at -70°C. The study areas were classified as follows by their geographical characteristics.

Maung district: Ban Wang Yen village where the majority of the land is used for occupational purposes or habitation and rice fields.

Sam Phran district: Ban Talad Jinda village where most of the land is covered by gardens.

Don Toom district: Ban Samkoen village where the area is covered by rice fields.

Kamphaeng Saen district: Ban Thung Look Nok, Ban Thung Kwang and Ban Thung Bau villages comprised of sugar cane fields.

Serological test

The indirect fluorescent antibody (IFA) test was performed as preliminary described using lassavirus (Josiah strain) infected Vero cell on spot slides kindly supplied by the US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701, USA (Jahrling *et al*, 1985; Elliott *et al*, 1982). The inactivated antigen slides were fixed with cold acetone for 10 minutes and dried at room temperature. Ten microliters of PBS diluted rat sera (1:16, 1:32 and 1:64) was then added on the spot slide. The serum positive control (I-A Souris 97-61 Lassa from Pasteur Institute Paris, France) was used as a reference control. After incubation of 30 minutes in a moisture chamber at 37°C, spots were washed in the pH 7.4 PBS for 10 minutes and rinsed with distilled water. The slides were then stained with 10 microliters of rabbit anti-mouse conjugated with FITC, then incubated in a moisture chamber for 30 minutes. After washing, the slides were mounted with buffer glycerol and examined by fluorescence microscope.

Statistical analysis

All data results were entered and analyzed using

SPSS statistical software for windows Version 7.5. Univariate analysis to test for statistical significance was done using χ^2 test for goodness of fit and Z-test for proportion.

RESULTS

Rodent characteristics and seroprevalence

All of 367 rodents were trapped from different geographical areas of Nakhon Pathom Province. Specimens were classified for species and nine were identified: *Bandicota indica*, *Bandicota savilei*, *Mus caroli*, *Mus castaneus*, *Rattus argentiventer*, *Rattus exulans*, *Rattus losea*, *Rattus norvegicus* and *Rattus rattus* (Table 1). The total seroprevalence against lassavirus antigen was 13.3% (49/367). Most of the seroprevalence was found among paddy field dike rodents especially *Bandicota savilei* (35.7%) and *Bandicota indica* (12%), while among the indoor-dwelling rodents, the most seroprevalence was found from *Rattus norvegicus* (31.5%).

Regarding the origin of the rodents, the seroprevalence among paddy field dike (N=194; 13.4% positive) and indoor rodents (N=173;13.3%) were not significantly different ($p>0.5$); there was no gender difference: males (N=217;13.4%), females (N=150;13.3 %) ($p>0.5$).

However the distribution of seroprevalence against lassavirus antigen found in Muang was sig-

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

Species	Total	Positive ^a
Paddy field dike rodents		
<i>Bandicota indica</i>	166	20 (12.0)
<i>Bandicota savilei</i>	14	5 (35.7)
<i>Mus caroli</i>	3	0 (0.0)
<i>Rattus argentiventer</i>	9	1 (11.1)
<i>Rattus losea</i>	2	0 (0.0)
Indoor rodents		
<i>Mus castaneus</i>	3	0 (0.0)
<i>Rattus exulans</i>	47	3 (6.4)
<i>Rattus norvegicus</i>	54	17 (31.5)
<i>Rattus rattus</i>	69	3 (4.3)
Total	367	49 (13.3)

^aNumber of positive (percentage)

Table 2

Lassa antigen antibody reacting sera from rodent species of Nakhon Pathom Province by district of origin.

Species	District			
	Muang	Sam Phran	Don Tum	Kamphaeng Saen
<i>Bandicota indica</i>	13/41 ^a	0/9	6/70	1/46
<i>Bandicota savilei</i>	4/11	0/1	1/2	-
<i>Mus caroli</i>	-	-	-	0/3
<i>Rattus argentiventer</i>	1/2	0/4	0/1	0/2
<i>Rattus losea</i>	0/1	0/1	-	-
<i>Mus castaneu</i>	-	0/3	-	-
<i>Rattus exulans</i>	1/4	0/22	1/12	1/9
<i>Rattus norvegicus</i>	14/32	3/8	0/7	0/7
<i>Rattus rattus</i>	1/7	2/48	0/10	0/4
Total	34/98	5/96	8/102	2/71
(%) positive	34.7 ^b	5.2	7.8	2.8

^apositive/total tested

^bp<0.0001

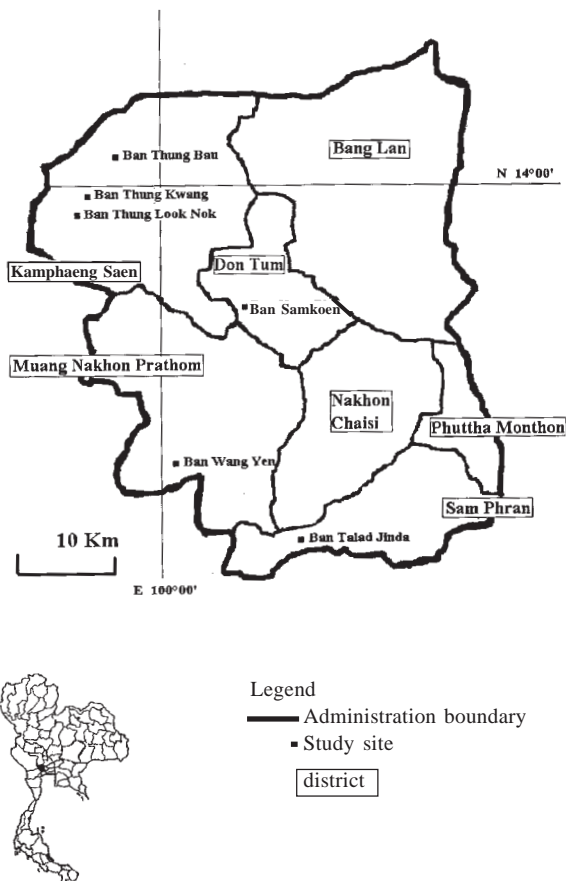


Fig 1—Study site: Nakhon Pathom Province.

nificantly different ($p < 0.0001$) from the other geographical areas of Nakhon Pathom. Among the different geographical areas, the highest seroprevalence against lassa virus was found in the rodent population collected from Muang district (34.7%), while in Don Tum district, Sam Phran district and Kamphaeng Saen district, the seroprevalence was respectively 7.8, 5.2 and 2.8%. In Muang district, most of the positive sera were found among *Bandicota indica* (13/41), *Rattus norvegicus* (14/32) and *Bandicota savilei* (4/11) (Table 2).

DISCUSSION

Despite the absence of difference in prevalence between paddy field dike rodents and indoor rodents, the highest seroprevalence was found among *Bandicota savilei*, *Bandicota indica* and *Rattus norvegicus*. These findings were consistent with an Arenavirus circulation in Thailand.

The IFA test which using lassa antigen is very sensitive and non-specific that can detect most of the reacting antibody in hosts infected by any arenavirus strain from the old world (Wulff and Lange, 1975).

A study in the Republic of Guinea (Meulen *et al*, 1996), reported that consumption of rodents' meat is a risk factor for rodent-to-human transmission of

lassavirus. In Thailand farmers and other low-income groups eat meat from rodent, trapped in the rice fields or sugar cane areas. Some rodents will be transferred to local markets like most paddyfield dike rodents *Bandicota indica*, *Bandicota savilei*, *Rattus argentiventer* and *Rattus losea* which have the highest antibody prevalence.

These preliminary findings of seroprevalence against an arenavirus found among rodents will hopefully lead to other serological surveys among high risk human populations who experience contact with rodents in targeted areas. It will encourage physicians to emphasize laboratory investigations in order to differentiate between the causes of fever unknown origin, meningitis and encephalitis cases in Thailand. Finally our research program an Emerging Viral Diseases with focus on virus isolation of a not yet identified arenavirus antigenically reacted the one arenavirus as predicted by the benign of arenavirus related circulation.

The existence of such arenavirus as been already speculated from the arenavirus and rodent co-evolution theory (Gonzalez and Duplantier, 1999).

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