

3. Title: Ecology of Arboviruses in Thailand

Principal Investigators: Douglas J. Gould, Ph.D.
Joe T. Marshall, Ph.D.
Skon Rohitayodhin, D.V.M.
Phillip K. Russell, Major MC

Associate Investigators: Somsak Pantuwatana, M.Sc.
Moufiled A. Moussa, Captain MSC

Objective:

To determine the ecologic factors which affect the maintenance and dissemination of arboviruses causing human disease in Thailand.

DESCRIPTION:

The area in and adjacent to the Red Cross horse farm at Bang Phra was selected as a site for the study of the ecology of Japanese encephalitis virus. A continuing program designed to measure avian population, migration and infection rates, rodent populations and infection rates; mosquito populations, breeding sites, biting habits and infection rates was begun in February 1966. The study is a cooperative program involving personnel of the Pasteur Institute and the department of entomology, and is an expansion of a program of virus isolation from mosquitoes carried on at Bang Phra since 1962.

Additional collections of vertebrate sera for arbovirus antibody studies are being made on a routine bases in the forested areas near route 23 south of Korat. Serologic surveys of near by US military personnel are being done in conjunction with the field studies.

Taxonomic studies on birds and rodents of Thailand are a significant part of the ecologic studies. Specimens are being sent to the US National Museum for verification of identification.

Experimental infections of cattle and water buffalo were studied to determine the potential of these species as hosts of JE and Chikungunya viruses.

Rodent sera collected in the city of Bangkok was tested for antibody to arboviruses.

PROGRESS:

Bang Phra Studies. Identification of 23 virus strains isolated between 1962 and 1966 from mosquitoes collected at Bang Phra has been completed. Sixteen of these strains were isolated by Dr. Skon Rohitayodhin at Bang Phra. All isolates came from 2 mosquito species, Culex tritaeniorynchus and Culex gelidus.

Of the 23 viruses identified, 21 are JE virus. Table 18 shows the distribution of these isolates by month and years. It is apparent that infected mosquitoes were present in the area 8 months of the year. Eleven of the JE strains were isolated from pools of Culex tritaeniorynchus and 10 strains from pools of Culex gelidus.

Table 18. Monthly Distribution of JE Virus Isolates from C. gelidus and C. tritaeniorynchus Collected at Bang Phra.

Year	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1962										2*	2	
1963							2				1	
1964					2	2						
1965			1	1	3	2	1		2			

* No. of JE virus strains isolated.

In addition to the JE viruses, two chikungunya strains were isolated, one from C. tritaeniorynchus in November 1962 and one from C. gelidus in March 1966. Three unknown viruses, not JE or chikungunya, are presently being investigated.

Since beginning the ecologic studies in February 1966, 628 birds have been netted, 95 recaptured. Rodents captured and marked total 132 with 7 recaptures. A total of 107 bats have been captured and banded with 20 recaptured. Over 450 blood specimens are being tested for virus and antibody to JE and chikungunya.

Experimental Infection of Thai Cattle and Buffalo with Japanese

Encephalitis and Chikungunya Viruses. The role of domestic cattle and water buffalo as reservoir or amplifying hosts of arboviruses in Thailand has not been defined. Previous findings of neutralizing antibody to JE and Chikungunya viruses in these animals stimulated further attempts to determine the potential of these species as possible ecologically significant hosts of these two agents. Attempts to infect buffalo with chikungunya virus and cattle with JE and Chikungunya viruses and to demonstrate infectivity for vector mosquitoes are described herein.

Young water buffalo, 6 to 12 months old, and young cattle, 5 to 8 months old were purchased from a local vendor. All animals came from herds in Central Thailand. The animals were kept in a screened shed prior to and during the experiments.

Virus strains isolated locally from human patients were used. Source and passage history are listed below:

<u>Virus</u>	<u>Source</u>	<u>Passage</u>
12908 Chikungunya	blood	BS C-1 -1
20875 JE	brain	BS-C-1 -3
21325 JE	brain	SM - 2

Mosquitoes used in these experiments were adult female Culex gelidus raised in the insectary from larva collected from natural breeding sites in Bangkok.

Animals were prebled and inoculated with virus. Infectivity titrations of the inoculum were performed by plaque method in LLC MK-2 cells. Blood specimens were taken daily for 10 days and tested for virus in suckling mice and BS-C-1 cell culture. Preinoculation and 10 days post inoculation sera were tested for hemagglutination-inhibiting (HI) and neutralizing (N) antibody. Mosquitoes were allowed to feed on each animal daily for ten days. An attempt was made to feed 30 mosquitoes on each animal each day, however an average of only 6 fed mosquitoes in each group was attained. The blooded mosquitoes from each lot were held in separate cages for 7 days then suspensions of ground mosquitoes were inoculated into mice and BS-C-1 tissue culture.

Three buffalo received 1.5×10^4 pfu of Chikungunya virus. Two were injected intravenously and one subcutaneously. No virus was detected in the blood during the 10 post inoculation days. Eighteen mosquito pools containing a total of 73 fed mosquitoes did not contain virus when tested in suckling mice and tissue culture. No antibody response was detected by HI or N tests. No chikungunya antibody was present prior to inoculation. Attempts to infect buffalo with JE virus were precluded by JE neutralizing antibody titers of 1:10 to 1:40.

Four cattle were inoculated with 60 pfu of 20875 JE virus. Serologic response is summarized in table 19. The two cattle, (No. 4 and 5) which received virus intravenously did not have a serologic response. The two cattle (No. 6 and 7) injected subcutaneously had serologic evidence of infection. Attempts to isolate virus from the blood of these cattle were negative in mice and tissue culture. Thirty nine mosquito pools containing a total of 246 fed mosquitoes were negative for virus.

One month later cattle number 4 and 5 were inoculated subcutaneously with 10^5 pfu of JE strain No. 21325. The relatively high dose given subcutaneously produced an antibody response but again the viremia tests were negative. Transmission to mosquitoes was not attempted.

Cattle number 6 and 7 were given 10^4 pfu of Chikungunya strain No. 12908 subcutaneously. As was the case with the buffalo, no viremia occurred and there was no antibody response.

The experiments described above indicate that cattle and water buffalo are not susceptible to infection with Chikungunya virus by peripheral inoculation. It is extremely doubtful that either species is a natural host of Chikungunya virus. The reported finding of chikungunya antibody in these species suggests a cross reaction with another group A arbovirus.

Table 19. Serologic Response of Cattle to Inoculation with Japanese Encephalitis Virus.

Cow No.	Dose	Route	HI Antibody		N Antibody	
			Day 0	Day 10	Day 0	Day 10
4	60 pfu ⁽¹⁾	IV	0 ⁽³⁾	0	0	0
5	60 pfn	IV	0	0	0	0
6	60 pfu	SC	0	40 ⁽⁴⁾	0	35 ⁽⁵⁾
7	60 pfu	SC	0	40	0	50
4	10^5 pfu ⁽²⁾	SC	0	160	0	35
5	10^5 pfu	SC	0	80	0	60

(1) JE strain No. 20875

(2) JE strain No. 21325

(3) 0 = less than 1: 10

(4) Reciprocal of HI titer vs 8 units JE antigen

(5) Reciprocal of 50% plaque reduction titer

Thai cattle appear to be readily infected by small doses of JE virus given subcutaneously. It is of interest that intravenous injection given by a method which insured minimal deposition of virus in the needle track did not produce infection. This may be explained by the fact that small doses of virus given intravenously are rapidly inactivated by non-specific antiviral substances present in the serum. Subcutaneous virus on the other hand may be less affected by serum components.

The failure to detect viremia in the four cattle which had serologic evidence of infection is highly significant. It strongly suggests that JE infected cattle are not infective for vector mosquitoes and hence play no role in dissemination of JE virus in nature.

Chikungunya HI Antibody in Rodents. Serum from 118 wild rodents trapped in Bangkok in September—October 1965 were tested for HI antibody to 3 arbovirus antigens; chikungunya, dengue-1 and Japanese encephalitis. Results are summarized in table 20.

Table 20. HI Antibody in Rodents.

<u>Species</u>	<u>No. Tested</u>	<u>Chik.</u>	<u>No. Positive Dengue-1</u>	<u>JE</u>
<u>Rattus norvegicus</u>	77	9	0	0
<u>Rattus rattus</u>	26	6	0	0
<u>Rattus exulans</u>	8	2	0	0
<u>Bandacota indica</u>	5	0	0	0
<u>Bandicota bangalensis</u>	2	0	0	0

The finding of HI antibody to chikungunya in rodents is surprising in view of negative findings previously reported from 1962 studies. Neutralization test and further testing to rule out nonspecific inhibitors are in progress. The likelihood of nonspecific inhibitory substances causing these results is small in view of negative reaction with the other antigens. Further investigations of the possible role of rodents as natural hosts of chikungunya virus will be carried out.