

Ecology of Arboviruses in Thailand

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OBJECTIVE: To investigate factors in ecology, particularly with respect to vertebrate populations, influencing and maintaining the transmission of arboviruses in nature.

DESCRIPTION: As outlined in previous Annual Reports, especially 1968.

PROGRESS: A serological survey of wild vertebrates at Bang Phra found considerable neutralizing antibody to JE in rodents, including Rattus rattus (40%), R. rajah (= surifer, 25%), Menetes berdmorei (33%), and Bandicota indica (47%). Neutralizing antibody to Wesselsbron (WESS) virus was detected in R. rattus (18%), M. berdmorei (5%), and B. indica (30%). Those data were obtained using the metabolic-inhibition test. Additional rodent sera have been tested now using tube neutralization and plaque tests in efforts to confirm the survey results and to detect antibody in animals collected at other places in Thailand.

Blood samples were taken by heart puncture and spun down minutes thereafter. Sera were kept at -20°C or colder temperatures until inactivation at 56°C in a waterbath for 1/2 hr. Sera then were diluted 1:5 for use in tube neutralization tests. After challenging with virus, serum-virus mixtures were incubated 1/2 hr at 37°C , and two culture tubes of BHK-21 cell monolayers received 0.1 cc of inoculum for each specimen. Tubes were maintained at 37°C while observing for cytopathic effect (CPE). If less than 50% CPE occurred in both tubes for a given specimen after seven days, antibody was considered to be present in the animal's sera. Equivocals resulted if only one of two tubes showed evidence of neutralization. Positive and equivocal sera were retested by plaque tests if sufficient sera was available. The MK-2 plaque reduction test was used according to the procedure described previously.

No evidence for neutralizing antibody to JE was found in rodent sera tested by tube neutralization (Table 1). In most cases, the amount of virus used to challenge sera was 80-100 TCID₅₀. However, the virus dose used to test the 56 sera of Rattus rattus collected at Bang Phra was excessive, and 53 of the samples were retested by plaque test with sera diluted 1:10. Plaque reduction in 29 sera was within the error expected of controls and thus was not significant. Seven specimens showed plaque reduction which was significantly different from controls at $p < .01$. The percent plaque reduction was 72% in one and 74% in another. Reduction of plaques may have been due to specific neutralizing antibody but non-specific plaque inhibition could have been present at the 1:10 dilution of sera used in the test. In any case, JE antibody does not appear to be as prevalent as the Bang Phra survey indicated. Rodents have not been implicated as hosts of JE in Japan, Malaysia or India, and it is doubtful that they function as natural hosts in Thailand.

Indications of WESS antibody were found in Rattus rattus collected at Bang Phra and in the area of the Sakaerat research station in Nakorn Ratchasima Province. Also, a positive result was obtained for one R. surifer collected at Sakaerat on 18 July 1969. The positive and equivocal R. rattus from Sakaerat were

distributed throughout the period June—December 1969. The percentage of Rattus rattus positives at Bang Phra was 18%, which was the percentage found in the previous survey. Positives and equivocal in the Bang Phra series were retested by plaque test (Table 2). Plaque reduction was significant in most cases at the 1:5 dilution of sera. At 1:10 dilution of sera, however, most specimens were negative. Four were significantly different from controls at $p < .01$, and a fifth was different at $p < .001$. These three specimens and the several solid positives in tube neutralization tests indicate that some neutralizing antibody to WESS virus may be present in rodents. Again, however, the involvement is not so great as the previous survey indicated. That rodents play a significant role in the ecology of WESS remains to be shown.

S—19—B Virus and Antibody Prevalence
in the Wrinkle-lipped Bat
(*Tardarida plicata*)

Shortly after the isolation of an unknown virus from dead bats collected in Saraburi Province (see elsewhere in this report), attempts were made: 1) to identify the virus and 2) to determine if antibody was present in living bats.

S—19—B was found to be either sensitive and is probably an arbovirus. Therefore S—19—B was tested against specific sera for several arboviruses known to occur in Thailand using the MK—2 plaque reduction technique (Table 3). No relationship to Chikungunya (CHIK), Sindbis (SIND), JE, Newcastle Disease (N.D.V.), Batai or Wesselsbron (WESS) was found. S—19—B formed big, clear plaques after three days of incubation at 37°C which were quite different from the small and hazy plaques produced by Dengue and Tembusu viruses given similar incubation. S—19—B plaques were intermediate in size between those of Chikungunya and Sindbis viruses. In terms of plaque morphology, S—19—B virus is related to group A. CF antigen has been prepared from guinea pigs with CF titers of 1:512 for use in further attempts to characterize S—19—B.

Tardarida plicata were mist-netted at evening exodus at a small cave on Khao Lam Phat, Kangkoi District, Saraburi Province. This cave is several hundred yards south and approximately 50 yards lower on the mountain than the large cave where S—19—B was originally isolated. The small cave may be a nursery cave. At the time of collection, several hundred—thousand T. plicata were resident there, whereas previous and later visits showed that T. plicata was not abundant. A second series of T. plicata was obtained from a large cave deep in the forest on Khao Phlong, near Amphur Phra Buddhabat, Saraburi Province.

Blood was taken from the heart and diluted to give 1:3 sera dilution prior to centrifugation, which was accomplished soon after bleeding. In the laboratory, sera were incubated at 56°C for 1/2 hr and diluted to 1:5 for use in BHK—21 tube neutralization tests, which were performed as described in the previous section. Sera (1:5) were challenged with 10—25 TCID₅₀ of S—19—B virus prepared in BHK—21 cell culture. Positives and equivocal were retested, where possible, in a second tube neutralization test.

Sera of male and female bats neutralized 25 TCID₅₀ of S—19—B virus (Table 4). A greater proportion of females were positive than males in the Khao Lam Phat population, whereas the reverse was true of the Khao Phlong population. Thus, both sexes are susceptible and probably are infected at similar frequencies in nature. Approximately 30% of each population evidenced prior contact with S—19—B. If equivocal are included as showing evidence of antibody, rates increase to 35%. Thus, contact with S—19—B is common. T. plicata, and probably all T. plicata populations in the Saraburi region are involved with this virus. Further studies of antibody prevalence using the MK—2 plaque test are in progress. It remains to be shown whether or not S—19—B virus occurs elsewhere in Thailand or in other countries where T. plicata is found.

Table 1. BHK-21 tube neutralization tests of rodent sera.

Location and Species	Period when animals collected.	Virus Dose in Tests (TCID ₅₀)		Number Positive over Number Tested		Number Equivocal over Number Tested	
		JE	WESS	JE	WESS	JE	WESS
Bang Phra, Cholburi Provinces: <u>Rattus rattus</u>	Nov. 1969	630	65	0/56	10/56	0/56	11/56
District, exclusive of Bang Phra Cholburi Province: <u>Rattus rattus</u> <u>Rattus (Lenothrix) surifer</u> <u>Menetes berdmorei</u>	Nov. 1969	80 and 100	100	0/17 0/3 0/7	0/6 — —	0/17 0/3 0/7	0/6 — —
Bangkok: <u>Rattus rattus</u> <u>Bandicota indica</u>	Dec. 1969	100	25	0/11 0/6	0/2 0/1	0/11 0/6	0/2 0/1
Khao Nam Tok, Kangkoi District, Saraburi Province: <u>Rattus rattus</u>	Dec. 1969	100	100	0/4	0/1	0/4	0/1
Pak Thong Chai District, Nakorn Ratchasima Province: <u>Rattus rattus</u> <u>Rattus (Lenothrix) surifer</u> <u>Rattus (Stenomys) sabanus</u> <u>Rattus (Maxomys) niviventer</u> <u>Menetes berdmorei</u> <u>Bandicota indica</u>	June-Dec 1969	80 and 100	100	0/46 0/29 0/13 0/7 0/5 0/5	1/20 1/14 0/5 0/2 0/2 0/4	0/46 0/29 0/13 0/7 0/5 0/5	5/20 0/14 0/5 0/2 0/2 0/4

Table 2. Comparison of BHK-21 tube neutralization test and MK-2 plaque test results.

Tube Neutralization Test Result (Sera 1:5)	<u>R. rattus</u> Serum No.	%Plaque Reduction (Sera 1:5)	% Plaque Reduction (Sera 1:10)
Equivocal WESS	3464	80	59*
	3467	85	1
	3469	73	0
	3473	88	32
	3479	27	—
	3488	59	0
	3503	88	18
	3508	71	1
	3512	34	—
	3514	68	64*
Positive WESS	3471	56	100**
	3472	88	64*
	3476	73	0
	3483	76	0
	3491	83	0
	3501	80	15
	3505	83	49

* = .01 > p > .001 ** = .001 > p

Table 3. S-19-B Virus versus specific sera in MK-2 plaque test.

Serum	Virus					
	S-19-B	Chik	Sind	JE	Batai	Wess
S-19-B (rabbit)	1.000*	<40	<10	<10	<10	<10
Chik (rabbit)	<10	640	≤10	<10	—	—
Sind (mouse)	<10	<10	10,240	—	—	—
JE (rabbit)	<10	<10	<10	640	<10	<10
N.D.V. (commercial)	<10	—	—	—	—	—
Batai (mouse)	<10	—	—	<10	> 640	<10
Wess (mouse)	<10	—	—	<10	<10	10,240

* Reciprocal of serum dilution giving 50% plaque reduction.

Table 4. S-19-B antibody prevalence in two populations of *T. plicata* at Saraburi Province.

Location—Date—Sex	Number Tested	A %Positive	B %Equivocal	A.+B.
Khao Lom Phat (8 Dec 69)				
Males	34	26	6	
Females	26	35	3	
Both	60	30	5	35
Khao Phlong (10 Dec 69)				
Males	41	39	2	
Females	41	15	14	
Both	82	27	8	35