

SEATO MEDICAL RESEARCH STUDY ON Rickettsial Diseases in Thailand.

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Objective:

To determine the distribution and seasonal variation of Rickettsial diseases in Thailand; identify arthropods and mammals which serve as vectors, reservoirs and alternate hosts; and serve as a consultative laboratory if requested.

Progress:

During this reporting period, studies on scrub typhus, murine typhus and Q-fever have been continued in Thailand. The current status of these projects is as described below.

1. Scrub Typhus. Surveys have been carried out in 22 provinces, chiefly along the northern, northeastern, eastern and southern borders of Thailand. In all areas, small mammals and their ectoparasites were collected, identified and processed for recovery of rickettsiae.

Table 1 presents the data on recovery of Rickettsiae tsutsugamushi from small mammals in various localities in Thailand. The agent has been recovered from some 11 species of mammal (see SMRL Annual report 1965), and in a single instance, from an avian predator (Accipitor badius) collected in the Arranyaprathet district.

Data on the chigger vectors are, as yet, incomplete pending conclusion of seasonal studies. The predominant chigger collected and the sole source of isolations to date in Thailand is Leptotrombidium (L) deliensis. R. tsutsugamushi has been isolated from L. (L) deliensis both by direct inoculations of chigger triturates into white mice and from mice upon which L. (L) deliensis collected in endemic areas have been allowed to feed. Although two additional chigger species which are confirmed vectors in Japan have been collected in Thailand (Leptotrombidium (L) scutellaris) in Nan and Loei provinces and L. (L) akamushi in Nakhornratchasima province) in significant numbers, the agent of scrub typhus has not been isolated from these two species. Enzootic scrub typhus is subject to seasonal fluctuations in Thailand. Table 2 presents data on the isolation of R. tsutsugamushi from small rodents and L. (L) deliensis collected in Ubol at different seasons. The data indicate a possible effect of climate (mainly rainfall and humidity) on the isolation rates.

Isolation of R. tsutsugamushi was attempted from blood obtained from 62 patients with "pyrexia of unknown origin" (P.U.O.) who had no obvious clinical origin for their fever, generally suspected of having malaria or typhoid. These attempts at isolation were positive for scrub typhus in six of 35 P.U.O. inpatients from Chiangmai, Samutsakorn and Korat hospitals and in four of 27 P.U.O. outpatients seen in Chiangrai and Arranyaprathet (Table 3).

In June 1965, a classical outbreak of scrub typhus occurred at Pak Thong Chai in Nakhornratchasima province which was investigated by project personnel. In June, 185 Thai soldiers carried out a training exercise in secondary forest and lalang grass areas. Subsequently, after an incubation period of 7.21 days, 41 (20.8%) of the 185 soldiers were admitted to the hospital presenting with fever and headache. A majority of the patients were hypotensive and leukopenic. Eschars with regional adenopathy were seen in about one half of the cases. Rash was seen in only 1 patient. All patients responded promptly to chloramphenicol. R. tsutsugamushi was isolated from 10 of 16 blood specimens which could be studied. While only one of thirty-four sera tested had a significant titre of OXK agglutinins (1:160), 33 of the 34 sera demonstrated specific rickettsial antibody in an indirect immunofluorescent test (Table 4). R. tsutsugamushi was isolated from three species of rodents and L. (L) deliensis collected in the maneuver area (Table 5).

Most strains of R. tsutsugamushi isolated in the course of this study have been found on antigenic analysis to be similar to those recognized in other countries. A few, however, are apparently related to a strain newly described by Dr. Elisberg (Table 6).

2. Murine Typhus. Many of the rodents trapped in the scrub typhus surveys, particularly Rattus rattus and R. exulans are host to numerous Xenopsylla cheopis. These fleas are well-known as vectors of R. mooseri, the agent of murine typhus. To date some 13 isolations of R. mooseri have been made in 5 areas of Thailand (Table 7).

Although no isolations of R. mooseri have, as yet, been made from human beings, a number of normal individuals residing in endemic murine typhus areas were bled and tested for complement fixing antibody to R. mooseri. Sera from 24 of 158 individuals tested, contained antibody to the agent of murine typhus (Table 8). The individuals with reactive sera gave a history of a previous febrile illness associated with "insect bite".

3. Q-fever The known association of Q-fever, stockyards and the meat industry prompted an investigation into the possibility of this disease being a cause of illness in Bangkok which is a focal center for the livestock industry of Thailand. The problem was approached first by attempting to isolate R. burneti from the blood of P.U.O. cases in the livestock industry, second by performing a serological survey for complement-fixing antibody to C. burneti in the sera of human beings and animals and third by isolation of C. burneti from ticks collected in and around slaughter houses. To date, no isolations of C. burneti have been made. Relatively few cases of P.U.O. have occurred in slaughter house workers and none of these have been related to infection with Q-fever. However, complement fixing antibody to the 9 mile strain of Q-fever antigen (Lederle) was detected in a significant number of sera collected from human beings and animals in the Bangkok area (Table 9). It is hoped that the serological observations will be confirmed by actual isolation of the agent in the near future.

Discussion:

It is clear that scrub typhus is widespread in Thailand, since it has been found in 20 of 22 provinces surveyed. In some of the areas it appears to be highly endemic. The seasonal variation in isolation rate appears to be consistent with that found in other areas (viz. increasing with conditions favorable to chigger populations). That considerable hazard to man exists in some areas is attested to by the epidemic cited, in which 41 (22.2%) of the 185 man company which had camped in an endemic area were stricken by a clinical illness consistent with scrub typhus, and that serological confirmation was obtained in 33 of 34 attempts. In contrast to experience elsewhere, it would appear that rash is not a prominent symptom of scrub typhus in Thailand. The reason for this is not known. Although the data on P.U.O. cases are limited so far, it would seem that as many as 15% of fevers of unknown origin in

endemic areas may be attributed to scrub typhus. This is of particular importance considering that sophisticated diagnostic tests are not readily available in many of these areas.

The military implications of these observations are obvious. Provision must be made in projected operations in endemic areas, especially in and after the rainy season, for vector control in bivouac areas and for rigorous discipline with regard to personal protective measures including but not limited to repellents and clothing impregnants.

The data regarding murine typhus are less nearly complete, but R. mooseri has been found in all five provinces where it was sought in commensal rats and ectoparasites. Further, the isolation rates are of the same order of magnitude as for scrub typhus. That this disease may be of some considerable importance in human disease is borne out by the finding of C.F. antibodies to this agent in 20.9% of 68 human sera in the Chiengrai area, and 11% of 90 sera from the Ubol area. These sera were obtained from residents in the area of rodent-flea surveys.

Work with Q fever has only recently been initiated, and caution is necessary in attempting to interpret the limited data available.

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Table I
DISTRIBUTION OF SCRUB TYPHUS IN THAILAND

LOCALITY		POOLS OF ANIMAL TISSUE TESTED	TISSUE POOLS POSI- TIVE FOR <u>RICKETTSIA</u> <u>TSUTSUGAMUSHI</u>	PER CENT POSI- TIVE
NORTH	CHIENGMAI	165	16	9.6
	CHIENGRAI	380	7	1.8
	NAN	58	—	—
NORTH-EAST	UDON	308	34	11.0
	NONGKAI	297	12	4.0
	NAKORNPANOM	753	10	1.3
	UBOL	546	63	11.5
	KORAT	64	14	22.0
CENTRAL	BANGKOK	16	2	12.5
	LOPBURI	153	4	9.6
	SAMUTPRAKARN	38	5	13.2
	SAMUTSAKORN	30	3	10
EAST	PRACHIN	124	9	7.2
	CHANBURI	57	1	1.7
SOUTH	PATTANI	89	3	3.3
	YALA	54	5	9.2
	NARATHIVA S	100	5	5.0
	17 PROVINCES	3232	193	5.9

Table 2

SEASONAL VARIATION IN ISOLATION OF
R. TSUTSUGAMUSHI AT UBOL, THAILAND

	JUNE (1963)	AUGUST (1963)	NOVEMBER (1965)	JANUARY (1963)	APRIL (1965)
MEAN* RAINFALL (MM)	216.7	284.4	12.5	0.7	71.7
MEAN* RELATIVE HUMIDITY (%)	79.8	82.4	73.1	68.5	68.8
<u>LEPTOTROMBIDIUM</u> <u>DELIENSIS (POOLS)</u>	0/14** (0%)	3/21 (15.7%)	3/10 (30%)	0/12 (0%)	NA ***
MAMMALS - TOTAL (POOLS)	14/80 (17.5%)	22/53 (41.5%)	12/37 (32.2%)	6/39 (15.4)	4/43 (9.3%)
<u>RATTUS EXLLANS</u>	0/2	/0	/0	0/5	0/24
<u>R. RATTUS</u>	4/33	5/14	4/7	0/10	0/3
<u>R. RAJAH</u>	4/17	10/23	7/20	4/12	2/10
<u>R. NINIVENTER</u>	/0	1/2	0/2	/0	/0
<u>R. BERDMOREI</u>	2/6	0/1	0/1	/0	/0
<u>R. CREMORI VENTER</u>	/0	0/1	/0	1/2	1/1
<u>TUPAIA GLIS</u>	2/11	6/9	1/7	1/10	1/5
<u>MENENTES BERDMOREI</u>	2/11	0/3	/0	/0	/0

* 10.Years mean (1951-1960). Source: Climatological data, Meteorological Department, Office of the Prime Minister, Bangkok, Thailand 1 July 1964.

** Numerator.number of isllations of R. tsutsugamushi Denominator.number of pools either chigger or mamal tissue) tested.

*** N.A. = Data not yet available.

Table 3

ISOLATION OF RICKETTSIA TSUTSUGAMUSHI FROM THE BLOOD OF UNSELECTED
PATIENTS WITH PYREXIA OF UNKNOWN ORIGIN.

LOCALITY IN THAILAND	DATE OF COLLECTION	NO. OF P.U.O. PATIENTS TESTED	NO. OF PATIENTS FROM WHICH <u>R. TSUTSUGAMUSHI</u> ISOLATED
CHIENGMAI	8/63	20	4
SAMUTSAKORN	4/65	14	1
CHIENGRAI	8/65	22	2
ARRANYAPRATHET	11/65	5	2
KORAT	1/66	1	1
TOTATS		62	10

Table 4

SUMMARY OF SEROLOGICAL INVESTIGATION OF
SCRUB TYPHUS OUTBREAK IN THAI MILITARY
PERSONNEL, PAK THONG CHAI, JUNE, 1965*

Military Personnel Category Number	Serum Collection	Results of Indirect Immunofluorescent Tests		
		Soldiers Tested	Number Positive**	Percent Positive
Ill-hospitalized 37	Two Specimens: During Disease	28	19(7)***	93
	Single Specimen: During Disease	7	6	86
Normal Exposed 88	Two Specimens: 18th & 33rd day After last exposure	25	4(1)***	20
	Single Specimen: 18-20th day after last exposure	27	3	11
	Single Specimen: 33rd day after last exposure	16	2	13

* Performed at WRATR by Dr. Bennett L. Elisberg. Chief Department of Rickettsial Diseases.

** Positive test on two specimens from same soldier signifies demonstration of a 4-fold or greater increase in antibody titer. On a single specimen a titer of 1:40 or greater is reported as positive.

*** Number in brackets denotes individuals with same antibody titers in both specimens.

Table 5

ISOLATION OF RICKETTSIA TSUTSUGAMUSHI FROM RATS AND CHIGGERS. PAK THONG CHAI
SCRUB TYPHUS OUTBREAK, JUNE 1965.

	NO. TRAPPED	NO. POOLS TESTED	NO. POOLS FROM WHICH <u>R. TSUTSUGAMUSHI</u> ISOLATED
<u>RATTUS RATTUS</u>	23	9	5
<u>R. RAJAH</u>	32	12	8
<u>R. NIVIVENTER</u>	1	1	1
<u>R. SABONUS</u>	2	2	0
<u>R. BERDMOREI</u>	1	1	0
<u>TUPAIA GLIS</u>	5	2	0
<u>LEPTOTHROMBIADIUM</u> <u>(L) DELIENSE</u>	365	5	2

Table 6
 ANTIGENIC CHARACTERIZATION OF STRAINS
 OF RICKETTSIA TSUTSUGAMUSHI ISOLATED IN
 THAILAND

	STRAIN DESIGNATION	NUMBER OF THAI STRAINS FALLING IN THIS GROUP
	KARP	20
	GILLIUM	20
	KATO	8
	UNKNOWN*	8
TOTALS	4	56

* May correspond to a newly recognized group of strains identified by Dr. Elisberg in other areas.

Table 7
 ISOLATION OF RICKETTSIA MOOSERI
 FROM COMMENSAL RATS (RATTUS RATTUS,
R. EXULANS AND RAT FLEAS (EXNOPSYLLA CHEOPIS)
 IN VARIOUS LOCALES IN THAILAND.

LOCALITY	SPECIMEN TESTED	TOTAL NUMBER TESTES	NUMBER OF POOLS TESTED	NUMBER OF POOLS TESTED FOR <u>R. MOOSERI</u>	SOURCE OF THE ISOLATION
CHIENGRAI	RATS	115	34	0	
	FLEAS	164	9	1	<u>Xenopsylla cheopis</u>
UBOL	RATS	95	23	4	4- <u>R. exulans</u>
	FLEAS	98	4	0	
NAN	RATS	17	4	2	2- <u>R. exulans</u>
	FLEAS	28	6	0	
LOEI	RATS	92	25	4	1 <u>Rattus</u> 3 <u>R. exulans</u>
	FLEAS	503	11	1	<u>X. cheopis</u>
SAMUTSAKORN	RATS	69	16	1	<u>R. exulans</u>
	FLEAS	26	7	0	

Table 8

COMPLEMENT FIXING ANTIBODIES TO
RICKETTSIA MOOSERI ANTIGEN IN SERA
 OF THAI NATIONALS RESIDING IN A MURINE
 TYPHUS ENDEMIC AREA

LOCALITY	DATE OF COLLECTION	NUMBER OF SERA TESTED	NUMBER OF SERA REACTIVE
CHIENGRAI	JULY-AUGUST 1965	68	14
UBOL	OCTOBER 1965	90	10

Table 9

COMPLEMENT FIXING ANTIBODIES TO NINE MILE
 STRAIN Q FEVER ANTIGEN (LEDERLE) IN SERA
 COLLECTED FROM SLAUGHTER HOUSE WORKERS AND
 VARIOUS ANIMALS IN THE VICINITY OF BANGKOK, THAILAND

SOURCE OF SERA	NUMBER TESTED	NUMBER REACTIVE*
WORKERS, BANGKOK SLAUGHTER HOUSE	381	4
COWS " " "	153	4
BUFFALOS " " "	75	0
GOATS " " "	48	3
SHEEP PHARMACEUTICAL FACTORY, BANGKOK	59	2
HORSES CHOLBURI AREA	114	0
DOGS GENERAL BANGKOK AREA	138	22

* Sera reactive at a titer of 1:10 or greater.