

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 46	Collection of Material for Rickettsial Isolation
Project No. 3A 025601 A 311	Military Medical Research Program S.E. Asia
Task 01:	Military Medical Research Program S.E. Asia
Subtask 01:	Military Medical Research Program SEASIA (Thailand)
Reporting Installation	US Army-SEATO Medical Research Laboratory APO 146, San Francisco, California Division of Medical Research Laboratories Department of Medical Entomology
Period Covered by Report:	1 April 1963 to 31 March 1964
Principal Investigator:	Captain Vichai Sangkasuvana*
Associate Investigators:	Major John E. Scanlon, MSC Mr. Kitti Thonglongya Dr. Bennett Elisberg**
Reports Control Symbol:	MEDDH-288
Security Classification:	UNCLASSIFIED

* Royal Thai Army Medical Service
**WRAIR

ABSTRACT

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This study has as its object the determination of the distribution and abundance of the rickettsiae in the wild mammals, arthropods and humans in Thailand. Small mammals were collected from many areas of Thailand,

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representing a wide variety of ecological situations. These ranged from the margins of a metropolitan area, to monsoon deciduous forest, to primary tropical evergreen forest. Tissues from these mammals, and ectoparasites (chiggers and ticks) removed from the hosts were inoculated in laboratory animals for recovery of rickettsiae.

Eighty nine strains of rickettsiae were recovered from mammal. tissues and seven strains from chiggers, chiefly Leptotrombidium deliense. Characterization of these strain is still in progress, but straining reaction and resistance to challenge by a known strain of scrub typhus rickettsiae leads to the conclusion that the agents isolated are this agent. Four strains of presumed scrub typhus rickettsiae were also recovered from humans with a relatively mild febrile disease in the Chiangmai area. Rickettsiae related to the agent of Indian tick typhus were isolated from ticks, and a strain of murine typhus rickettsiae was isolated from Rattus exulans. The amount of rickettsial infection in animals in Thailand, and perhaps in man, is greater than had heretofore been suspected.

Description: Small mammals are collected in various parts of Thailand, selected for their military importance, unusual terrain or history of rickettsial disease. The ectoparasites are removed from the hosts and the chiggers and ticks identified as far possible. The ectoparasites are frozen for subsequent inoculation, or inoculation, or inoculated in mice in the field. Mammal tissues are also ground in the field and inoculated in mice for recovery of rickettsiae. Mice returned to the laboratory for completion of isolation and passage work, and strains of rickettsiae isolated are subjected to challenge experiments with a known strain of rickettsiae. Humans living in the study area and workers exposed to unusual risk of rickettsial disease in the field are bled for serological examination, and suspected human cases are bled for isolation attempts.

Progress: The first virulent strain of rickettsiae found during these studies was isolated early in 1963. Since that time, the isolation program has accelerated rapidly, and by the end of the report period, eighty nine pools thought to contain scrub typhus rickettsiae had been detected. In addition, one strain of murine typhus and one strain of an agent related Rickettsia conorii was isolated for the first time in Thailand. Aliquots of all of the suspected pools have been forwarded to WRAIR for complete identification. The principal investigator departed for WRAIR in February to assist in the identification of the isolates. A list of the suspected scrub typhus isolates and the hosts from which they were derived is given in table 1. This represents a high rate of isolation, much higher than anticipated at the inception of the project.

Serological results obtained by cooperating workers at WRAIR have indicated a rather high degree of rickettsial activity in man and wild animals in Thailand, and the isolation rates are consistent with these results. Dr. Elisberg and his coworkers tested 194 human sera from Thailand by means of the fluorescent antibody technique and found that 13% of them were positive for scrub typhus at the 1:40 level (diagnostic) and 22% were positive at the 1:10 level (suggestive). In addition, CF tests indicated the presence of a member of the spotted fever group, the epidemic-murine typhus group and Q fever in humans and wild animals in any parts of the country. This was the first positive evidence for the existence of spotted fever-like agents and Q fever in Thailand. As noted above, a spotted fever-like agent was isolated from ticks (Rhipicephalus sp. and Ixodes sp. larvae) during these studies. On the basis of human and animal serology it is probably that infections by Rickettsial agents are widely spread in Thailand, but largely unrecognized at present.

The actual amount of overt human disease due to the rickettsiae in Thailand has not been accurately determined for any area as yet. However,

Table 1. Isolation of rickettsiae tentatively identified as Scrub typhus from small mammals, Thailand

<u>Species</u>	<u>No. of animals</u>	<u>Pools</u>	<u>Positive</u>
Rattus rattus	346	176	36
Rattus exulans	90	39	1
Rattus berdmorei	13	10	2
Rattus rajah	125	70	21
Rattus niviventer	14	11	1
Bandicota indica	106	64	15
Bandicota benghalensis	1	1	-
Callosciurus finlaysoni	1	1	-
Menetes berdmorei	69	39	4
Cannomys badius	3	2	-
Tupaia glis	131	72	9
Hylomys suillus	1	1	-
Herpestes javanica	4	2	-
Melogale personata	1	1	-
Paradoxurus hermaphroditus	1	1	-
Rousettus amplexicaudatus	5	2	-
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Total 16 species	911	491	89

isolations were made from four human scrub typhus cases at the McCormick Hospital, Chiangmai. Two of these were from the immediate vicinity of Chiangmai, the others were from the nearby villages of Sarapee and San Patong. Isolations of rickettsiae thought to be that of scrub typhus were made from rodents collected in each of these villages during the year. The cases from which isolations were made were quite mild, leading to the suspicion that much of the scrub typhus occurring in the country escapes notice. The infection appears to respond well and rapidly to antibiotic therapy, and there is a large amount of self-treatment practised in Thailand since drugs may be sold without prescription.

Even isolation were also made from chiggers removed from small mammal hosts during the year. These are the first known isolations from chiggers from Thailand. Preliminary data indicate that Trombicula deliensis is the major vector of scrub typhus in the areas surveyed. Feeding experiments with unengorged chiggers are planned to substantiate this fact, but these have not begun as yet. Chiggers were found to respond rapidly to rainfall in the dry deciduous forests of Thailand, as will be noted in the discussion of the field trips below.

Field studies

a. Bangkok and vicinity: A number of collections were made in the suburban areas around Bangkok, including: Prakanong, Prapadang and Mahachai. Some of areas visited are quite built up, but they were predominantly fruit garden areas. Rickettsiae were isolated from two pools of tissue from Rattus rattus at Prakanong; from three pools of R. rattus at Prapadang and one pool of R. rattus at Mahachai. Final identification of these rickettsiae is still in progress. Leptotrombidium deliensis was found on almost all of the rats examined in the area, and Laurentella indica and Walchia rustica were also common. Further collection are in planned to determine the extent of infection in mammals in the Bangkok area. The first isolation of scrub typhus in Thailand was reported from Ban Pong, west of Bangkok by Traub and others of the Walter Reed Army Institute of Research in 1955, from an area quite similar to those in which the present isolations were made.

b. Chiangmai and vicinity: The early collections in this area failed to produce virulent strains of rickettsiae. However, a number of strains have now been isolated from rodents collected at San Patong and Sarapee districts; from which the human cases noted above entered the hospital. Rickettsiae were isolated from 7 pools of R. rattus tissue and 14 pools of Bandicota indica tissue at San Patong. The latter is a large rat which frequents rice fields in Thailand, and which is frequently eaten by the villagers. At San Patong rickettsiae were also isolated from a

pool of chiggers taken from R. rattus. At Sarapee two isolations were made from tissues of R. rattus. Thus, isolations have been made from all of the elements involved in the transmission cycle of scrub typhus in the Chiangmai region; the chiggers, the wild animal reservoir and man. Further investigations are in progress in the Chiangmai area to determine the seasonal incidence of rickettsiae in man, chiggers and mammals. In addition, chiggers are being reared and it is planned to attempt to transmit rickettsiae to mice by the bite of unengorged chiggers when numbers of chiggers become available during the rainy season. It is extremely difficult to find chiggers on the ground or on mammal hosts during the dry hot season.

c. Chong Mek, Ubol Province: Considerable attention was paid to this area during the year, since the Royal Thai Army experienced an outbreak of a disease later diagnosed as scrub typhus while on maneuvers there in 1955. Three trips were made to Chong Mek during the year. The first (May-June) bracketed the end of the dry season and the beginning of the rainy season. The second trip (August) took place at the height of the rainy season. The third trip (November) covered the end of the rainy season. However, the rains were somewhat prolonged in 1963 and while rainfall was less than in August there were a number of rainy days during the two weeks spent in the area. A list of all of the mammals examined for rickettsiae during the three collecting trips will be found in Table 2. The Chong Mek area is situated along the Laos border, not far from the Mekong. This plateau area was originally covered with monsoon deciduous forest, much of which has been cut for agricultural purposes. Many patches of the remaining forest are ringed by fields of lalang and other grasses.

May to June Trip: On arrival, the area was found to be extremely dry, there having been essentially no rain since the preceding November. During the two week period, however, the rainy season opened and the ground rapidly became soaked. One hundred and fifty two animals were examined (Table 1) and 14 of 85 pools of mammal tissues were positive for rickettsiae. Fourteen pools of chiggers were inoculated in mice, but no positive pools were detected. Rattus rattus was the most common mammal collected, with R. rajah, Menetes berdmorei and Tupaia glis also quite abundant. Rattus exulans was collected during this period, but not subsequently. The most abundant chigger species was a new species of Leptotrombidium (temporarily designated No. 5). The chigger count per animal was quite low, even after a week of rain. A team from the USAMRU unit in Kuala Lumpur visited Chong Mek during the survey and attempted to find unengorged chiggers on the ground by the use of black plastic plates, a survey technique which has been successful in many parts of the world. Polished boots and dinner plates have been used for the same purpose, but no method produced unengorged chiggers in the Chong Mek area.

Total 2. Isolations of rickettsial from small mammals and chiggers, Chong Mek, Ubolrajathani, Thailand, 1963

Mammals	JUNE			AUGUST			NOVEMBER			TOTAL		
	Collected	Pools	Positive									
<i>Rattus exulans</i>	5(3.3)	2	0	-	-	-	-	-	-	5(1.6)	2	0
<i>Rattus rattus</i>	68(44.7)	33	4(12.1)	24(26.7)	14	5(35.7)	16(20.5)	7	4(57.1)	108(33.9)	54	13(24.1)
<i>Rattus rajah</i>	23(15.1)	17	4(23.5)	41(45.5)	23	10(43.5)	45(57.7)	20	7(35.0)	109(34.2)	60	21(35.0)
<i>Rattus niviventer</i>	-	-	-	2(2.2)	2	1(50.0)	2(2.6)	2	0	4(1.3)	4	1(25.0)
<i>Rattus berdmorei</i>	8(5.3)	6	2(33.3)	1(1.1)	1	0	1(1.3)	1	0	10(3.1)	8	2(25.0)
<i>Rattus cremeri- venter</i>	-	-	-	1(1.1)	1	0	-	-	-	1(.3)	1	0
<i>Bandicota bengalensis</i>	1(0.7)	1	0	-	-	-	-	-	-	1(.3)	1	0
<i>Tupaia glis</i>	24(15.8)	11	2(18.2)	15(16.7)	9	6(66.7)	13(16.7)	7	1(14.3)	52(16.3)	27	9(33.3)
<i>Menetes berdmorei</i>	21(13.8)	11	2(18.2)	6(6.7)	3	2(66.7)	-	-	-	27(8.5)	14	4(28.6)
<i>Callosciurus finlaysoni</i>	1	1	0	-	-	-	-	-	-	1(.3)	1	0
<i>Paradoxurus hermaphroditus</i>	1	1	0	-	-	-	-	-	-	1(.3)	1	0
Total	152	85	14(16.5)	90	53	24(45.3)	78	37	12(32.4)	319	173	50(28.9)
Chiggers	-	14	0	-	21	3(14.3)	-	10	3(33.3)			

* Percentage of total mammals collected during the period.

** Percentage pools positive for this species during the period.

August: The mammal collections during this period (table 1) were somewhat lower during the second trip, due largely to interference from the heavy rains. Approximately 85% of the mammals were chigger infested, a decided increase over the earlier trip. Perhaps more important, Leptotrombidium deliensis appeared in the collections. Positive chigger pools were detected (14.3%) where none were found in the previous collection, leading to the conclusion that L. deliensis is an important vector in the area. All of the positive pools contained this species, although none of them were free from other species. No individual attachments or pools were studied.

November: The November collections were intended to bracket the end of the rainy season. However, heavy rains continued in the area through most of the trip and the effect of drying was not observed. This is the time of the year in which cases occurred in Thai Army troops in Chong Mek. The chigger burden on the animals continued high, and L. deliensis continued to be the predominant species. Identification of the bulk of the many species collected on this trip is still in progress. Chigger pools and mammal tissues continued to show a high percentage of rickettsiae positives.

Khao Yai National Park, Nakorn Nayok and Nakorn Ratchasima Provinces: Khao Yai was recently developed as a National Park, and numbers of tourists and vacationers from Bangkok are starting to enter the area. It is on the edge of the Korat Plateau, and the height of the mountain chain (2500-4000 ft.) causes a considerable rain shadow effect. The forests on the slopes of the mountains making up the Park are of the evergreen type, considerably different from the monsoon forests of the Korat Plateau proper. Much of the park area is still virgin forests, interspersed with large areas of rolling hills covered with lalang and other grasses. These open areas presumably are due to human intervention, but this must have occurred some time in the past. The area is quite reminiscent of the region around Kuala Lumpur, Malaysia, far to the south. Studies were made here because of the unusual nature of the terrain, and the possible future exposure of large numbers of people in the Park. In addition, there are several small U.S. and Thai military installations in the Park and on its margins.

The survey was conducted in September, and it rained for at least part of each day. Ninety-two mammals were collected during the trip, including the very rare gymnuran, Hylomys suillus. Nine isolations of rickettsiae were made from 33 mammal tissue pools, but no rickettsiae were recovered from the 7 pools of chiggers inoculated in mice. Of particular interest was the relative abundance of Leptotrombidium akamushi at Khao Yai. This important chigger was extremely rare in our previous

Table 3. Rickettsiae isolations from small mammals,
Khao Yai National Park, Thailand, 1963

Mammal	Number Collected	Pools	Positive
<u>Hylomys suillus</u>	2	0	0
<u>Tupaia glis</u>	29	12	2 (16.7)*
<u>Rattus niviventer</u>	6	2	0
<u>Rattus rajah</u>	8	5	0
<u>Rattus rattus</u>	43	18	7 (38.9)
<u>Rattus sp.</u>	2	1	0
Total	90	38	9

* Percentage of pools positive for the species.

collections, only two specimens having been taken in Northern Thailand. at Khao Yai it was quite abundant on mammals captured in the lalang grass fields. The identification of all of the chiggers captured during the study, and a determination of their relative abundance in different habitats in the Park is still in preparation. The initial survey of the Park indicated that rickettsiae are present in the mammal population, and that both classical vectors of the disease in SE Asia are present. The area deserves additional attention, since the tropical evergreen forest environment is widespread in Thailand and neighboring countries, but generally difficult to reach due to isolation and lack of roads. The Khao Yai area can be reached in three hours by road from Bangkok, and routine helicopter service is planned for the Park in the near future

Mae Chan, Chiengrai Province: This is a relatively isolated area on the Thai border with Burma, and not far from the point where the borders of Thailand, Laos and Burma join. The area is of zoological interest because it drains into the Mekong River complex, while most of the rivers of Northern Thailand run southward to join the Menam Chao Phrya. The vertebrate fauna, particularly the birds which have been well studied, seem to have affinities with the Chinese species. Mae Chan is approximately 70 miles south of the border of the Chinese Province of Yunnan. The survey area was a flat plain, closely bordered with mountains. At the time of the survey (December) it was cold and dry, but slight rain fell during the period. The animal catch was very small, possibly because rice ready for harvest was still in the fields. Of the 30 pools of animal tissue inoculated, 4 yielded rickettsiae tentatively identified as those of scrub typhus. In addition, one strain of murine typhus was isolated from a pool of tissues from three Rattus exulans collected from row houses in the market area. These rats carried large numbers of the murine typhus flea vector, Xenopsylla cheopis. One of the scrub typhus strains also came from R. exulans, which is the first time we have isolated the rickettsiae from this rat.

This completes the summary of the field work during the year. Future plans include a detailed season study of typhus in the Chiengmai area with the assistance of the medical staff of McCormick Hospital. Many more of the border areas of Thailand, especially in the Northeast, will be surveyed. Additional surveys will also be made in the primary forest at Khao Yai.

Additional Note on Chiggers

During the year villagers, foresters and others residing in the various study sites were interviewed to determine if there was any local knowledge of chigger attacks. It developed that such knowledge was rather widespread. People in the Chong Mek, Khao Yai, Chiengrai and Chiengsan were well acquainted with chiggers, which they call "maeng daeng". This translates literally as "red bug", the same name which is used in

Japan (Japanese- "akamushi"). At Sarapee villagers were even aware that the nymphs and adults were associated with the chiggers, and that chiggers were abundant in the rainy season and early winter. It was also reported that workers conducting bamboo rafts down the Ping River below Chiangmai avoid stopping or sleeping at certain sandy spots along the river because of chigger attacks. Guides in Khao Yai reported that when they fished in certain sandy places they knew that they would be attacked by chiggers, and that would become ill with fever shortly thereafter. In the Lopburi area the chiggers were known as "sien din" or ground bugs, but were not thought to be particularly dangerous.

Distribution of Isolations

Since the inception of the project, rickettsiae have now been isolated from a number of places in Thailand. While many of the isolates thought to be scrub typhus are still being identified at WRAIR, the following list is given a general guide, subject to subsequent revision.

Scrub typhus

<u>Province</u>	<u>District</u>
Chiangmai	San Partong Sarapee Samoeng Hot
Ubolrajathani	Chong Mek Khlong Jium
Krungthep (Bangkok)	Prakanong
Samutprakarn	Prapadang
Chantaburi	Tha Mai Pong Nam Rawn
Chiengrai	Mae Chan Chieng San
Nakornrajsima	Pakchong (Khao Yai)

Indian Tick Typhus

Chiangmai	Hot
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Murine typhus

Chiengrai

Mae Chan

Additional notes on the mammals collected and on the various groups of ectoparasites found on them will be found in the report of Study Number 49.

Summary: Small mammals and ectoparasites (chiggers and ticks) were collected in several parts of Thailand during the year. Eighty-nine strains of rickettsiae tentatively identified as scrub typhus were recovered from the mammal tissues. Seven additional strains were isolated from chiggers, chiefly Leptotrombidium deliense. Four strains of rickettsiae were also isolated from patients diagnosed as having scrub typhus. The first Thailand isolations of an agent related to Rickettsia conorii was reported from ticks, and murine typhus rickettsiae were isolated from rodent tissues for the first time in Thailand. Serological evidence was obtained which indicates that human and wild animal infection with rickettsiae is much more widespread than had been thought, or as is indicated by the reported human cases of rickettsiosis. Lists of the localities where isolations were made, and the small mammal hosts from which isolations were made are given in the report. Aliquots of all of the strains have been forwarded to WRAIR for positive identification and for characterization of the strain differences. The principal investigator is presently participating in this portion of the project at WRAIR.

Conclusions: Rickettsiae which appear to be those of scrub typhus are found in the small mammals of many parts of Thailand. Rickettsiae were also recovered from chiggers and from febrile human cases, and it would appear that the disease should be more widespread as a clinical entity than present records indicate. The isolation of an agent related to Indian tick typhus, and of the rickettsiae of murine typhus considerably expands the known spectrum of rickettsiae in Thailand. The chiggers which probably serve as vectors of scrub typhus rickettsiae (Leptotrombidium sp.) showed a strong seasonal distribution pattern where studied in detail.