

Ectoparasite and *Rickettsia tsutsugamushi*
Studies in Thailand

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OBJECTIVE : To establish and describe the chiggers and ticks that are vectors or potential vectors of human pathogens in Thailand, and to determine the geographical distribution of *Rickettsia tsutsugamushi* in natural populations of chiggers in Thailand.

BACKGROUND : This is a continuation of previously reported studies (1). Earlier work during the period of 1961-1972 served as the basis for a number of publications, in which Lakshana (2) and Lekagul and McNeely (3) have established a firm taxonomic base on which epidemiological-ecological studies on *Rickettsia tsutsugamushi* in Thailand can proceed. Recent emphasis has shifted to the distribution of strains of *R. tsutsugamushi* that occur in vector chiggers in Thailand. However, problems still exist in identifying chigger specimens collected in Thailand, hence taxonomic studies on chiggers are continuing.

METHODS : Ectoparasites are collected from live trapped rodents and other small mammals by removal with forceps, by scraping or by holding the animals alive over a pan of water and allowing engorged ectoparasites to drop into the water. Engorged chiggers are normally preserved in alcohol and mounted on slides for study. Chiggers used for *R. tsutsugamushi* isolation attempts are preferably unengorged. Unengorged chiggers are usually found in leaf litter, on rotten logs and other favorable habitats frequented by rodents and other small mammals, and are easily collected by using 5" x 5" formica black plates. Collected unengorged chiggers are placed and kept alive in vials of water, which are then shipped to USAMRU-Kuala Lumpur for rickettsia studies. A technique using direct immunofluorescence has recently been developed to detect rickettsia in naturally infected mites (4). Using this technique, the internal contents of each unengorged chigger can be screened for nine different strains of *R. tsutsugamushi*, i.e., Karp, Gilliam, Kato TC 586, TA 678, TA 686, TA 716, TA 763 and TH 1817. After the internal contents of the chigger has been tested for rickettsia, its exoskeleton is mounted in Hoyer's mounting media on a slide for identification.

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RESULTS : Between 25 Oct. and 21 Dec. 1978, 1,379 unengorged chiggers of 12 species were collected in Thailand by the black plate method and sent to USAMRU-Kuala Lumpur for rickettsia detection and subsequent identification (Table 1). These specimens were collected in 2 provinces, Chiang Mai and Nakhon Ratchasima (two areas - Pak Chong and Sakaerat). The five most commonly collected species were *L. (L.) scutellare*, *L. (L.) deliense*, *L. (L.) miculum arvinum*, *Odontacarus* sp. and *L. (T.) paniculatum*, in descending order of abundance. Four new species, *Helenicula* sp., *L. (L.)* sp. D, *L. (L.)* sp. G and *Odontacarus* were also identified during this period, and represented 7.6% of the total specimens collected. Of the 12 species collected, 828 specimens of 10 species were screened in Kuala Lumpur for *Rickettsia tsutsugamushi*, and 6% (50/828) were positive (Table 2). A total of six species were positive for *R. tsutsugamushi* from the two provinces, with *L. (L.) deliense* and *scutellare* positive in both provinces, while *L. (L.) miculum arvinum* was positive only in Chiang Mai and *E. wichmanni*, *L. (T.) paniculatum* and *Odontacarus* sp. positive only in Nakhon Ratchasima Province. A total of 5.1% (20) of chiggers screened from Chiang Mai were positive while 6.8% (30) of chiggers screened from Nakhon Ratchasima were positive.

The sources and habitats for the chiggers screened for *R. tsutsugamushi* from Thailand during this period are shown in Table 3. Initially it would appear that the primary foci for infected chiggers are primary and secondary evergreen forests. However, only a few black plate collections were made from the other three basic habitats early regenerating evergreen, Pa Daeng (dry dipterocarp) forest and grass. Additional collections from all the habitats are needed before infection rates per habitat can be properly interpreted, however, four of the five habitats analyzed here contained infected chiggers. Probably most, if not all, of the basic habitats in Thailand contain chigger species harboring *R. tsutsugamushi*.

Several chigger species appear to be highly seasonal and found only during the cool season in Thailand. These species, *Helenicula* sp., *L. (L.) scutellare*, and *Odontacarus* sp., have been collected in Thailand only between the months of November to February. Of these, only *Helenicula* sp. was not found infected with *R. tsutsugamushi*. *Leptotrombidium (L.) scutellare* is a known vector of *R. tsutsugamushi* in other areas of Southeast Asia, but previous to this study, has not been found infected in Thailand.

Table 4 summarizes the chigger species captured in black plate collections in Thailand and screened for *R. tsutsugamushi* since this project was initiated in June 1977. Included are chigger-rickettsial data from the Pak Chong study described elsewhere in this Annual Report. At that time only *L. (L.) deliense* had been incriminated as a vector of *R. tsutsugamushi* in Thailand. One and half years later 8.2% (146) of 1,778 chiggers screened for *R. tsutsugamushi* infections have been found positive. Furthermore, 9 of 15 species screened contained rickettsial infections, indicating a much larger number of potential vectors of this human pathogen in Thailand than previously suspected. The role of at least seven of the nine infected species found here in the zoonotic maintenance of this pathogen is currently unknown. Species of the subgenus *Leptotrombidium* are usually considered of primary importance in the transmission of this pathogen to man (5) and five of the infected species found in this study belong in this subgenus. However, infected species of 3 other genera, *Eutrombicula*, *Microtrombicula*

and *Odontacarus*, and one species in the subgenus, *Leptotrombidium* (*Trombiculindus*, were also found. Generally, the species found infected with *R. tsutsugamushi* during this study fall into Nadchatram's (6) ecological group I, i.e., those species which effect man or have a better opportunity of coming in contact with him. According to the current interpretation of Nadchatram's groupings, only *Lept. (T.) paniculatum* and *Microtrombicula chamlongi* possibly would not fall into group I. However, these 2 species were not included in Nadchatram's study.

The large number of *R. tsutsugamushi* isolations made during this study were only possible because of the development of new techniques (4). Based on the attached data, the advantages of the MDFA technique in conjunction with the collection of unengorged chiggers on black plates are very obvious. Although the current project is scheduled for termination, these data suggest an obvious need for the continuation of similar studies.

A total of 7 new species of chiggers were identified during this study, of which 3 were found infected with *R. tsutsugamushi*. Currently, the descriptions and illustrations for 12 new species of the genus *Leptotrombidium* (*Lept.*) have been completed (including one new species found in this study) and those for 12 additional species are being completed in preparation for publication. The other 6 new species found in this study will be described and prepared for publication in the future.

A checklist of the Ticks of Thailand, containing 54 species in 10 genera is nearing completion. Of those, 23 have not been reported previously from Thailand. A host list is also included.

The biological and taxonomic efforts of this study are continuing and additional rickettsial isolation studies are anticipated.

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Table 1. Unengorged chiggers captured by black plate collections in Thailand between 25 Oct. - 21 Dec. 1978 and sent to USAMRU-Kuala Lumpur for rickettsia isolation.

Chigger Species	Black Plate Collection per Location	Chiang Mai (19)	Nakhon Ratchasima		TOTAL (63)
			Pak Chong (19)	Sakaerat (25)	
<i>Eutrombicula wichmanni</i>		-	-	23	23
<i>Helenicula</i> sp.		-	6	12	18
<i>Lept. (L.) deliense</i>		28	52	35	115
" " <i>fulleri</i>		1	-	-	1
" " <i>miculum arvinum</i>		77	3	2	82
" " <i>scanloni</i>		1	-	-	1
" " <i>scutellare</i>		394	-	591	985
" " <i>striatum</i>		-	-	1	1
" " sp. D ¹		-	1	1	2
" " sp. G ¹		3	-	-	3
<i>L. (Trom.) paniculatum</i>		-	5	61	66
<i>Odontacarus</i> sp.		-	81	1	82
TOTALS		504	148	727	1,379

¹ New undescribed species

Table 2. Source and species of Thai chiggers that were screened for *Rickettsia tsutsugamushi* from collections during the period 25 Oct. - 21 Dec. 1978

Location (Collection Dates) - Chigger Species	Specimens		
	Collected	Screened	Infected % (No.)
<u>Chiang Mai</u> (22 Nov.-4 Dec. 1978)	-	-	-
- <i>Lept. (L.) deliense</i>	28	24	8.3(2)
- " " <i>fulleri</i>	1	1	0
- " " <i>miculum arvinum</i>	77	56	1.8(1)
- " " <i>scanloni</i>	1	1	0
- " " <i>scutellare</i>	394	308	5.5(17)
SUBTOTALS	501	390	5.1(20)
<u>Nakhon Ratchasima</u> (25 Oct.-21 Dec. 1978)	-	-	-
- <i>Eutrombicula wichmanni</i>	23	15	13.3(2)
- <i>Helenicula</i> sp.	18	9	0
- <i>Lept. (L.) deliense</i>	87	64	10.9(7)
- " " <i>miculum arvinum</i>	5	5	0
- " " <i>scutellare</i>	591	234	7.3(17)
- " " sp. D	2	1	0
- " (<i>Trom.</i>) <i>paniculatum</i>	66	58	1.7(1)
- <i>Odontacarus</i> sp.	82	52	5.8(3)
SUBTOTALS	874	438	6.8(30)
TOTALS	1,375	828	6.0(50)

Table 3. Source and habitats for Thai chigger species screened for Rickettsia tsutsugamushi between 25 Oct. - 21 Dec. 1978.

Habitat - specimens Source - Species	Primary Evergreen		Secondary Evergreen		Early Regenerat- ing Evergreen		Dry Dipterocarp Forest		Grass	
	No. Screened	Infected %(No.)	No. Screened	Infected %(No.)	No. Screened	Infected %(No.)	No. Screened	Infected %(No.)	No. Screened	Infected %(No.)
Chiang Mai (22Nov.-4Dec.78)										
- <u>Lept. (L.) deliense</u>	-	-	24	8.3(2)	-	-	-	-	-	-
- " " <u>fulleri</u>	-	-	1	0	-	-	-	-	-	-
- " " <u>miculum arvinum</u>	54	1.9(1)	2	0	-	-	-	-	-	-
- " " <u>scanloni</u>	-	-	1	0	-	-	-	-	-	-
- " " <u>scutellare</u>	153	5.9(9)	155	5.2(8)	-	-	-	-	-	-
SUBTOTAL	207	4.8(10)	183	5.5(10)	-	-	-	-	-	-
Nakhon Ratchasima (25 Oct.-21Dec.78)										
- <u>Eutrombicula wichmanni</u>	-	-	-	-	-	-	-	-	15	13.3(2)
- <u>Helenicula</u> sp.	-	-	5	0	1	0	3	0	-	-
- <u>Lept. (L.) deliense</u>	19	0	41	14.6(6)	1	0	-	-	3	33.3(1)
- " " <u>miculum arvinum</u>	2	0	3	0	-	-	-	-	-	-
- " " <u>scutellare</u>	234	7.3(17)	-	-	-	-	-	-	-	-
- " " sp.D	-	-	-	-	1	0	-	-	-	-
- <u>Lept. (T.) paniculatum</u>	52	1.9(1)	5	0	-	-	1	0	-	-
- <u>Odontacarus</u> sp.	-	-	37	5.4(2)	15	6.7(1)	-	-	-	-
SUBTOTAL	307	5.9(18)	91	8.8(8)	18	5.6(1)	4	0	18	16.7(3)
TOTALS	514	5.4(28)	274	6.6(18)	18	5.6(1)	4	0	18	16.7(3)

Table 4. Summary of chigger species captured in black plate collections in Thailand and screened for during the period, June 1977 to December 1978.

No.	Species	Specimens		
		Collected	Screened	Infected % (No.)
1	<i>Asch. (L.) indica</i>	1	1	-
2	<i>Eutrombicula wichmanni</i> ¹	23	15	13.3(2)
3	<i>Gahrliopia (G.) mirabilis</i>	2	-	-
4	<i>Helenicula</i> sp. ²	18	9	-
5	<i>Lept. (L.) deliense</i>	2,018	929	9.4(87)
6	" " <i>fulleri</i>	22	1	-
7	" " <i>miculum arvinum</i> ¹	163	121	7.4(9)
8	" " <i>scanloni</i>	1	1	-
9	" " <i>scutellare</i> ¹	985	542	6.3(34)
10	" " <i>striatum</i>	1	-	-
11	" " sp. A ^{1,2}	119	39	17.9(7)
12	" " sp. B ^{1,2}	4	4	50.0(2)
13	" " sp. C ²	16	1	-
14	" " sp. D ²	4	2	-
15	" " sp. G ²	3	-	-
16	<i>Lept. (T.) paniculatum</i> ¹	77	59	1.7(1)
17	<i>Microtrombicula chamlongi</i> ¹	6	2	50.0(1)
18	<i>Odontacarus</i> sp. ^{1,2}	82	52	5.8(3)
19	<i>Siseca rara</i>	16	-	-
20	<i>Walchiella oudemansi</i>	68	-	-
21	" <i>traubi</i>	1	-	-
TOTALS		3,630	1,778	8.2(146)

¹ species not previously recorded as infected with *R. tsutsugamushi* in Thailand

² new undescribed species