

Epidemiological and Ecological Studies of Scrub
Typhus in Royal Thai Army Field
Training Facilities

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OBJECTIVES

1. To prospectively determine the susceptibility and the exposure risk of Royal Thai Army personnel to *Rickettsia tsutsugamushi* during field training exercises.
2. To determine the prevalence of *Rickettsia tsutsugamushi* in selected species of small mammals and chiggers from areas and habitats utilized by troops during training.
3. To determine if there is a seasonal effect influencing the susceptibility risk of Royal Thai Army personnel.
4. To evaluate the use of regional, habitat and seasonal data for predicting human exposure and risk potential to scrub typhus in Thailand.

BACKGROUND : In a previous report (1), the rationale for the establishment of a pilot project at Pak Chong, Nakhon Ratchasima Province, was presented. Preliminary results were presented in the report cited; the present report involves data accrued during this reporting period.

METHODS : Sixty-five soldiers undergoing Special Forces training at Pak Chong, Nakhon Ratchasima Province, contributed blood samples before and after their training. Training consisted of four weeks of classroom and field problems and four days of jungle bivouac approximately 15-20 km. from the base camp. The

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troop training includes daily contact with forested or grassy areas and a bivouac in similar areas. Total field exposure was calculated for each individual and background information on age, rank, occupation (both in and outside of the military), home, travel and previous medical history was taken. The latter of the two blood samples was collected two weeks after the end of training. The blood samples were tested by the Weil-Felix (2) and by the indirect immunofluorescence tests (3). Sulfonamide level determination was performed on 15 samples to determine the level of Fansidar usage as a possible confounding variable.

Rodents and other small mammals were live-trapped in each habitat associated with troop training, and often in the actual training areas. Traps were baited with bananas and normally placed in 2-3 habitats each night. All small mammals collected were identified to species, sexed, aged, bled via cardiac or retro-orbital puncture and examined for ectoparasites. Rodent rickettsial serology will be done at AFRIMS by the IFA technique (3). Attached chiggers were gently scraped off and placed in vials containing 70 per cent ETOH. Chiggers were counted and collated according to host species and habitat. Unengorged chiggers were collected in the troop training areas by black plates. Chiggers collected from black plates were kept alive in vials of water and sent to USAMRU-Kuala Lumpur, for rickettsia isolation by the micro direct fluorescent antibody (MDFA) technique (4).

RESULTS : All work this period was conducted in the base camp area at Ban Nong Tha Ku. This area is government owned, hilly and primarily covered with secondary evergreen forests, although more ecologically transient vegetation such as grass and early regenerating evergreen is also common. The basic soil type in the area is red-orange clay and the forest floors are fairly well preserved with considerable humus and leaf litter, indicating that fires are uncommon. Three basic habitats were identified in the area, i.e., tall grass, early regenerating evergreen and secondary evergreen. Because of troop field training exercises, trails were common in many areas of all 3 basic habitats, facilitating small mammal trapping.

Considerable precipitation fell in the study area during the period 24 October - 17 December 1978, as compared to the previous report period (1), particularly the first trapping period in June 1978.

During the last period of work in camp (December 1978) temperatures dropped considerably, with early morning lows on several days in the range of 8-12°C, and daytime temperatures in the range of 18-23°C. Warmer temperatures also occurred during that period.

Entomological-rodent trapping studies occurred during the period 24 October - 17 December 1978, and were conducted during 2 separate 10 day periods. The first period, 24 October - 2 November, corresponded with the first portion of training for the test soldiers, while the second period, 8-17 December, corresponded with the latter part of training for the test soldiers. The test soldiers were in frequent contact with the 3 basic habitats during both periods of this study. Although it was not possible to calculate the ratio of their exposure to each basic habitat, more training occurred in the grass and secondary evergreen habitats than in the early regenerating evergreen.

A total of 486 small mammals of 15 species were collected from 2,364 trap-days (traps x 24hr periods) during this study period (Table 1). The 3 basic habitats were not trapped equally (index trap-days): 53 per cent in secondary evergreen, 37 per cent in tall grass and 10 per cent in early regenerating evergreen. Accordingly, more animals were caught in secondary evergreen than in the tall grass and early regenerating evergreen respectively. Furthermore, trapping efficiency was higher in the secondary evergreen, (one animal per 4.3 trap-days) than in the tall grass (one animal per 5.5 trap-days) and the early regenerating evergreen (one animal per 6.5 trap-days).

Seven of the 15 species accounted for 96% (465/486) of the total number of small mammals captured: *Rattus surifer*, *R. rattus*, *Tupaia glis*, *R. sabanus*, *R. bukit bukit*, *Menetes berdmorei* and *R. koratensis*, in descending order of abundance, respectively. Most of these 7 species are commonly associated with a large number of chigger species in Thailand (5), including the most common vector of *R. tsutsugamushi*, *L. (Lept.) deliense*. Very obvious differences were detected in the numbers of species and individuals of given mammal species captured in the three habitats. While only 32.5 per cent (158/486) of the total animals were captured in the tall grass, 93 per cent (14/15) of the total animals were captured in the secondary evergreen, but only 67 per cent (10/15) of the species were captured there, while nearly 47 per cent (7/15) of the species were included in the 7.8 per cent (38/486) of the total animals captured in the early regenerating evergreen.

Rattus rattus, the species usually considered most closely associated with *L. (Lept.) deliense*, was most common (85 per cent, 74/87) in the tall grass habitat. An additional five species, *Bandicota savilei*, *Herpestes javanicus*, *Mus cervicolor*, *Mus* sp. and *Rattus losea*, were captured only in the grass. On the other hand, 95 per cent (21/22) of *Rattus koratensis*, 76 per cent (124/164) of *R. surifer*, 72 per cent (56/78) of *Tupaia glis*, 72 per cent (21/29) of *Menetes berdmorei* and 65 per cent (31/48) of *R. sabanus*, were collected in the secondary evergreen habitat. Only the insectivore-predator, *Hylomys suillus*, seemed to be fairly equally distributed in the 3 different habitats, although more specimens were collected in the early regenerating evergreen than would be expected.

A total of 148 unengorged chiggers were collected from 19 black plate (BP) collections during this period, of which 109 were screened and 8.3 per cent (9) were found positive for *Rickettsia tsutsugamushi* (Table 2) by the MDFA technique (4). The infected specimens included 14.3 per cent (6/42) of the *L. (Lept.) deliense* screened and 5.8 per cent (3/52) of the *Odontacarus* sp. screened. The last species is apparently new to science. Actually, six of the 19 BP Collections were made during the period 24 October - 2 November, and the remaining 13 BP collections were made during the period, 8-17 December. Chigger collection, habitat and rickettsial isolation data for these two periods are summarized in Table 3-4. These data show that chiggers infected with *R. tsutsugamushi* were collected during both periods and in two habitats, i.e., early regenerating evergreen (last period) and secondary evergreen (both periods). Furthermore, the chigger species composition (based only on BP collections) was completely different for the two periods. Only *L. (Lept.) miculum arvinum* and *deliense* were collected in the first period, while only *Helenicula* sp., *L. (Lept.)* Sp.D, *L. (Trom.) paniculatum* and *Odontacarus* sp. were collected during the last period.

Collections of engorged chiggers were made from all parasitized animals. These specimens have been slide mounted and are currently being identified. The rodent sera collected during this period and the previous annual progress report period are currently being examined for *R. tsutsugamushi* antibody levels.

Blood specimens on 65 soldiers were tested prior to the initiation of training and three (4.6%) were seropositive by the IFA technique. Fifty-nine of these soldiers (including the three with pre-existing antibody) were subsequently re-examined after training and none were positive at that time.

It is apparent from the lack of antibody against *R. tsutsugamushi* in this population (less than 5%) that the soldiers tested have not been exposed to scrub typhus in the recent past. The full duration of antibody retention is not known, but experimental infections in the silver-leaf monkey model have shown evidence of a low level of antibody up to two years following an infection (6). The loss of antibody in the three soldiers who had evidence of antibody prior to the initiation of training in a blood sample taken six weeks later attests to the transient nature of this antibody. The absence of infections in this human population, in spite of the close proximity to infected chiggers, indicates insufficient contact between the humans and the chiggers for infections to occur. Consequently, this area provides a poor site for the continuation of this project beyond the pilot stage.

Unfinished aspects of this project will be completed in the near future, at which time the studies in Pak Chong will be terminated.

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