

INVESTIGATION OF THE ROLE OF *BLANKAARTIA ACUSCUTELLARIS* (ACARI: TROMBICULIDAE) AS A VECTOR OF SCRUB TYPHUS IN CENTRAL THAILAND

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Abstract. Monthly collections of rodents were conducted in Phitsanulok Province, central Thailand in 1993 to investigate the role of the mite *Blankaartia acuscutellaris* as a vector of scrub typhus. Overall, a total of 41 rodents were collected and examined for the presence of the red colored larvae of *B. acuscutellaris* and yellow larvae of *Leptotrombidium deliense* and *Ascoshengastia* sp. A total of 787 *B. acuscutellaris* and 1390 yellow larvae were placed into pools, triturated and isolation of *Orientia tsutsugamushi* attempted in laboratory mice. The sera of 8 of the collected rodents had elevated antibodies to *O. tsutsugamushi* indicating active infections; however, *O. tsutsugamushi* was not isolated from rodent tissues or pools of larvae. The results of this survey suggest that *B. acuscutellaris* may not be an important vector of scrub typhus, but more studies are needed in endemic areas.

INTRODUCTION

Scrub typhus is a disease caused by the rickettsia *Orientia tsutsugamushi* (Hayashi) and is transmitted by trombiculid larvae, commonly called chiggers (Traub and Wisseman, 1974). The main vector of scrub typhus in Thailand is considered to be *Leptotrombidium deliense* (Walch) (Frances *et al.*, 1999), and recent studies have shown *L. imphalum* Vercammen-Grandjean and Langston and *L. chiangraiensis* Tanskul and Linthicum are important vectors in ricefield habitats in northern Thailand (Tanskul

et al., 1998; Tanskul and Linthicum, 1999).

A number of other species of trombiculid mites have been found to be infected with *O. tsutsugamushi*, but their role as vectors has not been confirmed (Frances *et al.*, 1999; Takada *et al.*, 1984). Tanskul *et al.* (1994) found that larvae of *Blankaartia acuscutellaris* Walch were infected with *O. tsutsugamushi* during a year long study in rice field habitats in Phitsanulok Province, central Thailand. They reported 7.3% of *B. acuscutellaris* were infected when tested by a direct fluorescent antibody (DFA) technique (Dohany *et al.*, 1978). They were able to demonstrate the occurrence of *O. tsutsugamushi* in rodents and a human, but did not attempt to isolate rickettsiae from chiggers. Nadchatram (1970) reported that *B. acuscutellaris* is a ground surface species usually associated with swampland habitat (including rice fields) and is relatively highly adaptable to fluctuating environmental conditions.

Because of the high numbers of DFA positive *B. acuscutellaris* in Phitsanulok, and because the species is known to bite humans,

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In conducting the research described in this report, the investigators adhered to the *Guidelines for Laboratory Facilities and Care*, as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

there is a high suspicion that this species may be an important vector of *O. tsutsugamushi* (Tanskul *et al*, 1994). Despite this, a colony of *B. acuscutellaris* naturally infected with *O. tsutsugamushi* has never been established, and *O. tsutsugamushi* has never been isolated from larvae of this species of mite. Here we describe studies to determine the role of *B. acuscutellaris* in the transmission of scrub typhus in Phitsanulok, central Thailand.

MATERIALS AND METHODS

Study site

The study was conducted within the grounds of the 31st Border Patrol Police Base, located 3 km southwest of the city of Phitsanulok, Phitsanulok Province, in central Thailand. The camp was fairly flat, with several large dams, and rice cultivation. The area had no large trees, but several areas had scrub bushes and grass. Local residents were permitted to fish in the ponds, graze cattle and raise poultry on the base. Rodent traps were placed throughout an area of 1 km² in grass and at the bases of small bushes.

Rodent collections

Between January 1993 and November 1993, 60 rodent wire traps (28 cm x 14 cm x 14cm) were placed in grass at the camp. Collections were not possible in February, May or October 1993. Traps were baited with a small piece of banana and were set in the evening and retrieved the next morning. All the collected rodents were retrieved and returned to the laboratory in Bangkok, held overnight, and then processed.

Laboratory procedures

In the laboratory, rodents were anaesthetised, then blood samples taken and chiggers removed into water. The larvae of *B. acuscutellaris* are bright red in color and easily distinguished from other species. All *B. acuscutellaris* were separated and pooled for isolation attempts in laboratory mice. Yellow

chiggers [*Leptotrombidium deliense*, (Walch), *Aschochoengastia indica* Hirst and *Aschochoengastia (Laurentella)* sp #4] from these rodents were also pooled and inoculated into mice.

Determination of infection in rodents

To determine if collected rodents were infected with *O. tsutsugamushi*, liver and spleen samples were removed, triturated to make a 20% solution in Snyder's Medium (Oaks *et al*, 1983), then immediately injected intraperitoneally into laboratory mice (0.2 ml per mouse). Triturates of larval mites collected attached to rodents were also prepared in Snyder's Medium and then injected into mice to determine infection with *O. tsutsugamushi*. Detailed methods used to determine infection in mice is shown in Frances *et al* (2000).

RESULTS AND DISCUSSION

A total of 41 rodents were collected during the study; 5 *Rattus rattus* (L.), 7 *Bandicota savilei* Thomas, 28 *Bandicota indica* (Bechstein) and 1 *Rattus argentiventer* (Robinson and Kloss). None of the liver/spleen isolation attempts were successful, however the serum of 8/41 (19.5%) was shown to have elevated antibodies to *O. tsutsugamushi*.

The inability to isolate *O. tsutsugamushi* from any of the rodents indicated that there was limited transmission of *O. tsutsugamushi*. The serum of eight rodents had elevated antibodies to *O. tsutsugamushi*, and five of these had high IgM titers (1:1,600), indicating an active infection (Strickman *et al*, 1994). Only two rodents with elevated antibodies to *O. tsutsugamushi* were infested with *B. acuscutellaris*, four with only yellow larvae (*L. deliense* and *Aschochoengastia* spp), and two did not have any attached larvae. These data indicate that the distribution of larvae in the study area was patchy.

The monthly collection of rodents, and *B. acuscutellaris* during the study is shown in Table 1. A total of 787 red larvae of *B. acuscutellaris* and 1,390 yellow larvae (*L. deliense*

Table 1

Collection of rodents with attached *Blancaartia acuscutellaris* at Border Patrol Police Camp 31, Phitsanulok Province, Thailand, 1993.

Month	Number of rodents collected	Number of rodents with attached <i>B. acuscutellaris</i>	Total number of <i>B. acuscutellaris</i> used for isolation attempts (No. pools)
January	4	2	77 (1)
February	NC		
March	4	3	251 (3)
April	3	2	47 (2)
May	NC		
June	4	2	60 (1)
July	11	7	74 (4)
August	5	3	121 (3)
September	7	4	70 (4)
October	NC		
November	3	3	87 (3)

NC = no collection.

and *Ascoshogastia* spp) were used for isolation attempts. Twenty-one batches (mean 35.8, range 5-200) of *B. acuscutellaris* and 23 batches (mean 60.4 per batch, range 5-200) of yellow larvae were inoculated into mice.

A previous study examined co-feeding of uninfected *B. acuscutellaris* with infected *L. deliense* (Frances *et al*, 2000). When single infected *L. deliense* were allowed to feed on a rat with *B. acuscutellaris* it was not possible to detect *O. tsutsugamushi* in *B. acuscutellaris* larvae. However, when groups of four or eight positive *L. deliense* were co-fed with *B. acuscutellaris* larvae, three of five batches of *B. acuscutellaris* were shown to acquire *O. tsutsugamushi*. In the current field study, *O. tsutsugamushi* was not isolated from rodent or *B. acuscutellaris* tissues, and so additional positive information on the role of *B. acuscutellaris* was not obtained. All of the larvae collected by Tanskul *et al* (1994) were tested for the presence of *O. tsutsugamushi* using a DFA method, and so they did not attempt to isolate rickettsiae from larvae. Frances *et al* (2000) showed that *B. acuscutellaris* acquire *O. tsutsugamushi* when feeding on rickettsiemic animals or when co-feeding with naturally infected larvae. The relatively high percentage

of *B. acuscutellaris* (7.3%) found positive by Tanskul *et al* (1994) may have been due to acquisition of rickettsiae during feeding.

The distinction between infected and infective mites has been described by several authors (Rapmund *et al*, 1969; Traub and Wisseman, 1974). A disadvantage of the DFA method is that it detects the occurrence of rickettsiae in the body of the animal, but it cannot distinguish if the larva is infective, and since the larva must be killed during the test, there is no way to confirm if the larva is infective.

The current study provides field evidence that *B. acuscutellaris* is not involved in the transmission of rickettsiosis in this habitat in central Thailand. However, additional studies in an area with higher densities of *B. acuscutellaris* are warranted to ascertain the role of this species as a vector of scrub typhus.

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