Short Communication

**ANTIGENIC CLASSIFICATION OF *RICKETTSIA TSUTSUGAMUSHI*
ISOLATES FROM SMALL MAMMALS TRAPPED IN DEVELOPING
OIL PALM COMPLEX IN PENINSULAR MALAYSIA***

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SUMMARY: Fifty-one *Rickettsia tsutsugamushi* isolates from small mammals collected in central Peninsular Malaysia were serologically characterized by direct immunofluorescence using eight prototype strains. Karp and Karp-related (TA763, TA686, TA716) antigens were found in 90.2% of the isolates.

Scrub typhus has been known to occur in Malaysia for over 50 years (Fletcher, Lesslar and Lewthwaite, 1928), but the importance of the disease was not fully recognized until recently when a study of febrile illnesses in rural Malaysia revealed a high incidence of infections, particularly in oil palm workers (Brown et al., 1976). The study yielded a number of human isolates from a Federal Land Development Authority (FELDA) oil palm complex at Bukit Mendi, Pahang, Peninsular Malaysia. Since rodents are common in these areas and serve as the primary hosts for the vector chiggers, the study afforded an opportunity to examine the relationship between strains existing in nature and those causing human infections. The human *Rickettsia tsutsugamushi* isolates from Bukit Mendi have been antigenically analyzed (Shirai et al., 1979). This report presents data on the isolates from rodents collected within the Bukit Mendi complex.

The study area has been previously described by Brown et al. (1976). Four trapping grids of 100 traps each were established. Three of the grids were located in the oil palm habitats containing 5- to 7-year-old trees, while the fourth was established on the forest fringe so that 50 traps were in the forest habitat and the other 50 in the oil palm habitat. Grids were trapped for four nights each month from January, 1976, through February, 1977. The trapped rodents were

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TABLE I

Isolation of Rickettsia tsutsugamushi organisms from different animals trapped at Bukit Mendi oil palm complex

Animals	Total	Positive (%)
<i>Callosciurus notatus</i>	14	2 (14.3)
<i>Chiropodomys gliroides</i>	1	0 (0)
<i>Echinosorex gymnurus</i>	4	0 (0)
<i>Rattus argentiventer</i>	23	6 (26.1)
<i>Rattus exulans</i>	4	1 (25.0)
<i>Rattus muelleri</i>	4	0 (0)
<i>Rattus sabanus</i>	1	0 (0)
<i>Rattus tiomanicus</i>	234	49 (20.9)
<i>Rattus whiteheadi</i>	5	0 (0)
<i>Sundasciurus lowii</i>	1	0 (0)
<i>Sundasciurus tenuis</i>	12	0 (0)
<i>Tupaia glis</i>	9	1 (11.1)
Total	312	59 (18.9)

identified and bled via cardiac puncture. The blood specimens were injected intraperitoneally into pairs of laboratory mice, and the isolations were confirmed by challenging the mice with the virulent Karp strain of *R. tsutsugamushi* (Brown *et al.*, 1976). Antigenic characterization of the isolates was performed by direct immunofluorescence. Of the eight prototype strains used in the test, four have been shown to share significant amounts of antigen: Karp, TA763, TA716 and TA686 (Shirai *et al.*, 1979).

During the study *Rattus tiomanicus* was the most commonly trapped animal (234/312) (Table I). *R. tsutsugamushi* organisms were isolated from five different animals: *R. tiomanicus*—20.9%; *Rattus argentiventer*—26.1%; *Rattus exulans*—25.0%; *Callosciurus notatus*—14.3%; and *Tupaia glis*—11.1%. Attempts to isolate organisms from seven other rodent species were unsuccessful. Seasonal fluctuations in rates of infections among the animals were not noted.

The serologic characterization of 51 isolates is shown in Table II. Eight other isolates were lost during reisolation attempts. Karp antigen and the Karp-related antigens (TA763, TA716 and TA686) were found singly or in combination with other antigens in 90.2% (46/51) of the isolates, while the Gilliam antigen was present in 23.5% (12/51) of the isolates. Kato antigen was not demonstrated in any of them. The different antigenic combinations appeared to exist rather uniformly throughout the grids, and the breakdown of the strain mixtures among the five different groups of animals from which the isolates were made demonstrated no specific pattern. Thirty-six of 51 (70.6%) isolates contained mixtures of two or more strains. The predominant prototype antigens occurring among the isolates were Karp and TA763 (Table III).

The current findings compare favorably with the high *R. tsutsugamushi* isolation rates from *Rattus* spp. which have been reported from other endemic

TABLE II

Antigenic characterization of isolates from animals trapped at Bukit Mendi oil palm complex

Isolates	Animals	
	Number	%
Karp	10	19.6
Gilliam	5	9.8
Karp, Gilliam	2	3.9
Karp, TA686	4	7.8
Karp, TA763	12	23.5
Karp, TH1817	1	2.0
Gilliam, TA716	1	2.0
TA686, TA716	1	2.0
TA716, TA763	1	2.0
Karp, Gilliam, TH1817	1	2.0
Karp, TA686, TA716	1	2.0
Karp, TA686, TA763	4	7.8
Karp, TA716, TA763	1	2.0
Karp, TA763, TH1817	2	3.9
Gilliam, TA716, TA763	2	3.9
TA686, TA716, TA763	2	3.9
Gilliam, TA686, TA716, TA763	1	2.0
Total	51	100.1

TABLE III

Frequency of Rickettsia tsutsugamushi prototype antigens found among the animal isolates

Prototype antigens	Number	Frequency*
Karp	38	74.5
TA763	25	49.0
TA686	13	25.5
Gilliam	12	23.5
TA716	10	19.6
TH1817	4	7.8
Kato	0	0
TA678	0	0

* Expressed as the percentage of isolates in which the respective antigen was identified.

areas (Trishnananda, Vasuvat and Harinasuta, 1964; Traub, Wisseman and Nur Ahmad, 1967; Muul, Lim and Walker, 1975). Ecological changes occurring with time may influence the infection rates within the population of small mammals. In an earlier study of a fully matured oil palm estate in Peninsular Malaysia, a low incidence of infection (0.5%) in *Rattus tiomanicus* was observed. In addition,

only 1.4% of all animals had demonstrable antibody to *R. tsutsugamushi* (Shirai *et al.*, 1978).

The study in the Bukit Mendi oil palm complex has demonstrated the predominance of Karp and Karp-related antigens in rodent isolates. These findings correlate with those found in the study with human isolates from the same sera (Shirai *et al.*, 1979). Additionally, the analysis of *R. tsutsugamushi* organisms identified in infected chiggers from the same area showed the presence of only Karp and Karp-related strains (unpublished data). This preponderance of Karp-related antigens is particularly important when examined in the context of vaccine strain selection. The study of additional endemic areas throughout the Asiatic-Pacific region could greatly aid the rational selection of antigens to be used as immunoprophylactic agents.

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