A SEROLOGICAL SURVEY OF SCRUB, TICK, AND ENDEMIC TYPHUS IN SABAH, EAST MALAYSIA

ANDREW C. TAYLOR, JEFFREY HII*, DARYL J. KELLY, DONALD R. DAVIS and GEORGE E. LEWIS, JR.

United States Army Medical Research Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia. *Vector Borne Diseases Control Programme, Department of Medical Services, Kota Kinabalu, Sabah, East Malaysia.

INTRODUCTION

Scrub typhus, the febrile illness caused by *Rickettsia tsutsugamushi*, seems to be more prevalent in Malaysia than is evidenced by official records (Brown *et al.*, 1976). Although its significance in terms of morbidity and mortality has declined since the introduction of rickettsiostatic antibiotics, scrub typhus continues to be of considerable economic importance in rural areas of Peninsular (West) Malaysia. This was demonstrated by a recent survey in Pahang in which 19% of all febrile patients admitted to a district hospital were shown to have scrub typhus (Brown *et al.*, 1984). That study also demonstrated that endemic (or murine) typhus, which is caused by *R. typhi*, was responsible for an additional 0.5% of all febrile illnesses, and tick typhus, which may be caused by any one of several members of the spotted fever group of rickettsiae (SFGR), for 0.3%. These last two findings were surprising; endemic typhus, which is transmitted by fleas, is usually considered to be an urban disease while tick typhus is a mild disease and therefore unlikely to require hospitalization.

In contrast to Peninsular Malaysia, very little is known about the prevalence of rickettsial diseases in East Malaysia. The mite *Leptotrombidium* (*Leptotrombidium*) *deliense*, a vector of *R. tsutsugamushi*, is found in areas along the west coast of Sabah (Dohany *et al.*, 1977). In addition, tick vectors of SFGR such as *Ixodes granulatus* and the *Dermacentor* and *Haemophysalis* species are prevalent particularly in forest areas in Sabah (Kohls, 1957; Marchette, 1966). A preliminary serological study of 28 febrile patients was performed in the Federal Territory of Labuan, an island off the Sabah mainland. Two patients were found to have serological evidence of tick typhus, while another 21 patients had SFGR specific antibody levels of $\geq 1:100$ (Shirai, A., pers. commun.).

We hypothesized that rickettsial diseases such as scrub typhus and tick typhus could be the cause of some of the many undiagnosed febrile illnesses occurring throughout rural East Malaysia. In collaboration with the Sabah Medical Department a seroepidemiological survey of selected rural populations was performed in order to define the prevalence of specific antibody to rickettsiae of the typhus, scrub typhus and spotted fever groups. In addition, paired acute and convalescent blood samples were collected from febrile patients attending health centers in the region in order to determine the relative frequency of the three rickettsial diseases studied. The health centers were selected, by the investigators, to represent a cross section of Sabah health centers.

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MATERIALS AND METHODS

Study area: Sabah, one of the 13 states in Malaysia (Fig. 1), covers an area of 29,388 sq. mi. on the northern part of the island of Borneo. Almost 80% of the country is hilly or mountainous and inhabited mostly by rural people. Central mountain ranges, from 4,000 to 6,000 ft in height, rise somewhat sharply from ranges of low hills near the western coast. Throughout the country, dense tropical forests and alluvial swampy coastal plains are intersected by numerous rivers and valleys. Physiographically, three distinct regions can be distinguished. The western lowland region occupying the narrow coastal strip west of the Crocker range, the central uplands region consisting of an extensive area of hill masses with large valley areas, and the eastern lowlands which includes the Kinabatangan basin and extensive delta areas. The 1980 census determined the population to be 1.002 million; the total population is now estimated to be 1.25 million of which 74% are rural people.

The state is characterized by a tropical wet climate with a monthly mean maximum temperature of 29.6-32.2°C and a minimum of 22.8-23.3°C. A high relative humidity (80-90%) prevails throughout the year. Mean annual rainfall varies from 80 to 100 in, with the wet seasons normally occurring from November to February and from June to August.

Study sites and population: The antibody prevalence surveys were performed by the staff of the Vector-Borne Diseases Control Programme in conjunction with malaria and filariasis mass blood surveys. Random cross-sectional surveys were made in a total of 837 individuals. Studies were conducted in seven different rural areas of Sabah - Tawau district (4 villages; Serundong Laut, Ulu Kalabakan, Kalabakan Scheme, Luasong Camp; n=689) and on Banggi island (3 villages: Meliyu, Pangkalan Darat, Kapitangan; n=148) (see Table 1, Fig. 1). The race, age and sex of each participant are summarized in Table 2.

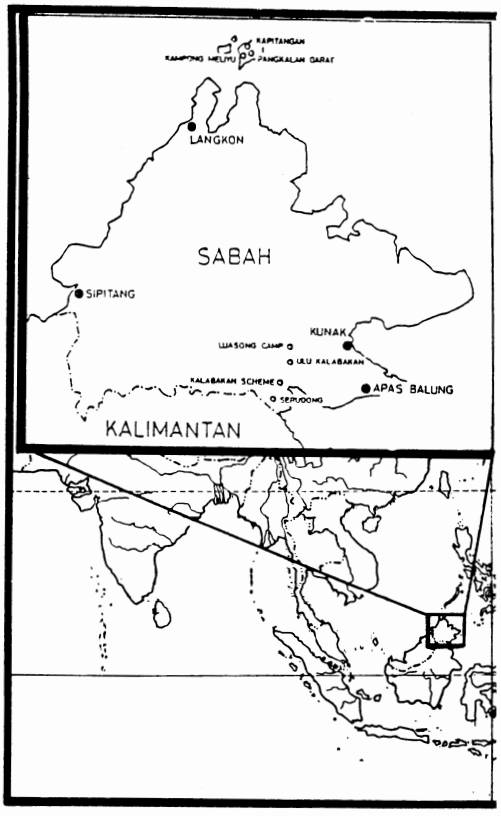

Fig. 1—Map of Sabah, East Malaysia, showing location of survey sites. (O) indicate villages, (●) indicate health centers.

In order to determine the relative frequency of rickettsial diseases a second population was surveyed. Paired (acute and convalescent) serum samples were collected from 383 febrile patients attending any one of the four health centers (Table 1, Fig. 1,) from July to November 1983. The mean duration between the first and second collection was 2 weeks. In addition to name, age, sex and occupation, limited clinical data were also recorded for each patient. The sex and age distribution are summarized in Table 2.

Table 1

Details of study sites in Sabah, East Malaysia.

Village	Survey date	Annual Number of PUO cases*	Type of health facility available	Ecotype	Principal occupation
Serundong Laut	Dec 83	NA**	1-1 1/2 hr to Kalabakan	Mangrove river swamp type scrub	Shifting cultivation
Ulu Kalabakan	Dec 83	NA	3/4 hr to Kalabakan	As above, cocoa plantations	Estate work lumbering
Kalabakan Scheme	Dec 83	1329	VGSC***	Cleared forest, resettlement scheme	Cocoa estate
Luasong Camp	Dec 83	NA	Health dispensary	Cleared forest, softwood forest	Logging, cocoa
Meliyu	Mar 84	NA	1 hr to dispensary	Coastal, coconut	Fishing
Kapitangan	Mar 84	NA	As above	Secondary forest	Hunting, shifting cultivation
Pangkalan Darat	Mar 84	NA	As above	Secondary forest	As above
Apas Balung	Jul-Nov 83	1177	Health dispensary	Palm oil, rubber & cocoa plantation	Estate work
Kunak	Jul-Nov 83	622	Health dispensary	As above	As above
Langkon	Jul-Nov 83	2046	Health dispensary	Palm oil estate	As above
Sipitang	Jul-Nov 83	1446	Health dispensary	Coastal rural town administrative and trade center	Fishing, rubber, timber, cocoa

* PUO = pyrexia of unknown origin
** NA = not available
*** VGSC = village group sub-center

Table 2

Sex, race and age distribution of the 1,220 patients studied in Sabah, East Malaysia.

Characteristic	Rural villages (n = 837) (%)	Health Centers (n = 383) (%)
Sex:		
Male	396 (47)	177 (46)
Female	441 (53)	206 (54)
Race:		
Tidong	190 (23)	NA
Murut	167 (20)	NA
Indonesian	109 (13)	NA
Iban	61 (7)	NA
Filipino	58 (7)	NA
Banggi Dusun	75 (9)	NA
Others	177 (21)	NA
Age (Years):		
< 9	289 (34)	197 (51)
10-19	181 (22)	86 (22)
20-29	151 (18)	58 (15)
30-39	90 (11)	21 (6)
> 40	126 (15)	21 (6)

NA = Not available.

Health services in the study areas were provided through rural health dispensaries and village group subcenters (VGSC). Medical assistants in these dispensaries provide basic outpatient care, limited inpatient care, health education at the village level and tuberculosis and malaria treatment. Generally, they coordinate preventive and curative services over a wide area, with one health center serving a catchment area population of about 7,000. In addition, mobile teams of satellite VGSCs provide maternal and child welfare services to outpatients, visit most villages regularly and serve a population of 2,000. In 1983, the most common cause of dispensary outpatient first attendance was pyrexia of unknown origin (134,857 cases). Although some traditional medicine is still practiced at the village level, recourse to government health services is usual in the event of fever or other acute illness, particularly for the children. As far as we know, there is a better awareness of the origin and methods of treatment of malaria than filariasis, scrub and tick typhus.

Most of the rural people are involved in farming, lumbering, hunting or fishing. Recently, some of the rubber and large areas of secondary rain forest have been cleared and planted with oil palm, cocoa and softwood trees. Much of the local population continues to live in small villages, often with small holdings on the jungle fringe, and subsists on

small scale rice growing and shifting cultivation. Lalang grass is quite widespread, and washing in streams and rivers is commonly practiced.

Sample collection and testing: Blood samples obtained by finger prick were collected on filter paper (Whatmans No. 4) (Gan *et al.*, 1972) and allowed to dry for one hour before being sealed in plastic bags. Samples were mailed to the United States Army Medical Research Unit-Malaysia (USAMRU-M) for evaluation. Upon arrival at the USAMRU-M the specimens were stored at 4°C. Testing for antibodies specific to the eight prototype strains of *R. tsutsugamushi* (Elisberg *et al.*, 1978), Thai TT-118 strain (Robertson *et al.*, 1973) and *R. typhi* was performed by the microimmunofluorescent antibody method using fluorescein conjugated horse anti-human whole globulin (Progressive Laboratories Inc., Baltimore, MD, U.S.A.) (Robinson *et al.*, 1976). All samples were screened at a dilution of 1:50. Those found to be positive were subsequently titrated to the endpoint. A titer of $\geq$1:50 was accepted as evidence of prior exposure to the infecting agent (Brown *et al.*, 1981). Either a fourfold rise in titer between paired samples, or, in the case of scrub typhus, a single titer of $\geq$ 1:400 in a febrile patient, was considered evidence of active infection (Brown *et al.*, 1983).

Statistical analysis: Statistical analysis of the data was by the Chi square method, with Yates modification for small numbers where appropriate.

RESULTS

Antibody prevalence survey: Of 837 blood samples collected in the antibody prevalence surveys (Table 3), 72 (8.6%) had SFGR specific antibody at a titer of $\geq$1:50; in 64, the titer was 1:50 and in 8 samples it was 1:100. Seven samples (0.8%) had *R. tsutsugamushi* specific antibody titers of $\geq$ 1:50; 5 at 1:50, 1 at 1:100 and 1 at 1:200. In Luasong Camp, 52 of 315 (16.5%) people surveyed had SFGR specific antibody at a titer of $\geq$ 1:50, a rate significantly higher than that of the remaining population($p<0.001$). There was no statistical difference in the occurrence of positive SFGR antibody titers between the sexes or between age groups in either the total study population or the Luasong Camp population ($p>0.10$). The seven individuals that demonstrated elevated antibody titers to *R. tsutsugamushi* came from 5 different villages. Five were adult males, one a woman, and one a young

Table 3

Rickettsial antibody titers in 837 residents of Sabah, East Malaysia.

Reciprocal titer	Number (%) of subjects with antibody* to SFGR**	*R. tsutsugamushi*
< 50	765 (91.4)	830 (99.2)
50	64 (7.6)	5 (0.6)
100	8 (1.0)	1 (0.1)
200	-	1 (0.1)
Total Positive*** (%)	72 (8.6)	7 (0.8)

* 2 subjects had antibody to both tick and scrub typhus rickettsiae.
** Spotted fever group rickettsia.
*** A positive titer is $\geq$ 1:50.

girl. In none of the blood samples tested was antibody to typhus group rickettsiae detected.

Febrile disease survey: In none of the 383 febrile patients from which paired blood samples were collected was there serological evidence of active infection with any of the rickettsial organisms studied. Thirty of the patients (7.8%) had a SFGR specific antibody level of 1:50, and 2 patients (0.5%) had *R. tsutsugamushi* specific antibody at a titer of 1:50. There was no evidence of antibody to typhus group rickettsiae in any of the samples tested. Sixteen of the 97 (16.5%) patients studied at the Apas Bulung Health Centre had SFGR specific antibody levels of 1:50. This was significantly more frequent than in the remaining population ($p < 0.001$).

The two forest dwelling populations surveyed (Apas Bulung and Luasong Camp) had nearly four times the rate of occurrence of SFGR antibody (16.5%) of the other groups (4.5%).

DISCUSSION

The overall rate of antibody to SFGR in our antibody prevalence survey study population was 8.6%, while the rate of *R. tsutsugamushi* specific antibody was only 0.8%. No serological evidence of an active case of scrub, tick or endemic typhus was found in the febrile population studied. Therefore, although tick typhus may have been a common cause of illness among our study population, the low prevalence of antibody to *R. tsutsugamushi* and typhus group rickettsiae suggests that scrub typhus and endemic typhus were an extremely uncommon cause of febrile illness.

There is a high rate of SFGR antibody in wildlife samples collected from forest areas in Sabah (Marchette, 1966). Thus we were not surprised to find that forest dwellers had a higher SFGR antibody prevalence than rural peoples.

It is important to note that scrub typhus is apparently not a serious problem in Sabah, even though Sabah is within the scrub typhus endemic Pacific region. Although it is known that vectors of scrub typhus do occur in the region and are therefore likely to feed on human hosts, we can only presume that the density of the vectors is low and/or that *R. tsutsugamushi* infection among the vectors is at a very low level.

Assuming that the subpopulations studied reflect conditions within the general rural population, the results should have considerable relevance to public health planners/providers in Sabah. Rickettsial diseases are a major cause of febrile illness in rural areas of Peninsular Malaysia demanding serological investigation and often presumptive treatment with a tetracycline (Brown *et al.*, 1984). This seems not to be the case in Sabah, except in forest dwellers, where the diagnosis of tick typhus should be considered.

SUMMARY

A seroepidemiological survey of 837 people and 383 febrile patients was performed in rural areas of Sabah. We determined that the rickettsial diseases scrub typhus and endemic typhus were uncommon causes of febrile illness, as was tick typhus, except in forest dwelling peoples. The rate of occurrence of SFGR specific antibody was 16.5% among 412 forest dwellers, indicating that tick typhus may be a frequent cause of illness in this population.

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