ANTIBODY PREVALENCE OF *ORIENTIA TSUTSUGAMUSHI*, *RICKETTSIA TYPHI* AND TT118 SPOTTED FEVER GROUP RICKETTSIAE AMONG MALAYSIAN BLOOD DONORS AND FEBRILE PATIENTS IN THE URBAN AREAS

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Abstract. The seroprevalence of *Orientia tsutsugamushi* (OT), *Rickettsia typhi* (RT) and TT118 spotted fever group rickettsiae (SFGR) among blood donors and febrile Malaysian patients in the urban areas was determined. Of the 240 blood donors, 5.4%, 9.2% and 1.7% had either present or previous exposure to OT, RT and SFG rickettsiae, respectively. Patients admitted to an urban hospital had high seroprevalences of OT (43.5%) and RT (22.9%), as compared to SFGR (11.6%). Antibody levels suggestive of recent infections of scrub typhus, murine typhus and tick typhus were detected in 16.8%, 12.7% and 8.2% of patients respectively. No significant difference was noted in the distribution of rickettsial antibodies among urban patients from 2 geographical locations. However, the serologic patterns of rickettsial infection in the urban areas were different form those of rural areas.

INTRODUCTION

Rickettsial infections are major causes of febrile illnesses throughout the Asia-Pacific region (WHO, 1993). Scrub typhus infection, which is caused by *Orientia tsutsugamushi* (OT), is endemic in the western Pacific region and has been reported as the most frequent infection among febrile hospitalized patients in rural areas of Malaysia (Cadigan *et al*, 1972; Saunders *et al*, 1980; Brown *et al*, 1984). Murine typhus, caused by infection with a typhus group rickettsiae, *Rickettsia typhi* (RT), was once considered a sporadic and uncommon cause of illness in Malaysia. There is no information on the etiologic agent of spotted fever (tick typhus) in Malaysia. A recent serosurvey of febrile patients from eight health centers in rural areas of Malaysia showed that a total of 51.4% of the patients had antibodies against at least one of these rickettsiae, indicates the endemicity of rickettsial infections in this country (Tay *et al*, 2000). Among these patients, antibody to spotted fever group rickettsiae (SFGR) was most prevalent (42.5%), followed by RT (28.1%) and OT (24.9%). In another study (Tay *et al*, 1999), 23.3%, 3.0% and 57.3% of healthy rubber estate workers had antibodies detected against OT, RT and SFGR, respectively. Both studies demonstrated the high prevalence of SFGR in the rural parts of Malaysia.

Limited prevalence data is available on the prevalence of rickettsial infections in urban areas in this and other countries (Strickman *et al*, 1994) as rickettsiae particularly scrub typhus and tick typhus are thought to be associated with agricultural sectors and observed in areas of rural transmission. This study was carried out to determine the seroprevalence of OT, RT and SFG rickettsiae among blood donors and febrile patients from the urban areas and to compare with those of rural areas.

MATERIALS AND METHODS

Serum samples

Sera were collected from 240 blood donors

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and 292 febrile patients from Kuala Lumpur Hospital (KLH, located at the capital city of Malaysia, Kuala Lumpur) from 1998 to 1999.

Indirect immunoperoxidase (IIP) assay

An IIP assay as described by Kelly *et al* (1988) was used for the determination of specific IgG and IgM antibody levels against three prototype strains of OT (mixture of serotypes Karp, Kato and Gilliam), RT (Wilmington strain) and TT118, an unclassified SFGR. Acetone-fixed antigen slides dotted with whole cell rickettsiae were supplied by the Rickettsial Laboratory of the Institute for Medical Research, Malaysia. Positive reactions of IIP assay were indicated by the observation of brownish-stained rickettsiae in the respective antigenic smear, whereas no organisms could be seen in negative reaction. Specific IgG and IgM titers were determined and titers equal or greater than 1:50 were considered positive.

RESULTS

Table 1 shows the antibody prevalence of blood donors and febrile patients to each rickettsial group and the respectively reciprocal geometric mean antibody titers. Of the 240 blood donors, 35 (14.6%) were seropositive to at least 1 rickettsial group, of which 5.4%, 9.2% and 1.7% had either present or previous exposure to OT, RT and SFGR, respectively. IgM antibodies against OT and SFGR were detected in 6(2.5%) and 2 (0.8%) patients, respectively. None had IgM antibody detected against RT. IgG antibody against OT, RT and SFGR were detected in 7 (2.9%), 22 (9.2%) and 1 (0.4%) patients, respectively. The reciprocal geometric mean titers for each type of antibodies was lower than 100 except for IgG antibody against OT (361.2).

Of the 292 febrile patients admitted to Kuala Lumpur Hospital, 189 (64.7%) had either

Table 1
Seroprevalence of OT, RT and SFGR among blood donors, febrile patients and rubber estate workers.

Rickettsial antibody	No. (%) blood donors	RGMT	No. (%) urban patients[a]	RGMT	No(%) rural patients[b]	RGMT	No(%) rubber estate worker[c]	RGMT
Seropositive to at least one rickettsial group	35 (14.6)	-	189 (64.7)	-	821 (51.4)	-	198 (66.0)	-
OT	13 (5.4)	-	127 (43.5)	-	397 (24.9)	-	70 (23.3)	-
IgG	7 (2.9)	361.2	124 (42.5)	237	337 (21.2)	440.3	25 (8.3)	187.5
IgM	6 (2.5)	49.9	49 (16.8)	217	179 (11.2)	222.9	55 (18.3)	136.2
RT	22 (9.2)	-	67 (22.9)	-	449 (28.1)	-	9 (3.0)	-
IgG	22 (9.2)	68.3	63 (21.6)	284	348 (21.8)	105.4	6 (2.0)	141.0
IgM	0 (0.0)	-	37 (12.7)	446	290 (18.2)	143.0	8 (2.7)	91.5
SFGR	4 (1.7)	-	34 (11.6)	-	678 (42.5)	-	172 (57.3)	-
IgG	1 (0.4)	99.7	12 (4.1)	168	559 (35.2)	183.5	64 (21.3)	148.0
IgM	2 (0.8)	49.9	24 (8.2)	99.7	498 (31.3)	248.7	160 (53.3)	153.8
Total	240		292		1,596		300	

RGMT = reciprocal geometric mean titers.
OT, *O. tsutsugamushi*; RT, *R. typhi*; SFGR, spotted fever group rickettsiae, TT118.
[a]from Kuala Lumpur Hospital; [b]refer to Tay *et al* (2000); [c]refer to Tay *et al* (1999).

Table 2
Distribution of positive sera towards OT, RT and SFGR among blood donors, febrile patients and rubber estate workers.

Rickettsial antibody	No. (%) blood donor	No. (%) patients (KLH)	No. (%) patients (IH)	No. (%) rural patients[a]	No(%) rubber estate worker[b]
OT only	9 (25.7)	97 (51.3)	300 (68.8)	68 (8.3)	23 (11.6)
RT only	20 (57.1)	46 (24.3)	50 (11.5)	58 (7.1)	3 (1.5)
SFGR only	2 (0.8)	10 (5.3)	27 (6.2)	198 (24.1)	120 (60.6)
OT, RT only	2 (0.8)	12 (6.3)	7 (1.6)	17 (2.1)	0 (0.0)
OT, SFGR only	2 (0.8)	15 (7.9)	27 (6.2)	106 (13.2)	46 (23.2)
RT, SFGR only	0 (0.0)	6 (3.2)	20 (4.6)	168 (20.5)	5 (2.5)
OT, RT, SFGR only	0 (0.0)	3 (1.6)	5 (1.1)	206 (25.1)	1 (0.5)
No. positive sera	35	189	436	821	198

OT = *O. tsutsugamushi*; RT = *R. typhi*; SFGR = spotted fever group rickettsiae, TT118; KLH = Kuala Lumpur Hospital; IH = Ipoh Hospital.
[a]refer to Tay *et al* (2000); [b]refer to Tay *et al* (1999).

previous or recent exposure to at least 1 rickettsial group. A total of 43.5% of the patients had either IgG and IgM detected against OT. This was followed by the seroprevalence of RT (22.4%) and SFGR (11.6%). Using IgM as an indicator for recent infections, 16.8% and 12.8% of the patients might have recent exposure to OT and RT respectively. The highest geometric mean antibody titer was noted with IgM antibodies against RT (548.1).

To determine whether there was any difference in the distribution of rickettsial antibodies as a result of geographical variation, the IIP results and demographic data of 436 febrile patients from another urban hispital, Ipoh Hospital (located at the capital city of Perak State, north of Kuala Lumpur) were analyzed. Table 2 shows the distribution of positive sera (irrespectively of IgG and IgM) to OT, RT and SFG rickettsiae among blood donors and patients. Among blood donors with positive IIP findings, the majority of positive sera (57.1%) had antibodies against RT detected. Among urban patients from the 2 hospitals who were seropositive to at least 1 rickettsial group, antibodies against OT were most common, followed by antibodies against RT. Antibody prevalence to SFGR was the lowest. Antibodies against OT and SFGR were detected most frequently compared to other sera with more than 1 type of ricketssial antibodies detected.

Table 3 shows the antibody prevalence among gender and various age and ethnic groups of patients attending Kuala Lumpur Hospital. Only 125 patients with detail demographic data from KLH were included in the analysis. The mean age of patients was 35.1 years (range 9-78) and the ethnic distribution was as follows: Malay, 7; Chinese, 1; Indian, 2. The male/female ratio was 1:1. There was no significant association between antibody prevalence of OT, RT and SFGR and gender ($p > 0.05$) except that the IgM antibodies against RT were two times more prevalent in females. All age groups had evidence of exposure to rickettsial infections, with IgM antibodies detected most commonly among those aged 41-60 years old for all the three rickettsial groups. Patients aged >60 years had higher seroprevalence of OT (91.7%). Antibody levels suggesting most recent exposure to OT were noted mostly 60-70% of the three races. IgM antibodies against RT and SFGR were detected more frequently among the Indians (30.8%).

Table 4 shows the antibody prevalence among gender and various age and ethnic groups of patients attending to Ipoh Hospital. A total

Table 3
The seroprevalence of OT, RT and SFGR among f febrile patients attending to Kuala Lumpur Hospital.

Characteristic	No. (%) patients	No. (%) of patients reactive to					
		OT		RT		SFGR	
		IgG	IgM	IgG	IgM	IgG	IgM
Sex							
Male	64 (51.2)	47 (73.4)	14 (21.9)	20 (31.3)	8 (12.5)	5 (7.8)	7 (10.9)
Female	61 (48.8)	41 (67.2)	14 (23.0)	21 (34.4)	14 (23.0)	2 (3.3)	6 (9.8)
Total	125 (100.0)	88 (70.4)	28 (22.4)	41 (32.8)	22 (17.6)	7 (5.6)	13 (10.4)
Age group (years)							
≤20	23 (18.4)	15 (65.2)	6 (26.1)	8 (34.8)	2 (8.7)	1 (4.3)	1 (4.3)
21-30	32 (25.6)	24 (75.0)	6 (18.8)	7 (21.9)	3 (9.4)	0 (0.0)	5 (15.6)
31-40	28 (22.4)	16 (57.1)	4 (14.3)	9 (32.1)	6 (21.4)	3 (10.7)	2 (7.1)
41-50	18 (14.4)	15 (83.3)	7 (38.9)	6 (33.3)	5 (27.8)	2 (11.1)	3 (16.7)
51-60	12 (9.6)	7 (58.3)	1 (8.3)	9 (75.0)	4 (33.3)	1 (8.3)	1 (8.3)
>60	12 (9.6)	11 (91.7)	4 (33.3)	2 (16.7)	2 (16.7)	0 (0.0)	1 (8.3)
Ethnic group							
Malay	82 (65.6)	60 (73.2)	21 (25.6)	21 (25.6)	12 (14.6)	3 (3.7)	11 (13.4)
Chinese	17 (13.6)	12 (70.6)	4 (23.5)	8 (47.1)	2 (11.8)	2 (11.8)	1 (5.9)
Indian	26 (20.8)	16 (61.5)	3 (11.5)	12 (46.2)	8 (30.8)	2 (7.7)	1 (3.8)

OT = *Orientia tsutsugamushi*; RT = *Rickettsia typhi*; SFGR = spotted fever group rickettsiae, TT118
Note: Only 125 patients with detail demographic data were included in the analysis.

Table 4
The seroprevalence of OT, RT and SFGR among febrile patients attending to Ipoh Hospital.

Characteristic	No. (%) patients	No. (%) of patients reactive to					
		OT		RT		SFGR	
		IgG	IgM	IgG	IgM	IgG	IgM
Sex							
Male	226 (51.8)	161 (71.2)	65 (28.8)	47 (20.8)	32 (14.2)	34 (15.0)	24 (10.6)
Female	210 (48.2)	158 (75.2)	70 (33.3)	31 (14.8)	13 (6.2)	24 (11.4)	20 (9.5)
Total	436 (100.0)	319 (73.2)	135 (31.0)	78 (17.9)	45 (10.3)	58 (13.3)	44 (10.1)
Age group (years)							
≤20	61 (14.0)	34 (55.7)	26 (42.6)	15 (24.6)	7 (11.5)	9 (14.8)	12 (19.7)
21-30	34 (7.8)	21 (61.8)	9 (26.5)	10 (29.4)	7 (20.6)	3 (8.8)	5 (14.7)
31-40	48 (11.0)	28 (58.3)	21 (43.8)	13 (27.1)	9 (18.8)	7 (14.6)	8 (16.7)
41-50	58 (13.3)	44 (75.9)	23 (39.7)	10 (17.2)	8 (13.8)	9 (15.5)	4 (6.9)
51-60	71 (16.3)	53 (74.6)	21 (29.6)	12 (16.9)	8 (11.3)	7 (9.9)	6 (8.5)
>60	164 (37.6)	139 (84.8)	35 (21.3)	18 (11.0)	6 (3.7)	23 (14.0)	9 (5.5)
Ethnic group							
Malay	338 (77.5)	273 (80.8)	114 (33.7)	38 (11.2)	20 (5.9)	40 (11.8)	26 (7.7)
Chinese	43 (9.9)	18 (41.9)	9 (20.9)	18 (41.9)	10 (23.3)	8 (18.6)	8 (18.6)
Indian	43 (9.9)	18 (41.9)	11 (25.6)	20 (46.5)	13 (30.2)	9 (20.9)	9 (20.9)
Others	12 (2.8)	10 (83.3)	1 (8.3)	2 (16.7)	2 (16.7)	1 (8.3)	1 (8.3)

OT = *Orientia tsutsugamushi*; RT = *Rickettsia typhi*; SFGR = spotted fever group rickettsiae, TT118

of 436 patients were included in the analysis. The mean age of patients who were seropositive to at least 1 rickettsial group was 49.3 years (range 1-87) and the ethnic distribution was as follows: Malay, 8; Chinese, 1; Indian, 1. The male/female ratio was 1:1. There was no significant association between antibody prevalence of OT, RT and SFGR and gender ($p > 0.05$), except that the IgM antibodies against RT was two times more prevalent in males (Table 4). All age groups had evidence of rickettsial infections, with IgM antibodies against OT, RT and SFGR most commonly detected among those aged 31-50 years old. The prevalence of IgG to OT increased with age. Overall, antibody levels suggesting most recent exposure to OT were noted mostly among the Malays (33.7%), while the IgM prevalence of RT and SFGR were highest among the Indians (Table 4).

DISCUSSION

The morbidities and mortalities due to rickettsial infections in Malaysia, are thought to be underestimated. The nonspecific pattern of illnesses, the difficulty in seeing a rash on dark skinned patients and the widely use of tetracycline for empiric treatment for most febrile illness interfere with the recognition of these rickettsial infections. Scrub typhus was still a public health problem whereas antibodies to RT and SFGR seem to be more widespread than OT in the rural areas of Malaysia (Tay *et al*, 1999; 2000). While the rural transmission of rickettsial diseases has received much attention, there was little information on the seroprevalence of rickettsial infections among the urban dwellers.

The seroprevalence of rickettsiae among blood donors and urban patients in this study reflects the exposure of urban dwellers to various rickettsiae. Among the blood donors, there was a higher seroprevalence of RT compared to other rickettsiae. The RT infection (murine typhus) is distributed worldwide and is prevalent mostly in unhygienic, crowded living environment of urban areas where the most important rat reservoirs (*Rattus* spp) and flea vector (*Xenopsylla cheopsis*) are found. However, RT has also been isolated from jungle rats in a rural area in Malaya (Marchette, 1966) and a high seroprevalence (28.2%) had been reported among rural febrile patients (Table 1) and among rubber estate workers during a dry season (Tay *et al*, 1999).

No significant difference was noted in the distribution of rickettsial antibodies among patients from 2 geographical locations. However, the serologic patterns of rickettsial infections in the urban areas were different from those of rural areas (Table 1 and Table 2). There was a significant lower prevalence of SFGR but a higher seroprevalence of OT and RT among the urban patients as compared to the rubber estate workers (Tay *et al*, 1999) and rural patients (Table 1). A low seroprevalence of mixed rickettsial antibodies was also noted among urban patients as compared to those from rural areas (Table 2).

Rickettsial disease is viewed as an aberration incidental to the ecosystem in which the rickettsiae normally exist (Walker and Fishbein, 1991). The high prevalence of OT infections among urban patients noted in this study is interesting as most of the earlier reports of human infection with OT described transmission in agricultural or undeveloped areas (Audy, 1961; Cadigan *et al*, 1972; Saunders *et al*, 1980; Brown *et al*, 1984). It was uncertain whether the urban dwellers had acquired OT infection from the vicinity of their living environment or through their visit to rural areas of Malaysia. With the rapid economic development, deforestation and urbanization in Malaysia, the close proximity of agricultural habitats and urban development may allow the transmission of rickettsial diseases in the urban areas. The increase in leisure activities such as hiking, jungle tracking and eco-tourism may have also exposed urban dwellers to OT and other rickettsiae.

There was a low prevalence of SFGR among urban patients when compared to rural dwellers (Table 1). It is not determined in this study whether the agent responsible for anti-

body in urban area was the same as the one that causes disease in the rural areas. Isolation and characterization of SFGR from both areas is necessary.

No significant association of prevalence of rickettsial antibody with gender and age of urban patients was noted in this study (Table 3 and Table 4) as well as for the rural people (Tay *et al*, 1999, 2000). The increase of antibody prevalence (both IgM and IgG) of OT with age in this study could be due to increased contact with transmission foci, repeated inoculation with rickettsiae during many years, or long-term persistence of antibody due to inapparent and chronic infection (Smadel *et al*, 1952). The high prevalence of OT infections in the urban and rural areas indicates a need for improved surveillance and the institution of appropriate control strategies.

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