RESEARCH NOTE

SEROLOGICAL EVIDENCE OF NATURAL INFECTION OF WILD RODENTS (*RATTUS* SPP AND *TUPAIA GLIS*) WITH RICKETTSIAE IN MALAYSIA

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INTRODUCTION

Scrub typhus, murine typhus and tick typhus are some rickettsial infections which have been reported in Malaysia. The importance of small rodents as host for various arthropods which transmit *Rickettsia* spp has been documented in many reports. In Malaysia, surveys in scrub typhus endemic areas have demonstrated a high prevalence of infection in various small mammalian species (Walker *et al,* 1973; Muul *et al,* 1975) and in some species over 50% of rodents had experienced a scrub typhus infection (Walker *et al,* 1973). There is limited local information on the infection of rodents with other rickettsiae.

A preliminary study on arthropod-borne rickettsioses in Malaysia (Ho *et al,* 1997) has shown a high seroprevalence of tick typhus in certain locations of the country, including a small village in Selangau, Sarawak. This report presents the results of a serological survey to determine the prevalence of *Orientia tsutsugamushi, Rickettsia typhi* and TT118 rickettsiae (an unclassified spotted fever group rickettsiae) in wild rodents in Selangau, Sarawak.

Rodents were captured alive in wire mesh traps set near human habitations and secondary forest. Captured rodents were anesthetized with ether and bled by cardiac puncture using a 23 gauge needle and a 3 ml syringe. Serum was collected following centrifugation of blood at 3,000 rpm and stored at –20°C until assayed for antibody.

Sera from various *Rattus* spp were assayed for rickettsial antibodies by the indirect immunofluorescent antibody (IFA) microassay as described by Robinson *et al* (1976). Three rickettsial species *ie: O. tsutsugamushi, R. typhi* and TT118 rickettsiae were prepared as antigen spots on teflon-coated slides. Two fold dilutions of each serum with starting dilution of 1:20 were added to individual antigen spots and incubated in a humid chamber for 30 minutes at 37°C. After washing, a fluorescein-conjugated anti-rat antibody (Kirke- gaard and Perry labs) was added to each spot. Slides were then incubated for 30 minutes, washed, and examined with a fluorescent microscope. Sera having titers of ≥ 1:40 were considered positive. For sera collected from *Tupaia glis,* a laboratory-prepared fluorescein-conjugated rabbit anti-tupaia immunoglobulins was used.

Sera from a total of 31 *Rattus* spp and 10 tree shrews, *T. glis* were tested and the results of the antibody assays are shown in Table 1. Antibodies towards TT118 rickettsiae were detected from 29 (70.7%) of the rodents, *ie* 21 (67.7%) from *Rattus* spp and 8(80%) from *T.glis*. All rodents were seronegative to *O.tsutsugamushi* except for 2 (4.9%) *R. muelleri*. All rodents were seronegative to *R. typhi* except for 1 (2.4%) *R.muelleri*. Titers of the reactive *Rattus* sera ranged from 1:20 to 1:320 and those of the *T.glis* ranged from 1:20 to 1:80. In this study, there was a low prevalence of *O.tsutsugamushi* (4.9%) and *R. typhi* (2.4%) in the wild rodents compared with those of TT118 spotted fever group rickettsiae (70.7%). The low prevalence of *O.tsutsugamushi* has also been reported in small mammals in a mature oil palm estate in Peninsular Malaysia (Shirai *et al,* 1978). The prevalence of this organism could be varied due to different geographical locations and the distribution of infected chiggers and host.

Although previous publication showed low prevalence of tick typhus complement-fixing antibodies in small mammals (Marchette, 1966), we

Table 1

Prevalence of antibody against TT118 rickettsiae in wild rodents in Selangau, Sarawak.

Species	No. tested	Antibody titers				
		20	40	80	160	320
Rattus exulans	2			1		
R.diardi	2			1		
R.muelleri	10	1	3#	3	1@	2*
R.inas	15	5	3	5	1	1
R.whiteheadi	1				1	
R.tiomanicus	1				1	
Tupaia glis	10	2	7	1		
Total	41	8	13	11	4	3

#Antibody to *O. tsutsugamushi* was also detected from a wild rat with titer of 1: 80.
@Antibody titer to *O. tsutsugamushi* was 1: 160.
*Antibody to *R. typhi* was also detected from a wild rat with titer of 1:40.

reported a high seroprevalence of TT118 spotted fever group rickettsiae in both *Rattus* spp and *T.glis* in this study. The high prevalence of SFG rickettsiae had also been reported in wild rats collected in the 1970s from two regions in Thailand (Tamaki *et al,* 1996) and in small rodents captured in an area in Hokkaido (Okabayashi *et al,* 1996).

The causative agents of tick typus in Malaysia had not been isolated yet. No febrile period or scrotal reaction could be demonstrated on initial isolation in guinea pig and attempts to serially pass the organism in guinea pigs and hamsters were unsuccessful (Marchette, 1966); it is thus suspected that the rickettsiae is of low virulence. TT118 strain isolated from ticks in Chiang Mai, Thailand has been widely used as antigen for diagnosis of tick typhus in this region. Several reports had shown that TT118 or its antigenically related organisms are endemic in rural areas of Thailand and Malaysia (Strickman *et al,* 1994; Ho *et al,* 1997).

Serologic evidence suggests that wild rodents in this area are highly infected with TT118 spotted fever rickettsiae or some antigenically related rickettsiae. In addition, hemolymph preparations from ticks collected from vegetation in this area were found positive by direct immunofluorescence test (Tay *et al,* 1996). The presence of wild rodents, and ticks as reservoirs and vectors in this area implies that natural transmission of tick typhus may occur at a high rate. This may be an explaination for the high seroprevalence of tick typhus in this area.

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