SEROLOGIC EVIDENCE OF INFECTION WITH EHRlichiae AND SPOTTED FEVER GROUP RICKETTSIAE AMONG RESIDENTS OF GAG ISLAND, INDONESIA

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Abstract. The causative agents of scrub and murine typhus are considered endemic to Indonesia. However, the presence of spotted fever group rickettsiae and ehrlichiae have not been previously described in this country. During an investigation of arthropod-borne diseases on Gag Island, located northwest of the island of New Guinea in eastern Indonesia, the prevalence of antibody to the etiologic agents of monocytic ehrlichiosis, spotted fever rickettsiosis, and scrub and murine typhus were determined. Analysis of 55 blood samples from residents of Gag Island showed seroreactivity to antigen preparations of Ehrlichia chaffeensis (7 of 48, 14.6%), two spotted fever group rickettsiae: Rickettsia rickettsii (5 of 48, 10.4%) and R. conorii (10 of 49, 20.4%), Orientia tsutsugamushi (5 of 33, 9.4%), and R. typhi (1 of 48, 2.1% [by an indirect immunofluorescence assay] and 1 of 50, 2.0% [by an enzyme-linked immunosorbent assay]). These results show serologic evidence of infection with ehrlichiae and spotted fever group rickettsiae for the first time in Indonesia in a location where the prevalence of antibody to O. tsutsugamushi and R. typhi was lower.

INTRODUCTION

Rickettsial diseases are endemic to Indonesia.\(^1\) Murine typhus (Rickettsia typhi) has been reported on many of the larger islands of the archipelago, including Java,\(^2\)–\(^7\) Sumatra,\(^8\),\(^9\) Bali,\(^4\),\(^10\) Muna,\(^4\) and Irian Jaya.\(^11\) The closely related etiologic agent of epidemic typhus, R. prowazekii, does not occur in Indonesia\(^2\),\(^4\),\(^12\),\(^13\) due to the absence of the arthropod vector, the human louse Pediculus humanus humanus.\(^2\) Scrub typhus (Orientia tsutsugamushi) has been recognized throughout Indonesia, especially during World War II when the occurrence of the disease on the island of New Guinea and neighboring islands was found to be a major impediment for military forces.\(^14\)–\(^16\) In addition to New Guinea, scrub typhus has been reported on the islands of Java,\(^5\),\(^7\),\(^17\)–\(^19\) Sumatra,\(^8\),\(^9\) Borneo,\(^20\) and Sulawesi.\(^21\) Other rickettsiae and Rickettsia-like organisms, such as spotted fever group rickettsiae and ehrlichiae, have not been previously reported occurring in Indonesia,\(^1\),\(^3\)\(^\text{-}\)\(^2\)\(^2\) although they have been reported in the surrounding Asia-Pacific region, including Australia,\(^12\),\(^23\)–\(^25\) Malaysia,\(^22\),\(^26\)–\(^29\) Thailand,\(^30\)–\(^34\) Japan,\(^32\),\(^35\)–\(^38\) India,\(^12\),\(^31\) Pakistan,\(^30\),\(^33\) Nepal,\(^31\) China,\(^39\),\(^40\) Mongolia,\(^3\) and Taiwan.\(^32\)

This report was the outcome of an investigation to determine the presence and level of arthropod-borne diseases on Gag Island, a small, isolated island located northwest of the Province of Irian Jaya, Indonesia. An investigation on malaria prevalence and parasite drug resistance has been reported elsewhere.\(^43\) Herein we describe, for the first time, serologic evidence of life-long inhabitants of Indonesia infected with spotted fever group rickettsiae and ehrlichiae. Additionally, we report a lower prevalence of antibody to O. tsutsugamushi and a near absence of seroreactivity to R. typhi among residents of Gag Island, Indonesia.

MATERIALS AND METHODS

Study population. Gag is a small island located in the Halmahera Sea, northwest of Irian Jaya and along the eastern edge of the Maluku Island Chain in the Republic of Indonesia (Figure 1). Approximately, 500 indigenous people lived on this island at the time of the survey, most of them in the village of Gambir. In addition, approximately 200 non-indigenous mining personnel were temporarily residing on the island. The local inhabitants were primarily subsistence farmers and fishermen. Gambir is located on the coast along the northeast side of the island, surrounded by sago swamp, coconut groves, and native forest. Most people lived in well-constructed, cement homes; however, the houses still allowed easy access to rodents and arthropod vectors. Four hundred fifty-six residents of Gambir and 112 mining employees were screened for malaria and lymphatic filariasis. Of these, 80 villagers and three mining employees were enrolled in a study of in vivo resistance of malaria parasites to chloroquine and Fansidar® (F. Hoffmann-La Roche, Basel, Switzerland).\(^41\) From these 83 individuals, 55 sera (53 from villagers and two from mining employees) were available for testing of exposure to rickettsiae and rickettsia-like organisms.

Human blood was collected by venipuncture, separated, and the serum was stored at -70°C. For the indirect immunofluorescence assay (IFA), the serum was assayed for reactivity to R. typhi, R. rickettsii, and E. chaffeensis as describe by the manufacturer (MRL Diagnostics, Cypress, CA). Sera were also assessed for reactivity to R. typhi, R. conorii, and O. tsutsugamushi with an enzyme-linked immunosorbent assay (ELISA) format previously described.\(^8\)

This study was reviewed and approved by the Committee for the Protection of Human Subjects of the U.S. Naval Medical Research Unit No. 2 and the Communicable Disease Research Center, National Institutes of Health, Research and Development (Jakarta, Indonesia). Informed consent was obtained from all human adult participants and from parents or legal guardians of minors.

RESULTS

Fifty-five human serum specimens from Gag Island were examined for serologic reactivity to E. chaffeensis, R. rickettsii, R. conorii, O. tsutsugamushi, and R. typhi antigen preparations. The seroreactivity prevalence is shown in Table 1. Agreement was found between the results obtained with different assays (IFA and ELISA) for detecting antibodies to
spotted fever group and murine typhus rickettsiae. All five sera reactive to the IFA R. rickettsii antigens (geometric mean titer [GMT] = 169) were also reactive to the ELISA R. conorii antigens (GMT = 2,111). In addition, five other sera were also reactive to R. conorii ELISA antigens but at a lower mean titer (GMT = 400).

Reactivity to R. typhi antigen preparations was found in only one serum sample of the 48 tested by the IFA, and in the same sample of 50 sera tested by the ELISA. This same sample was also reactive against the R. conorii and E. chaffeensis antigen preparations. Seroreactivity to E. chaffeensis agents by the IFA was found in seven of 48 sera analyzed (14.6%, GMT = 256). Five of 53 sera (9.4%) showed reactivity against O. tsutsugamushi by the ELISA. No associations between sera reactive to Ehrlichia antigens and those reactive to Rickettsia or Orientia antigens were found. All seroreactivity was found among Gag Island residents; the two mining employees were non-reactive to the rickettsial antigens tested.

DISCUSSION

These results provide evidence for rickettsial and rickettsial-like infections among humans residing on Gag Island. Moreover, the data provide evidence of infection with spotted fever and ehrlichial agents in Indonesia for the first time. Because both agents, or specific antibody to them, have been reported in various other locations in the Asia-Pacific-Australia region,22–40 it is not surprising that reactivity to these agents would be found among resident, in-country Indonesians. This is especially true for spotted fever group rickettsiae since specific antibody to strain TT-118 was found among 8.6% of 837 people surveyed in Sabah,27 and two of 39

FIGURE 1. The Indonesian archipelago showing the geographic location of Gag Island near Irian Jaya (Indonesian New Guinea). Gag Island, with an area of 56 km² and low elevation interior hills, contains only one village, Gambir (black area), which is located near Gambir Bay.
Haemaphysalis conigera ticks collected in Sarawak contained rickettsiae in their hemolymph that were reactive to fluorescein isothiocyanate–labeled antibody to *R. conorii.* Both of these studies were performed on the Malaysian portion of the Island of Borneo that is shared with Indonesia. Moreover, ticks known to be capable of transmitting rickettsial agents (*Ixodes* spp, *Dermacentor* spp, *Haemaphysalis* spp, *Rhipicephalus sanguineus*) have been reported throughout Indonesia.\(^\text{42–45}\) although no ticks were detected on 12 peridomestic murid rodents collected in Gambir at the time of this study (Bangs MJ, unpublished data).

Relatively few studies of *ehrlichiae* in Asia have been reported despite the increasing evidence for their presence elsewhere in the world. The first *ehrlichial* organisms isolated from fever patients in the 1950s in Japan were *E. sennetsu,*\(^\text{46}\) now referred to as *Neorickettsia sennetsu.*\(^\text{47}\) Another agent related to *N. sennetsu,* the SF agent, was isolated in Japan in 1962 from *Stellantechmus falcatus.*\(^\text{48}\) In addition, *E. muris* strains, which are closely related to *Ehrlichia canis,* have been isolated from mice and *Haemaphysalis* ticks,\(^\text{37}\) and HF and Anan strains have been isolated from *Ixodes* ticks in Japan.\(^\text{38}\) Except for Malaysia, where *N. sennetsu* has been reported,\(^\text{29}\) other countries have yet to report the presence of the Japanese strains. However, *E. canis* has been found in dogs from Singapore and Vietnam,\(^\text{49–51}\) and more recently, human *ehrlichiosis* has been reported in Thailand.\(^\text{34}\) Further investigations on seroprevalence and isolation of *ehrlichial* agents are clearly needed throughout Asia. We found that seven of 48 human sera contained antibodies reactive with *E. chaffeensis* antigen preparation by an IFA procedure. It is unknown at this time whether the seven individuals were previously infected with *E. chaffeensis* or a closely related species, or what mammalian reservoir is the natural host for this agent (e.g., rodent or dog). There are no deer on Gag Island.

Murine and scrub typhus are endemic diseases in Indonesia.\(^\text{3}\) Murine typhus endemicity varies throughout the Indonesian archipelago with the highest prevalence found in eastern Java and the lowest in the less populated islands of eastern Indonesia.\(^\text{4}\) The variation in endemicity is mainly due to differences in rodent and/or vector distribution/densities between and within islands. This variation in geographic distribution of disease has also been seen in other countries.\(^\text{28,32}\) Thus, the low seroprevalence of antibodies to *R. typhi* found among the study population (one seroreactive individual) and the lack of rat fleas ectoparasites from 12 collected *Rattus* spp. from Gambir (Bangs MJ, unpublished data) support the observations that murine typhus occurs rarely in eastern Indonesia.

Scrub typhus was first identified in New Guinea and rural areas of Java\(^\text{11}\) and Sumatra.\(^\text{8}\) Its impact was greatly felt by immunologically naive military forces, which encountered *O. tsutsugamushi* in New Guinea and neighboring islands.\(^\text{15,16}\) Post-World War II investigations have shown that a low endemic level of scrub typhus still prevails in the New Guinea area\(^\text{11,43}\) as well as throughout the Indonesian archipelago.\(^\text{5–7,52,53}\) We report for the first time evidence of antibody to *O. tsutsugamushi* among human inhabitants of Gag Island.

In conclusion, we have found serologic evidence of rickettsial and ehrlichial agents present among inhabitants of Gag Island. This is the first report of human antibodies reactive to spotted fever rickettsiae and ehrlichia antigen preparations detected in Indonesia, although spotted fever rickettsiae and ehrlichiae have been described elsewhere in Asia and Australia.

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REFERENCES


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* IFA = indirect immunofluorescence assay; ELISA = enzyme-linked immunosorbent assay
† Number of samples of a total of 55 human serum samples that were available for testing.
‡ Number of samples that were seroreactive to rickettsial antigen preparations.
§ GMT = geometric mean titer of antigen-specific antibody in the seroreactive samples.


