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Molecular survey of tick microbiome and tick-borne pathogens in ixodid ticks and rodents collected in Sarawak, Malaysian Borneo
(マレーシアボルネオ島サラワク州におけるマダニ微生物叢ならびにマダニ
およびネズミ類でのマダニ媒介性病原体の分子調査)

Ticks are obligate hematophagous arthropods, feeding on a wide range of animal hosts, including humans. Tick-borne diseases (TBDs), which afflict humans and animals, are caused by infectious agents such as bacteria, viruses, and protozoa, transmitted by tick bites. TBDs are regarded as a potential emerging threat to public health in Malaysia. Ticks also harbor largely non-pathogenic microorganisms, which have been implicated in tick survival, development, and reproduction. Understanding the microbial structure in ticks is crucial to unravel the dynamics of pathogen colonization and transmission. In addition, precise identification of tick species is crucial for epidemiological investigation, prevention, and control of TBDs in general. To gain insight into the tick microbiome and understand the status of tick-borne pathogens (TBPs) in Sarawak, Malaysian Borneo, where such studies are limited, tick and rodent samples were collected from two primary forests and an oil palm plantation for the molecular screening. Overall, nine species of ticks from four genera were collected, in which seven species, *Ixodes granulatus*, *Haemaphysalis hystricis*, *Haemaphysalis shimoga*, *Dermacentor compactus*, *Dermacentor steini*, *Dermacentor atrosignatus*, and *Amblyomma testudinarium*, were successfully identified based on phenotypic and genotypic identification. This study provides the first molecular evidence of multiple tick species in Sarawak and the first report of *H. shimoga* in Malaysia. Next, *I. granulatus* ticks and rodent spleens were screened for *Borrelia* spp., in which *Borrelia burgdorferi* sensu lato and Relapsing fever (RF) *Borrelia* were detected in feeding *Ixodes* ticks and rodents.

This study presented new geographical records of *Borrelia yangtzensis* and *Borrelia miyamotoi* and the first evidence in Malaysia of *B. miyamotoi*, a causative agent for RF in humans. Next, the tick microbiome was identified using Next-generation sequencing (NGS), which revealed significant differences between tick species, developmental stages, and infection status. Symbionts that dominating in each tick species and developmental stage were also identified. Furthermore, zoonotic TBPs such as *Rickettsia heilongjiangensis* and *Ehrlichia erwingii*, which cause spotted fever and ehrlichiosis, respectively, in humans, were identified from tick samples. TBPs screening using rodent spleen samples revealed *Ehrlichia chaffeensis* and *Coxiella* sp. closely related to *Coxiella burnetii* were detected, and both are known to cause disease in humans. Overall, this study identified multiple bacteria species from the genera of *Anaplasma*, *Ehrlichia*, *Borrelia*, *Bartonella*, *Coxiella*, *Francisella*, and *Rickettsia* from tick and rodent samples. These findings provide important insights into potential pathogen species circulating in ixodid ticks and rodents in Sarawak, which warrant future investigation on prevalence and risks. Finally, tick-borne protozoal screening using ticks and rodent samples revealed detection of a *Babesia* sp. genotype closely related to *B. macropus* identified in eastern grey kangaroos in Australia from two *H. shimoga* ticks. Phylogenetically, *Babesia* sp. in this study clustered with marsupial-associated *Babesia* spp. in *Babesia* sensu stricto clade. Given that of general lack information from this region, this thesis has contributed to the imperative data and insights on tick species, tick microbiome, and TBPs of bacteria and protozoa in Sarawak, Malaysian Borneo. This data is fundamental and valuable for the upcoming research development from this region.

(498 words)