

MINI-REVIEW: ZONOTIC AGENTS FROM TICKS IN MALAYSIA

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ABSTRACT

Ticks serve as vectors of multiple pathogenic bacteria, viruses and piroplasms with medical and veterinary importance worldwide. Close contact with animal hosts infested with ticks may result in transmission of zoonotic pathogens from animal reservoirs to humans. In Malaysia, past exposure to tick-borne pathogens such as *Anaplasma*, *Ehrlichia*, *Babesia* and other rickettsial infections have been reported in both human and animals. Research into the presence and transmission of tick-borne zoonotic pathogens is crucial in safeguarding human health. In our laboratory, we aim to study the presence of tick-borne pathogens by using molecular techniques and next-generation sequencing (NGS). The detection of bacteria and viruses are performed in ticks collected from farms, indigenous people (Orang Asli) villages and forests such as the national parks in Malaysia. The bacterial communities in these ticks were investigated by 16S rRNA amplicon sequencing on NGS platform. Early findings revealed the presence of diverse community of bacteria, including pathogens and endosymbionts. On-going efforts include expanding the microbiome investigations into ticks from other regions in Malaysia, as well as to intensify the discovery of tick-borne viruses. This review summarizes the current knowledge of potential tick-borne zoonotic agents in Malaysia, as well as the recent findings in the investigation of the bacterial and viral agents of ticks from Malaysia undertaken at the Tropical Infectious Diseases Research and Education Centre (TIDREC).

Keywords: Tick-borne diseases, zoonoses, 16s amplicon sequencing, infectious diseases

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INTRODUCTION

Ticks are competent vectors for the transmission of a number of pathogenic bacteria, viruses and piroplasms. Ticks are obligate parasites, as they feed on multiple hosts throughout their life cycles. Animals, including wildlife and livestock, are the usual hosts for ticks. As ticks are commonly found in forested areas, human populations living or working in or near to forested areas are at risk of exposure to tick bites, as well as the transmission of zoonotic agents from the animal reservoirs in the forested areas. Bacterial infections transmitted by ticks, including rickettsial spotted fever, relapsing fever and Lyme disease, have been largely reported worldwide with many intensive investigations into the pathogenesis, transmission dynamics and the disease burdens (Parola *et al.*, 2005; Kernif *et al.*, 2016). These diseases affect both developing countries such as Thailand and Malaysia as well as developed countries including Japan and the United States (Uchida *et al.*, 1986; Sirisanthana *et al.*, 1994; Hook *et al.*, 2015; Kernif *et al.*, 2016). Tick-borne bacterial infections are also of economic importance as it may serve as a threat to livestock industries. Tick-borne diseases such as Theileriosis and Anaplasmosis affects cattle and goats, causing poor growth, reducing herd fertility and dairy yields.

Tick-borne viral infections, on the other hand, are currently regarded as emerging diseases of concern to humans, specifically for Crimean-Congo Haemorrhagic Fever (CCHF), which is widely reported in Asia, Middle East, Europe and Africa (World Health Organization, 2013) and creeping in to wider geographical areas in which the disease was previously absent, such as the Great Britain and southern Iran (England *et al.*, 2016; Farhadpour *et al.*, 2016). More recently, outbreaks involving a novel tick-borne virus, Severe Fever with Thrombocytopenia Syndrome virus (SFTSV), were reported in China, Japan and Korea (Kim *et al.*, 2013; Yun *et al.*, 2014). This virus causes a haemorrhagic fever in humans, and

has been shown to be carried by a number of animal hosts living in close contact with humans, including goats, cattle and dogs (Zhao *et al.*, 2012). Infections from tick-borne viruses are normally associated with high mortality, up to 30% for SFTS and 40% to CCHF (Kim *et al.*, 2013; World Health Organization, 2013). The occurrence of these diseases can be subtle initially with only isolated incidences. Explosive outbreak could suddenly occur resulting in difficulties in transmission control and inability of the current healthcare infrastructure to cope with due to under-preparedness. Continuous surveillance for tick-borne infections are necessary to safeguard public health as well as the economic interests and food security of a country.

Current Knowledge of Tick-Borne Diseases in Malaysia

Initial studies on rickettsial diseases in Malaysia were undertaken as early as 1900s (Huxsoll, 1978). However, much attention had been directed to Scrub typhus, which is a rickettsial disease primarily transmitted by mites. Due to the similarities in the clinical manifestations (i.e. fever and rashes) of different rickettsial infections, and the lack of definitive clinical diagnostic tests, it was difficult to clearly differentiate the exact rickettsial species causing these diseases (Lim, 2014).

There have been more recent studies in Malaysia suggesting possible exposure of the rural populations to both mites and tick-borne rickettsial infections (Tee *et al.*, 1999; Sagin *et al.*, 2000; Tay *et al.*, 2000). However, efforts into investigating the presence of potential etiological agents to rickettsial diseases in ticks and the animal reservoirs were only undertaken in the recent years. The use of molecular techniques has tremendously facilitated the identification of specific rickettsial agents from ticks and animals, including novel rickettsial species. Molecular detection based on polymerase chain reaction (PCR) amplification of specific gene sequences of the rickettsial bacteria from ticks or tissues of animal hosts has enabled identification of the exact bacterial species from different tick species or animal hosts. Novel spotted fever group *Rickettsia* sp. genetically related to *Rickettsia tamurae*, as well as novel *Anaplasma* sp. and *Ehrlichia* sp., have been detected from *Amblyomma helvolum* and *Amblyomma varanense* ticks collected from *Python molurus* and *Naja sumatrana* snakes in Sepang and Johor respectively (Kho *et al.*, 2015b). *Anaplasma* sp. and *Bartonella bovis* were detected in *Rhipicephalus microplus* and *Haemaphysalis bispinosa* ticks respectively, as well as the associated cattle hosts in Malaysian farms (Tay *et al.*, 2014, Kho *et al.*, 2015a). *Ehrlichia* sp., *Anaplasma* sp. and *Coxiella burnetti* have been reported in *Rhipicephalus sanguineus* ticks and the associated canine hosts

in separate studies (Koh *et al.*, 2015; Watanabe *et al.*, 2015). *Rickettsia felis* has also been detected in the blood of the long-tailed macaques (*Macaca fascicularis*), although the infections remain subclinical and the role of ectoparasites in the infection is unclear (Tay *et al.*, 2015). Despite having found multiple rickettsial species in ticks and the animal hosts, the transmission dynamics between ticks and the animal hosts, as well as the risks of transmission to humans, remained uninvestigated. Furthermore, the data on tick-borne viral infections are still severely lacking in Malaysia. A number of viruses nevertheless, have been isolated from ticks in Malaysia, including the Lanjan virus from a pool of *Dermacentor auratus* ticks and the Langat virus from *Ixodes* sp. (Smith, 1956; Tan *et al.*, 1967). However, these viruses have not been reported to cause human diseases. More studies are needed nevertheless, as most of the earlier studies did not have the benefit of the various advanced technologies now available.

Towards this end, our centre together with our collaborators have undertaken initiatives to conduct surveillance activities for tick-borne pathogens in Malaysia. Earlier findings from our laboratory suggested a low but detectable presence of tick-borne encephalitis virus (TBEV) positive sera among a small group of farm workers (Mohd Shukri *et al.*, 2015). This study raised the concern that tick-borne viral infections were under-reported, despite an earlier study reporting no positive TBEV infections among patients with encephalitis admitted to Ministry of Health hospitals in Peninsular Malaysia and Sabah (Thayan *et al.*, 2004). Thus, this points to the need for enhanced surveillance of tick-borne diseases among the human populations, as well as the animal reservoirs and ticks.

Apart from seroprevalence studies, molecular detections of potential tick-borne pathogens in ticks collected from rural areas in Malaysia are also currently in progress. Most recently, we performed virus detection in *Ixodes* sp. ticks collected from wild, small mammals sampled in Pulau Tioman and Taman Negara Pulau Pinang, using reverse transcription-PCR. Viruses were not detected, therefore suggesting the absence of tick-borne viruses in the tick samples. Nonetheless, it was noted that the sample size was small and involving only island sites. Future surveillance study and research on ticks sampled from more areas are necessary in exploring the prevalence of tick-borne viruses in Malaysia.

Next Generation Sequencing in Unveiling the Microbiome of Ticks

One of the approaches for the investigation of tick-borne pathogens is to utilize the next generation sequencing (NGS) platforms. It is particularly invaluable for the investigation of the bacterial communities associated with arthropods

including ticks. The use of NGS will allow for the screening of large numbers of ticks, as well as the detection of non-targeted bacteria which will be otherwise undetected using species or gene-specific PCR amplification. A number of studies have reported the use of NGS in revealing the microbiome of ticks worldwide, especially in ticks that have been implicated in diseases transmission such as *Ixodes persulcatus* and *Amblyomma americanum* (Kurilshikov *et al.*, 2015; Fryxell & DeBruyn, 2016).

For NGS, DNA is commonly prepared from ticks using commercially available DNA extraction kits (Carpi *et al.*, 2011; Hawlena *et al.*, 2013; Kurilshikov *et al.*, 2015). These DNA can be directly sequenced using a method known as shotgun metagenomic sequencing that will reveal both host and microbiome sequences (Carpi *et al.*, 2011; Hawlena *et al.*, 2013). Alternatively, a short region of the bacterial 16s rRNA sequence could be amplified from each sample by PCR and sequenced (Carpi *et al.*, 2011; Khoo *et al.*, 2016a). The DNA library, either the shotgun library or PCR amplicons, could then be sequenced on commonly available NGS platforms, including Ion Torrent PGM, Roche 454 or the Illumina MiSeq platforms (Carpi *et al.*, 2011; Hawlena *et al.*, 2013; Kurilshikov *et al.*, 2015). Barcoding sequences allow for the sequencing of multiple samples in a single run, allowing for screening of large numbers of samples. The bacterial sequences can then be analyzed using established metagenomic sequencing analyses pipelines such as QIIME or MEGAN (Huson *et al.*, 2007; Caporaso *et al.*, 2010). Community analyses, such as alpha and beta diversity, could also be investigated for the bacterial communities for separate tick populations, which could provide insights for the interaction between commensal and pathogenic bacteria in the tick host.

As an initial study, we sequenced the bacterial 16s rRNA amplicons obtained from 20 tick samples, consisting of individual adults and pooled larvae or nymphs, collected from dogs, cats and chicken in two different Orang Asli settlements in Perak (Khoo *et al.*, 2016a). These samples consisted of ticks from three species, *Haemaphysalis wellingtoni*, *Haemaphysalis hystrix* and *H. bispinosa*, commonly found in the rural areas of Malaysia. Our findings revealed up to 392 known bacterial genera, with a large number of environmental bacteria such as *Bacillus*, *Staphylococcus* and *Pseudomonas*. Potential tick-borne pathogens were also identified, including *Rickettsia felis*, the etiological agent for cat-flea typhus that can cause diseases in humans and cats. Bacteria from the *Coxiella* genus were also identified in these samples. Follow-up studies using larger number of ticks revealed the presence of *Coxiella burnetii*, the agent for Q fever, detected in *H. hystrix* and *Dermacentor steini* (Khoo *et al.*, 2016b). A number

of *Coxiella*-like bacteria, a group of bacteria genetically related to *C. burnetti*, were also detected in these ticks.

Coxiella-like bacteria have been reported as endosymbionts in *A. americanum* ticks (Jasinskas *et al.*, 2007; Zhong *et al.*, 2007). Recently, human infections with *Coxiella*-like bacteria was reported in France, from patients with experience of tick-bites (Emmanouil *et al.*, 2016). Given that this study used ticks collected from the forested areas near the Orang Asli settlements, the risk of zoonotic disease transmission to the Orang Asli is highly probable and demands further investigation. The screening for tick bacterial communities in Malaysia is currently on-going in our laboratory, in which larger number of ticks of different species, host and from different geographical locations are included. The findings from this study is expected to provide the information on the bacterial communities of ticks in selected areas of Malaysia, which will be crucial in formulating control and healthcare strategies for preventing and mitigating infections in humans.

CONCLUSIONS AND FUTURE PERSPECTIVES

Tick-borne diseases are of public and veterinary health importance globally, as exemplified from the recent outbreaks of SFTSV in East Asia, and the extended battle against borrelial diseases in the North America and Europe. In Malaysia, the occurrence of tick-borne diseases is still under-investigated and under-appreciated. Microbiome studies in our laboratory, together with the molecular studies from ticks and animals hosts from other researchers have readily showed the presence of various tick-borne bacterial agents, including *Rickettsia*, *Coxiella*, *Anaplasma* and *Bartonella* in ticks and the animal hosts. Despite the lack in discovery of tick-borne viruses, evidence of past exposures to TBEV among human populations also suggests the risk of tick-borne viral diseases in Malaysia. More thorough studies into the transmission dynamics and the associated risk factors of tick-borne diseases are still lacking to date. Risk factors including geographical factors, seasonality, climate or other environmental determinants, animal host range, and human activities are all contributing factors to the spread and transmission of tick-borne zoonotic agents and merits further investigations. In Malaysia, the rapid change in land use, including the conversion of forests to farmlands or plantations and the spread of urbanization to forest-fringe areas,

resulted in increased encroachment into animal, especially wildlife habitats. This lead to greater opportunities for humans-animals interactions contributing to possible zoonotic disease transmission. The establishment of prevention and protection strategies against the spread of tick-borne and other zoonotic diseases, and hence will rely on the joint efforts between agencies and expertise in the medical, veterinary and environmental health in enhancing research in this area, expanding the knowledge base and improving the education and healthcare among the affected communities. Therefore, a One Health approach here would prove beneficial and effective.

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