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Research Note

Molecular detection of *Anaplasma platys* and *Babesia gibsoni* in dogs in Malaysia

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Abstract. This study reports for the first time molecular detection of *Anaplasma platys* infection in 4 (13.3%) of 30 Malaysian dogs investigated. A low occurrence (3.3%) of *Babesia gibsoni* was also noted, being detected in one of the 30 dogs. *Rickettsia*, *Bartonella*, *Orientia tsutsugamushi*, and *Ehrlichia* DNA were not detected in the dog blood samples. The role of *A. platys* as an agent of canine anaplasmosis and its transmission through *Rhipicephalus sanguineus* ticks merits further investigation.

Anaplasma platys (formerly *Ehrlichia platys*) is an obligate intracellular bacteria of the family *Anaplasmataceae* which causes infectious canine cyclic thrombocytopenia (ICCT) in dogs. The organism was first reported in 1978 in Florida (Harvey *et al*., 1978). Since then, it has been reported in many geographical regions (Arraga-Alvarado *et al*., 2003; de la Fuente *et al*., 2006). *Anaplasma platys* can be transmitted through the bite of an infected tick (*Rhipicephalus sanguineus*) (Arraga-Alvarado *et al*., 1997; Harvey, 1993; Hibler *et al*., 1986). Although ICCT is considered a benign disease without obvious clinical signs (Arraga-Alvarado *et al*., 2003) by most investigators, severe infections such as uveitis and pancytopenia have been reported (Glaze *et al*., 1986; Arraga-Alvarado, 1992).

Identification of *A. platys* morulae, seen as inclusion bodies in blood smears, is challenging due to the cyclic parasitemia nature of the disease (Breitschwerdt, 2000). Serological approaches are useful to determine the degree of exposure but are not able to differentiate the causative species as cross-reactivity of *A. platys* with other *Anaplasma* has been reported (Beaufils *et al*., 2002). Therefore, molecular method is essential for accurate detection of *A. platys* when parasitemia is low and for strain differentiation of the infecting strain. Up to now, there was no report yet on *A. platys* infection in dogs in Malaysia.

Canine babesiosis causes hemolytic anemia, thrombocytopenia, lethargy, and splenomegaly in infected dogs. The disease has been classified as a clinically significant tick-borne haemoprotozoan disease worldwide (Irwin, 2009). Based on microscopic analysis of blood smears, a survey conducted between 1973 and 1981 in Selangor, Malaysia, documented the identification of *Babesia gibsoni* and *Babesia canis* from 17.7% and 1.1% of dogs, respectively (Rajamanickam *et al*., 1985). The availability of advanced molecular methods such as polymerase chain reaction

(PCR) assays is expected to facilitate the detection and identification of babesia circulating in the dog population in Malaysia.

This study was performed to detect the presence of *Anaplasma* and *Babesia* in dogs surrendered to an animal shelter in Selangor, Malaysia. The dogs were mostly local mongrels which came from the households in Klang Valley. As little data is available on vector-borne diseases of dogs in Malaysia, PCR assays targeting *Rickettsia*, *Bartonella* and *Orientia* were also included for testing of the blood samples. A recent study in our laboratory demonstrated the detection of *Rickettsia felis*, *Bartonella henselae and Bartonella clarridgeiae* in *Ctenocephalides felis* fleas, suggesting fleas as potential vectors of rickettsioses and bartonellosis in this country (Mokhtar & Tay, 2011). In addition, the presence of antibody against scrub typhus (causative agent: *Orientia tsutsugamushi*) and murine typhus (causative agent: *Rickettsia typhi*) in dogs have also been documented (Huxsoll *et al.*, 1977).

In this study, EDTA-anticoagulated blood samples were obtained from dogs and DNA was extracted using QIAamp tissue kit. Two PCR assays were performed for amplification of *A. platys* DNA, i.e. a 16S rRNA PCR assay, using primer pair EHR 16SD/EHR 16SR which amplified *Anaplasma* and *Ehrlichia* species (Parola *et al.*, 2000; Martin *et al.*, 2005) and a *Anaplasma* species-specific nested PCR assay, using outer primers fD1 and EHR 16SR and inner primers PLATYS- F and PLATYS-R (Inokuma *et al.*, 2001). For detection of *Babesia* spp., primer pair Babesia-F/Babesia-R was used to amplify *Babesia* 18S rDNA gene (Inokuma *et al.*, 2003). PCR assays targeting spotted fever and typhus group rickettsiae, *Bartonella* spp. and *O. tsutsugamushi* were attempted as described previously (Norman *et al.*, 1995; Labruna *et al.*, 2004; Liu *et al.*, 2006).

Purification of PCR products was carried out using LaboPass PCR Purification Kit (Cosmo Genetech, Korea) as described by the manufacturer. Purified PCR products were sequenced using primer pairs EHR 16SD/EHR 16SF and PLATYS- F/PLATYS-R for *Anaplasma* and primer pair Babesia-F/Babesia-R for *Babesia*. The obtained sequences were aligned with BioEdit Sequence Alignment Editor Software (version 7.0.5.3) and compared for similarity with sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) program (National Center for Biotechnology Information, National Institute of Health). The neighbour-joining method of MEGA software (version 4.0) was employed to determine the phylogenetic status of the *Anaplasma* based on the partial 16S rRNA sequences.

Anaplasma DNA was detected from four (13.3%) dogs by the *Anaplasma* 16S rRNA PCR assay. Sequence analysis of all four amplicons (designated as B2, B4, 1_1, and 1_7) using primer pair PLATYS-F/PLATYS-R shows 100% (501 nt/501 nt) similarity with that of the *A. platys* strain Hd83 (GenBank accession no. GQ395385) reported from Praia city, Cape Verde (Götsch *et al.*, 2009). Figure 1 is the neighbor-joining phylogram which shows the placement of the Malaysian *Anaplasma* specimens with *A. platys* strain Hd83, among other established *Anaplasma* species.

Babesia DNA was detected in one blood sample. Sequence analysis of the partial 18S rRNA gene demonstrates 99% (567 nt/568 nt) similarity to that of *B. gibsoni* genotype Asia 2 (GenBank accession no. AF175301). Hence, we conclude a low occurrence (3.3%) of *B. gibsoni* in this study.

This study reports for the first time the detection of *A. platys* infection in 4 (13.3%) Malaysian dogs. The role of *A. platys* as an agent in canine anaplasmosis has not been thoroughly investigated in Malaysia. *Ehrlichia canis*, a closely related member of *Anaplasma*, was not detected in this study, although the organism has been reported from 0.2% of dogs by blood smear analysis (Rajamanickam *et al.*, 1985), and 15% dogs by serological tests in a recent study (Rahman *et al.*, 2010). Coinfection of *Rickettsia*, *Bartonella*, *O. tsutsugamushi* was not detected in the dog samples in this study. More extensive epidemiological studies will be required to determine the distribution of *A. platys* and *B. gibsoni* in

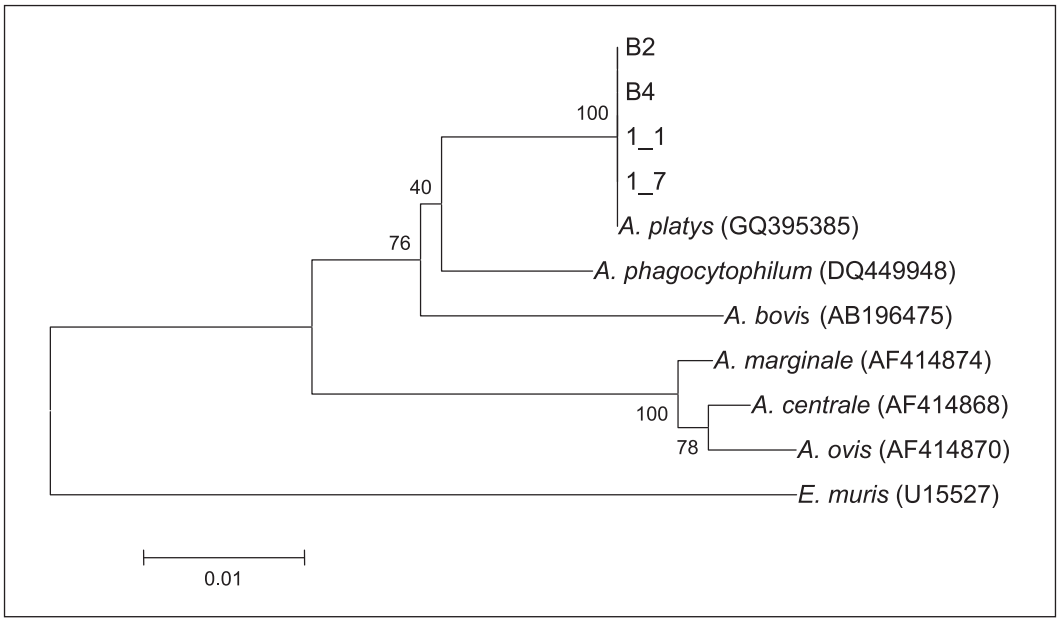

Figure 1. Neighbor-joining phylogram based on partial *16S rDNA* sequences shows the phylogenetic placement of B2, B4, 1_1 and 1_7 specimens among established *Anaplasma* species. *Ehrlichia muris* was used as the outgroup. Bootstrap analysis was performed with 1000 replications. Numbers in brackets are GenBank accession numbers. Scale bars indicate nucleotide substitutions per site

Malaysia. In addition, good tick control (particularly, of *R. sanguineus*) is important to prevent the spread of canine anaplasmosis and babesiosis.

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