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ARTHROPODS AND MEDICAL ENTOMOLOGY - ORIGINAL PAPER

Ticks and associated pathogens from dogs in northern Vietnam

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Abstract

The medical and veterinary significance of ticks and tick-borne pathogens (TBPs) in tropical and subtropical zones is well recognized. Although ticks and TBPs are known to occur in Southeast Asia, limited data is available in the international literature for some countries, such as Vietnam. The aim of this study was to investigate the species of ticks and TBPs associated with dogs in northern Vietnam. Out of 359 dogs enrolled in this study, 26.2% ($n = 94$) were infested by 466 ticks (i.e., 287 males, 139 females, 30 nymphs, and 10 larvae). All ticks were morphologically identified as *Rhipicephalus sanguineus* sensu lato, and some of them genetically characterized as belonging to the tropical lineage. A total of 302 ticks were molecularly screened for the detection of selected TBPs. Three ticks were positive for *Hepatozoon canis*, one for *Ehrlichia canis*, and one for *Babesia vogeli*, representing the first molecular characterization of these pathogens in Vietnam. In conclusion, the tropical lineage of *R. sanguineus* s.l. is the dominant tick taxon infesting dogs from northern Vietnam, where different TBPs are circulating.

Keywords Ticks · Vietnam · Dogs · Companion animals · Pathogens · Vector-borne diseases

Introduction

Canine vector-borne diseases (CVBDs) are a group of infectious diseases caused by diverse pathogens (e.g., bacteria, helminths, and protozoa), which are transmitted by arthropod vectors, including ticks (Otranto et al. 2009). In Southeast Asia (SEA), *Rhipicephalus sanguineus* sensu lato (s.l.), *Rhipicephalus microplus*, and *Haemaphysalis bispinosa* are the main tick species parasitizing dogs (Nuchjangreed and Somprasong 2007; Wells et al. 2012; Tran and Nguyen 2014). In particular, *R. sanguineus* s.l. ticks are the most commonly found on dogs in the SEA (Irwin and Jefferies 2004), playing a major role as a vector of pathogenic bacteria and protozoa which can be transmitted to animals (particularly dogs) and humans (Dantas-Torres 2008). For example, in Malaysia, *Hepatozoon canis*, *Babesia gibsoni*, *Babesia vogeli*, and *Coxiella burnetii* were molecularly detected in *R. sanguineus* s.l. from dogs (Watanabe et al. 2015; Prakash et al. 2018a, b). Additionally, *Anaplasma platys* and *Ehrlichia canis* were found in *R. sanguineus* s.l. parasitizing dogs in the Philippines (Ybañez et al. 2012), with up to 3.9% and 21.8% dogs molecularly positive to *E. canis* from Thailand and Cambodia, respectively (Liu et al. 2016; Inpankaew et al. 2016). Tick-borne diseases may cause clinical conditions (e.g., lethargy, anemia, and thrombocytopenia), as reported for *B. vogeli* in dogs from the Philippines (Ybañez et al. 2017). Some zoonotic TBPs were also reported in SEA, including *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, which has been molecularly detected in ticks from Malaysia (Koh et al. 2018). Despite few published reports about ticks and TBPs in dogs (Phan et al. 1977; Kolonin 1995; Petney et al. 2007; Tran and Nguyen 2014), a limited number of studies on canine TBPs are available in Vietnam. In addition, molecular techniques have never been used in Vietnam for species delineation of canine TBPs (e.g., *B. vogeli*, *E. canis*, and *H. canis*), and local veterinarians rely mostly on serological tests and cytological examination of stained blood smears for diagnosing those infections. Since a large number of dogs are illegally imported from neighboring countries to Vietnam for human consumption (Chin 2018), and without any veterinary control, these dogs could represent a further source of spreading infectious diseases (Ngo et al.

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Table 1 Primers and target genes of pathogens investigated by PCR in this study

Pathogens	Primer	Target	Fragment size	References
Babesia spp.	PIRO-A: AATACCCAATCCTGACACAGGG PIRO-B: TTAAATACGAATGCCCCCAAC	18S rDNA	408 bp	Olmeda et al. (1997)
Anaplasma spp./*Ehrlichia* spp.	EHR16SD: GGT ACC YAC AGA AGA AGT CC EHR16SR: TAG CAC TCA TCG TTT ACA GC	16S rDNA	345 bp	Martin et al. (2005)
Hepatozoon spp.	HepF: ATACATGAGCAAAATCTCAAC HepR: CTTATTATTCCATGCTGCAG	18S rDNA	666 bp	Inokuma et al. (2002)

2011), including those transmitted by ticks. Therefore, the aim of this study was to investigate the species of ticks and TBPs associated with dogs from northern Vietnam.

Materials and methods

From March to May 2018, a total of 359 (211 females and 148 males) privately owned dogs living in Hanoi (northern Vietnam) and rural areas of neighboring provinces (i.e., Phutho, Vinhphuc, Bacgiang, Hanam) were visited and checked for tick infestation. Dogs were individually checked by manual inspection for 10 min each. All ticks found were removed and placed into labeled tubes, individualized per dog, containing 70% ethanol. Ticks were divided per developmental stage (i.e., larval, nymph, adult) and gender (female and male) and identified using morphological keys (Walker et al. 2005; Keirans 2009).

To confirm tick identification, a partial fragment of the 16S rRNA gene was amplified by using the primers RHS16SF and RHS16SR (Burlini et al. 2010). PCR was used to detect *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., and *Hepatozoon* spp. from ticks. Primers, amplified target genes, and PCR conditions have been described elsewhere (Olmeda et al. 1997; Inokuma et al. 2002; Martin et al. 2005) and are shown in Table 1.

PCR products were subjected to electrophoresis in a 2% agarose gel stained with GelRed (VWR International PBI, Milano, Italy) and viewed on a GelLogic 100 gel documentation system (Kodak, New York, USA). Amplicons were purified by mixing 10 μl of the PCR product with 0.5 μl of *Escherichia coli* exonuclease I (Exo I; MBI, Fermentas, Lithuania), 1 μl of shrimp alkaline phosphatase (SAP), and 0.5 μl of SAP reaction buffer (MBI, Fermentas, Lithuania) to remove primers and unincorporated dNTPs. This mix was then incubated at 37 °C for 20 min then at 85 °C for 15 min according to the manufacturer's protocol. PCR purified products were sequenced using the Taq DyeDoxyTerminator Cycle Sequencing Kit (v.2, Applied Biosystems) in an automated sequencer (ABI-PRISM 377). Sequences were aligned using Geneious program and compared among them and with those available in GenBank database by Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). The percentage of nucleotide variation among sequences of a given taxon was calculated by pairwise comparison (Kimura 2 parameter model) of the entire sequences herein determined using the Geneious 11.1 software.

Statistical analysis

The frequency of tick infestation was compared in relation to dog housing conditions (indoor versus outdoor) using chi-square test, which was performed using IBM SPSS Statistics 20.0 software. Differences were considered statistically significant at p value < 0.05.

Table 2 Number of dogs positive for ticks and their developmental stages collected from dogs living indoor and outdoor

Areas	Dogs		Ticks				Total specimens collected
	Outdoor	Indoor	Larvae	Nymphs	Adults		
					Male	Female	
Hanoi	6/26	41/155	–	6	150	72	288
Phutho	3/10	7/9	–	3	52	23	78
Vinhphuc	17/73	0/10	3	2	60	26	91
Bacgiang	7/31	4/21	–	1	16	12	29
Hanam	9/19	0/5	7	18	9	6	40
Total	42/159	52/200	10	30	287	139	466

Results and discussion

Ninety-four (26.2%; 95% CI = 21.85–31.04) dogs were infested by ticks with a mean intensity of 4.96 (95% CI = 3.88–6.89). No significant difference was found in the frequency of tick infestation in dogs living indoor (26%) and outdoor (26.4%) ($p > 0.05$). Of the 466 ticks collected, 426 (91.4%) were adults (287 males and 139 females), 30 (6.4%) nymphs, and 10 (2.2%) larvae (Table 2). All ticks found in this study were morphologically identified as *R. sanguineus* s.l., the most common group of ticks found on dogs worldwide (Dantas-Torres et al. 2013). In particular, specimens of *R. sanguineus* s.l. genetically characterized belonged to the tropical lineage (99% nucleotide identity, GenBank accession numbers MF425981 and MF351600). The consensus sequence has been submitted in the GenBank database (accession number MH674443). Some species of the genus *Haemaphysalis* (i.e., *H. mageshimaensis*, *H. quadriaculeata*, *H. spinigera*, *H. canestrinii*, *H. papuana*, *H. hystricis*) were found on dogs in Vietnam in a previous survey (Kolonin 1995), but none of them was identified in this study. Of the 466 ticks, 302 samples were screened for TBPs based on the number of ticks collected from each dog (i.e., all ticks when < 10/dog and 10% when > 10/dog). Totally, five ticks were positive for at least one pathogen. In particular, two males and one female were positive for *H. canis*, one female for *E. canis*, and one male for *B. vogeli*.

Here, we report the occurrence of *H. canis*, *E. canis*, and *B. vogeli* in Vietnam for the first time in the international literature, through molecular investigation in *R. sanguineus* s.l. tick vector. Indeed, in a previous study performed on worldwide-collected *R. sanguineus* s.l., none of the ticks from southern Vietnam scored positive for the investigated TBPs (i.e., *Cercopithifilaria* spp., *H. canis*, and *A. platys*) (Latrofa et al. 2014). In spite of the scant scientific literature on this subject, the circulation of TBPs is known among veterinary practitioners in Vietnam. However, the limitations in terms of diagnostic procedures and scientific data may alter the perception of local veterinary practitioners about the risk of infection by TBPs in dogs. For instance, canine ehrlichiosis was first reported in US military dogs during the Vietnam War, when this disease affected and killed hundreds of animals (Kelch 1984). Additionally, a serological survey conducted in 1972 reported the occurrence of antibodies to selected vector-borne pathogens in military dogs after serving in Vietnam (Alexander et al. 1972). Finally, a molecular study on TBPs in ticks collected on wild pigs and from the environment in southern Vietnam showed the presence of an *Ehrlichia* sp., which clustered with *E. chaffeensis*, the agent of human monocytic ehrlichiosis (Parola et al. 2003).

This study should increase awareness among dog owners and veterinary practitioners regarding the importance of ticks and their transmitted pathogens, toward a timely diagnosis and treatment of canine TBPs in Vietnam. In addition, our findings should prompt further investigations of CVBDs to better assess their distribution and risk factors in SEA.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval Informed consent and agreement were obtained from dog owners before sampling ticks. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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