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Original article

# Tick-borne haemoparasites and Anaplasmataceae in domestic dogs in Zambia

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#### ABSTRACT

Tick-borne diseases (TBDs), including emerging and re-emerging infectious diseases, are important threats to human and animal health worldwide. Indeed, the number of reported human and animal infectious cases of novel TBD agents has increased in recent decades. However, TBDs tend to be neglected, especially in resourcelimited countries that often have limited diagnostic capacity. The aim of this molecular survey was to detect and characterise tick-borne pathogens (Babesia, Theileria, and Hepatozoon parasites and Anaplasmataceae bacteria) in domestic dogs in Zambia. In total, 247 canine peripheral blood samples were collected in Lusaka, Mazabuka, Monze, and Shangombo. Conventional PCR to detect the selected pathogens was performed using DNA extracted from canine blood. One hundred eleven samples were positive for protozoa and 5 were positive for Anaplasmataceae. Sequencing of thirty-five randomly selected protozoa-positive samples revealed the presence of Babesia rossi, Babesia vogeli, and Hepatozoon canis 18S rDNA. Based on these sequences, a multiplex PCR system was developed to yield PCR products with different amplicons, the size of which depended on the parasite species; thus, each species could be identified without the need for sequence analysis. Approximately 40% of dogs were positive for H. canis. In particular, the positive rate (75.2%) of H. canis infection was significantly higher in Shangombo than in other sampling sites. Multiplex PCR assay detected B. rossi and B. vogeli infections in five and seven dogs, respectively, indicating that this approach is useful for detecting parasites with low prevalence. Sequencing analysis of gltA and groEL genes of Anaplasmataceae revealed that two and one dogs in Lusaka were infected with Anaplasma platys and Ehrlichia canis, respectively. The data indicated that Zambian dogs were infected with multiple tick-borne pathogens such as H. canis, B. rossi, B. vogeli, A. platys, E. canis and uncharacterized Ehrlichia sp. Since some of these parasites are zoonotic, concerted efforts are needed to raise awareness of, and control, these tick-borne pathogens.

#### 1. Introduction

Ticks are the second most common blood sucking arthropods next to

mosquitoes. They not only cause anaemia in their animal hosts, but also carry and transmit a wide variety of viruses, bacteria, and protozoa, some of which cause tick-borne diseases (TBDs) (de la Fuente et al.,

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Fig 1. Dog sample collection sites in Zambia.

2008; Otranto et al., 2014). These TBDs not only include multiple existing infectious diseases, but also comprise emerging and re-emerging infectious diseases. An example of one such emerging TBD is severe fever with thrombocytopaenia syndrome, which was reported to be endemic to China in 2011 and which poses serious threats to human and animal health (Parola et al., 2005; Yu et al., 2011). Moreover, in the past two decades, the number of reported cases of infection with novel TBDs in humans and animals has increased (Kernif et al., 2016).

Given that some TBDs in humans are zoonoses, it is important to identify the tick-borne pathogens in pets, livestock, and wild animals and elucidate the factors that determine their prevalence. The most common tick-borne protozoan pathogens of dogs are Babesia and Hepatozoon (Homer et al., 2000; Baneth et al., 2003). These haemoparasites live in mammalian blood cells and cause severe diseases and sometimes death in infected animals (Schnittger et al., 2012). Specifically, Babesia gibsoni, Babesia canis, Babesia rossi, and Babesia vogeli are causative agents of canine babesiosis (biliary fever). B. gibsoni is distributed in Asia, North America, Europe, and northern and eastern Africa (Farwell et al., 1982; Jefferies et al., 2003; Lobetti, 1998). B. canis is transmitted by Dermacentor reticulatus, which is prevalent in Europe (Solano-Gallego and Baneth, 2011). The other two species, B. rossi and B. vogeli, are mainly transmitted by Haemaphysalis leachi and Rhipicephalus sanguineus sensu lato (s.l.), respectively (Apanaskevich et al., 2007; Criado-Fornelio et al., 2003). The distribution of B. rossi is restricted to sub-Saharan Africa, while that of B. vogeli is worldwide (Europe, Africa, Asia, South and North America) (Oyamada et al., 2005; Matjila et al., 2008). Hepatozoon canis and Hepatozoon americanum are the agents of canine hepatozoonoses that range from being asymptomatic with low levels of parasitaemia to a severe life-threatening illness characterised by high levels of parasitaemia, fever, anaemia, and lethargy (Baneth et al., 2000; Baneth et al., 2003). These two Hepatozoon species are genetically and geographically distinct (Baneth et al., 2000). H. canis is distributed in Africa, southern Europe, the Middle East, and Asia (Baneth and Vincent-Johnson, 2006) and is mainly transmitted by R. sanguineus s.l. and Haemaphysalis longicornis (Dantas-Torres et al., 2012; Murata et al., 1995). H. americanum is found in the Americas and is transmitted by Amblyomma maculatum (Mathew et al., 1998). Recently, new Hepatozoon spp. were reported in dogs and wildlife in Turkey and Brazil (Aydin et al., 2015; Soares et al., 2017).

Anaplasma and Ehrlichia species are obligate intracellular bacteria that belong to the family of Anaplasmataceae. These tick-borne pathogens infect humans and animals all over the world. Anaplasma platys is primarily found in dogs with cyclic thrombocytopaenia (Harvey et al., 1978). In addition, new Anaplasma species that are closely related to Anaplasma phagocytophilum, which causes human granulocytic anaplasmosis, have been detected in canine blood (Inokuma et al., 2005). Ehrlichia canis is the causative agent of canine ehrlichiosis, which is transmitted by *R. sanguineus* s.l. (Groves et al., 1975; Aguiar et al., 2007). While *E. canis* was initially thought to be pathogenic in canines only, it was eventually also detected in human patients with the typical clinical findings of ehrlichiosis (Perez et al., 2006).

Only a few studies have examined haemoparasites and Anaplasmataceae in domestic and wild dogs in Zambia. Baba et al. (2012) described the case of a dog that was exported from Zambia to Japan and was infected with *E. canis*, while Nalubamba et al. (2011) showed that of 1196 samples from domestic dogs in Lusaka, 2.4% were positive for *Babesia* parasites. Williams et al. (2014) reported that 11 wild and eight domestic dogs in the Eastern and Western Provinces of Zambia were all negative for *Babesia* infection. However, they did find that 65% of wild dogs and 13% of domestic dogs were infected with *Hepatozoon*. Recently, Vlahakis et al. (2018) described the first molecular evidence of *A. platys* in domestic dogs in Lusaka.

To better understand the infection status and distribution of tickborne pathogens in Zambian domestic dogs, we subjected blood samples from 247 domestic dogs living in four districts of Zambia to our newly developed multiplex PCR assay, which differentiates between the main tick-borne canine haemoparasites. This molecular survey showed that some Zambian dogs are infected with *Anaplasma, Ehrlichia, Babesia*, and especially *Hepatozoon*.

#### 2. Materials and methods

#### 2.1. Ethics

All procedures were performed in accordance with the guidelines established by the Animal Experiment Committee of the Graduate School of Veterinary Medicine, Hokkaido University (Sapporo, Japan). Download English Version:

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