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Molecular epizootiology of canine hepatozoonosis in Croatia ☆

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ABSTRACT

An epizootiological survey was conducted to investigate the prevalence of hepatozoonosis in a population of 924 apparently asymptomatic dogs from different regions of Croatia. DNA was isolated from canine blood and screening PCR on the 666 bp fragment of 18S rRNA revealed that 108 (11.8%) of dogs were infected. Positive samples were confirmed by partial sequencing of the 18S rRNA gene. The consensus sequences, derived from various sequence data sets, were compared with sequences of 18S ssrRNA of *Hepatozoon* spp. available in GenBank. The alignments revealed 106 *Hepatozoon canis* and two *Hepatozoon sp.* sequences. Among *H. canis* isolates, we found a certain amount of heterogeneity, while both *Hepatozoon sp.* isolates were identical to the Spanish isolate (Accession No. AY600625) from *Clethrionomys glareolus*. On the basis of eight commonly mutated nucleotide positions in the partial 18S rRNA gene sequence, we divided the *H. canis* isolates into five groups. The results obtained indicate a higher prevalence and significance of hepatozoonosis in Croatia than previously believed and demonstrate that the organisms belonging to *H. canis* that infect European dogs are genetically very heterogeneous.

1. Introduction

Apicomplexan blood parasites of the genus *Hepatozoon* are among the most biodiverse and prevalent haemogregarines and have been described from a wide range of mammals, amphibians and reptiles, as well as from various haematophagous arthropods, which serve as both the vectors and definitive hosts of the parasites. The life cycle of *Hepatozoon* seems to lack the salivary transmission between hosts commonly associated with other vector-borne diseases. Instead, transmission takes place when the intermediate host ingests the definitive host, an invertebrate containing *Hepatozoon* oocysts. Sexual stages, with sporogony, take place in different blood-sucking arthropods and asexual phases occur in the endothelial cells of various organs or in the parenchymal cells of the liver (Baneth et al., 2007). Presently, two *Hepatozoon sp.* causing canine hepatozoonosis have been described: *Hepatozoon canis*, a relatively widespread protozoan, and *Hepatozoon americanum*, which seems to be present only in North America (Baneth et al., 2000).

Hepatozoon americanum is the causative agent of canine hepatozoonosis in the United States (USA), and is transmitted by the tick *Amblyomma maculatum* (Vincent-Johnson et al., 1997a; Baneth et al., 2003). The agent causes a distinct clinical syndrome in dogs characterised by fever, lethargy, weight loss, stiffness, signs of pain, paralysis and an ocular discharge (Vincent-Johnson et al., 1997a,b; Macintire et al., 2001). *Hepatozoon canis* is the cause of Old World canine hepatozoonosis and has been reported from South America, the USA, southern Europe, the Middle East, Africa and the Far East. The main vector of the disease is the brown dog tick, *Rhipicephalus sanguineus* (Baneth et al., 2001, 2003). The pathogenicity of *H. canis* is thought to be low because subclinical infections are common, usually causing a mild disease that affects the spleen, lymph nodes and bone marrow, resulting in anaemia and lethargy (Baneth and Weigler, 1997; Gavazza et al., 2003). Gametocytes in circulating white blood cells are often an incidental finding in dogs without clinical signs and the disease is easily diagnosed by observation of the blood film (Baneth et al., 1996).

Hepatozoon canis is a protozoan parasite that infects domestic and wild carnivores, including canids, such as the domestic dog (Baneth et al., 2007), the domestic cat (Rubini et al., 2006), the red fox (*Vulpes vulpes*) (Criado-Fornelio et al., 2003a,b, 2006, 2007a; Fishman et al., 2004; Majláthová et al., 2007) and the crab-eating fox (*Cerdocyon thous*) (Alencar et al., 1997). Although well investigated in the USA (Macintire et al., 1997; Allen et al., 2008), South America (Gondim et al., 1998; O'Dwyer et al., 2001, 2006; Paludo et al., 2003, 2005; Rubini et al., 2005, 2008; Criado-Fornelio et al., 2006, 2007a,b; Forlano et al., 2007; Eiras et al., 2007; Rubini et al., 2008; Yabsley et al., 2008; Mundim et al., 2008), Sudan (Oyamada et al., 2005), Nigeria (Sasaki et al., 2008),

☆ Note: Nucleotide sequence data reported in this paper are available in the GenBank, EMBL and DDBJ databases under the Accession Nos.: FJ497009–FJ497024.

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Table 1
Isolates of *Hepatozoon canis* from various parts of the world.

Animal species	Isolate	Genbank Accession No.	Origin	Diagnostic method (% positive)	Tissue	Clinical signs	References
Dog			Malaysia	Microscopy (1.2%)	Blood	Randomly selected	Rajamanickam et al. (1985)
Dog			Israel	Microscopy	Blood	Symptomatic	Baneth et al. (1995)
Dog			Israel	ELISA (33%)	Blood	Asymptomatic	Baneth et al. (1996)
Crab-eating fox (*Cerdocyon thous*)			Brazil	Microscopy	Blood	Symptomatic	Alencar et al. (1997)
Dog			USA	Microscopy	Sceletal muscle	Symptomatic	Mancitire et al. (1997)
Dog			Israel	Microscopy	Blood	Smptomatic + asymptomatic	Baneth et al. (1997)
Dog			Brazil	iycroscopy	Blood, biopsate	Symptomatic	Gondim et al. (1998)
Dog			Japan	Serology (4.2%)	Blood	Asymptomatic	Inokuma et al. (1999)
Dog		AF176835	USA	PCR	Blood		Mathew et al. (2000)
Dog		AF206669	Israel	PCR	Blood		Baneth et al. (2000)
Dog			Brazil	Microscopy (39.2%)	Blood	Randomly selected	O'Dwyer et al. (2001)
Dog	Fukuoka	AF418558	Japan	Microscopy PCR	Blood	Smptomatic + asymptomatic	Inokuma et al. (2002)
Dog			Japan	PCR	Blood	Asymptomatic	Inokuma et al. (2002)
Dog			Brazil	Microscopy (5.9%)	Blood	Asymptomatic	O'Dwyer et al. (2004)
Fox (*Vulpes vulpes*)			Israel	ELISA (24%)	Blood	Asymptomatic	Fishman et al. (2004)
Dog			Turkey	Microscopy	Blood	Symptomatic	Voyvoda et al. (2004)
Dog			Greece	ELISA	Blood	Symptomatic	Mylonakis et al. (2004)
Dog	Dog 8 Dog 33 Dog 26 Dog 12 Dog 47 Dog 60 Dog 13 Dog 78 Dog 68	DQ111751 DQ111755 DQ111754 DQ111752 DQ111756 DQ111758 DQ111753 DQ111757 DQ111759	Sudan	PCR	Blood	Asymptomatic randomly selected	Oyamada et al. (2005)
Dog		AY864679 AY864678 AY864677 AY864676	Brazil	Microscopy PCR	Blood	Symptomatic	Paludo et al. (2005)
Dog	Isolate 1 Isolate 2	DQ198378 DQ198379	Brazil	PCR (67.7%) Microscopy (22.6%)	Blood	Symptomatic	Rubini et al. (2005)
Dog	Spain 2	AY461378	Spain	PCR (26.6%)	Blood	Symptomatic	Criado-Fornelio et al. (2006)
Fox (*Vulpes vulpes*)	Curupira 3 Curupira 1 Curupira 4	AY461375 AY461376 AY471615	Brazil	PCR (69.2%)	Spleen	Killed in a hunt	Criado-Fornelio et al. (2006)
Fox (*Vulpes vulpes*)	Spain 1 (85%) Spain 3 (15%)	AY150067 AY731062	Spain	PCR (90%)	Liver Spleen	Killed in a hunt	Criado-Fornelio et al. (2003a) Criado-Fornelio et al. (2003b) Criado-Fornelio et al. (2006)
Dog	Selcuk Aydin Bodrum Marmaris Manisa Kusadasy	DQ060329 DQ060328 DQ060327 DQ060326 DQ060325 DQ060324	Turkey	PCR (25.8%) Microscopy (10.6%) IFAT (36.8%)	Blood	Asymptomatic or moderate symptoms	Karagenc et al. (2006)
Cat	Isolate 2 Isolate 1	DQ315566 DQ315565	Brazil	PCR	Blood	Symptomatic	Rubini et al. (2006)
Dog			Thailand	Microscopy (2.6%) PCR (11.4%)	Blood	Randomly selected	Jittapalapong et al. (2006)
Cat			Thailand	Microscopy (0.7%) PCR (32.3%)	Blood	Randomly selected	Jittapalapong et al. (2006)
Dog	Dog 1 Dog 2 Dog 3	DQ439543 DQ439540 DQ439544	Venezuela	Microscopy (13%) PCR (44.7%)	Blood	Asymptomatic randomly selected	Criado-Fornelio et al. (2007a)
Dog	Thailand 1 Thailand 2	DQ519358 DQ519357	Thailand	PCR (30%)	Blood	Symptomatic	Criado-Fornelio et al. (2007a)
Fox (*Vulpes vulpes*)	Spain 4 Spain 5	DQ439541 DQ439542	Spain	PCR (35%)	Spleen Blood	Asymptomatic	Criado-Fornelio et al. (2007a)
Dog		DQ071888	Brazil	PCR	Blood	Naturally and experimentaly infected	Forlano et al. (2007)
Dog		Identical to AY461375	Brazil	PCR(44%) qPCR(56%)	Blood	Asymptomatic	Criado-Fornelio et al. (2007b)
Dog	Spain 6	EF650846	Spain	PCR(3.5%) qPCR(20%)	Blood	Asymptomatic tick-exposed	Criado-Fornelio et al. (2007b)
Dog		Identical to DQ060324	Venezuela	PCR(0%) qPCR(13.3%)	Blood	Asymptomatic	Criado-Fornelio et al. (2007b)
Dog		99% identical to AF176835	Argentina	Microscopy (83.3%) PCR (100%)	Blood	Asymptomatic	Eiras et al. (2007)