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Original article

Genetic diversity of *Hepatozoon* spp. in *Hydrochoerus hydrochaeris* and *Pecari tajacu* from eastern Amazon

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ABSTRACT

This study aimed to identify and characterize genetically species of the genus *Hepatozoon* detected in *Hydrochoerus hydrochaeris* (capybaras) and *Pecari tajacu* (collared peccaries) from two localities from the Eastern Amazon. Blood samples from 196 free-living *H. hydrochaeris* from Marajó Island and 109 *P. tajacu* kept in captivity in Belém, Pará, were collected and analyzed for the presence of *Hepatozoon* spp. Partial sequences of the 18S rRNA gene were obtained and analyzed in comparison to others available in the NCBI database. Our results demonstrated a high prevalence of *Hepatozoon canis* in both mammals and the existence of four haplotypes of *Hepatozoon* spp., three of *Hepatozoon canis* and one of *Hepatozoon cuestensis*, found only in *H. hydrochaeris*. In addition, these data increase the genetic diversity of *H. canis* from the Eastern Amazon, as well as reporting, for the first time, the infection of mammals by *H. cuestensis* and *P. tajacu* by *H. canis*.

1. Introduction

Hydrochoerus hydrochaeris (capybaras) and *Pecari tajacu* (collared peccaries) are widely distributed in South America and are among the Brazilian wild mammals that are of great economic importance due to the appreciation of their meat and to the interest of the international leather industry (Mayor et al., 2010; Ojasti, 1991). In nature, these animals appear to play an important role in maintaining the life cycle of apicomplexan protozoa such as *Toxoplasma gondii* (Abreu et al., 2016; Thois et al., 2003), *Eimeria* spp. (Wilber et al., 1996) and *Hepatozoon canis* (Criado-Fornelio et al., 2009).

In wild mammals from Brazil, *H. canis* has been detected in *Pseudalopex vetulus* (hoary fox), *Cerdocyon brachyurus* (maned wolf) (André et al., 2010), *Dusicyon thous* (crab-eating fox), *Pseudalopex gymnocercus* (pampas fox) (Criado-Fornelio et al., 2006), *Hydrochoerus hydrochaeris* (capybara) (Criado-Fornelio et al., 2009) and *Leopardus pardalis* (ocelot) (Braz and Umeda, 2015). Considering that the genus *Hepatozoon* belongs to the group of hemogregarines, one of the characteristics of species of this genus is its heteroxenous life cycle involving an intermediate vertebrate host and a definitive invertebrate hematophagous host (Baneth and Shkap, 2003).

Unlike most of the pathogens that are transmitted by an arthropod vector through the salivary glands during the blood repast, the main route of infection of the intermediate host by *Hepatozoon* spp. is oral ingestion of the definitive host containing mature polysporocyst oocysts (Baneth et al., 2007; Baneth, 2011; Desser, 1993). Thus, geographical distribution of the final host and the existence of reservoirs, such as some wild animals, can determine the dispersion patterns of *Hepatozoon* spp. (Dantas-Torres, 2010; Najm et al., 2014). In fact, foxes and golden jackals appear to have great importance in the distribution of *H. canis* (Duscher et al., 2013; Farkas et al., 2014; Imre et al., 2015; Najm et al., 2014).

The worldwide occurrence of *H. canis*, including in places where there is no report of the existence of the tick vector *Rhipicephalus sanguineus* sensu lato, has reinforced the concept that other species of ticks have vectorial competence for *H. canis* (Majláthová et al., 2007; Mitková et al., 2014; Mitková et al.,2016). In Brazil, despite the high abundance of *R. sanguineus* sensu lato (Araújo et al., 2015; Dantas-Torres et al., 2009; Soares et al., 2006), studies carried out in an attempt to detect *H. canis* infection did not find any evidence of the presence of this protozoan (Demoner et al., 2013; Forlano et al., 2005, Gomes et al., 2010).

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Table 1
Hepatozoon spp. 18S rRNA sequences used for phylogenetic analysis plus additional information retrieved from the GenBank database.

Strain	GenBank accession No.	Species	Host	Reference
Brazil	KU729738	*H. canis*	Dog	Gomes et al. (2016)
Iran	KT736298	*H. canis*	Dog	Unpublished
Turkey	KX588232	*H. canis*	Dog	Unpublished
Croatia	HM212626	*H. canis*	Fox	Dezdek et al. (2010)
Brazil	KU729737	*H. canis*	Dog	Gomes et al. (2016)
Brazil	EF622096	*H. canis*	Capybara	Criado-Fornelio et al. (2009)
Hungray	KJ572976	*H. canis*	Golden Jackal	Farkas et al. (2014)
Croatia	FJ497022	*H. canis*	Dog	Vojta et al. (2009)
Czech Republic	KU597242	*H. canis*	Tick	Hamšíková et al. (2016)
Brazil	KC342527	*H. cuestensis*	Rattlesnake	O'Dwyer et al. (2013)
Brazil	KC342524	*H. cuestensis*	Rattlesnake	O'Dwyer et al. (2013)
USA	AF176836	*H. americanum*	Dog	Mathew et al. (2000)
Brazil	KU729739	*H. americanum*	Dog	Gomes et al. (2016)
Japan	AB771547	*H. felis*	Iriomote cat	Unpublished
Brazil	KY684005	*H. felis*	Ocelot	Soares et al. (2017)
Italy	KY649445	*H. silvestris*	Domestic cat	Giannelli et al. (2017)
Bosnia and Herzegovina	KX757031	*H. silvestris*	European wild cat	Hodžić et al. (2017)
Bosnia and Herzegovina	KX757032	*H. silvestris*	European wild cat	Hodžić et al. (2017)
Croatia	KT274177	*H. ayorgbor*	Wood mouse	Unpublished
Croatia	KT274178	*H. ayorgbor*	Yellow-necked fieldmouse	Unpublished
Ghana	EF157822	*H. ayorgbor*	Ball python	Sloboda et al. (2007)
Canada	AF130361	*H. catesbianae*	No data available	Carreno et al. (1999)
Morocco	KU680464	*Hepatozoon* sp.	Desert wall gecko	Tomé et al. (2016)
Morocco	KU680466	*Hepatozoon* sp.	Moorish gecko	Tomé et al. (2016)
Brazil	AY461377	*Hepatozoon* sp.	Fox	Criado-Fornelio et al. (2006)
Brazil	KC127679	*Hepatozoon* sp.	Fox	Almeida et al. (2013)
Brazil	KY684007	*Hepatozoon* sp.	Caiman	Soares et al. (2017)
Brazil	KJ413113	*Hepatozoon* sp.	Caiman	Unpublished
Brazil	KY684006	*Hepatozoon* sp.	Turtle	Soares et al. (2017)
Brazil	KY684004	*Hepatozoon* sp.	Paca	Soares et al. (2017)
Australia	AY252110	*Hepatozoon* sp.	Slaty grey snake	Ujvari et al. (2004)
Australia	AY252105	*Hepatozoon* sp.	Water python	Ujvari et al. (2004)
China	KF939622	*Hepatozoon* sp.	King rat snake	Unpublished
Brazil	KU667308	*Hepatozoon* sp.	Wild rodent	Demoner et al. (2016)
Chile	FJ719813	*Hepatozoon* sp.	Monito del monte	Merino et al. (2009)

In addition, there is no consensus so far about which tick species is in fact eco-epidemiologically important in the transmission of *H. canis* in Brazil. While O'Dwyer et al. (2001) indicated *Amblyomma cajennense* sensu lato as a potential vector, Demoner et al. (2013) refuted this possibility and suggested the existence of more than one strain of *Amblyomma ovale* capable of becoming infected by *H. canis*. The detection of mature oocysts of *H. canis* in the haemocoel of the cattle tick, *Rhipicephalus microplus*, collected from a dog and identification of DNA of the protozoan in this tick suggest that *R. microplus* can be a possible vector of *H. canis* (Miranda et al., 2011), however the experimental infestation of *H. canis*-infected dogs with *R. microplus* was not successful (Demoner et al., 2013).

Most of our understanding of the disease caused by some species of the genus *Hepatozoon* comes from studies with domestic dogs (Aydin et al., 2015; Eiras et al., 2007; Ewing et al., 2000; Harvey et al., 2016; Li et al., 2008). In fact, the infection of wild animals by *Hepatozoon* spp. has been poorly studied in Brazil, principally in some regions, such as the Amazon. Thus, the objective of this study was to investigate the occurrence and genetically characterize the species of *Hepatozoon* found in *H. hydrochaeris* of extensive livestock farming and *P. tajacu* kept in captivity in the State of Pará, Brazil.

2. Materials and methods

2.1. Samples and DNA extraction

A total of 196 blood samples from *H. hydrochaeris* raised by extensive livestock farming on Marajó Island (State of Pará, Brazil) and 109 from *P. tajacu* maintained in captivity in the Brazilian Agricultural Research Cooperation (EMBRAPA Western Amazon) were collected into tubes containing ethylenediaminetetraacetic acid (EDTA). Total DNA of each sample was extracted from a 300μL aliquot of the blood by using a standard phenol-chloroform procedure, as described by Sambrook et al. (1989). DNA quality was checked by electrophoresis in an agarose gel, and the DNA was then quantified using the Qubit 2.0 fluorometer (Thermo Fisher Scientific).

2.2. PCR amplification and DNA sequencing

Molecular diagnosis of *Hepatozoon* spp. was undertaken according to Gomes et al. (2016) which is based on the partial amplification through nested PCR of the 18S rRNA gene of *Hepatozoon* spp. Briefly, we used HepF and HepR primers (Inokuma et al., 2002) in the first round of PCR followed by a second round of PCR with HepNF and HepNR primers (Gomes et al., 2016) whose amplification produced a fragment of approximately 300bp.

In order to properly identify the *Hepatozoon* species, positive samples identified after molecular diagnosis, according to Gomes et al. (2016), were submitted to another nested PCR assay to amplify a larger fragment (~670bp) of the 18S rRNA than the one amplified in the molecular diagnosis protocol. In this second assay, the first round of amplification included: NBA1 (5′GGTTGATCCTGCCAGTAGT3′) (Criado-Fornelio et al., 2003) and HPF2 (5′GACTTCTCCTTCGTCT AAG3′) (Criado-Fornelio et al., 2006) primers, while the second round PCR included HepF and HepR primers. First round PCR was carried out in 25μL reactions with 10–20 ng of the DNA template, 2.5mM $MgCl_2$, 0.125mM each of deoxyribonucleotide triphosphates (dNTPs), 10mM Tris-HCl, 50mM KCl, 0.2μM of each primer, and 1 U Taq DNA polymerase (Invitrogen). The amplification reaction consisted of 40 cycles of 30 s at 95 °C, 30 s at 58 °C, and 2 min at 72 °C, preceded by 10 min at 95 °C and followed by 10 min at 72 °C. Second-round PCR was carried out under the same conditions of the first round PCR used here to the