Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Diversity of piroplasmids among wild and domestic mammals and ectoparasites in Pantanal wetland, Brazil

Keyla Carstens Marques de Sousa[a], Marina Pugnaghi Fernandes[a], Heitor Miraglia Herrera[b], Carla Roberta Freschi[c], Rosangela Zacarias Machado[a], Marcos Rogério André[a,*]

[a] Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brazil
[b] Universidade Católica Dom Bosco, Campo Grande, MS, Brazil
[c] Imunodot Diagnósticos Ltda, Jaboticabal, SP, Brazil

ARTICLE INFO

Keywords:
Babesia
Cytauxzoon
Dogs
Phylogenetic analysis
Theileria
Rangelia
Wildlife

ABSTRACT

Piroplasmoses are one of the most prevalent arthropod-borne diseases of animals. The present work aimed to investigate the occurrence of piroplasmid in wild mammals, domestic dogs and ectoparasites in southern Pantanal region, central-western Brazil. For that purpose, blood or tissue samples from 31 *Nasua nasua*, 78 *Cerdocyon thous*, 7 *Leopardus pardalis*, 42 dogs, 110 wild rodents, and 30 marsupials, and 1582 ticks were submitted to PCR assays for piroplasmid targeting 18SrRNA and hps70 genes. Seven dogs, one *C. thous,* five *L. pardalis*, three *N. nasua*, six wild rodents, eight *Amblyomma parvum*, two *Amblyomma sculptum* and one *Amblyomma ovale* were positive for piroplasmid-PCR assays. Genotypes closely related to *Babesia vogeli* were detected in six dogs and five wild rodents. While genotypes closely related to *Babesia caballi* were detected in one *C. thous*, one dog, one *A. ovale* and one *A. sculptum,* genotypes closely related to *Babesia bigemina* and *Babesia bovis* were detected in four *A. parvum* ticks. Four sequences obtained from *A. parvum*, three coatis and one wild rodent were closely related to *Theileria equi. Cytauxzoon* spp. was detected in four ocelots. The present study revealed that wild and domestic animals in Brazilian southern Pantanal are exposed to different piroplasmid species.

1. Introduction

Piroplasmid (Piroplasmida) are apicomplexan protozoa including the genera *Babesia*, *Theileria*, *Cytauxzoon* and *Rangelia* (Yabsley and Shock, 2013). These agents are tick-borne protozoans that parasitize blood cells of numerous wild and domestic vertebrates worldwide (Alvarado-Rybak et al., 2016). These parasites have a great economic and veterinary impact, being considered the second most commonly parasites found in the blood of mammals after trypanosomes (Schnittger et al., 2012). In the vertebrate hosts, the infection is usually characterized by fever, anemia and hemoglobinuria, and in severe cases, can lead to death (Kuttler, 1988). Although some of these parasites can cause diseases in animals and humans, the vectors are still unknown for many piroplasm species (Kjemtrup et al., 2000; Hersh et al., 2012).

Previously, the classification of piroplasmids relied only on host of origin, size and shape of trophozoites (small or large) and the number of merozoites within erythrocytes. However, the identification based on host origin has been invalidated, since many of these parasites are not host-specific (Penzhorn, 2006; Criado-Fornelio et al., 2003; Yabsley and Shock, 2013). Besides, the diagnosis based only on direct observations of blood smears does not always allow species identification and usually molecular assays are necessary in order to identify the etiological agent involved (Criado-Fornelio et al., 2003). In the last few years, the advent of molecular techniques has contributed to an expressive increase in the number of studies reporting infection with piroplasmids in wild animals worldwide (Alvarado-Rybak et al., 2016).

In Brazil, there are few reports concerning the seroprevalence and molecular detection of piroplasmid in wild carnivores. For instance, André et al. (2011) found a seroprevalence of 31.7% and 10.3% against *B. vogeli* antigen among wild felines and canids maintained in captivity, respectively. Additionally, André et al. (2011) detected a genotype closely related to *B. leo* in a neotropical wild cat (*Oncifelis colocolo*) and Cape genet (*Genetta tigrina*) also maintained in captivity in zoos in the state of São Paulo, Brazil. In addition to this, fatal cases of cytauxzoonosis were reported in two lions maintained in capitivity in a zoo in the

* Corresponding author at: Laboratório de Imunoparasitologia, Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias Júlio de Mesquita Filho (UNESP), Campus de Jaboticabal, Via de Acesso Prof. Paulo Donato Castellane, s/n, Zona Rural, CEP: 14884-900, Jaboticabal, São Paulo, Brazil.
E-mail address: marcos_andre@fcav.unesp.br (M.R. André).

http://dx.doi.org/10.1016/j.ttbdis.2017.09.010
Received 8 April 2017; Received in revised form 11 September 2017; Accepted 15 September 2017

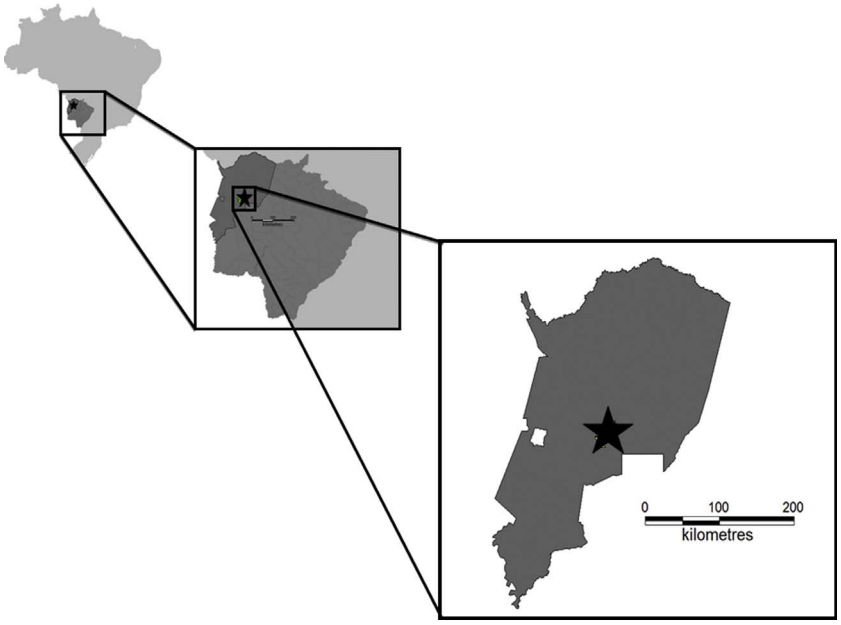

Fig. 1. Capture sites. Map of Mato Grosso do Sul State, central-western Brazil, showing the Pantanal region, where mammals' blood and spleen samples and ticks were collected in the present study.

state of Rio de Janeiro (Peixoto et al., 2007). Besides, André et al. (2009) detected *Cytauxzoon* DNA closely related to *Cytauxzoon felis* in asymptomatic neotropical felines also maintained in captivity in zoos in the cities of São Paulo and Brasília. Furthermore, *Cytauxzoon* sp. has also been molecularly detected in domestic cats from the states of Rio de Janeiro (Maia et al., 2013) and Mato Grosso do Sul (André et al., 2015).

Due to the lack of information about the epidemiology and transmission routes of piroplasms among wild animals in Brazil, the present study aimed to investigate the occurrence of piroplasmids in wild mammals and domestic dogs and their respective ectoparasites in the region of Pantanal, state of Mato Grosso do Sul, central-western Brazil.

2. Materials and methods

2.1. Study area

The fieldwork was conducted at the Nhumirim ranch (56°39′ W, 18°59′ S), located in the central region of the Pantanal, municipality of Corumbá, state of Mato Grosso do Sul, central-western Brazil (Fig. 1). This region is characterized by a mosaic of semi-deciduous forest, arboreal savannas, seasonally flooded fields covered by grasslands with dispersed shrubs and several temporary and permanent ponds. The Pantanal is the largest Neotropical floodplain, being well known for its rich biodiversity. Two well-defined seasons are recognized in that region: a rainy summer (October to March) and a dry winter (April to September) (Sousa et al., 2017a, 2017b).

2.2. Biological sampling

Between August 2013 and March 2015, a total of 256 animals were captured in the central region of the Pantanal, municipality of Corumbá, state of Mato Grosso do Sul: 158 carnivores, among them 78 crab-eating foxes (*C. thous*), 31 coatis (*N. nasua*) and seven ocelots (*L. pardalis*); 140 small mammals, among them 110 wild rodents (77 *Thrichomys fosteri*, 25 *Oecomys mamorae* and 8 *Clyomys laticeps*) and 30 wild marsupials (14 *Thylamys macrurus,* 11 *Gracilinanus agilis*, 4 *Monodelphis domestica* and 1 *Didelphis albiventris*). Additionally, 42 blood samples from domestic dogs cohabiting the same studied area were collected. Blood samples were collected from carnivores and domestic dogs by puncture of the cephalic vein and stored in Vacutainer® tubes with EDTA and without EDTA, in order to obtain total blood and serum samples for molecular and serological assays, respectively. Spleen samples were collected from small mammals and stored in absolute ethanol (Merck®, Kenilworth, Nova Jersey, USA) for molecular assays. All blood and serum samples were stored at −20 °C. The DNA extraction and serological assays were performed one week after the captures. All animal captures were in accordance with the licenses obtained from the Brazilian Government Institute for Wildlife and Natural Resources Care (IBAMA) (license numbers 38145, 38787-2) and endorsed by the Ethics Committee of FCAV/UNESP University (Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus Jaboticabal) n° 006772/13 (Sousa et al., 2017a, 2017b).

One thousand five hundred and eighty-two ticks parasitizing the sampled mammals were collected, of which 1033 (65.2% [115 adults and 918 nymphs]) belonging to *A. sculptum* Berlese, 241 (15.2% [78 adults and 163 nymphs]) belonging to *A. parvum* Aragão, 32 (2%) *A. ovale* Koch adults, one (0.06%) *Amblyomma tigrinum* Koch adult, one (0.06%) *Rhipicephalus (Boophilus) microplus* (Canestrini) adult, one (0.06%) *Rhipicephalus sanguineus* s.l. (Latreille) adult, four (0.2%) *Amblyomma auricularium* (Conil) nymphs, and 269 (17%) *Amblyomma* larvae. Besides, a total of 80 *Polygenis* (*Polygenis*) *bohlsi bohlsi* (Wagner) fleas were collected (Sousa et al., 2017a, 2017b).

2.3. Giemsa-stained blood smears

Blood smears were performed using peripheral blood collected from wild carnivores and domestic dogs, fixed with methanol and stained with Giemsa (Giemsa stain, modified, Sigma-Aldrich®, St. Louis, MO, USA).

2.4. Enzyme-linked immunosorbent assay (ELISA)

In order to detect IgG antibodies to *B. vogeli*, canids (*C. thous* and dogs) serum samples were individually tested by an ELISA assay using a commercial kit (IMUNODOT, Diagnósticos Ltda®, Jaboticabal, SP, Brazil), according to the manufacturer's instructions.

2.5. DNA extraction

DNA was extracted from 200 µL of each whole blood (158 wild