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Occurrence and molecular characterization of hemoplasmas in domestic dogs and wild mammals in a Brazilian wetland

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ABSTRACT

Hemotropic mycoplasmas are known to cause anemia in several mammalian species. The present work aimed to investigate the occurrence of *Mycoplasma* spp. in wild mammals, domestic dogs and their respective ectoparasites, in southern Pantanal region, central-western Brazil. Between August 2013 and March 2015, 31 *Nasua nasua*, 78 *Cerdocyon thous*, seven *Leopardus pardalis*, 42 dogs, 110 wild rodents, and 30 marsupials were trapped and ectoparasites (ticks and fleas) found parasitizing the animals were collected. Mammals and ectoparasites DNA samples were submitted to conventional PCR assays for *Mycoplasma* spp. targeting 16S rRNA and *RnaseP* genes. Twenty-four *N. nasua*, three *C. thous,* two domestic dogs, one *L. pardalis* and one wild rodent were positive for 16S rRNA PCR protocols. Fourteen *N. nasua* samples were also positive in *RnaseP* PCR. No marsupial or arthropod showed positivity for *Mycoplasma* spp. The phylogenetic analyses based on 16S rRNA gene showed that all sequences obtained from dogs, two sequences obtained from *C. thous* and ten sequences obtained from *N. nasua* showed to be closely related to *Mycoplasma haemocanis/Mycoplasma haemofelis* species. Genotypes closely related to '*Candidatus* Mycoplasma haemominutum' and *Mycoplasma haemomuris* were detected in the *L. pardalis* and in the wild rodent, respectively. Probably a novel *Mycoplasma* genotype, closely related to a sequence obtained from a Brazilian capybara was detected in 14 *N. nasua*, based on a concatenated phylogenetic analysis of 16S rRNA and *RnaseP* genes. The present study revealed that wild animals in southern Pantanal region, Brazil, are exposed to different species of hemoplasmas.

1. Introduction

Hemotropic mycoplasmas (hemoplasmas) are epicellular erythrocytic bacteria lacking cell wall. In contrast to several mucosal mycoplasmas, hemoplasmas have never been grown successfully in culture so far. These pathogens are known to be the causative agents of infectious anemia in several mammalian species and may induce acute hemolysis in some cases (Tasker, 2010). The disease is characterized by anorexia, lethargy, dehydration, weight loss and in some cases, can lead to death (Willi et al., 2007). Furthermore, hemotropic mycoplasmas are considered emergent zoonotic agents, mainly in immunocompromised individuals or those highly exposed to arthropod vectors (dos Santos et al., 2008; Maggi et al., 2013a).

The transmission of hemoplasmas between domestic cats and dogs seems to occur mainly by bloodsucking arthropods, such as ticks and fleas, blood transfusion, contaminated fomites and transplacentally (Seneviratna et al., 1973; Messick, 2003; Lappin et al., 2006). In addition to this, infections through biting and fighting are considered another possible routes of hemoplasma transmission (Tasker, 2010).

Although hemoplasmas have been detected in domestic cats (Braga et al., 2012; Miceli et al., 2013; André et al., 2014; Santis et al., 2014) and dogs (Ramos et al., 2010; Alves et al., 2014; Valle et al., 2014; Soares et al., 2016) from several localities in Brazil, few reports have documented the occurrence of hemoplasma species in wild animals. For

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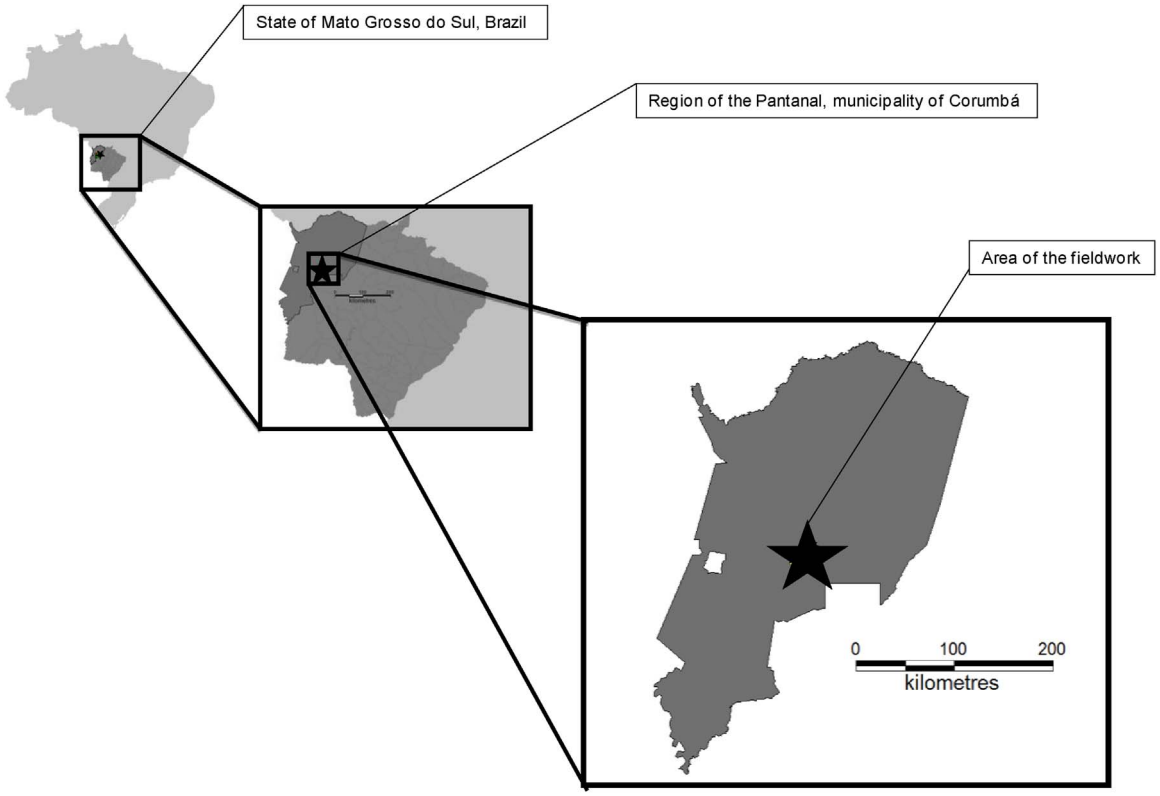

Fig. 1. Capture sites. Map of Mato Grosso do Sul State, central-western Brazil, showing the Pantanal region, where animals samples were collected in the present study.

instance, hemoplasmas have been detected in wild carnivores maintained in captivity in zoos (Willi et al., 2007; Guimarães et al., 2007; André et al., 2011), wild rodents (Vieira et al., 2009; Conrado et al., 2015; Gonçalves et al., 2015), monkeys (Bonato et al., 2015), and deer (Grazziotin et al., 2011) in Brazil.

Due to the lack of information about the epidemiology and routes of transmission of hemoplasmas among the wildlife from Brazil, the present study aimed to investigate the occurrence of hemotropic mycoplasmas 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. Material and methods

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 the studied region: a rainy summer (October to March) and a dry winter (April to September) (Sousa et al., 2017).

Between August 2013 and March 2015, a total of 256 mammals were captured in the central region of the Pantanal, municipality of Corumbá, state of Mato Grosso do Sul, including 158 carnivores, among 78 crab-eating foxes (*C. thous*), 31 coatis (*N. nasua*) and seven ocelots (*L. pardalis*); 140 small mammals, among 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*). Forty-two blood samples from domestic dogs cohabiting the same studied area were also collected. All animal captures were in accordance with the licenses obtained from the Brazilian Government Institute for Wildlife and Natural Resources Care (IBAMA) (license numbers38145, 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., 2017).

One thousand five hundred and eighty-two ticks parasitizing the sampled mammals were collected, including 1033 (65.2% [115 adults and 918 nymphs]) *Amblyomma sculptum* Berlese specimens, 241 (15.2% [78 adults and 163 nymphs]) *Amblyomma parvum* Aragão specimens, 32 (2%) *Amblyomma 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. Furthermore, a total of 80 *Polygenis* (*Polygenis*) *bohlsi bohlsi* (Wagner) fleas were also collected (Sousa et al., 2017).

DNA was extracted from 200 µL of each whole blood (158 wild carnivores and 42 domestic dogs) and 10 mg of spleen (140 small mammals) samples using the QIAamp DNA Blood Mini kit (QIAGEN®, Valencia, CA, USA), according to the manufacturer's instructions. The amount of tick DNA extracted was 523, of which 228 (43.5%) were from adults, 256 (48.9%) pooled nymphs, and 39 (7.4%) from pooled larvae. While DNA extraction from ticks was processed in pools for nymphs (up to 5 individuals) and larvae (up to 10 individuals), the adults were processed individually. A total of 39 pooled fleas samples were submitted to DNA extraction. The fleas DNA extraction was also performed in pools consisting of up to five individuals. Ticks and fleas were macerated and submitted to DNA extraction, using the same kit before mentioned (Sousa et al., 2017).

In order to verify the presence of amplifiable DNA in the samples, internal control PCR assays targeting fragments of mammalian glyceraldehyde-3-phosphatedehydrogenase (GAPDH), ticks mitochondrial 16S rRNA and fleas cytochrome-c oxidase subunit I (Cox-1) genes were performed (Table 1). All 298 DNA animal samples amplified the predicted product for GAPDH gene. Out of 523 sampled ticks, 31