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BRIEF COMMUNICATION

DETECTION OF *Leptospira* spp. AND *Brucella abortus* ANTIBODIES IN FREE-LIVING JAGUARS (*Panthera onca*) IN TWO PROTECTED AREAS OF NORTHERN PANTANAL, BRAZIL

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SUMMARY

This study aimed to assess the exposure of free-living jaguars (*Panthera onca*) to *Leptospira* spp. and *Brucella abortus* in two conservation units in the Pantanal of Mato Grosso, Brazil. The presence of antibodies in blood samples of eleven jaguars was investigated using autochthonous antigens isolated in Brazil added to reference antigen collection applied to diagnosis of leptospirosis by Microscopic Agglutination Test (MAT). The Rose Bengal test was applied for *B. abortus* antibodies. Two (18.2%) jaguars were seroreactive for the *Leptospira* spp. antigen and the serovar considered as most infective in both animals was a Brazilian isolate of serovar Canicola (L01). All jaguars were seronegative for *B. abortus*. These data indicate that the inclusion of autochthonous antigens in serological studies can significantly increase the number of reactive animals, as well as modify the epidemiological profile of *Leptospira* spp. infection.

KEYWORDS: Zoonotic diseases; *Leptospira* spp.; *B. abortus*; Serology; *Panthera onca.*

INTRODUCTION

The jaguar (*Panthera onca*) is the largest felid in the Americas, where retaliation from ranchers due to livestock predation, illegal hunting tourism activity, and habitat loss associated with agricultural expansion are threats to the species in the Pantanal biome, an important area for jaguar conservation in the long-term[4].

Leptospirosis is a zoonosis of worldwide distribution and global importance[1]. The incidence of human infection is higher in the tropics where conditions for its transmission are favorable, but the disease occurs in both industrialized and developing countries[2]. The genus *Leptospira* is divided into 20 species based on DNA hybridization studies[2], and these 20 species are classified into more than 280 serovars, according to their antigenic relatedness[5], which affect various vertebrate hosts and remain in the environment by a dynamic process through a variety of domestic and wild animals. Leptospires are shed in the urine of carrier animals and the transmission is strongly affected by environmental conditions[2]. In Brazil, serological surveys have shown exposure to *Leptospira* spp. in various captivity and free-living wild species, of which the serovars Castellonis, Hardjo[10], and Copenhageni[15] were the most likely to cause infection in captive jaguars. Pomona was the most prevalent serovar found in free-living sampled jaguars[7,21]. In addition to the death of a female puma in Rio de Janeiro's zoo which showed clinical signs of leptospirosis and titers ≥ 400 to serovar Pomona by MAT[15], the high titers found in free-living neotropical felids in the same biome studied[12] suggest that *Lepstospira* spp. exposure may affect the conservation of wild felids. Due to the fact that transmission occurs mainly in wet environments and that wild animals are relevant in leptospirosis epidemiology, studies are necessary in the Pantanal region to clarify the potential impact of *Leptospira* spp. exposure on wild populations.

The occurrence of brucellosis in humans is highly dependent on the occurrence of the disease in animals' reservoirs, including wildlife[8]. The main clinical signs of *Brucella abortus* in wild mammals are abortion, orchitis, epididymitis and infertility[24]. In Brazil, antibodies against *B. abortus* have been detected in free-living and captive white-lipped and collared peccaries[11,13,18,21], in free-living and captive maned wolves (*Chrysocyon brachyurus*)[16], in a free-living jaguar in the Atlantic Forest[21] and in another in the Emas National Park[12]. The present study aimed to detect antibodies to *Leptospira* spp. and *B. abortus* in jaguars from two conservation units in the Pantanal region, Brazil.

MATERIAL AND METHODS

The studied areas comprised two federal conservation units (Taiamã

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Ecological Station - 16° 50' 34.31" S, 57° 35' 03.70" W, and Pantanal Matogrossense National Park - 17° 50'47. 33" S, 57° 24'12.67" W) in the Pantanal of Mato Grosso, considered one of the largest floodplains in the world. Annual rainfall ranges from 1,200 to 1,300 mm across the region, defining dry and rainy seasons with seasonal fluctuations in water level, which have great influence on ecological processes[17]. The average annual temperature is 25 °C[3].

Eleven jaguars were captured between July 2010 and November 2012, under license granted by the Authorization System and Biodiversity Information - SISBIO, numbers: 30896-1 and 18699-1, immobilized with a combination of tiletamine and zolazepam (Zoletil 100®, Virbac SA, Carros-Cedex, France) and fitted with radio-collars. After clinical examination and collection of biological samples, all animals were released at the same site at which they were captured. Sera blood samples were frozen and stored at -20 °C until testing and analysis at the Biological Samples' Bank of National Research Center for the Conservation of Carnivorous Mammals of Brazil (CENAP/ ICMBio).

Serum samples were examined for different leptospiral antibodies by Microscopic Agglutination Test (MAT)[6] with the cut off 1:100 dilution against the following pathogenic serovars: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whiticombi, Cynopteri, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Mini, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Andamana, Patoc and Sentot, which were cultivated in modified EMJH medium. In addition to the reference strains, eleven Brazilian isolates of *Leptospira* spp. were used in this study: Brasiliense serovar isolated from *Didelphus marsupialis* (Strain 4B), Pomona serovar isolated from domestic *Sus scrofa* (Strain M7/87), Guaricura serovar isolated from *Bubalus bubalis* (Strain M4/98), Copenhageni serovar isolated from *Rattus norvegicus* (Strain M9/99), Canicola serovar isolated from *Canis familiars* (Strain L01), Canicola serovar isolated from domestic *Sus scrofa* (Strain L04), Canicola serovar isolated from *Bos Taurus* (Strain L014), Bananal serovar isolated from *Hydrochaeris hidrochaeris* (Strain 2A CAP), Bananal serovar isolated from *Hydrochaeris hidrochaeris* (Strain 21A CAP), Pomona serovar isolated from domestic *Sus Scrofa* (Strain Gr6) and M110/06 isolated from *Cerdocyon thous* (probably a new species). The positive sera were titrated by testing serial twofold serum dilutions and the reciprocal of the highest serum dilution that showed 50% agglutinated leptospira was defined as the serum titer[20].

For brucellosis, serum samples were examined by the Rose Bengal test (RBT) for screening and 2-mercaptoethanol test (2-ME) as a confirmatory test[14]. The antigen used was an inactive suspension of *B. abortus* 1119-3 produced by the Institute of Technology of Paraná, Brazil.

RESULTS

All animals were considered adults based on tooth wear and color, ranging from four to 10 years old. Only two (18.2%) jaguars tested were seroreactive for *Leptospira* spp. antigen by MAT, one from each conservation unit of this study. The serovar considered as most infective in both animals was a Brazilian isolated antigen, serovar Canicola (L01) with titers = 3200. One of the seropositive animals reacted only to serovars of Brazilian isolate antigens (Canicola L01, T = 3200. Canicola L04, T = 800; Canicola L014, T = 400), and the other animal showed a low titer for serovar Copenhageni (10A), the only reaction for antigen of the reference collection (Canicola L01, T = 3200; Canicola L04, T = 400; Canicola L014, T = 400; Copenhageni 10A, T = 200: Copenhageni M9/99, T = 200). Both animals were in good overall health at the time of capture and no clinical signs were correlated with the infections dealt with in the present paper. All eleven jaguar serum samples were negative for *B. abortus* antigen by RBT.

DISCUSSION

Despite the absence of clinical signs at the time of capture, high antibody titers to serovar Canicola (L01) isolated from *Canis familiaris* in the state of Paraná, southern Brazil, were detected in two jaguars highlighting the importance of using local antigens in serological surveys, usually carried out only with collection of reference antigens performed by MAT. This high titer (T = 3200) also suggests a recent or frequent contact with this or a closely related agent, which probably circulates in these two regions of the northern Pantanal, despite the low frequency of positive animals found in this study (18.2%). Although the specificity of MAT is good, there is significant serological cross-reactivity among serovars that may result in an equal or even higher antibody titer[19]. Therefore, serological tests may suggest, but not definitively identify, the infecting serovar, and isolation of the agent and molecular analysis should be required.

Serological surveys are commonly performed in studies of wildlife exposure to pathogens in South America to determine whether wild animals have been exposed to an antigen, due to the concern of disease transmission across the interface between wildlife and domestic animals. However, local Brazilian antigens have rarely been tested in studies involving free-living species in serological investigations for leptospiral infections. VIEIRA *et al.* (2013)[23] did not detect seropositivity to serovar Canicola (L01) using local strains in a study of exposure to *Leptospira* spp. in wild mammals from the southern Pantanal of Mato Grosso do Sul, and the only other antigen isolated in Brazil of serovar Canicola (L014) was detected in *Cerdocyon thous.* In the present study, the results showed high antibody titers for serovar Canicola, of which the main natural reservoir is the dog, suggesting an occasional contact between jaguars and domestic dogs. This is a concern for the conservation of wild carnivores and requires further investigations in the Pantanal region. Furthermore, the inclusion of autochthonous antigens in serological inquiries should be considered because they can significantly increase the number of reactive animals, as well as modify the epidemiological profile of infections, likewise observed in a study conducted on cattle by SARMENTO *et al.* (2012)[22].

The serological method used to detect *B. abortus* antibodies in the present study is recommended by the Brazilian Department of Livestock Health[14] and is also performed in serological surveys for brucellosis in wildlife[7,21]. FURTADO (2010)[7] reported the contact of a jaguar with *Brucella* sp. in the Pantanal biome, suggesting that predation of cattle infected with *B. abortus* may explain seroconversion. In the present work, although all samples were negative for antibodies against *B. abortus*, zoonotic diseases included in sanitary control programs need to be further investigated in wildlife to assist decision-makers in the development of effective action plans. The negativity of exposure to *B. abortus* in jaguars from these two conservation units suggests a low level of environmental anthropogenic alteration, commonly related to livestock

areas, with consequent predation of cattle. However, the validation of diagnostic techniques, such as serology, requires careful analysis of the isolation of *Brucella* agent, especially when it comes to wildlife[9], in order to determine the possible transmission chain of the disease and the role of certain species in the maintenance of the agent in the environment.

Despite the low number of reactive animals for *Leptospira* antigens and the absence of individuals positive for *B. abortus* antibodies in the present study, more extensive investigations are necessary to determine the likelihood of the impact of infection by these pathogens on the health and reproductive parameters of wild populations. These data are important for the development of management plans of protected areas, as well as for an evaluation of the role played by this species in the epidemiological cycle of this important zoonotic disease.

Preventive measures for *Leptospira* spp. infection, such as water treatment for consumption and chemoprophylaxis, can also be recommended for people who visit these areas for professional reasons or for recreational activities.

RESUMO

Detecção de anticorpos para *Leptospira* spp. e *Brucella abortus* em onças-pintadas (*Panthera onca*) de vida livre em duas áreas protegidas no Pantanal Norte, Brasil

Este estudo teve como objetivo avaliar a exposição de onças-pintadas de vida livre (*Panthera onca*) para *Leptospira* spp. e *Brucella abortus* em duas unidades de conservação no Pantanal de Mato Grosso, Brasil. A presença de anticorpos em amostras de sangue de onze onças foi investigada utilizando antígenos autóctones isoladas no Brasil adicionais a coleção de antígenos de referência aplicada usualmente ao diagnóstico da leptospirose pelo teste de soroaglutinação microscópica (MAT). Para os anticorpos de *B. abortus*, foi utilizado o teste de Rosa Bengala. Duas onças-pintadas (18,2%) foram reagentes para *Leptospira* spp. e o sorotipo considerado como o mais provável pela infecção em ambos os animais foi um isolado brasileiro do sorovar Canicola (L01). Todas as onças-pintadas foram soronegativas para *B. abortus*. Estes dados indicam que a inclusão de antígenos autóctones em estudos sorológicos pode aumentar significativamente o número de animais reativos, assim como modificar a caracterização do sorotipo mais prevalente.

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