Exposure of free-ranging wild carnivores, horses and domestic dogs to *Leptospira* spp in the northern Pantanal, Brazil

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Leptospirosis is a zoonotic disease affecting most mammals and is distributed throughout the world. Several species of domestic and wild animals may act as reservoirs for this disease. The purpose of this study was to assess the exposure of free-ranging wild carnivores, horses and domestic dogs on a private reserve located in the northern Pantanal (Brazil) and the surrounding areas to Leptospira *spp from 2002-2006, 75 free-ranging wild carnivores were captured in the Pantanal and serum samples were collected. In addition, samples from 103 domestic dogs and 23 horses in the region were collected. Serum samples were tested for the presence of* Leptospira *antibodies using the microscopic agglutination test. Thirty-two wild carnivores (42.7%) were considered positive with titres ≥ 100, and 18 domestic dogs (17.5%) and 20 horses (74.1%) were also found to be positive. Our study showed that horses, dogs and several species of free-ranging wild carnivores have been exposed to* Leptospira *spp in the Pantanal, suggesting that the peculiar characteristics of this biome, such as high temperatures and an extended period of flooding, may favour bacterial persistence and transmission. In this region, wild carnivores and horses seem to be important hosts for the epidemiology of* Leptospira *species.*

Key words: *Leptospira* spp - free-ranging wild carnivores - public health - conservation - serology - Pantanal

Leptospirosis is a zoonotic disease caused by members of the pathogenic spirochete genus *Leptospira*, which includes more than 16 genetically identified species and at least 260 serovars that are traditionally categorised into serogroups (Levett 2001). Leptospires are distributed throughout the world and are maintained in nature by a wide variety of wild and domestic animals (Vijayachari et al. 2008). Wild animals are a potential source of infection for humans and domestic animals and the reverse may also occur (Leighton & Kuiken 2001).

Recent studies involving serological screenings of free-ranging and captive wild animals have shown seroreactivity to *Leptospira* spp in Brazil (Souza Júnior et al. 2006, Corrêa et al. 2004, Lilenbaum et al. 2004), including the Pantanal region (Mathias et al. 1999, Girio et al. 2004, Freitas et al. 2010).

In addition to the possibility of *Leptospira* spp causing asymptomatic infections in free-ranging wild animals, this pathogen may cause lethal infections, increasing mortality rates in wild populations (Gulland et al. 1996) and therefore representing a risk to biodiversity conservation.

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The purpose of this study was to assess the exposure of free-ranging wild carnivores, horses and domestic dogs on a private reserve and the surrounding areas in the northern Pantanal to *Leptospira* spp.

MATERIALS AND METHODS

Study area - The Private Natural Heritage Reserve - Social Service of Commerce (RPPN/SESC Pantanal, 106,000 ha) is a private reserve located in the Pantanal of Barão de Melgaço (16º17'S 56º57'W), approximately 100 km from Cuiabá, the capital of the state of Mato Grosso, Brazil.

The RPPN/SESC Pantanal is seasonally flooded and contains a mosaic of vegetation types, including forest and former pasturelands. The area has distinct rainy (October-March) and dry (April-September) seasons and it belongs to climate type Aw, according to the Köppen classification system (dry winters and rainy summers) (Nimer 1989). The mean annual precipitation varies between 1,100-1,200 mm (Antunes 1986). The mean temperature in January (middle of the wet season) is 27-28ºC and in July (middle of the dry season) is 23-24ºC (Antunes 1986). Roads are used only by the park guards and researchers. Motor vehicles are used only during part of the dry season. During most of the year, transportation is by horses or horse-drawn carts only.

Study population and sampling - The study and captures were performed from January 2002-August 2006. Wild carnivore samples included 43 crab-eating foxes (*Cerdocyon thous*), eight maned wolves (*Chrysocyon brachyurus*), one bush-dog (*Speothos venaticus*), 13 crab-

eating raccoons (*Procyon cancrivorous*), four ocelots (*Leopardus pardalis*) and seven pumas (*Puma concolor*). Most wild carnivores were captured using baited box traps, with the exception of the pumas, which were captured with the help of trained hound dogs and an experienced local person. Box traps were placed along dirt roads in the Reserve area where vehicles are rarely used. The traps were activated for periods lasting approximately 10 days in all seasons of the year. Captures were distributed along the Reserve's area, with slightly higher concentrations in the central region and close to the northern border. Animals were sedated with a combination of tiletamine and zolazepam (Zoletil®, Virbac SA, Carros-Cedex, France) for blood collection and clinical examination. In total, 103 domestic dogs from settlements located adjacent to the northern border of the Reserve and 23 horses used for transportation throughout most of the Reserve area primarily were physically constrained for blood collection and clinical examination. The area from where these horses originated was not determined. Blood samples were allowed to clot and then centrifuged within 24 h of collection. Sera were removed, frozen and stored at -20ºC until tested.

The necessary permits for trapping and the subsequent procedures were obtained by the Brazilian government environmental agency [Brazilian Institute of Environment and Renewable Natural Resources (IBAMA)] (license 02027.007051/03-70). Procedures were approved by the Bioethics Commission of the Faculty of Veterinary Medicine and Zootechny of the University of São Paulo (protocol 370/2003).

Detection of antibodies to Leptospira spp - Antibodies to *Leptospira* spp were detected by the microscopic agglutination test (MAT) (Faine et al. 2000). Samples with antibody titres (T) ≥ 100 were considered seroreactive. Antigens used in this test included the following pathogenic serovars of *Leptospira* spp: Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes, Sentot, Wolffi (*Leptospira interrogans*), Castellonis, Javanica, Mini, Tarassovi, Whitcombi (*Leptospira borgpetersenii*), Butembo, Cynopteri, Grippotyphosa, (*Leptospira kirschneri*), Panama (*Leptospira nogushii*), Brasiliensis and Shermani (*Leptospira santarosai*). Hyperimmune sera produced in rabbits obtained from BgvV Laboratory (Berlin, Germany) and presenting a minimum anti-serum T of 12,800 were used as positive controls. Sera that previously showed negative results for the MAT with the same antigens used in this study were used as negative controls.

Data analysis - Frequencies of occurrence and the respective confidence intervals were calculated for all carnivores as a group and separately for domestic dogs and horses using the Minitab 14 software.

RESULTS

The frequency of seroreactive wild carnivores was 42.67% (31.21, 54.12). The frequency of seroreactive crab-eating foxes was 39.53% (17/43) for serovars Autumnalis (2 individuals with T = 1,600, 1 with T = 800, 1 with T = 200, 2 with T = 100), Pyrogenes (1 with T = 3,200, 2 with T = 1,600, 1 with T = 200), Canicola (2 with T = 400, 1 with T = 200, 1 with T = 100), Canicola/Pyrogenes (1 with T = 800), Pomona (1 with T = 100) and Australis (1 with T = 100). The frequency of seroreactive maned wolves was 37.5% (3/8) for serovars Autumnalis (1 with T = 800), Pyrogenes (1 with T = 200) and Canicola (1 with T = 100). The frequency of seroreactive crab-eating raccoons was 50% (6/12) for serovars Canicola (1 with T = 1,600, 1 with T = 100), Pomona (1 with T = 800, 1 with T = 400) and Pyrogenes (1 with T = 800, 1 with T = 400). The frequency of seroreactive pumas was 28.57% (2/7) for serovars Autumnalis (1 with T = 1,600) and Bratislava (1 with T = 1,600). The frequency of seroreactive ocelots was 75% (3/4) for serovars Pomona (1 with T = 800), Canicola (1 with T = 400) and Pyrogenes (1 with T = 100). The only bush dog sampled was seroreactive for three serovars (Autumnalis, Pomona and Hardjo) and showed the same T for each (T = 800).

Among the domestic dogs, 18 (17.48%) were seroreactive (10.02, 24.93) to *Leptospira* spp serovars Pyrogenes (1 with T = 800, 3 with T = 400, 3 with T = 200), Autumnalis (3 with T = 200), Canicola (2 with T = 200), Hebdomadis (2 with T = 200), Sentot (2 with T = 200), Wolffi (1 with T = 400), Hardjo/Wolffi (1 with T = 200) and Canicola/Icterohaemorrhagiae/Pyrogenes (1 with T = 200).

Among the wild horses, 19 (70.37%) were seroreactive (53.72, 88.89) to *Leptospira* spp serovars Bratislava (1 with T = 400, 2 with T = 200, 1 with T = 100), Icterohaemorrhagiae (3 with T = 200, 1 with T = 100), Autumnalis (1 with T = 400, 2 with T = 200), Hebdomadis (1 with T = 800, 1 with T = 200), Pyrogenes (1 with T = 400), Bratislava/Autumnalis (1 with T = 200), Autumnalis/Castellonis/Icterohaemorrhagiae (1 with T = 200), Bratislava/Autumnalis/Icterohaemorrhagiae (1 with T = 200, 1 with T = 100) and Bratislava/Autumnalis/Pomona (1 with T = 200).

DISCUSSION

The detection of exposed wild carnivores, dogs and horses in our study shows the complexity of the *Leptospira* spp epidemiologic chain in the Pantanal. Previous serosurveys of free-ranging animals to *Leptospira* spp in this biome have also detected seroreactivity in the sampled populations (Mathias et al. 1999, Girio et al. 2004, Freitas et al. 2010). The peculiar characteristics of this biome, such as the extended period of flooding and elevated temperatures throughout the year, seem to favour the persistence and transmission of *Leptospira* species.

Wild carnivores and horses presented high seroreactivity frequencies. Other studies involving *Leptospira* spp serosurveys in Pantanal wildlife have detected seroreactive frequencies of 24% (4 of 17) (Mathias et al. 1999) and 9.7% (4/41) (Girio et al. 2004) in pampas deer (*Ozotoceros bezoarticus*) and 70% (49/70) in white-lipped peccaries (*Tayassu pecari*) (Freitas et al. 2010). Wild carnivores routinely cover vast areas to obtain their dietary requirements, passing frequently through flooded sites. They may also be exposed to *Leptospira* spp by consuming infected prey (Reilly et al. 1970), such as rodents, which are considered important *Leptospira* spp

reservoirs and are frequently preyed on by carnivores. In addition, horses travel long distances in the Reserve, especially during the wet season, when motor vehicles are unable to use the dirt roads and horses are required for transportation. Thus, both these groups of animals seem to be highly exposed to *Leptospira* spp.

In contrast, domestic dogs presented a relatively low seroreactivity frequency compared to the other groups tested in this study. One possible explanation is most dogs are kept as pets in the region, with the exception of the ones used for hunting. Therefore, most dogs usually remain close to houses and dry areas during flood periods.

Reserve guards, field researchers and village inhabitants also have an increased risk of exposure to *Leptospira* spp, especially during the wet season, when they normally require horses for transportation and their legs and feet are in contact with water for long time periods (personal observation). As leptospirosis is an important zoonosis and wild and domestic animals have been exposed to the agent in the region, it is important to assess the exposure of humans who work or visit the RPPN/SESC Pantanal or live in the surrounding areas.

Few of most frequently detected serovars in our study were similar to those detected in other studies in the Pantanal. While we detected Canicola (9), Pyrogenes (8) and Autumnalis (8) among wild carnivores, Pyrogenes (8) and Autumnalis (3) among dogs and Icterohaemorrhagiae (5) and Autumnalis (4) among horses, previous research has found different serovars in these animals. Girio et al. (2004) detected Pomona, Wolffi, Icterohaemorrhagiae and Copenhageni, Mathias et al. (1999) detected Hardjo, Mini and Wolffi and Freitas et al. (2010) have detected Icterohaemorrhagiae, Autumnalis, Pomona, Bataviae and Copenhageni. Although these analyses were all performed in the Pantanal, our study site was located in MT, in the northern Pantanal, while the previous studies were performed in the state of Mato Grosso do Sul. The nearest of these was located in the region of Corumbá (Freitas et al. 2010), which is approximately 400 km southwest of our study site. This difference might explain our frequent detection of serovars such as Canicola and Pyrogenes, which were not detected in other studies.

These results suggest that the serovars detected in our study have been circulating in the northern region of the Pantanal. However, the possibility of cross-reactivity among serovars in the MAT should not be discarded (Kingscote 1986).

The antibody T detected in our study were higher in wild carnivores than in horses and dogs. While we detected a $T \geq 800$ in 15 carnivores, including a crab-eating fox with an antibody T of 3,200 for Pyrogenes and seven individuals with T of 1,600 (4 crab-eating foxes: 2 for Pyrogenes and 2 for Autumnalis; 2 pumas: 1 for Pyrogenes and 2 for Autumnalis, and one crab-eating raccoon: Canicola), only one horse (Hebdomadis) and one dog (Pyrogenes) presented T of 800. This result might be explained by the more recent and/or frequent exposure of wild carnivores to *Leptospira* species. In addition, T in our study were higher than those of other studies with wild animals in the Pantanal. For pampas deer, T ranging from 100-200 were detected in four seroreactive animals (Mathias et al. 1999), and Girio et al. (2004) reported T ranging from 100-800 in 64 seroreactive individuals of several wild and domestic animal species, with the exception of a sheep (*Ovis aries*), which had a reported T of 3,200. Among 49 seroreactive peccaries, Freitas et al. (2010) detected three animals with a T of 1,600 (Autumnalis, Canicola, Autumnalis/Icterohaemorragiae) and two with a T of 800 (Autumnalis and Copenhageni).

Mammalian species that are highly susceptible to infection by *Leptospira* spp serovars but manifest little or no clinical disease may act as maintenance hosts of one or more serovars in an ecosystem. Other mammals that are normally less susceptible to infection but suffer clinical disease more frequently are potential accidental hosts (Leighton & Kuiken 2001). The high frequency of seroreactive wild carnivores and horses suggests that they may act as "maintenance hosts" for specific serovars in the RPPN/SESC Pantanal region. However, further studies are needed to more clearly identify differences in seroreactivity frequencies between species of carnivores, determine whether they develop clinical disease and identify serovars using molecular tools among other analyses needed to test these hypotheses.

The crab-eating fox was the most frequently sampled species and presented a considerably high seroreactivity frequency. The crab-eating raccoon has a close phylogenetic relation with the North American raccoon, which is considered a *Leptospira* spp reservoir in the state of Illinois, USA (Mitchell et al. 1999). The high proportion of exposed crab-eating raccoons (6/12) and crab-eating foxes in our study suggests that they may play a role in the maintenance of the agent in the Pantanal region. However, future studies involving the experimental infection of these species with controlled parameters, such as an inoculation dose, shedding time and T of shed leptospiras, among others, are needed to clarify the possible role of these animals in the epidemiology of *Leptospira* spp in the wild.

A puma died in Rio de Janeiro's Zoo after showing clinical signs of leptospirosis (Lilenbaum et al. 2004). Pathological lesions were also suggestive of *Leptospira* spp exposure and serology showed an antibody T of 400 for serovar Pomona. The detection of seropositive wild felids, including two pumas with antibody T of 1,600 for serovars Bratislava and Autumnalis and three ocelots with antibody Ts of 800, 400 and 200 for serovars Pomona, Canicola and Pyrogenes, respectively, suggests that populations of wild felids species in our study region might be affected by *Leptospira* spp exposure. This is a concern for the conservation of wild felids and other carnivores in the Pantanal and should be further investigated.

Horses presented the highest seroreactivity frequency to *Leptospira* spp (74.07%) among all species sampled in our study. Therefore, their role in the maintenance of the agent in the RPPN/SESC Pantanal should not be overlooked. However, the total number of horses in the Reserve is approximately 30, significantly lower than the number of individuals of different species of wild mammals. Nonetheless, there is a significant population of horses and livestock in the areas surrounding the Reserve.

The role of these animals in the epidemiology of *Leptospira* spp in the region should be further investigated.

Our study shows that horses, dogs and several species of free-ranging wild carnivores have been exposed to *Leptospira* spp in the region of the RPPN/SESC Pantanal, suggesting that the peculiar characteristics of the Pantanal biome, such high temperatures and an extended flooding period, may favour *Leptospira* persistence and transmission. Furthermore, these data suggest that wild carnivores and horses are important for epidemiology of the agent, and our results also indicate the potential impact of *Leptospira* spp exposure on the conservation of wild carnivores in the Pantanal. However, further studies should be conducted to clarify these issues. To our knowledge, this is the first report of free-ranging crab-eating raccoons and ocelots exposed to *Leptospira* spp.

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