

Occurrence of antibodies anti -*Toxoplasma gondii*, *Neospora caninum* and *Leptospira interrogans* in a captive deer herd in Southern Brazil

Ocorrência de anticorpos anti -*Toxoplasma gondii*, *Neospora caninum* e *Leptospira interrogans* em um rebanho de cervídeos de cativeiro do sul do Brasil

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Abstract

A large number of Brazilian zoos keep many endangered species of deer, however, very few disease surveillance studies have been conducted among captive cervids. Blood samples from 32 Brazilian deer (*Blastocercus dichotomus*, *Mazama nana* and *Mazama americana*) kept in captivity at Bela Vista Biological Sanctuary (Foz do Iguaçu, Brazil) were investigated for 10 ruminant pathogens, with the aims of monitoring deer health status and evaluating any potential zoonotic risk. Deer serum samples were tested for *Brucella abortus*, *Leptospira* (23 serovars), *Toxoplasma gondii*, *Neospora caninum*, bovine viral diarrhoea virus, infectious bovine rhinotracheitis virus, foot-and-mouth disease virus, western equine encephalitis virus, eastern equine encephalitis virus and Venezuelan equine encephalitis virus. Antibodies against *T. gondii* (15.6%), *N. caninum* (6.2%) and *L. interrogans* serogroup Serjoe (3.1%) were detected. The serological results for all other infectious agents were negative. The deer were considered to be clinically healthy and asymptomatic regarding any disease. Compared with studies on free-ranging deer, the prevalences of the same agents tested among the captive deer kept at the Sanctuary were lower, thus indicating good sanitary conditions and high-quality management practices at the zoo.

Keywords: Deer, *Leptospira*, *Neospora caninum*, serosurvey, *Toxoplasma gondii*, zoo.

Resumo

Um grande número de zoológicos brasileiros abriga espécies de cervídeos ameaçados de extinção, entretanto, estudos de vigilância de doenças em cervídeos de cativeiro são escassos. Amostras de sangue de 32 cervídeos brasileiros (*Blastocercus dichotomus*, *Mazama nana* e *Mazama americana*), mantidos em cativeiro no Refúgio Biológico Bela Vista (Foz do Iguaçu, Brasil), foram investigados para 10 patógenos de ruminantes, visando monitorar o estado de saúde dos cervídeos e avaliar a presença de agentes zoonóticos. As amostras de soro foram testadas para *Brucella abortus*, *Leptospira* (23 sorovares), *Toxoplasma gondii*, *Neospora caninum*, diarréia viral bovina, rinotraqueíte infecciosa bovina, febre aftosa, encefalomielite equina do oeste, encefalomielite equina do leste e encefalomielite equina venezuelana. Foram detectados anticorpos para *T. gondii* (15,6%), *N. caninum* (6,2%) e para *L. interrogans* sorogrupo Serjoe (3,1%). As sorologias apresentaram resultado negativo para as demais doenças. Os cervídeos foram considerados clinicamente sadios e assintomáticos para doenças. Comparados aos estudos de populações de vida livre, as soroprevalências para os mesmos agentes testados foram menores para os cervídeos mantidos no Refúgio, indicando as boas condições sanitárias e a qualidade das práticas de manejo no zoológico.

Palavras-chave: Cervídeo, *Leptospira*, *Neospora caninum*, sorologia, *Toxoplasma gondii*, zoológico.

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Disease surveillance has an essential role in the management of wild animals in captivity. Zoo animals are susceptible to a wide range of infectious agents, due to their closer contact with humans and other captive animals, as well as their confinement in herds, depending on the species (MURAKAMI et al., 2012). Moreover, living conditions in captivity differ greatly from the conditions of animals living in the wild. Captive animals may be more exposed to stress and behavioral changes, which might lead to fragile health status (PEARSON et al., 2015). A large number of Brazilian zoos are responsible for captive deer conservation and breeding programs, however, disease surveillance studies among captive deer are scarce because of handling difficulties and high mortality.

Serological and molecular studies among free-ranging Brazilian deer have suggested that deer are exposed to a wide spectrum of parasitic, bacterial and viral pathogens, such as *Toxoplasma gondii* (FERREIRA et al., 1997), *Neospora caninum* (TIEMANN et al., 2005a), *Babesia* spp. and *Theileria* spp. (SILVEIRA et al., 2011), *Ehrlichia chaffeensis* and *Anaplasma* spp. (MACHADO et al., 2006; SACCHI et al., 2012), *Mycoplasma ovis* (GRAZZIOTIN et al., 2011a), *Leptospira* spp. (VIEIRA et al., 2011) and foot-and-mouth disease virus (ARAUJO et al., 2010). To date, only two studies related to neosporosis and mycoplasmosis investigations have been conducted among captive deer in Brazil (TIEMANN et al., 2005b; GRAZZIOTIN et al., 2011b). In the present work, we investigated the serological profile of a ruminant pathogen panel in a population of deer (*Blastocerus dichotomus*, *Mazama nana* and *Mazama americana*) kept in captivity at Bela Vista Biological Sanctuary (BVBS), with the aims of monitoring deer health status and evaluating any potential zoonotic risk. Considering that BVBS maintains one of the largest captive herd of *Mazama* spp. in Brazil and that little is known about their health status, this population study may provide relevant information on deer management, diseases surveillance and zoonotic risk for other conservation units.

BVBS (25° 26' 57" S and 54° 33' 18" W) is an environmental conservation unit in the Parana river basin, located in the area of the Itaipu Binacional hydroelectric power plant (Foz do Iguaçu, Brazil) (Figure 1). The sanctuary is committed to protection, maintenance, rehabilitation and reintroduction of threatened wild animals, along with breeding programs for these species. The region is considered to be a World Heritage Site (UNESCO/WHC, 2015) and is home to a diversity of endangered species, including deer. The marsh deer (*B. dichotomus*) has been listed as a vulnerable species since 1982 because of past and current habitat losses (DUARTE et al., 2008a), while insufficient information is available for the Brazilian dwarf brocket deer (*M. nana*) and red brocket deer (*M. americana*) (APRIL & DUARTE, 2008; DUARTE et al., 2008b). Similarly, little is known regarding deer exposure to infectious agents, or regarding the role that captive deer might play as reservoirs for such agents.

Serum samples were collected from 32 animals (22 *M. nana*, six *B. dichotomus* and four *M. americana*) kept at BVBS. All the deer are kept in areas demarcated by metal fences, in small groups of the same species. A group of six marsh deer and another group of three dwarf brocket deer are located in the zoo area for exhibition in distinct enclosures apart from one another, while the other 23 deer

(20 Brazilian dwarf brocket and three red brocket deer) are kept alone or in small groups (3-5 animals) in the breeding area, away from the zoo touristic route. Animals were chemically immobilized using a blowgun whose darts contained an association of 10% ketamine hydrochloride (7 mg/kg) and 2% xylazine hydrochloride (0.5 mg/kg) for *M. nana* and *M. americana*, whereas 20% zolazepam (3 mg/kg) and 2% xylazine hydrochloride (0.5 mg/kg) were administered for *B. dichotomus*. Blood samples were collected by means of jugular venipuncture using vacuum tubes without anticoagulant. The samples were clotted and centrifuged, and the serum samples were stored at -20 °C until assayed. This study was approved by the federal regulatory agency IBAMA (Brazilian Institute for the Environment and Renewable Resources) under the protocol number 22.158-2 (November 12, 2009) and was conducted in accordance with IBAMA's ethical rules.

All serological tests were conducted at the Biological Institute of São Paulo, Brazil, according to the established and internationally recommended protocols of the World Organization for Animal Health regulations (OIE, 2008). Serum samples were tested for antibody activity against 10 infectious agents. Serum neutralization assays were used to detect antibody activity against bovine viral diarrhea virus (BVD) (NADL strain) (EDWARDS, 1990), infectious bovine rhinotracheitis virus (IBR) (herpesvirus type 1, Los Angeles strain) (BITSCH, 1978), western equine encephalitis virus (WEE) (Tatuí strain), eastern equine encephalitis virus (EEE) (TR25717 strain) and Venezuelan equine encephalitis virus (VEE) (OIE, 2008). Cutoff titers of 1:10 for BVD, 2 (1:4) for IBR and 10 for EEE, WEE and VEE were used. Agar gel immunodiffusion was performed for foot-and-mouth disease virus (FMD): samples were considered positive when a line appeared between two consecutive wells (MCVICAR & SUTMOLLER, 1970). Presence of antibodies against 23 *Leptospira* serovars (see Table 1 for details) was tested by means of the microscopic agglutination test (MAT) (OIE, 2008) and against *Brucella abortus* 1119-3 strain by means of the complement fixation test (CFT) (OIE, 2008) and rose Bengal test (RBT) (MORGAN et al., 1969). Serum samples giving titers greater than or equal to 1:100 were considered positive for

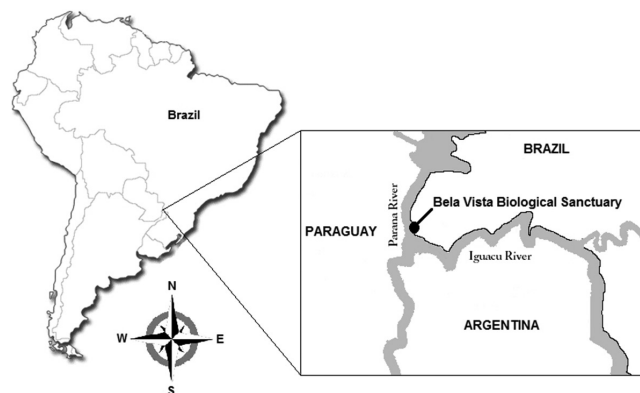


Figure 1. Sampling location for captive deer in Brazil. Bela Vista Biological Sanctuary (25° 26' 57" S and 54° 33' 18" W) belongs to the world's largest hydroelectric power plant (Itaipu Binacional) and is located in a large environmental conservation area shared by Paraguay, Argentina and Brazil.

Table 1. Prevalence of ten infectious disease agents in the Brazilian deer population.

Pathogen tested ^a	Method ^c	<i>B. dichotomus</i>	<i>M. nana</i>	<i>M. americana</i>
BVD virus	SN	0/6	0/22	0/4
IBR virus	SN	0/6	0/22	0/4
FMD virus	AGID	0/6	0/22	0/4
<i>Leptospira</i> spp. ^b	MAT	1/6	0/22	0/4
<i>Brucella abortus</i>	RBT, CT	0/6	0/22	0/4
<i>Neospora caninum</i>	ELISA	1/6	1/22	0/4
<i>Toxoplasma gondii</i>	IFAT	2/6	2/22	1/4
EEE virus	SN	0/6	0/22	0/4
WEE virus	SN	0/6	0/22	0/4
VEE virus	SN	0/6	0/22	0/4

^aBVD, Bovine viral diarrhea; IBR, Infectious bovine rhinotracheitis; FMD, Foot and mouth disease; EEE, Eastern encephalitis equine; WEE, Western encephalitis equine; VEE, Venezuelan encephalitis equine. ^bList of *Leptospira* spp. tested in this study according to their serogroup and serovar (within parenthesis) designation: a) *Leptospira borgpetersenii* serogroup Ballum (serovar castellonis), Javanica (javanica), Mini (mini), Tarassovi (tarassovi), and Celledoni (whitcombi); b) *Leptospira interrogans* serogroup Australis (serovar australis and bratislava), Autumnalis (autumnalis), Bataviae (bataviae), Canicola (canicola), Icterohaemorrhagiae (icterohaemorrhagiae and copenhageni), Hebdomadis (hebdomadis), Pomona (pomona), Pyrogenes (pyrogenes), Sentor (sentor), and Serjoe (hardjo and wolffi). c) *Leptospira kirschneri* serogroup Autumnalis (butembo), Cynopteri (cynopteri), Grippotyphosa (grippotyphosa), Panama (panama), and Shermani (shermani). ^cSN, serum neutralization; AGID, agar gel immunodiffusion; MAT, microscopic agglutination test; RBT, rose Bengal test; CT, complement fixation; ELISA, enzyme-linked immunodiffusion assay; IFAT, indirect fluorescent antibody test.

Leptospira spp., whereas 20 international complement fixation test units per mL and any visible reaction were considered positive in the CFT and RBT, respectively. An ELISA test for antibodies against *N. caninum* (HerdChek *Neospora caninum* Antibody Test Kit, IDEXX Laboratories Inc., Maine, USA) was used according to manufacturer's instructions. As a confirmatory assay, positive samples were then tested by means of a *N. caninum* strain NC-1 indirect fluorescent antibody test (IFAT) (CONRAD et al., 1993) using anti-bovine IgG (cutoff titer of 1:25). The serological analysis on *T. gondii* was performed by means of IFAT using anti-bovine IgG antibodies (cutoff titer of 1:64) as recommended by manufacturer (Imunoteste[®] *Toxoplasma gondii*, IMUNODOT Diagnósticos Ltda., Jaboticabal, Brazil). Positive and negative controls of bovine origin were used for all tests.

All the deer were in good body condition with no evidence of diseases at the time of physical examination and blood sampling. Remarkably, all the animals recovered well from the anesthesia procedure and no mortality was registered. Regarding the serological panel tests, 15.6% (5/32), 6.2% (2/32) and 3.1% (1/32) of the animals were positive for *T. gondii*, *N. caninum* and the *L. interrogans* serogroup Serjoe, respectively. All the serum samples were negative for antibody activity against *B. abortus*, BVD, IBR, FMD, EEE, WEE and VEE (Table 1).

A wide range of animals may be infected by the apicomplexan protozoa *N. caninum* and *T. gondii*. The definitive hosts for *N. caninum* are dogs and wild canids (DUBEY et al., 2011; MUNHOZ et al., 2013) while for *T. gondii*, cats and wild felids (ARAMINI et al., 1998; DUBEY & PROWELL, 2013) are the definitive hosts. Oocysts shed in the environment by the definitive hosts may be ingested by susceptible animals such as wild ruminants causing abortion in sheep, goats and cattle (MORENO et al., 2012; HOAR et al., 1996). Although there is no evidence of abortion in deer, several serological studies have indicated that these wild ruminants might be reservoirs for both protozoa (DUBEY et al., 2009). Among 32 captive deer at BVBS, 15.6% and 6.2% were positive for *T. gondii* and *N. caninum*,

respectively. These prevalences were lower than in other serological surveys conducted in Brazil. In relation to *T. gondii*, 27.2% (18/66) of the free-ranging marsh deer in the Pantanal region (marshland at 19° 50' S and 56° 33' W) and 12.2% (5/41) of the pampas deer (*Ozotoceros bezoarticus*) in a national park (dry region at 18° 15' S and 52° 53' W) were seropositive (FERREIRA et al., 1997). Among 150 *Mazama* spp. in captive herds and zoos located in different regions of Brazil, 42% were positive for *N. caninum* (TIEMANN et al., 2005b) while among free-ranging pampas deer, 13% (3/23) of the animals in a national park and 75% (12/16) of the animals in the Pantanal region were positive (TIEMANN et al., 2005a). The prevalences found at BVBS may be related to the proximity between the deer's zoo areas and the other wild mammals' zoo areas, including canids and felids, which if infected by *N. caninum* and *T. gondii*, might be the source of infection for zoo herbivores. Previous serological studies showed that 66.6% (38/57) of the felids at BVBS were exposed to *T. gondii* (ULLMANN et al., 2010). In addition, the housekeeping staff observed sporadic presence of feral cats in the zoo area, which could be playing a role in the oocysts dissemination. Nonetheless, we cannot rule out the possibility of congenital transmission, which has already been demonstrated in relation to *N. caninum* among white-tailed deer (DUBEY et al., 2013).

Leptospirosis has been a management concern in zoos in which there is greater animal density than would be expected in nature (LELU et al., 2015). Infected deer may show subclinical or clinical disease evolving to death (FAIRLEY et al., 1984; TONIN et al., 2011). Moreover, these animals have been also indicated as reservoir for *Leptospira* spp. (JORI et al., 2014). A serological comparison between two distinct pampas deer populations showed that there was no exposure to *Leptospira* spp. in the population from a dry region (Emas National Park, Goiás, Brazil) while 9.7% (4/41) (GIRIO et al., 2004) and 24% (4/17) (MATHIAS et al., 1999) of the animals from the wetland region (Pantanal, Mato Grosso do Sul, Brazil) were seropositive for the serogroups Serjoe and Mini. In contrast, none of the ten *M. americana* investigated were positive

in Pantanal (GIRIO et al., 2004). At BVBS, only one animal (3.1%) was positive for the serogroup Serjoe. A previous serosurvey for *Leptospira* spp. conducted among felids at BVBS showed that 3.5% (2/57) of the animals were positive for the serogroups Cynoptery and Autumnalis (ULLMANN et al., 2012). Although these findings showed distinct serogroup patterns between felids and deer, it is not known whether the felids are transmitting the agent to the deer via shedding into the environment. Regarding the low prevalence and lack of clinical signs of disease in the deer herd and also in the felids, leptospirosis does not seem to pose an animal management concern at BVBS. However, since maintenance of *Leptospira* spp. in the environment is related to humid weather conditions but the blood collections were made during the dry season, seropositivity rate may vary over time. Since leptospirosis may affect humans and is easily transmitted, serological indications should be taken into consideration in zoonosis prevention programs in zoos.

Regarding BVBS management practices and building areas, deer maintained for zoo exhibition are separated from those placed in the breeding area. These areas are distant from one another, preventing direct and indirect contact among groups kept in distinct locations. At the zoo area, 4/9 (44.4%) of deer were *T. gondii*, *N. caninum* and *L. interrogans* serogroup Serjoe seropositive, while only 3/23 (13%) of deer showed anti-*Toxoplasma* and anti-*Neospora* antibodies at the breeding area. Regarding to animal origin, 25/32 (78%) of the animals were born in captivity comprising 6/25 (24%) of the seropositive animals whereas only 1/7 (14%) of those free-living born animals was seropositive and this individual was located in the zoo area. The distinct seroprevalence between groups could be possibly related to the occasional presence of feral cats in the zoo area that might be reservoirs of *T. gondii* for the zoo animals and to the proximity between the enclosures of the crab-eating fox (*Cerdocyon thous*), jaguar (*Panthera onca*) and ocelot (*Leopardus pardalis*) to the deer's enclosures. In contrast, the 23 deer distributed in 13 enclosures separated by fences at the breeding area have no proximity with other species. The overall low seroprevalence of deer for the tested agents at BVBS compared to other previous studies is possibly related to several effective management practices, including the distance between zoo and breeding areas, limited animal transfers between both areas, distinct housekeeping staff teams for zoo and for breeding areas, stringent animal isolation during quarantine for all new arrivals from other institutions or from wildlife, periodic fecal and hematological examinations as well treatments for parasites, periodic pest control, hygiene and health care procedures while handling different species during the workday, the cleanness of enclosures and the periodic housekeeping staff supervision.

Zoos, conservation units and breeding stations are responsible for genetic conservation of wild animals, especially with regard to endangered species. Management strategies for captive animals in such settings are essential for preventing diseases and animal losses. In our serological surveillance, we found that captive deer have been exposed to *T. gondii*, *N. caninum* and *L. interrogans* serogroup Serjoe at some point during their lives. Previous studies showed that felids from the same zoo areas were also exposed to *T. gondii* and *Leptospira* spp. (ULLMANN et al., 2010; ULLMANN et al., 2012). In these studies and ours, tested felids

and deer were asymptomatic indicating that these animals could be playing roles as reservoirs of the mentioned pathogens. Within our studied deer population, those animals located in the breeding areas showed lower positivity compared to the ones located in the zoo areas. It was possibly due to the greater distance between deer and other animals in the breeding area compared to the zoo area and no presence of feral cats, decreasing the risk of inter-species transmission. Moreover, the seroprevalence rates found for deer at BVBS were lower than those previously reported in other deer populations and may reflect the good sanitary conditions and high-quality zoo practices. Finally, more disease surveillance studies in other captive deer populations will be important for comparative analysis and to establish the most suitable deer management protocol.

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