SHORT COMMUNICATIONS

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Pathogen Surveillance in Free-ranging Giant Otters (*Pteronura brasiliensis*) in Three Ecoregions of Brazil

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ABSTRACT: The giant otter (*Pteronura brasiliensis*) is the largest river otter in the world and globally classified as endangered. Little information is available regarding pathogen exposure in this species due to low densities and the challenges associated with capturing individuals in the wild. Serological assays were used to investigate exposure of 10 free-ranging giant otters from different Brazilian ecoregions to canine distemper virus (CDV), parvovirus, rabies virus, *Leptospira* spp., smooth *Brucella*, and *Toxoplasma gondii*. Molecular assays were conducted to assess the presence of *Hepatozoon* spp., *Cytauxzoon* spp., and *Babesia* spp. Serum antibodies were detected for CDV (5/10), canine parvovirus (2/10), rabies virus (1/7), *Leptospira* spp. (2/10), and *T. gondii* (3/10). None of the giant otters were exposed to smooth *Brucella*. Of the individuals that received molecular testing, 2/6 were positive for *Hepatozoon* spp., and 0/6 were positive for *Babesia* spp. or *Cytauxzoon* spp. These results provide the first data on pathogen exposure in free-ranging giant otters. Long-term monitoring of these populations is essential to determine which pathogens are endemic and which may pose a conservation threat to giant otters.

Key words: Canine distemper virus, Conservation Medicine, infectious disease, Mustelidae, parvovirus, *Toxoplasma gondii*.

Giant otters (*Pteronura brasiliensis*) are the largest river otters in the family Mustelidae, serving as top predators of aquatic ecosystems and important bioindicators of the conservation status of rivers, lakes, and swamps. Classified as endangered by the IUCN, they are endemic to South America; currently, in Brazil, they occur in the Amazon, Cerrado, and Pantanal ecoregions (Groenendijk et al. 2023). Historically hunted for their pelts, giant otters are now threatened by habitat loss, water body contamination, and human conflict (Leuchtenberger et al. 2018). Studies on their ecology and conservation have been conducted (Duplaix et al 2015; Leuchtenberger et al. 2018), but many knowledge gaps remain regarding their health and pathogen exposure.

All otters species (subfamily Lutrinae) are susceptible various pathogens, including canine distemper virus (CDV), parvovirus, *Toxoplasma gondii*, and *Leptospira* spp. (White et al. 2013; Echenique et al. 2018; Sanders et al. 2020). In California, USA, *T. gondii* has caused mortality in southern sea otters (*Enhydra lutris nereis*); however, it does not seem to have negatively affected American river otters (*Lontra canadensis*) in North Carolina, USA (Shapiro et al. 2019; Sanders et al. 2020), underscoring the importance of evaluating pathogen-host relationships in

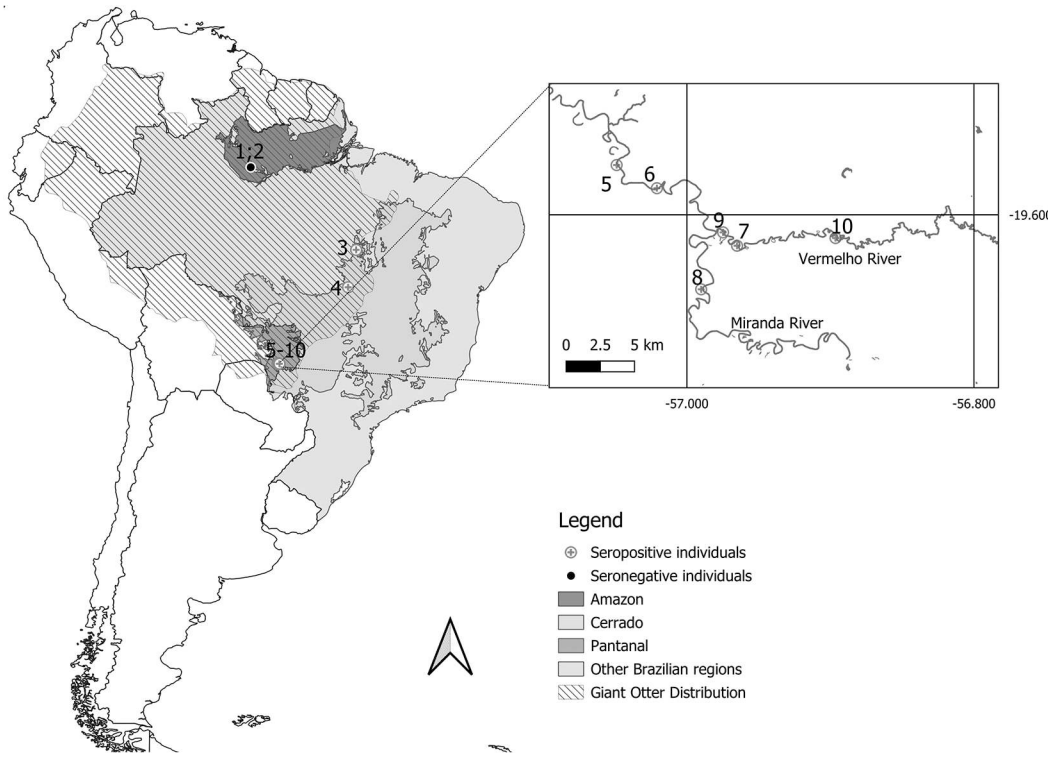

FIGURE 1. Geographic distribution of five capture sites for 10 free-ranging giant otters (*Pteronura brasiliensis*; labeled by individual otter identification numbers) across three Brazilian ecoregions (Amazon, Cerrado, Pantanal). Symbols indicate whether at least one individual sampled at a given site tested positive for one or more investigated pathogens, or if all individuals tested negative for the surveyed pathogens. Pathogens screened included canine distemper virus (CDV), parvovirus, rabies virus, *Leptospira* spp., *Toxoplasma gondii*, smooth *Brucella*, *Hepatozoon* spp., *Babesia* spp., and *Cytauxzoon* spp. Animals captured in the Pantanal are presented in detail in the inset box, which highlights the spatial distribution of individual capture sites along the Miranda and Vermelhor rivers. (Refer to Table 1 for additional details regarding captured individuals and observed pathogens).

the context of species, geography, and environmental conditions. We aimed to investigate the exposure of free-ranging giant otters in Brazil to pathogens of potential conservation concern (CDV, parvovirus, rabies virus, *Leptospira* spp., *T. gondii*, smooth *Brucella*, and *Hepatozoon*, *Babesia*, and *Cytauxzoon* spp.), to establish baseline data for this endangered species.

We conducted this study at five sites across three ecoregions in Brazil (Fig. 1): site 1) the Balbina Hydroelectric Power Plant reservoir (BHPP; 1°55′18″S, 59°29′11″W), located in the Uatumã-Trombetas moist forest, in the Amazon ecoregion; site 2) the Cantão State Park (CSP; 9°21′32″S, 49°58′36″W); and site 3) the Meandros do Araguaia Environmental Protection Area (MAR; 12°38′51″S, 50° 41′04″W), both protected areas situated along the Araguaia River in the Cerrado ecoregion; site 4) the Miranda River (MR; 19°34′36″S, 57°2′45″W); and site 5) its tributary, the Vermelho River (VR; 19°20′24″S, 57°0′36″W), both in the Pantanal ecoregion.

Between August 2007 and July 2017, 10 free-ranging giant otters from different social groups were captured during the dry seasons across the study areas (two in BHPP, one in CSP and one in MAR, three in MR and three in VR). We captured otters using a funnel-shaped net trap installed at the entrance of dens (Silveira et al. 2011). We chemically immobilized otters with a combination of tiletamine-zolazepam (Zoletil; Virbac, Carros-Cedex, France) with a mean dose of 3 mg/kg intramuscularly, administered with a

syringe. When necessary, this was supplemented with additional doses of tiletamine-zolazepam, ketamine, or ketamine–midazolam. Once fully immobilized, heart rate, respiratory rate, and rectal temperature were monitored at 10-min intervals. Otters were weighed, their dental arches were analyzed, neck coat patterns were recorded, and thorough physical examinations were conducted. Otters were classified as adults (>2.5 yr), subadults (1.5–2 yr), or juveniles (6–18 mo), based on tooth wear and body weight as described (Groenendijk et al. 2005). Blood samples were collected by cephalic, jugular, or caudal (tail) venipuncture with a (22-gauge needle into plain and EDTA vacuum tubes (BD Vacutainer, Curitiba, Paraná, Brazil). After the procedures, giant otters were kept in shaded, quiet cages for monitored recovery and released at the capture site once fully responsive.

At the field laboratory, blood samples without anticoagulant were centrifuged for 5 min at 1,200 × G, and the resulting serum was separated and aliquoted. Blood samples containing anticoagulant were also aliquoted as whole blood in 0.6 mL microcentrifuge tubes. Samples were stored at −20°C for 10 mo to 4 yr prior to analysis.

This study represented a collaborative effort to optimize the use of rare biological samples, which are limited due to the challenges and low success rates of capturing giant otters. Therefore, analyses for individuals 1–7 and 8–10 were performed in different laboratories due to shipping limitations at the time. Consequently, distinct methodologies may have been applied to assess exposure to the same pathogen, reflecting necessary methodological adjustments to logistical limitations rather than intentional differences in study design. Not all analyses were performed for all individuals.

For individuals 1–7, antibodies against *T. gondii* were detected using the modified agglutination test. Individuals with titers ≥1:25 were considered positive (Dubey and Desmonts 1987). For individuals 8–10, *T. gondii* antibodies were detected using the indirect fluorescent antibody test (IFAT). Antigen slides previously prepared with *T. gondii* (Imunoteste *Toxoplasma gondii*; Imunodot Diagnósticos Ltda, Jaboticabal, São Paulo, Brazil.) were used. The IFAT used an anti-canine IgG conjugate, and samples were considered positive when antibodies were detected in a serum dilution of 1:32.

Antibodies against *Leptospira* spp. were detected using the microscopic agglutination test (Faine 1982) against the following serovars: Australis, Autumnalis, Bataviae, Bratislava, Canicola, Castellonis, Copenhageni, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Tarassovi, and Wolffi. Additional serovars were tested beyond those listed above: Andamana, Butembo, Cynopteri, Panama, Patoc, Sentot, Shermani, and Whitcombi for individuals 1–7; and Djasiman for individuals 8–10. Animals that reacted to ≥1 serovar were considered seropositive. The serovar with the highest antibody titer in each sample was considered to be the most probable infecting serovar for that individual.

Sera were tested for smooth *Brucella* using the Rose Bengal test and *Brucella abortus* (stain 1119-3) as the antigens (Lage et al. 2006). Serum from *B. abortus*-infected cattle was used as the positive control.

For individuals 1–7, antibodies to CDV were detected using a serum neutralization microscopic test (NMT; Appel and Robson 1973). Titers ≥8 were considered positive (Courtenay et al. 2001). Antibodies against parvovirus were detected using the hemagglutination inhibition test (Carmichael et al. 1980), with titers ≥80 considered positive (de Almeida Curi et al. 2010). This test does not differentiate between feline parvovirus, canine parvovirus (CPV), and mink enteritis virus (Barker and Parrish 2001). For individuals 8–10, a dot-blot ELISA was used to detect IgM antibodies against CDV and CPV, with IgM scores (titers) of 1 (10) or 2 (50) considered weakly positive, scores (titers) of 3 (250) considered medium positive, and scores (titers) of 4–6 (≥1,250) considered strongly positive.

For individuals 1–7, antibodies against rabies virus were detected using the rapid fluorescent focus inhibition test (Smith et al. 1996). Titers ≥ 0.10 IU/mL were considered positive (Jorge et al. 2010). This test was not conducted on otters 8–10.

Molecular tests were performed for six giant otters to detect *Hepatozoon* spp., *Cytauxzoon* spp., and *Babesia* spp. For individuals 3, 4, and 6, DNA extraction followed Rubini et al. (2005) using 200 µL whole blood aliquots processed with the GFX genomic blood DNA purification kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and eluted in 100 µL of Tris-EDTA (hydroxymethyl aminomethane-ethylenediaminetetraacetic acid) buffer. Specific primers were used for each pathogen: Hep-F (5′-TACATGAGCAAAATCTCAAC-3′) and Hep-R (5′-CTTATTATTCCATGCTGCAG-3′) for *Hepatozoon* spp., and Piro-F (5′-TGACAC AGGGAGGTAGT-3′) and Piro-R (5′-CAA CAAAATAGAACCAAAG-3′) for *Cytauxzoon* spp. and *Babesia* spp. The PCR assays followed protocols described by Inokuma et al. (2002) for *Hepatozoon* spp., and Furtado et al. (2017) for *Cytauxzoon* and *Babesia* spp. For otters 8–10, DNA was extracted from whole blood samples stored on filter paper (FTA card for DNA extraction, MGM Assessoria Biologica Ltda, Curitiba, Paraná, Brazil), following Araújo et al. (2009). The DNA concentration and integrity were evaluated using spectrophotometry (NanoDrop ND-1000; Thermo Fisher Scientific, Waltham, Massachusetts, EUA) and agarose gel electrophoresis (1%). The same primers (Hep-F and Hep-R) and PCR conditions described by Inokuma et al. (2002) were used for *Hepatozoon* spp. For *Babesia* spp., PIRO-A-F (5′-AATACC CAATCCTGACACAGGG-3′) and PIRO-B-R (5′-TTAAATACGAATGCCCCCAAC-3′) primers were used, as described Carret et al. (1999). For *Cytauxzoon* spp., ITS1-F (5′-CGATCG AGTGATCCGGTGAATTA-3′) and ITS1-R (5′-GCTGCGTCCTTCATGATGTG-3′) primers were used, as described (Brown et al. 2010).

The individual characteristics of the giant otters are summarized in Table 1. All individuals appeared to be of appropriate body weight and free of clinical signs of systemic disease. Physical examination revealed scars (individual 4), dental damage and an enlarged scent gland (individual 6), a recent tail injury (individual 7), bilateral conjunctival hyperemia (individual 8), and lesions on the interdigital membranes of all digits, a lesion on the tip of the tail, a fracture of the right upper lateral incisor with necrotic pulp, and wear of the left upper premolar with pulp exposure (individual 9; Fig. 2). All captured giant otters returned to their original groups immediately after release and exhibited no behavioral changes.

Individual results for serological and molecular assays are presented in Table 1. Overall, 3/10 giant otters were seropositive for *T. gondii* (titers 25–200) and 2/10 were seropositive for *Leptospira* spp. (titers 100–200), with the most likely infecting serovars being Pomona (n=1) and Autumnalis (n=2). Of the six tested, one individual from the Cerrado (CSP) was antibody-positive for rabies virus. Additionally, 5/6 giant otters from the Pantanal were seropositive for CDV (titers 8-16 by NMT; 50-6,250 by ELISA), and 2/6 were seropositive for parvovirus (specifically canine parvovirus). Among the individuals tested for *Hepatozoon* spp., 2/6 were infected. None of the giant otters tested had antibodies against smooth *Brucella*, *Cytauxzoon* spp., or *Babesia* spp. Both individuals from the Amazon were negative for all pathogens.

The limited sample size (n=10) was consistent with the low population density and endangered status of *P. brasiliensis*, which restrict opportunities for capturing this species in the wild. Nevertheless, our data provide valuable baseline information for health assessments in this threatened species. Free-ranging giant otters from different Brazilian ecoregions showed exposure to 6/9 pathogens investigated, and 8/10 individuals exhibited evidence of exposure to at least one pathogen. Given the small sample size and the single sampling event for each inidividual, it was impossible to infer population-level impacts of these pathogens.

Detection of antibodies against *T. gondii* in giant otters from the Cerrado and Pantanal indicated prior exposure to this protozoan. Although the potential threat to Brazilian giant otters remains unclear, infection by this parasite warrants monitoring due to possible sublethal effects, such as reproductive impairment. Eye disorders in giant otters were first recorded in the Pantanal in 2015 and again in 2019 (Soresini et al. 2025; Supplementary Material Fig. S1), which could be consistent with toxoplasmosis. Isolation and characterization of the parasite

TABLE 1. Capture data and results of serologic and molecular tests for 10 free-ranging giant otters (*Pteronura brasiliensis*; ID = identification number) captured between August 2007 and July 2017 at five different study sites in the Amazon (BHPP), Cerrado (CSP, MAR), and Pantanal (MR, VR) ecoregions in Brazil, including body length, weight, and estimated age, and showing measured titer values considered positive and/or positive (P) or negative (N) results (NA = data not available).

					Capture data			Serologic tests[a]						Molecular tests		
ID	Sex[b]	Age Class[c]	Site[d]	Ecoregion	Date	Length (cm)	Weight (kg)	*T. gondii*	*Leptospira*	*Brucella*	Rabies	CDV	Parvovirus	*Hepatozoon*	*Babesia*	*Cytauxzoon*
1	F	A	BHPP	Amazon	February 2012	149	21	N	N	N	N	N	N	NA	NA	NA
2	F	A	BHPP	Amazon	February 2012	161	22.2	N	N	N	N	N	N	NA	NA	NA
3	M	A	CSP	Cerrado	August 2007	172	26	25	N	N	0.13	N	N	P	N	N
4	F	A	MAR	Cerrado	March 2009	152.5	20	N	Pomona (200) Autumnalis (100)	N	N	N	N	P	N	N
5	M	A	MR	Pantanal	July 2010	183	32.9	25	N	N	N	16	N	NA	NA	NA
6	M	A	MR	Pantanal	November 2009	180	29.5	N	Autumnalis (200)	N	N	N	N	N	N	N
7	M	A	VR	Pantanal	July 2010	171[e]	30.5	200	N	N	N	8	N	NA	NA	NA
8	M	A	MR	Pantanal	August 2016	178	26	N	N	N	NA	6,250	50	N	N	N
9	F	A	VR	Pantanal	October 2016	170	29	N	N	N	NA	50	10	N	N	N
10	F	S	VR	Pantanal	July 2017	162.5	21.8	N	N	N	NA	6,250	N	N	N	N

[a]*T. gondii* = *Toxoplasma gondii*; Pomona and Autumnalis refer to the serovars of *Leptospira interrogans* detected, titer values in parentheses; CDV = canine distemper virus.
[b]M = male, F = female.
[c]A = adult (>2.5 yr), S = subadult (1.5–2 yr).
[d]CSP = Cantão State Park, MAR = Meandros Araguaia River Park, MR = Miranda River, BHPP = Balbina Hydroelectric Power Plant, VR = Vermelho River.
[e]Total body length may have been affected by tail lesion.

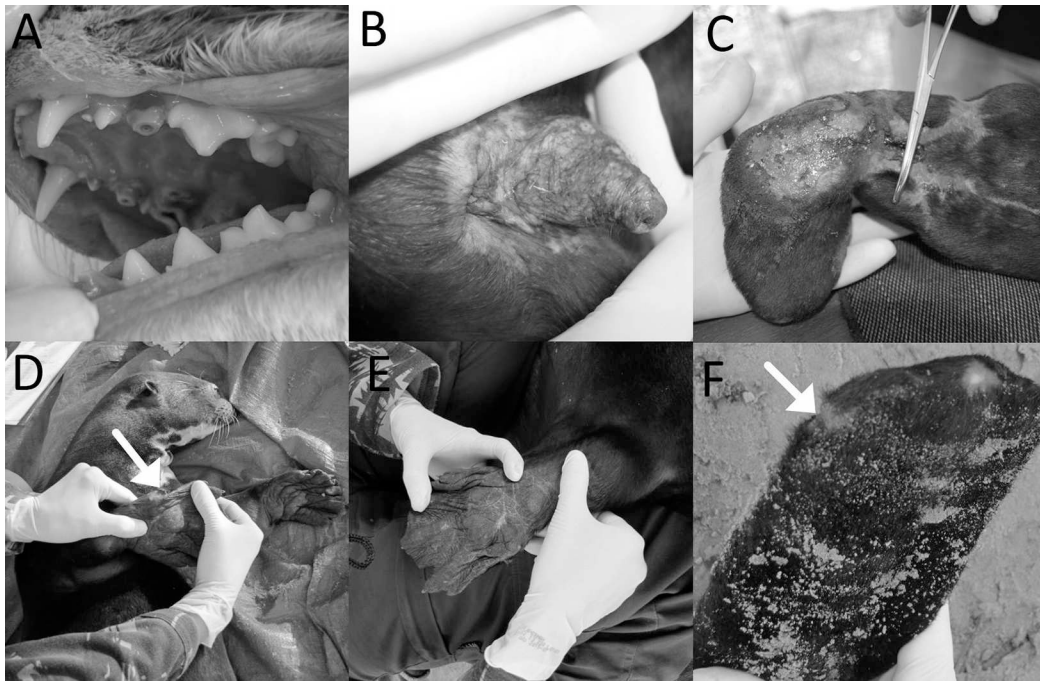

FIGURE 2. Physical injuries and lesions observed in captured giant otters (*Pteronura brasiliensis*) from the Pantanal ecoregion in Brazil. A. Fractured teeth and B. enlarged scent gland, both observed in individual 6. C. Fractured tail, observed in individual 7. D. Lesion on the interdigital membrane of a thoracic limb. E. Area with absence of part of the membrane and F. lesion on the tail probably caused by a piranha bite, all three observed in individual 9.

from affected animals is important, as *T. gondii* strains circulating in Brazil tend to be more virulent to humans and animals than those from other regions (Dubey et al. 2012).

As semi-aquatic animals, giant otters from the Cerrado and Pantanal were probably exposed to *Leptospira* spp. through contact with contaminated waters. The low antibody titers detected may indicate past exposure or a latent infection. The seropositive giant otter from the Pantanal showed no alterations in movement patterns, suggesting it was not clinically affected. Given the zoonotic potential of *Leptospira* spp. in aquatic ecosystems, continued monitoring is recommended.

No evidence of exposure to smooth *Brucella* was detected in giant otters tested in this study. Antibodies to *Brucella* spp. have been reported in northern sea otters (*Enhydra lutris kenyoni*) from Washington and California, USA (White et al. 2013).

The giant otter from the Cerrado that tested positive for rabies virus antibodies probably experienced a nonlethal exposure. The animal exhibited no clinical signs during capture and moved normally during 12 mo of monitoring. As has been observed in other wild carnivores (Jorge et al. 2010), this individual may have developed neutralizing antibodies without manifesting disease, suggesting rabies virus circulation in the region.

The exposure of giant otters from the Pantanal to CDV is consistent with previous reports of infection in Neotropical otters (*Lontra longicaudis*) from southern Brazil (Michelazzo et al. 2022). Differences in CDV titers probably reflected the diagnostic methods used, with ELISA detecting higher but less specific antibody levels than the neutralization test. Further molecular testing is needed to characterize and compare the virus strains circulating in these populations.

Two giant otters from the Pantanal were exposed to parvovirus, which has also been reported in Neotropical otters in southern Brazil (Echenique et al. 2018). Testing fresh

fecal samples from otter latrines for parvovirus combined with molecular characterization is recommended, along with monitoring offspring survival and mortality rates.

Among the vector-borne pathogens surveyed, *Hepatozoon* spp. was only detected in giant otters from the Cerrado, whereas *Babesia* spp. and *Cytauxzoon* spp. were not found. *Hepatozoon* spp. infection in otters has rarely been investigated, with only a single report in Neotropical otters (Alves et al. 2025). The spread of vector-borne diseases may be facilitated by climate change, through increasing temperatures, altered precipitation patterns, and shifts in host composition (Socha et al. 2022).

Giant otters from the Amazon showed no evidence of pathogen exposure, whereas those from the Pantanal had the highest pathogen detection rates. Human activity in the Amazon is lower than along Pantanal rivers, where tourism, artisanal fishing, and otters coexist in close proximity (Oliveira et al., 2015; Tomás et al., 2019). Such interactions inevitably bring domestic animals into closer contact with wildlife, elevating the risk of exposure to pathogens relevant to conservation and health.

The impact of these pathogens on giant otters, particularly immunosuppressed individuals or those inhabiting degraded environments, remains unknown. Giant otters living in suboptimal habitats may exhibit poor body conditions or unusual prey consumption (Leuchtenberger et al. 2015, 2020). Nevertheless, the animals evaluated in this study appeared healthy, suggesting individuals that develop clinical disease and subsequently die may not be detected in time for sampling, potentially leading to underestimation of pathogen impacts.

Long-term monitoring is essential to evaluate the clinical significance of these pathogens, elucidate the role of giant otters in pathogen transmission, and identify which agents are endemic or represent emerging threats to the species. Our findings also highlight the importance of monitoring pathogen spillover within a One Health framework.

Capture and sampling procedures were approved by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), under permit numbers 12794 (MR), 13183 (CSP), 17960 (MAR), 27396 (BHPP), and 48515 (MR and VR), and were conducted in accordance with the Embrapa Pantanal Committee on Ethics for the Use of Animals in Research (protocol no. 007/2015). We thank the Memphis Zoo, U.S. Forest Service, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Earthwatch Institute, Jaguar Conservation Fund, and Instituto Nacional de Pesquisa da Amazônia for financial support in Cantão State Park and Meandros do Araguaia Environmental Protection Area. G.S., G.M., and C.L. thank the Universidade Federal de Mato Grosso do Sul and the Embrapa Pantanal for logistic support during fieldwork carried out in the Pantanal. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, Doctoral Scholarship 1461215 awarded to G.S. CNPq also provided a fellowship to G.M. (308934/2008-9) and a scholarship to C.L. (476939/2008-9) and supported two projects (485890/2013–5 and CNPq/Fundect Pronex 006/2015). We thank Instituto Chico Mendes, Cantão State Park, and Meandros Araguaia Environmental Protection Area for the necessary project permits. We are grateful for the field assistance of Waldomiro de Lima e Silva, Sidnei José Benício, Fabiano Aguiar da Silva, Tiago Boscarato, Eduardo Freitas, Guilherme Ribeiro Lana, Normezy and Nildon A. Pinto Ataíde, and Luis C. L. C. Silva and Mario A. F. Rego for veterinary assistance during the capture and tagging procedures in Cantão State Park, in Meandros Araguaia Environmental Protection Area or in the Pantanal. We are grateful to Edu Fragoso for providing us with a photographic record of an unhealthy giant otter. We thank the Houston Zoo for financial support for complementary laboratory analysis.

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