

Exposure of dogs and wild carnivores to canine distemper virus, canine parvovirus, *Leishmania infantum*, and *Toxoplasma gondii* in the Xingu River basin, Brazilian Amazon: Prevalence, spatial distribution, and association with land cover types

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ABSTRACT

Infectious diseases transmitted by domestic dogs can have important consequences for wildlife health. This study aimed to investigate the exposure to four selected pathogens in dogs and wild carnivores in six municipalities in the Xingu River Basin (Pará state, Brazil). The prevalence of positive animals, their spatial distribution, and the association with land cover types were analyzed. Blood samples from 298 dogs and 11 free-ranging wild carnivores were tested through serological diagnoses. The seroprevalence to canine distemper virus, canine parvovirus, *Leishmania infantum*, and *Toxoplasma gondii* was 68.6, 75.4, 14.8, and 47.1%, respectively. The seroprevalence to canine distemper virus and *T. gondii* was significantly higher in dogs than in wild carnivores. Spatial analyses revealed a broad distribution of seropositive animals, except for animals seropositive to *L. infantum*, which were concentrated in the southern region close to the margins of the Xingu River. Spatial clusters of seropositive animals were detected for all tested pathogens, indicating areas with a greater risk of exposure. Positive results for canine distemper virus, *T. gondii*, and *L. infantum* were associated with different types of land cover, and thus were considered risk factors for pathogen exposure. The high seroprevalence of dogs to canine distemper virus and to canine parvovirus are concerning, suggesting risks of transmission to wild species inhabiting areas close to the surveyed communities.

KEYWORDS: epidemiology, wildlife, risk factor, spatial analysis, zoonosis

Exposição de cães e carnívoros selvagens ao vírus da cinomose canina, vírus da parvovirose canina, *Leishmania infantum* e *Toxoplasma gondii* na bacia do Rio Xingu, Amazônia brasileira: prevalência, distribuição espacial e associação com tipos de cobertura do solo

RESUMO

Doenças infecciosas transmitidas por cães domésticos podem ter consequências importantes na saúde de animais silvestres. Este estudo objetivou investigar a exposição a quatro patógenos em cães e carnívoros silvestres em seis municípios da bacia do Rio Xingu (Pará, Brasil). A prevalência de animais positivos, sua distribuição espacial e a associação com tipos de cobertura do solo foram analisados. Amostras de sangue de 298 cães e 11 carnívoros silvestres de vida livre foram testados por diagnósticos sorológicos. As soroprevalências para o vírus da cinomose canina, vírus da parvovirose canina, *Leishmania infantum* e *Toxoplasma gondii* foram 68,6, 75,4, 14,8 e 47,1%, respectivamente. As soroprevalências para o vírus da cinomose canina e *T. gondii* foram significativamente maiores em cães do que em carnívoros silvestres. Análises espaciais revelaram uma ampla distribuição de animais soropositivos, exceto aqueles soropositivos para *L. infantum*, os quais se concentraram na região sul próximo às margens do Rio Xingu. *Clusters* espaciais de animais soropositivos foram detectados para todos os patógenos estudados, indicando áreas com maiores risco de exposição. Resultados positivos para o vírus da cinomose canina, *T. gondii* e *L. infantum* estiveram associados a diferentes tipos de cobertura do solo, que, portanto, foram considerados fatores de risco para a exposição aos patógenos. As elevadas soroprevalências de cães para o vírus da cinomose canina e para o vírus da parvovirose canina são preocupantes, sugerindo riscos de transmissão para espécies silvestres que habitam áreas próximas das comunidades investigadas.

PALAVRAS-CHAVE: epidemiologia, animais selvagens, fatores de risco, análise espacial, zoonose

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INTRODUCTION

Infectious diseases transmitted from domestic dogs to wild animals occur worldwide and have important implications for biodiversity conservation (Woodroffe *et al.* 2012). Infections do not necessarily cause host death but also symptoms that lead to a decline in populations, directly or indirectly, due to reduced competition capacity for hunting or escape from predators, infertility, altered secondary sex ratios and movement patterns, morbidity, and increased susceptibility to other infectious diseases (Preece *et al.* 2017). Canine distemper virus (CDV) in lions, *Panthera leo* (Linnaeus, 1758) (Mourya *et al.* 2019), canine parvovirus (CPV) in wolves, *Canis lupus* (Linnaeus, 1758) (Mech *et al.* 2008), and the rabies virus in African wild dogs, *Lycaon pictus* (Temminck, 1820) (Canning *et al.* 2019) are examples of pathogens that can cause diseases with substantial mortality rates in wildlife, including endangered species. These diseases are called the “Big Three” due to strongly negative impact on wild carnivores (Knobel *et al.* 2014). Wild canids are particularly susceptible to diseases carried by dogs since both animal groups are closely related (Woodroffe *et al.* 2004). The transmission routes of these events are difficult to elucidate (Blasio *et al.* 2019), but they most likely occur through direct contact between dogs and wildlife (Curi *et al.* 2010).

Disease transmission by dogs to wild species can be high under particular circumstances. Isolated communities of low socioeconomic status may have fewer healthy dogs that can become major reservoirs for the transmission of diseases to humans and sympatric wildlife (Fung *et al.* 2014). Factors associated with this condition may include limited resources to seek veterinary care services (including deworming and immunization), low access to transportation, lack of awareness and information regarding animal welfare, poor-quality food provided to dogs, and cultural habits. In addition, these threats to wildlife can have greater impacts in areas that are biodiversity hotspots, where diverse and numerous species occur. The Amazon region perfectly illustrates the abovementioned situation, with thousands of impoverished communities living in remote areas in one of the most biodiverse ecosystems in the world.

Among the diverse pathogens found in dogs, CDV is one of the most important that impairs wildlife health (Knobel *et al.* 2014). Several cases and outbreaks have been reported among diverse animal species worldwide, making canine distemper probably the most threatening disease of dog origin affecting wild carnivores (Knobel *et al.* 2014; Mourya *et al.* 2019). In Brazil, CDV outbreaks of large magnitude have not been described. However, severe cases have occasionally been reported, including in a hoary fox, *Lycalopex vetulus* (Lund, 1842), a lesser grison, *Galictis cuja* (Molina, 1758), coatis, *Nasua nasua* (Linnaeus, 1766), and felids (*Puma concolor* Linnaeus, 1771, *Leopardus wiedii* Schinz, 1821,

and *Herpailurus yagouaroundi* Geoffroy Saint-Hilaire, 1803) (Megid *et al.* 2010; Megid *et al.* 2013; Michelazzo *et al.* 2020; Viana *et al.* 2020).

The present study was part of a project on the control of endemic diseases transmissible to wildlife, conducted during the construction of the Belo Monte Hydroelectric Dam (BMHD) in Pará state, Brazil. This project targeted communities that inhabit areas near the BMHD, and aimed to assess the level of exposure of domestic and wild animals to the following pathogens: CDV, CPV, *Leishmania infantum* (Nicolle, 1908) (synonym to *Leishmania chagasi*), and *Toxoplasma gondii* (Nicolle & Manceaux, 1908). Other goals included the spatial distribution of positive and negative animals and their association with different land cover types.

MATERIAL AND METHODS

Study area

Six municipalities located in the lower Xingu River were surveyed between November 2011 and September 2015: Altamira, Porto de Moz, Senador José Porfírio, Anapu, Brasil Novo, and Vitória do Xingu (Figure 1). The geographical characteristics of the region are typical of the Amazon biome, with dense tropical forests, equatorial climate, high precipitation levels, low population density, and high deforestation levels. At the time of our survey, most communities were of low socioeconomic status with inadequate sanitary conditions.

Animals and samples

Blood samples were collected from 298 dogs and transported to the BMHD station for storage (-20°C) until laboratory diagnosis. Most dogs were apparently healthy, but several showed clinical abnormalities of multiple origins, including malnutrition, ectoparasites, apathy, dermatitis, old bone fractures, and pelvic paralysis. Vaccination status was reported by the owners of 107 dogs but only against rabies. Most dogs roamed freely and had access to the forest environment and surrounding community areas.

Eleven free-ranging wild carnivores were captured from wildlife rescue activities during vegetation suppression in the areas destined for the water reservoir (Table 1). The animals that required captive management actions for biometric data collection, tagging, and translocation were sampled for this study. Blood samples were collected when the animals were chemically restrained.

The location of the wild carnivores and dogs was georeferenced using a GPS device for later spatial analyses. All procedures were approved by the Brazilian Institute of Environmental and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA, process 02001.001848/2006-75).

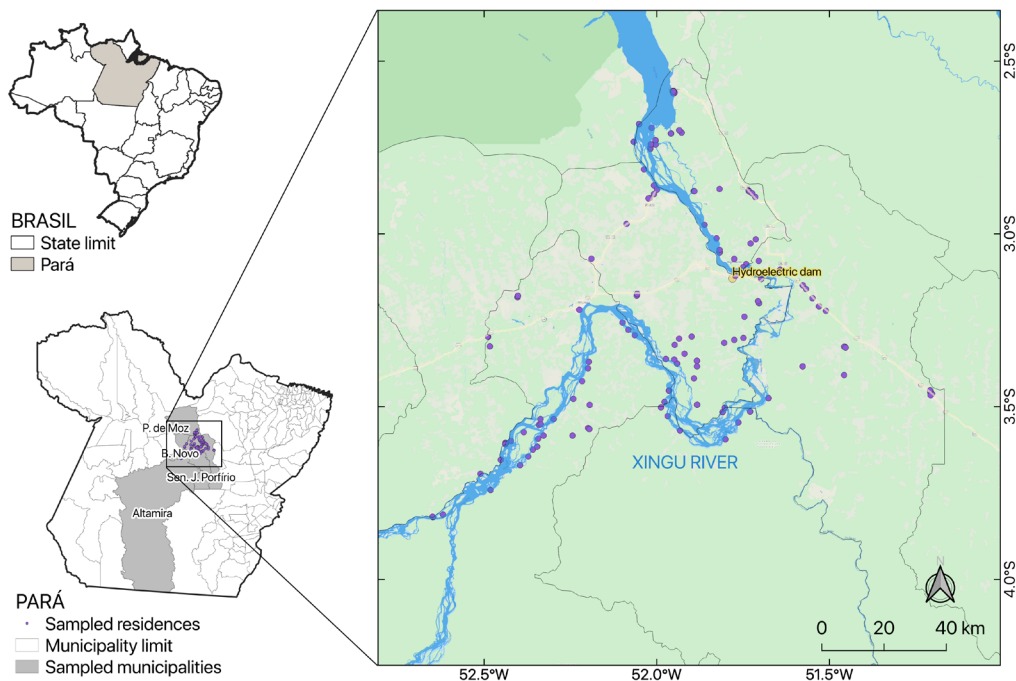


Figure 1. Map of the study region where dogs and wild carnivores were sampled for diagnosis of infectious diseases in the lower Xingu River basin, Pará state, Brazil. This figure is in color in the electronic version.

Table 1. Data from wild carnivores surveyed in the lower Xingu River basin (Pará state, Brazil) and results of serological tests for canine distemper virus (CDV), canine parvovirus (CPV), *Leishmania infantum*, and *Toxoplasma gondii*.

Species	Common name	Family	Sampling date	Age class	Sex	Weight (kg)	Author id code	Laboratory diagnosis (serological titer)			
								CDV	CPV	<i>L. infantum</i>	<i>T. gondii</i> *
<i>Cerdocyon thous</i> (Linnaeus, 1766)	Crab-eating fox	Canidae	10 Sep 2012	Adult	M	5.16	203	Negative	Positive (40)	Negative	Negative
<i>Speothos venaticus</i> (Lund, 1842)	Bush dog	Canidae	14 Jan 2012	Adult	M	4.98	31	Positive (40)	Positive (40)	Negative	Negative
			14 Jan 2014	Subadult	M	3.2	451	Negative	Negative	Negative	Positive
<i>Eira barbara</i> (Linnaeus, 1758)	Tayra	Mustelidae	21 Oct 2011	Young	F	2.18	97	Negative	Negative	Negative	Negative
			17 May 2014	Subadult	M	2.9	502	Positive (40)	Positive (40)	Negative	Negative
<i>Lontra longicaudis</i> (Olfers, 1818)	Neotropical otter	Mustelidae	09 Nov 2012	Young	F	4.3	369	Negative	Positive (40)	Negative	Negative
			03 Sep 2013	Young	M	3.73	441	Negative	Positive (40)	Negative	Negative
<i>Potos flavus</i> (Schreber, 1774)	Kinkajou	Procyonidae	10 Apr 2013	Adult	F	1.66	330	Negative	Positive (40)	Negative	Negative
			02 Jun 2015	Adult	F	1.5	666	Negative	Negative	Negative	Negative
<i>Procyon cancrivorus</i> (G. Cuvier, 1798)	Crab-eating raccoon	Procyonidae	27 Dec 2014	Cub	M	0.3	595	Negative	Negative	Negative	Negative

*Endpoint titer could not be performed (32 was used as the cutoff point)

Laboratory diagnosis of pathogens

Exposure to CDV, CPV, *L. infantum*, and *T. gondii* was assessed through antibody detection using serological tests performed by the TECSA[®] laboratory (Belo Horizonte, Minas Gerais state, Brazil). Detailed information on the laboratory tests and protocols are protected by the laboratory’s privacy policy and cannot be published. Because of the limited sample availability for laboratory tests, diagnosis of CDV, CPV, and *T. gondii* could not be performed in 44 domestic dogs.

Diagnosis of CDV and CPV was performed by the detection of IgG antibodies using immunochromatography tests with a dilution 1:40 as the cut-off titer. Exposure to *L. infantum* was investigated using indirect immunofluorescent antibody tests (IFATs) and enzyme-linked immunosorbent assays (ELISA) to detect IgG. For IFATs, a dilution of 1:40 was considered the cut-off point, whilst the ELISA is a qualitative test that yields negative or positive results. Exposure to *L. infantum* was considered when an animal tested positive in at least one of the two tests. Exposure to *T. gondii* was

assessed using IFATs to detect IgM and IgG antibodies with a dilution of 1:32 as the cut-off point. Exposure to *T. gondii* was considered when an animal tested positive in at least one of the two tests. Dogs and wild carnivores were tested using the same diagnostic techniques, which employed anti-dog conjugates. Except for *T. gondii*, the endpoint titers of seropositive samples were determined. Standardized serological tests for different wild species are extremely scarce; therefore, we used laboratory tests that employed conjugates against the immunoglobulins of domestic dogs, which are phylogenetically close to wild carnivores, as in previous epidemiological studies (Clifford *et al.* 2006; Brandão *et al.* 2020).

Spatial analysis

Cluster analyses were performed independently for each pathogen to assess the spatial prevalence of seropositive animals and identify areas with high risk of exposure. The animals were classified as cases (seropositive) and controls (seronegative), and the rates of pathogen prevalence within circles of various sizes were calculated using statistical modeling and the *Bernoulli* distribution. This test compares the hypothesis that the risk of being positive is greater within the circle with the hypothesis that the risk is equal inside and outside the circle, and significance was set at 5%. The scan statistics method was applied as described by Kulldorff and Nagarwalla (1995) using the SaTScan™ 9.5 software. Prevalence inside and outside the clusters was compared using the chi-square test or Fisher’s exact test using R v.3.6.1. The circle with the maximum value of the likelihood ratio was considered the most probable cluster (Kulldorff 1997; Wheeler 2007; Pfeiffer *et al.* 2008) and was represented on the map using the QGIS v.3.8 software.

Association with land cover types

The habitat of each animal’s origin was explored as risk factors for pathogen exposure and were investigated by comparing with laboratory tests results. The analyzed variables consisted of the types of land cover and soil use (LCSU) present in the studied region, such as forest formation, tree farming, floodplain, pasture, agriculture (soybean, sugar cane, and rice), urban infrastructure, and water bodies. Data were retrieved from the MapBiomias v.5.0 platform (as raster image of 30 m resolution, 4.0 collection), which makes the catalog of images of Brazil with different categories of LCSU publicly

available (MAPBIOMAS 2022). QGIS v.3.8 software was used to create a 3 km radius buffer area around each animal’s origin (28.3 km²), which was assumed to be their home range (Jesus *et al.* 2023). The proportion of each LCSU type of in the raster image within the buffer area of each animal was calculated. The variables were compared with the laboratory tests results (seropositive or seronegative) for each disease. The normality of data was analyzed using the Shapiro–Wilk test, followed by the non-parametric Mann–whitney U test, both performed in R v.3.6.1.

RESULTS

The seroprevalence of CDV, CPV, *L. infantum*, and *T. gondii* was 68.6, 75.4, 14.8, and 47.1%, respectively. Seroprevalence for CDV and *T. gondii* was significantly higher in dogs than in wild carnivores. CPV was the pathogen with the highest prevalence in all specimens tested and in each animal group (Table 2).

Thirty-nine dogs were seropositive to *L. infantum* using both ELISA and IFAT, whereas four dogs were seropositive using ELISA and three using IFAT. Regarding the IFAT for *T. gondii*, 122 seropositive animals were reactive to IgG antibodies, and two (dogs) were reactive to both IgM and IgG. Among dogs, the serological titers for CDV were 40 (n = 97), 80 (n = 21), 160 (n = 30), and ≥ 320 (n = 32); for CPV, they were 40 (n = 64), 80 (n = 28), 160 (n = 44), and ≥ 320 (n = 58); and for *L. infantum* (IFAT), they were 40 (n = 15), 80 (n = 26), and 160 (n = 1).

The spatial analysis revealed a wide geographical distribution of animals seropositive to CDV, CPV, and *T. gondii*. All animals seropositive to *L. infantum* were concentrated in the southern region of the study area, in localities close to the margins of the Xingu River (Figure 2). Significant clusters of seropositive animals were detected for all pathogens tested. *L. infantum* was the only pathogen with three clusters, with one cluster (Cluster 1) presenting the highest relative risk (9.1) among all tested pathogens (Table 3). This result indicates a 9.1-fold higher risk of becoming exposed inside the cluster than outside, despite the prevalence inside the cluster being only 24.1%. The prevalence in CDV and CPV clusters was 100 and 97.8%, respectively.

Table 2. Results of serological tests for canine distemper virus (CDV), canine parvovirus (CPV), *Leishmania infantum*, and *Toxoplasma gondii* in dogs and wild carnivores in six municipalities of the lower Xingu River basin (Pará state, Brazil).

Pathogens	Dogs			Wild carnivores			Difference between prevalence*	Total		
	N	Positive	% (CI 95%)	N	Positive	% (CI 95%)		N	Positive	% (CI 95%)
CDV	254	180	70.8 (64.8 - 76.3)	11	2	18.2 (2.3 - 51.7)	< 0.001	265	182	68.6 (62.7 - 74.2)
CPV	254	194	76.3 (70.6 - 81.4)	11	6	54.5 (23.4 - 83.2)	0.19	265	200	75.4 (69.8 - 80.5)
<i>L. infantum</i>	298	46	15.4 (11.5 - 20.0)	11	0	0 (0.0 - 28.5)	0.25	309	46	14.8 (11.1 - 19.3)
<i>T. gondii</i>	254	124	48.8 (42.5 - 55.1)	11	1	9.1 (0.2 - 41.3)	0.023	265	125	47.1 (41.0 - 53.3)

N: Number of animals tested. % (CI 95%): confidence interval. *p value.

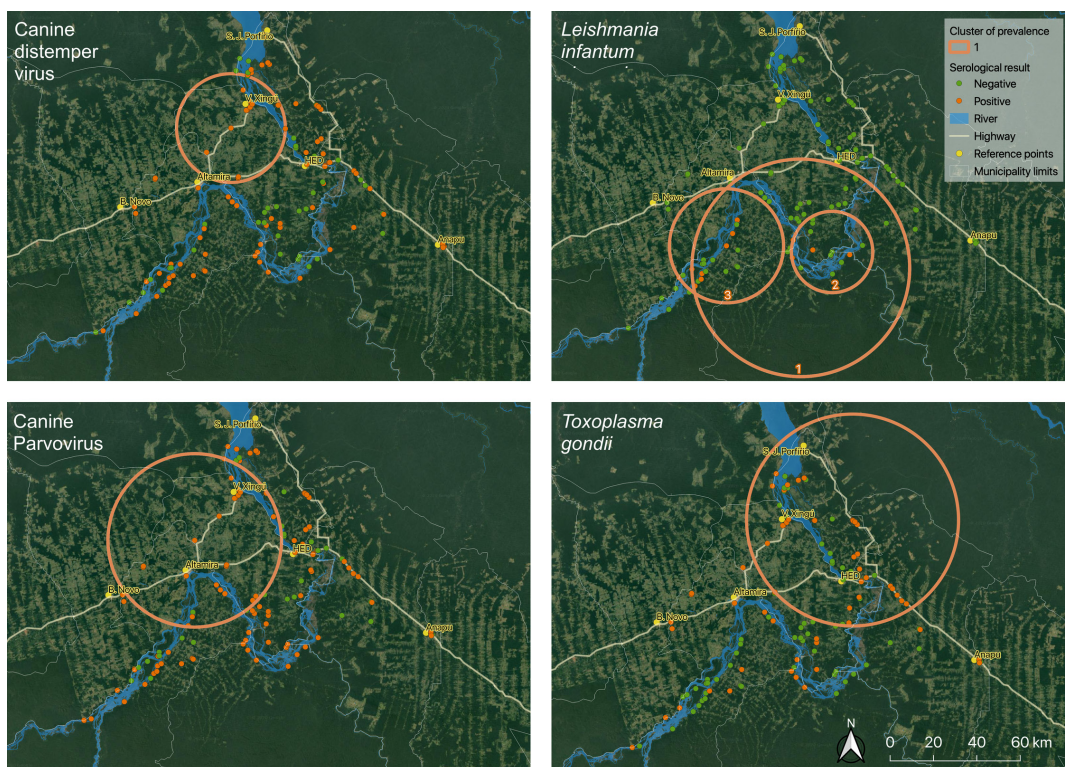


Figure 2. Spatial clusters of dogs and wild carnivores seropositive to canine distemper virus, canine parvovirus, *Leishmania infantum*, and *Toxoplasma gondii* in the lower Xingu River basin, Pará state, Brazil. This figure is in color in the electronic version.

Table 3. Data from spatial clusters of dogs and wild carnivores seropositive to canine distemper virus (CDV), canine parvovirus (CPV), *Leishmania infantum*, and *Toxoplasma gondii* in six municipalities of the lower Xingu River basin (Pará state, Brazil).

Clusters data	CDV	CPV	<i>L. infantum</i>			<i>T. gondii</i>
			Cluster 1	Cluster 2	Cluster 3	
Prevalence inside the cluster	100% (23/23)	97,8% (44/45)	24,1% (35/145)	28,8% (23/80)	37,8% (14/37)	62,1% (77/124)
Expected cases	15.8	34	19.2	10.6	4.9	58
Relative risk	1.5	1.4	9.1	3.9	3.9	1.9
Log likelihood ratio	9.1	10.2	16.6	10.3	8.6	11.1
P-value	0.014	0.003	<0.001	0.003	0.016	0.001
Prevalence outside the cluster	65.7% (159/242)	88.6% (156/176)	26.7% (4/15)	8.3% (16/192)	10.2% (25/244)	73.4% (47/64)

Animals seropositive to CDV were associated with areas of pasture ($p = 0.015$) and a few water bodies ($p < 0.03$), but not with floodplains and forest formations (Figure 3; Table 4). Seropositivity to *L. infantum* was positively associated with areas of floodplains ($p < 0.001$) and forest formations ($p < 0.001$) and negatively associated with pastures ($p < 0.001$); the only variable that did not reach statistical significance was water bodies. Seropositivity to *T. gondii* was positively associated with pasture areas ($p < 0.001$) and negatively associated with floodplains ($p < 0.001$), forest formations ($p < 0.001$), and water bodies ($p < 0.001$). Seropositivity to CPV did not show significant association with floodplains, pastures, forest formations or agricultural areas. Agricultural areas, urban infrastructure, and tree farming areas showed a median of 0% at the buffers of seropositive and seronegative dogs; thus, they were not considered in the analysis. It is important

to note that LCSU types such as urban infrastructure, agriculture, and tree farming are rare in the studied region, and their median area around seropositive and seronegative dogs for all pathogens was equal to zero (Table 4). Thus, although some types were significant ($p < 0.05$) in the analysis, we did not consider them as representative.

DISCUSSION

The high seroprevalence of CDV and CPV in dogs was surprising and indicates the broad circulation of these pathogens. The small number of wild carnivores sampled hinders major conclusions at the population level; thus, the following discussion focuses mainly on dogs. Seroprevalence studies in Brazil are scarce, especially in the Amazon region, which limits comparisons with our results. The lack of data is

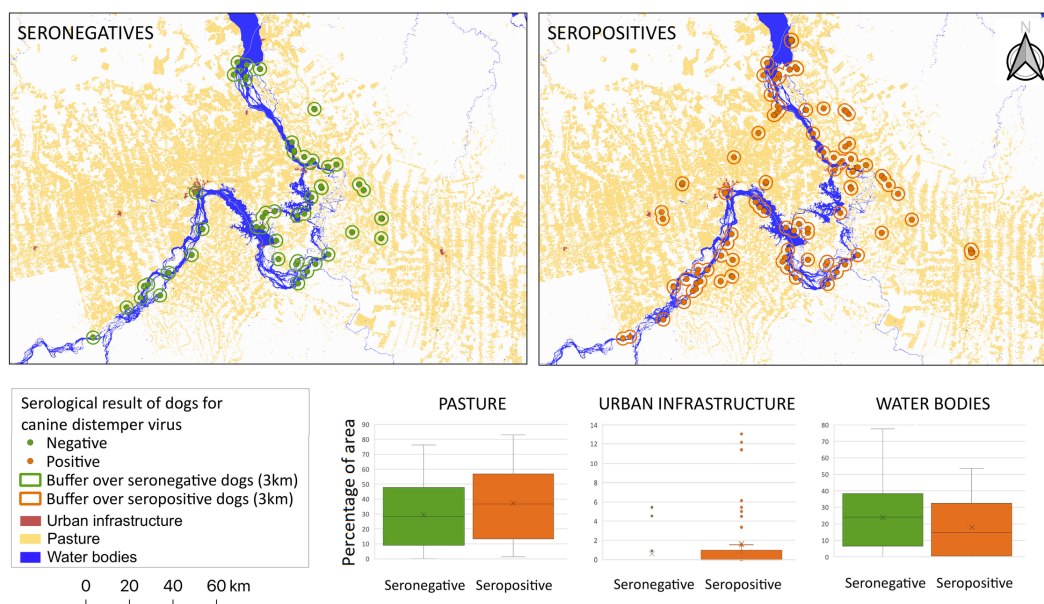


Figure 3. Correlation between the occurrence of dogs seropositive to canine distemper virus with three land cover types in the lower Xingu River basin, Pará state, Brazil. The results of the remaining land cover types are omitted. This figure is in color in the electronic version.

Table 4. Association of the type of land cover with serological tests for canine distemper virus (CDV), canine parvovirus (CPV), *Leishmania infantum*, and *Toxoplasma gondii* in dogs and wild carnivores in six municipalities of the Xingu River basin (Pará state, Brazil).

Coverage	CDV				CPV				<i>L. infantum</i>				<i>T. gondii</i>			
	Median of area (%)		MW-U	p-value	Median of area (%)		MW-U	p-value	Median of area (%)		MW-U	p-value	Median of area (%)		MW-U	p-value
	+	-			+	-			+	-			+	-		
River floodplain	0.3	0.86	7468	0.883	0.89	0.285	6197	0.572	5.17	0.57	1593	<0.001	0.57	1.73	6417	<0.001
Pasture	36.7	28.4	6149	0.015	32.7	33.8	6357	0.790	9.16	35.1	1419	<0.001	43.6	22.6	5037	<0.001
Forest formation	43.2	42.7	7059	0.393	43.9	39.6	6346	0.774	54.5	40.9	1503	<0.001	38.5	45.3	6635	<0.001
Water bodies	14.9	24.1	6308	0.031	16.6	20.7	6156	0.521	30.5	16.2	3049	0.148	6.49	28.6	6176	<0.001
Agriculture	0	0	8306	0.006	0	0	6929	0.26	0	0	4098	0.0818	0	0	8689	0.906
Urban	0	0	9279	<0.001	0	0	7701	0.002	0	0	2821	0.005	0	0	10653	<0.001
Tree farming	0	0	7311	0.133	0	0	6460	0.791	0	0	4130	<0.001	0	0	8308	0.012

+: positive. -: negative. MW-U: Mann Whitney U statistic.

particularly noticeable in the case of spatial analyses and risk factor assessments. This is one of the first studies to explore diverse pathogens in a large sample size of domestic dogs in the Brazilian Amazon.

Severe cases of CDV infection in free-ranging carnivores have been described in Brazil (Megid *et al.* 2010; Megid *et al.* 2013; Michelazzo *et al.* 2020; Viana *et al.* 2020). Antibodies against CDV in unvaccinated dogs have been reported with prevalence rates of 66 and 71.4% in Goiás and Minas Gerais states, respectively (Curi *et al.* 2010; Furtado *et al.* 2016), indicating high exposure levels and the presence of infected animals as potential sources of infection. The reported rates are very similar to the seroprevalence detected in the present study (70.8%). Such a high frequency of exposure is alarming, suggesting recurrent infections in the population and their possible role as a source of infection for wild species. One of the few assessments in the Brazilian Amazon detected

27% (27/101) of dogs seropositive to CDV in Pará state (Whiteman *et al.* 2007). In the Bolivian Amazon, Fiorello *et al.* (2004) found an expressive seroprevalence of 92% in dogs from the borders of Madidi National Park. Comparison of prevalence rates between studies should be cautious due to methodological differences. Nonetheless, studies have shown that CDV is widely distributed in Brazil and other South American countries and should be the focus of great concern for wildlife conservation. Although CDV seroprevalence was higher in dogs than in wild carnivores, we should also consider that wild carnivores can maintain enzootic cycles of CDV and transmit it to susceptible dogs (Prager *et al.* 2012).

Although CPV is less life-threatening to wildlife than CDV, carnivores have been affected by CPV in southeastern Brazil and northeastern Argentina (Bucafusco *et al.* 2019; Spera *et al.* 2020, Spera *et al.* 2021). The detected 75.4% seroprevalence corroborates the results of previous studies,

demonstrating that CPV is highly prevalent in Brazilian dog populations. In southeastern Brazil, seroprevalences as high as 100 and 97% were found in dogs living close to environmentally protected areas (Curi *et al.* 2010; Curi *et al.* 2016). Studies on CPV in the Brazilian Amazon region are rare. Courtenay *et al.* (2001) reported that 13% (3/23) of dogs from Marajó, Pará state, Brazil, were seropositive; and a seroprevalence of over 90% was found in the Bolivian Amazon (Fiorello *et al.* 2004, Fiorello *et al.* 2006). Therefore, CPV is a common pathogen in dogs in Brazil, with evidence of particularly high exposure rates in some areas at the interface between domestic and wild species.

Leishmania infantum is the etiological agent of visceral leishmaniasis (VL), a zoonotic disease of great concern to human and animal health worldwide, with domestic dogs acting as the main reservoir. Brazil is endemic for VL, with varying levels of endemism across its immense territory. VL is expanding geographically in the Amazon biome, especially in Pará state. The 14.8% seroprevalence that we detected corroborates previous investigations in dogs from Pará, which reported 23.3 and 43% seropositivity (Valadas *et al.* 2010; Silveira *et al.* 2012). Additionally, among the six municipalities surveyed, two (Vitória do Xingu and Anapu) had sporadic cases of human VL according to the Notifiable Diseases Information System (SINAN, 2021) (i.e., the two municipalities accounted for five cases of VL between 2016 and 2019). Our results confirm infection by *L. infantum* in dogs from Pará state; moreover, indicate the presence of this parasite in remote areas occupied by communities of low socioeconomic status.

Similar to *L. infantum*, seroprevalence studies of *T. gondii* in dogs are especially important in a public health context. The frequency of antibodies in dogs indicates the level of environmental contamination by parasitic oocysts, thus indicating the risk of human exposure (Olbera *et al.* 2020). *Toxoplasma gondii* is one of the most studied parasites in dogs, and Brazil, including the Amazon biome, has broad serological assessments of this parasite. Seroprevalence in Brazilian dogs ranges from 3.1 to 91% (Dubey *et al.* 2012), and populations with no evidence of exposure are very rare, endorsing the generalist nature and wide geographical distribution of this parasite. The 47.1% seroprevalence detected in the present study is not surprising and corroborates previous studies that showed substantial rates of 69.8 and 52.0% seropositivity in the Amazon (Valadas *et al.* 2010; Minervino *et al.* 2012). These numbers indicate high levels of environmental contamination by *T. gondii* and, thus, an elevated risk for human infection by this pathogen.

The wide geographical distribution of animals seropositive to CDV, CPV, and *T. gondii* revealed that these pathogens were present in several localities of the surveyed region, and probably had a broad circulation in the animal population.

Clusters of seropositive animals were detected for all the tested pathogens, indicating areas of elevated risk of exposure in the subpopulations of the animals sampled. This probably results from multiple factors that lead to higher transmission rates inside the areas comprised by the clusters. Additionally, cluster location differed among pathogens, probably reflecting epidemiological particularities. However, CDV and CPV clusters overlapped mostly between Altamira and Vitória do Xingu, which may be due to common epidemiological features of CDV and CPV, including dogs serving as the main reservoirs of these viruses and transmission through direct contact or contact with bodily fluids (Thompson *et al.* 2020). The spatial distribution of *L. infantum* was heterogeneous, with seropositive animals concentrated in the southern region. The origin of the geographical pattern of this disease is uncertain, but several factors are known to influence the prevalence of *L. infantum* in a particular region, such as the dispersion of sand flies (Oliveira *et al.* 2016) and dog density (Chamaillé *et al.* 2010). The detected areas of high risk of exposure can assist preventive interventions directed towards these pathogens and guide future research.

Since CDV is transmitted mainly through direct contact between animals, the association between exposure rates and land cover types is difficult to elucidate. Most studies on CDV risk factors have explored individual characteristics of animals, such as age, sex, and vaccination status, while the influence of the environment has been poorly assessed. The effect of environmental characteristics is likely related to the complex patterns of animal movements and contact rates. Areas with pastures likely provide open spaces that facilitate animal encounters through visual contact, thereby increasing CDV transmission rates. In contrast, water bodies (like rivers and lakes) are inappropriate for interactions among dogs.

The positive association of *T. gondii* with pasture areas was unexpected. *Toxoplasma gondii* oocysts are susceptible to ultraviolet radiation (Pinto-Ferreira *et al.* 2021), and open spaces such as pastures allow high penetration of sunlight on the ground surface, probably decreasing oocyst survival and infection rates in terrestrial animals. Pastures are resource-poor habitats for most animals, and dogs and wild carnivores that live close to pasture areas likely need to increase their home ranges. Dogs from rural areas can roam over long distances in search of mating opportunities, to hunt, form social groups, and mark territories. This may increase the risk of *T. gondii* exposure in a similar way that stray dogs have a greater chance of being seropositive than housed dogs (Liu *et al.* 2012). The negative association of *T. gondii* exposure with forest formations and floodplains were not expected. These areas are characterized by denser vegetation and higher humidity levels than pastures, benefiting oocyst survival. It is possible that the region we surveyed has a naturally low prevalence of *T. gondii* in the wild environment, and the detected high

seroprevalence resulted from peridomicile exposure where domestic cats are more abundant.

Several factors may increase the incidence of *L. infantum*, including human-induced changes in environmental conditions that alter the vector (*Lutzomyia* spp.) habitat (Sevá *et al.* 2017). Floodplains and forest formations were risk factors for *L. infantum* exposure, suggesting that these habitats are suitable for the parasite vector. Forested areas were likely inhabited by wild animals that served as competent hosts for *L. infantum*, thus increasing the odds of exposure in this type of environment. Regarding floodplains, high humidity levels and organic matter in the soil may increase habitat suitability for sand fly larvae, and thus increase vector abundance, density, and feeding frequency in dogs. Previous studies have reported similar results, with increased densities of sand flies following the rainy seasons (França-Silva *et al.* 2005; Lara-Silva *et al.* 2015). However, in the norther region of this study, many seronegative dogs lived close to the Xingu River (Figure 2, between the BMHD and Senador José Porfirio). The low seroprevalence in this region – despite proximity to the river – is probably associated with pasture coverage in the animal buffer area, which was negatively correlated with positive results for *L. infantum*.

The small sample size of wild carnivores limits the discussion at the population level, as indicated by the wide range of the confidence intervals. Nonetheless, some useful pieces of information can be drawn from these results. First, the high prevalence rates for CDV and CPV indicate a high exposure level to these viruses that should cause concern. Second, CPV seropositivity was detected in five of the six carnivore species, suggesting exposure to this virus among diverse animal taxa. Finally, some of the sampled species, like bush dogs and kinkajous, are very difficult to access, and even results from a few animals may provide valuable epidemiological information.

Although the transmission chain for CDV and CPV could not be determined in the present study, domestic dogs are likely to be the origin of exposure among the studied carnivores, as discussed elsewhere (Woodroffe *et al.* 2004; Fiorello *et al.* 2006). CPV infection occurs mostly through contact with a contaminated environment, while CDV infection occurs more frequently through direct contact between infected and susceptible hosts (Fiorello *et al.* 2004). Therefore, the wild carnivores surveyed probably became exposed to CPV by sharing the same territories as dogs (in peridomestic or wild areas), while exposure to CDV presumably occurred during agonistic or social interactions with dogs. Transmission risk can be higher for dogs in poor body conditions (Fung *et al.* 2014) that roam freely in the environment (Hughes and Macdonald, 2013), conditions that were observed in most communities surveyed during sampling.

Studies on CDV and CPV in Brazilian Amazon wildlife are almost nonexistent. Serological evidence of infection has been described in wild carnivores from southeast and central-west Brazil (Nava *et al.* 2008; Curi *et al.* 2012; Furtado *et al.* 2013; Furtado *et al.* 2016), including seropositivity rates above 90% (Curi *et al.* 2012). Thus, wild populations may present high levels of exposure to these viruses in different Brazilian biomes. Additional investigations are required to assess the infection status and morbimortality by these pathogens in wildlife, including molecular diagnostic tests and comparisons with health parameters.

Broad assessments of VL in Brazilian wildlife, using serological and molecular tests (Humberg *et al.* 2012; Paiz *et al.* 2015), have demonstrated the diversity of species exposed to or infected by *L. infantum* and their possible role as reservoirs. However, most studies performed in the Amazon region have focused on detecting *Leishmania* species responsible for cutaneous leishmaniasis (such as *L. braziliensis* Vianna, 1911 and *L. amazonensis* Lainson & Shaw, 1972), while investigations of VL in wildlife from this biome remain limited. The few records of *L. infantum* infection include a southern tamandua, *Tamandua tetradactyla* (Linnaeus, 1758) from Pará state, Brazil (Araújo *et al.* 2013), and two red howler monkeys, *Alouatta seniculus* (Linnaeus, 1766) from French Guiana (Medkour *et al.* 2019). A noteworthy study conducted in Marajó Island (Pará state, Brazil), detected a high prevalence of crab-eating foxes infected by *L. infantum*, but a low infectiousness rate for sandflies compared to domestic dogs, indicating that these foxes were not important sources of infection (Courtenay *et al.* 2002). These authors also suggested that foxes became infected in the peridomestic environment, where domestic dogs serve as the main reservoirs.

Wild carnivores seropositive for *L. infantum* were not detected in this study, indicating low levels of exposure. However, an epidemiological bias due to the small sample size should be considered. Given the 15.4% seroprevalence detected in dogs, wildlife could possibly become exposed to *L. infantum* in the studied region. A larger number of specimens could provide more accurate data on VL seroprevalence in the Amazon wildlife.

The construction of hydroelectric dams has considerable negative impacts, such as deforestation of extensive areas, high mortality rates in wildlife, changes in the vegetation composition, and migration of traditional communities. These profound modifications succeeded the period of the study and probably increased the threat to wildlife and public health. Actions from diverse segments of the government and society are needed to mitigate the impacts of landscape modifications, hydroelectric dams, and the presence of domestic animals in this biodiversity hotspot.

CONCLUSIONS

This study represents one of the largest epidemiological surveys conducted on domestic dogs in the Brazilian Amazon. Exposure to CDV, CPV, *T. gondii*, and *L. infantum* was detected in dogs in the lower Xingu River basin. The high seroprevalence of CDV in dogs is concerning and highlights the risk of transmission to local wildlife. A wide spatial distribution of seropositive animals was observed for CDV, CPV, and *T. gondii*, whilst animals seropositive to *L. infantum* were concentrated near the Xingu River. Exposure to CDV and *T. gondii* was more frequent in areas of pasture, while exposure to *L. infantum* was positively associated with floodplains and forest formations. Clusters of seropositive animals were observed for all investigated pathogens, indicating specific areas with higher risk of exposure.

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DATA AVAILABILITY

The data that support the findings of this study are available, upon reasonable request, from the corresponding author (Felipe Fornazari).

