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Original Contribution

Exposure of Free-Ranging Wild Carnivores and Domestic Dogs to Canine Distemper Virus and Parvovirus in the Cerrado of Central Brazil

Mariana Malzoni Furtado,[1,2] Erika Midori Kida Hayashi,[2] Susan Dora Allendorf,[3] Claudio José Coelho,[4] Anah Tereza de Almeida Jácomo,[1] Jane Megid,[3] José Domingues Ramos Filho,[4] Leandro Silveira,[1] Natália Mundim Tôrres,[1,5] and José Soares Ferreira Neto[2]

[1]*Jaguar Conservation Fund/Instituto Onça-Pintada, Mineiros, GO, Brazil*
[2]*Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária, São Paulo, SP 05508-270, Brazil*
[3]*Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, SP, Brazil*
[4]*Laboratório Biovet, Vargem Grande Paulista, SP, Brazil*
[5]*Instituto de Biologia, Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil*

Abstract: Human population growth around protected areas increases the contact between wild and domestic animals, promoting disease transmission between them. This study investigates the exposure of free-ranging wild carnivores and domestic dogs to canine distemper virus (CDV) and parvovirus in Emas National Park (ENP) in the Cerrado savanna of central Brazil. Serum samples were collected from 169 wild carnivores, including the maned wolf (*Chrysocyon brachyurus*), crab-eating fox (*Cerdocyon thous*), hoary fox (*Pseudalopex vetulus*), puma (*Puma concolor*), ocelot (*Leopardus pardalis*), pampas cat (*Leopardus colocolo*), jaguarundi (*Herpailurus yagouaroundi*), striped hog-nosed skunk (*Conepatus semistriatus*) and coati (*Nasua nasua*), and from 35 domestic dogs living on rural properties bordering ENP. Serological tests showed that 10.6% of wild carnivores (maned wolves, crab-eating foxes and ocelots) and 71.4% of domestic dogs were exposed to CDV, and 56.8% of wild carnivores, including all species sampled except coatis, and 57.1% of domestic dogs were exposed to parvovirus. This report is the first to indicate that the free-ranging pampas cat, jaguarundi and striped hog-nosed skunk are exposed to parvovirus. CDV and parvovirus deserve attention in ENP, and it is extremely important to monitor the health of carnivore populations and perform molecular diagnosis of the viruses to determine the possible involvement of the domestic dog in their transmission.

Keywords: crab-eating fox, conservation Medicine, infectious disease, maned wolf, serosurvey, virus, wild canids, wild felids

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Correspondence to: Mariana Malzoni Furtado, e-mail: marianafurtado@jaguar.org.br

Introduction

Wild carnivores are threatened by habitat fragmentation, hunting and loss of prey (Redford 1992; Pedersen et al. 2007). Infectious diseases are considered a threat, and the growth of human populations and consequent increase in domestic animals around protected areas enhance the opportunities for disease transmission among wild and domestic animals (Cleaveland et al. 2001; Daszak et al. 2000; Bengis et al. 2002).

Wild carnivores play an important role in the ecosystems where they occur, and their decline may result in loss of ecosystem balance (Murray et al. 1999; Macdonald and Kays 2005). They are potentially susceptible to several viral diseases that can severely affect carnivore populations, such as canine distemper virus (CDV) and parvovirus (Fowler 1986; Fiorello et al. 2004; Mccarthy et al. 2007).

CDV has been responsible for significant population declines in free-ranging carnivores such as black-footed ferrets (*Mustela nigripes*) in the United States (Williams et al. 1988), African wild dogs (*Lycaon pictus*) in Kenya (Alexander and Appel 1994) and lions (*Panthera leo*) in the Serengeti (Roelke-Parker et al. 1996). In Brazil, exposure to CDV has been reported in free-ranging pumas (*Puma concolor*), jaguars (*Panthera onca*), maned wolves (*Chrysocyon brachyurus*), crab-eating foxes (*Cerdocyon thous*), ocelots (*Leopadus pardalis*) and raccoons (*Procyon cancrivorus*) (Jorge 2008; Nava et al. 2008; Curi et al. 2012; Furtado et al. 2013).

Parvovirus was responsible for a pandemic among wild wolves and coyotes in the late 1970s in the United States (Thomas et al. 1984; Mech and Goyal 1993; Gese et al. 1997; Mech et al. 2008). In Yellowstone National Park, parvovirus was responsible for a 30% decline in the wolf population. It is also related to the mortality of pups, limiting the growth of grey fox populations (*Canis lupus*) (Smith and Almberg 2007). In Brazil, the exposure of free-ranging crab-eating fox, maned wolves, raccoons, tigrina (*Leopardus tigrinus*), ocelots, pumas and bush dog (*Speothus venaticus*) to parvovirus has already been reported (Filoni et al. 2006; Jorge 2008; Curi et al. 2010, 2012).

To better understand the circulation of CDV and parvovirus in Brazil, this study investigates the exposure of free-ranging wild carnivores and domestic dogs (*Canis familiaris*) to these viruses in the region of Emas National Park, an important protected area in the central Brazilian Cerrado savanna.

Methods

Study Area

Emas National Park (ENP) is situated in central Brazil, in the extreme southwest of the state of Goiás (18°19′S, 52°45′W) (Fig. 1). At 132,000 ha, it is among Brazil's most representative reserves of the Cerrado, the second largest biome that covers 21% of the country's area and protects large tracts of grassland plains and small patches of shrub fields, marshes and riparian forest (Klink and Machado 2005). ENP is situated in one of the most productive agricultural areas of central Brazil, where soybean, corn and sugar cane plantations dominate the landscape, and the native vegetation surrounding the park has been converted to extensive agricultural lands.

Animals and Blood Sample Collection

The blood samples used in this project were part of the Wild Carnivore Population Long Term Monitoring Program in the region of Emas National Park conducted by the non-governmental organization Jaguar Conservation Fund (Jácomo et al. 2004, 2009; Furtado et al. 2006, 2007; Silveira et al. 2009).

Between November 2000 and July 2008, 169 wild carnivores were captured in ENP and its surrounding areas. Small- and medium-sized carnivores were captured using leg-hold traps or metal cage traps with live pigeons or chickens as bait (Silveira et al. 2009). Pumas were captured using trained hounds. The animals were anesthetized with a combination of tiletamine and zolazepam (Zoletil®) at specific doses for each species. Blood samples were collected by puncture of the saphenous, cephalic or jugular vein. At the time of capture, physical examination of the animals was conducted. All wild carnivores sampled appeared to be in good or excellent physical condition, with adequate body weight and an absence of clinical signs of apparent illness. Among the animals captured, 91 were fitted with radio collars and subsequently monitored via radio-telemetry (Jácomo et al. 2009; Oliveira et al. 2010).

Samples were collected from 35 domestic dogs living on rural properties surrounding ENP between May 2002 and October 2003 with the prior authorization of their owners. Two of these domestic dogs were captured inside the park in the same metal cage traps used to capture wild carnivores.

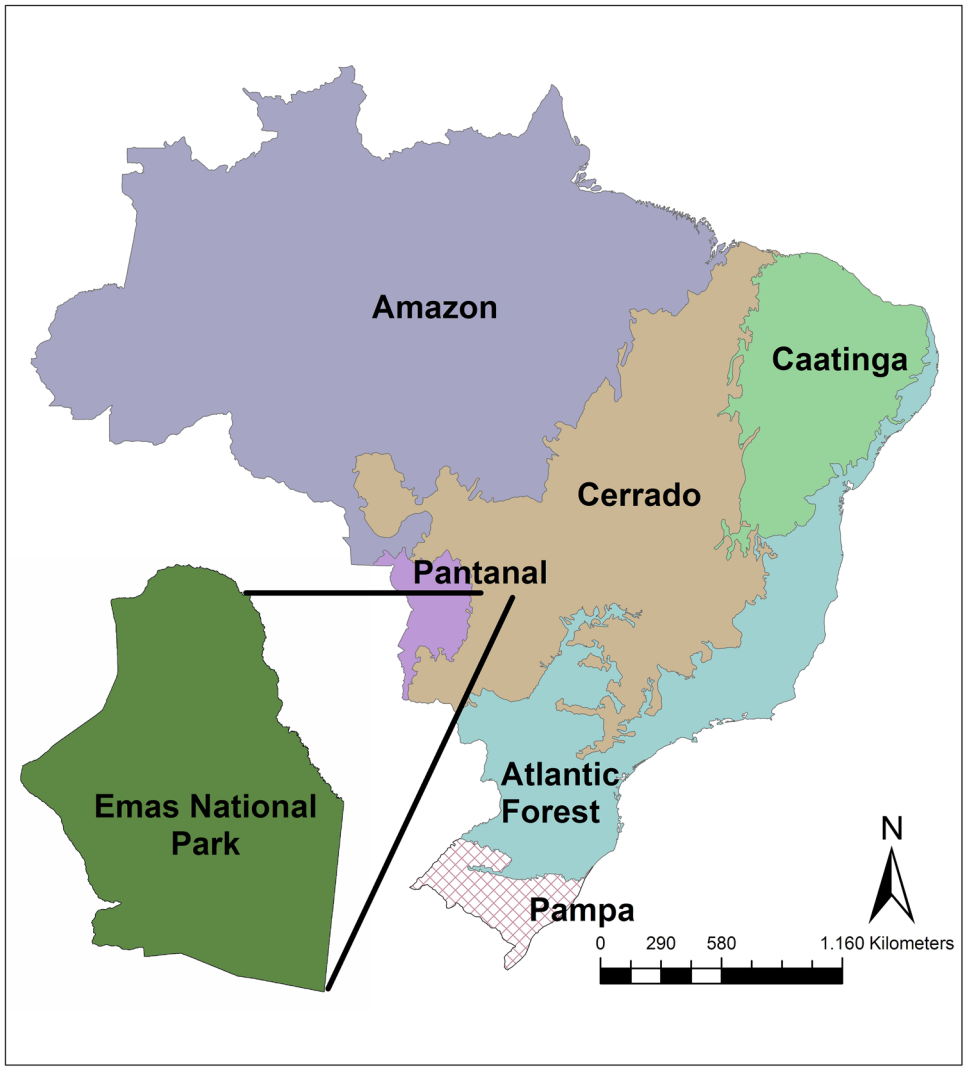

Figure 1. Location of Emas National Park in the Cerrado of central Brazil.

The samples were transported to the field laboratory, where blood was centrifuged for 5 min at 1200×*g*. The serum was removed from the clot tube, divided into aliquots and stored at −20°C. Samples were transported, on average for 12 h, in a cooler with ice packs to São Paulo-SP and stored at −20°C until analysis.

Laboratory Analyses

Antibodies against CDV were detected using the serum neutralization microscopic test (Appel and Robson 1973) adapted to use chicken fibroblasts (Biazzono et al. 2001). Tests were performed at the Biovet laboratory in Vargem Grande Paulista, São Paulo, Brazil. Titers ≥8 were considered positive (Courtenay et al. 2001).

Antibodies against parvovirus were detected using a hemagglutination inhibition test (Carmichael et al. 1980) performed at the Department of Hygiene and Veterinary Public Health, Faculty of Veterinary Medicine and Animal Science of the Paulista State University of Julio de Mesquita Filho (UNESP), Botucatu-SP, Brazil. This test cannot differentiate among feline parvovirus, canine parvovirus and mink enteritis virus (Barker and Parrish 2001). Titers ≥80 were considered positive (Curi et al. 2010).

Neither test has been validated for use in wild carnivores because of potential inaccuracy or cross-reaction with other agents (Greiner and Gardner 2000). Currently, there are no serological tests validated for the wild carnivores sampled in our study, but the tests developed for domestic dogs and cats are widely used in the testing of wild carni-

Table 1. Canine Distemper Virus and Parvovirus Results in Wild Carnivores and Domestic Dogs Sampled in the Region of Emas National Park, Brazil, Between November 2000 and May 2008.

Species		Canine distemper virus				Parvovirus			
Common name	Scientific name	Exam.	Posit.	%	Titer	Exam.	Posit.	%	Titer
Maned wolf	*Chrysocyon brachyurus*	70	9	12.9	8–16	69	34	49.3	80–1280
Crab-eating fox	*Cerdocyon thous*	58	7	12.1	8–32	59	40	67.8	80–1280
Hoary fox	*Pseudalopex vetulus*	5	0	0	–	5	5	100.0	320–1280
Skunk	*Conepatus semistriatus*	4	0	0	–	4	4	100.0	80–320
Coati	*Nasua nasua*	1	0	0	–	1	0	0	–
Jaguarundi	*Herpailurus yagouaroundi*	1	0	0	–	1	1	100.0	320
Pampas cat	*Leopardus colocolo*	15	0	0	–	15	5	33.3	80–640
Ocelot	*Leopardus pardalis*	11	2	18.2	8	11	5	45.5	80–160
Puma	*Puma concolor*	4	0	0	–	4	2	50.0	80–160
	Total	169	18	10.6	8–32	169	96	56.8	80–1280
Domestic dog	*Canis familiaris*	35	25	71.4	8–128	35	20	57.1	80–1280

Exam. examined, *Posit.* positive, *Titer* variation of titers.

vores (Malmlov et al. 2014; Orozco et al. 2014a, b; Nelson et al. 2012; Curi et al. 2012; Furtado et al. 2013; Nava et al. 2008; Fiorello et al. 2007; Deem and Emmons 2005).

Temporal Patterns of Infection

To search for trends in seroprevalence over time, we analyzed the percentage of wild carnivores seropositive for CDV and parvovirus through the years. Because our sample size varied from year to year, we used only the years with large sample sizes of 22–33 captures, from 2002 to 2007.

Handling procedures were consistent with the Ethical Principles in Animal Research adopted by the Bioethics Commission of the School of Veterinary Medicine and Animal Science of University of São Paulo and were approved by permit number 2017/2010. The Chico Mendes Institute for Biodiversity Conservation/ICMBio granted field permit number 13,249 for the work in ENP.

Results

Maned wolves, crab-eating foxes, ocelots and domestic dogs were found to be seropositive for CDV, with titers ranging between 8 and 32 for wild carnivores and 8 and 128 for domestic dogs. All species sampled, except the coati, were found to be seropositive for parvovirus, with titers ranging between 80 and >1280. Detailed results are displayed in Table 1.

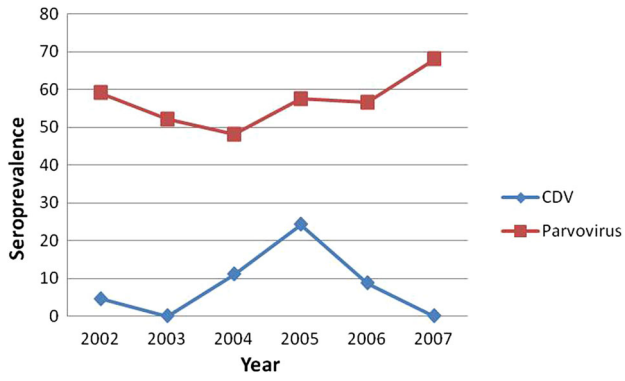

Figure 2. Annual seroprevalence rates for wild carnivores in ENP.

The seroprevalence of CDV varied across the years: the wild carnivores sampled had no cases of infection in 2003, while CDV infected the wild population sometime in 2004 and disappeared again in 2007. For parvovirus, the seroprevalence varied from 48.1 to 68.0% through 2002 and 2007 (Fig. 2).

Figures 3 and 4 illustrate the movement of wild carnivores that tested seropositive for CDV and parvovirus, respectively, and rural properties with domestic dogs seropositive for these viruses.

Discussion

CDV

Our results show the circulation of CDV in the region of ENP in the Brazilian Cerrado biome. Free-ranging maned

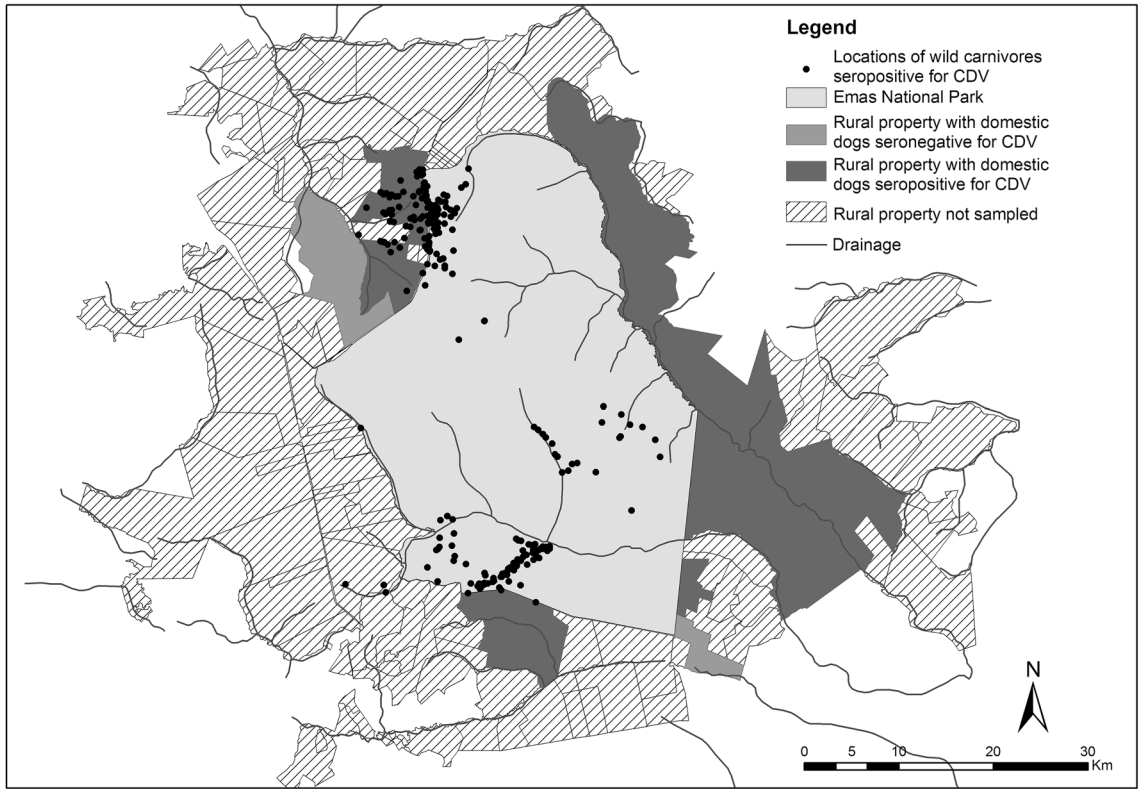

Figure 3. Movement of wild carnivores seropositive for CDV and rural properties with domestic dogs seropositive/seronegative for CDV in the region of Emas National Park, central Brazil.

wolves, ocelots and crab-eating foxes were exposed to CDV, but domestic dogs were more commonly exposed to the virus. On the other hand, hoary foxes, striped hog-nosed skunks, coati, jaguarundi, pumas and pampas cats did not show evidence of CDV exposure.

The exposure of maned wolves, crab-eating foxes and ocelots to CDV had already been described in studies from other Brazilian biomes (Jorge 2008; Megid et al. 2009; Santos 2010; Curi et al. 2012) and other South American countries (Deem and Emmons 2005; Orozco et al. 2014a, b; Ferreyra et al. 2009; Fiorello et al. 2007). Unlike our study, hoary foxes and pumas from Brazil were already reported to be exposed to the virus (Jorge 2008; Nava et al. 2008; Megid et al. 2010; Santos 2010). However, none of these studies was associated with significant population declines, nor was our study.

The lower levels of antibody titers detected in wild carnivores from ENP do not imply infection, but may represent previous natural exposure to CDV. As we used serodiagnosis, we cannot discard the possibility of false positives due to cross-reaction with other agents and nonspecific inhibition. However, the test used in this study is widely used in wild carnivores worldwide (Curi et al. 2010, 2012; Nava et al. 2008; Deem and Emmons 2005; Fiorello et al. 2007; Orozco et al. 2014a, b; Gehrt et al. 2010; Furtado et al. 2013).

The pattern of CDV seroprevalence along the years suggests a discrete outbreak: after its absence in 2003, it reaches a peak, declines and disappears from the wild population in 2007. No mortality or morbidity of wild carnivores was observed during this period. The extent of an outbreak apparently depends on the size of the susceptible host population. But as our serological survey included just few wild carnivorous and our sample size varied from year to year, we cannot state if there are patterns for CDV along the years. Also we cannot relate these data with the domestic animals, which were sampled only in 2002 and 2003.

Domestic dogs living in the surrounding areas of ENP were highly exposed to CDV. Because most of them were not vaccinated for CDV (Furtado et al. 2013), detection of antibodies is most likely due to natural exposure to the virus. Thus, domestic dogs could have acted as a source of CDV to wild carnivores in ENP, but close contact between

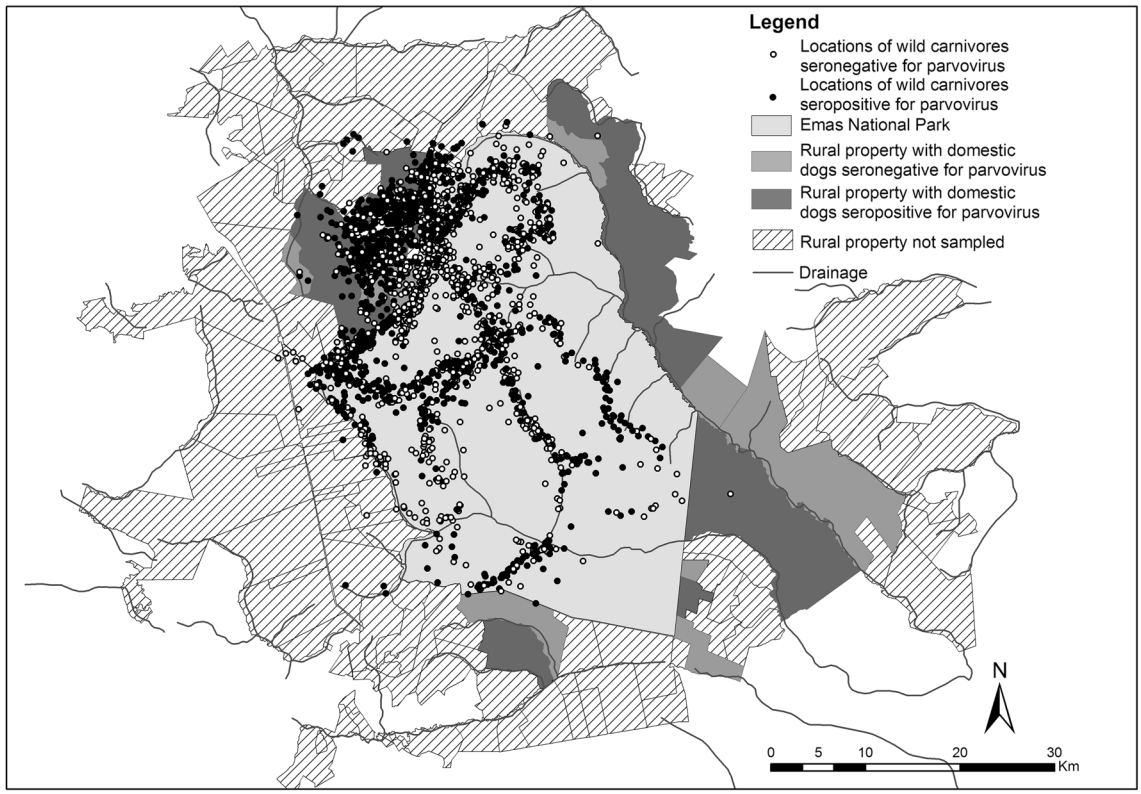

Figure 4. Movement of wild carnivores seropositive and seronegative for parvovirus and rural properties with domestic dogs seropositive/seronegative for parvovirus in the region of Emas National Park, central Brazil.

them would be required to cause infection. CDV is transmitted by direct contact with the nasal and oral secretions or urine of infected animals, or aerosols, and although excreted for long periods, the virus is rapidly inactivated outside its host (Deem et al. 2000; Arns et al. 2007; Kennedy-Stoskopf 1999). Megid et al. (2009, 2010) had suggested that domestic dogs might act as a source of CDV infection in wild canids in Brazil after detecting similar phylogenetic results for the viruses.

Although we cannot state the mode of CDV transmission for wild carnivores in our study, the situation of ENP potentially enables the transmission of the pathogen between wild carnivores and domestic dogs: (1) ENP is entirely bordered by farms with domestic dogs seropositive for CDV; (2) ENP has no physical barrier against wild carnivores, and seropositive wild animals used farms with seropositive domestic dogs as part of their home range (Fig. 2); and (3) most domestic dogs move freely and enter the park on occasion. However, we have no information on direct contact between domestic dogs and wild carnivores, which could suggest that factors other than direct contact with domestic dogs could also influence CDV transmission to wild carnivores. Some authors have suggested that wildlife plays an important role in maintaining CDV infection in the wild regardless of domestic dogs (Craft et al. 2008; Woodroffe and Donnelly 2011; Prager et al. 2012; Woodroffe et al. 2012). To confirm the mode of CDV transmission in ENP, future research should involve molecular diagnosis and phylogenetic analysis of the virus.

Parvovirus

Our results indicate the circulation of parvovirus in the region of ENP, and both wild carnivores and domestic dogs were equally exposed to the virus. All species of wild carnivores sampled in ENP, except coatis, were exposed to parvovirus. This report is the first to indicate the exposure of the free-ranging striped hog-nosed skunk, jaguarundi and pampas cat to the virus.

Parvovirus has previously been reported in maned wolves, crab-eating foxes, hoary foxes, ocelots, pumas and jaguarundis in Brazil (Jorge 2008, Curi et al. 2010, 2012; Santos 2010; Hübner et al. 2010; Filoni et al. 2012) and other South American countries (Deem and Emmons 2005; Orozco et al. 2014a; Fiorello et al. 2007; Ferreyra et al. 2009). There are different species of parvovirus that infect

several carnivorous species but they are all limited to their taxonomic units. Although a human variant of the parvovirus does exist, its transmission is limited to the human species. As in the canine and feline families, the parvovirus crosses over different genus within the family groups, but is limited to these families (Flanagan et al. 2012).

The high levels of antibody titers found in maned wolves, crab-eating foxes and hoary foxes in this study (80 to >1280) suggested an active response to parvovirus at the time of sampling.

The wild carnivore population showed constant and high levels of seroprevalence for parvovirus. The rates of parvovirus did not vary significantly across the years, consistent with a pattern of chronic infection. Parvovirus is probably endemic in the region of ENP; although we have no information about mortality or morbidity related to this virus during this period, the virus probably has low pathogenicity and prolonged infectiousness.

Domestic dogs living in the areas surrounding ENP were highly exposed to parvovirus. Considering that most domestic dogs in the region are not vaccinated for parvovirus (Furtado et al. 2013), the antibody titers detected in this study most likely stem from natural exposure to the agent. Parvovirus can survive for long periods in the environment and is transmitted through feces, so direct contact with infected animals is not necessary (Gordon and Angrick 1986). As most of the domestic dogs in the region of ENP are seropositive for parvovirus, and wild carnivores use the farms as part of their home ranges (Fig. 3), domestic dogs could serve as a source of infection for the virus. On the other hand, the wild carnivores seropositive for parvovirus also used the interior of ENP as part of their home range and are quite widely distributed throughout the park (Fig. 3). Thus, we cannot conclusively state the form of parvovirus transmission in the region or how the agent is being maintained in this environment.

Given the impact of parvovirus on wolves (Wydeven et al. 1995; Fournier-Chambrillon et al. 2004; Mech et al. 2008) and its ability to reduce the reproductive output of wild canids (Gese et al. 1997; Mech et al. 2008), it is extremely important to conduct molecular diagnosis and phylogenetic analysis of this virus in ENP.

Conclusion

CDV and parvovirus deserve attention in the region of ENP, especially due to the possibility of the involvement of domestic dogs in their transmission. We consider the following to be extremely important: (1) conducting molecular diagnosis and phylogenetic analysis of these viruses; (2) health monitoring of carnivore populations in the region of ENP; (3) vaccination of domestic dogs against CDV and parvovirus; (4) more rigorous supervision of ENP to prevent the entry of domestic animals into the park; and (5) educating the human populations around the park regarding the risks that domestic animals may represent for wildlife. We also suggest that infectious disease should be considered in wild carnivore management plans.

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