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Wild canids, domestic dogs and their pathogens in Southeast Brazil: disease threats for canid conservation

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Abstract Wild canids are under many pressures, including habitat loss, fragmentation and disease. The current lack of information on the status of wildlife health may hamper conservation efforts in Brazil. In this paper, we examined the prevalence of canine pathogens in 21 free-ranging wild canids, comprising 12 *Cerdocyon thous* (crab-eating fox), 7 *Chrysocyon brachyurus* (maned wolf), 2 *Lycalopex vetulus* (hoary fox), and 70 non-vaccinated domestic dogs from the Serra do Cipó National Park area, Southeast Brazil. For wild canids, seroprevalence of antibodies to canine parvovirus, canine adenovirus, canine coronavirus and *Toxoplasma gondii* was 100 (21/21), 33 (7/21), 5 (1/19) and 68 (13/19)

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percent, respectively. Antibodies against canine distemper virus, *Neospora caninum* or *Babesia* spp. were not found. We tested domestic dogs for antibodies to canine parvovirus, canine distemper virus and *Babesia* spp., and seroprevalences were 59 (41/70), 66 (46/70), and 42 (40/70) percent, respectively, with significantly higher prevalence in domestic dogs for CDV (P < 0.001) and *Babesia* spp. (P = 0.002), and in wild canids for CPV (P < 0.001). We report for the first time evidence of exposure to canine coronavirus in wild hoary foxes, and *Platynossomun* sp. infection in wild maned wolves. Maned wolves are more exposed to helminths than crab-eating foxes, with a higher prevalence of Trichuridae and Ancylostomidae in the area. The most common ectoparasites were *Amblyomma cajennense*, *A. tigrinum*, and *Pulex irritans*. Such data is useful information on infectious diseases of Brazilian wild canids, revealing pathogens as a threat to wild canids in the area. Control measures are discussed.

Keywords Brazilian Cerrado · Canid conservation · Disease · Seroprevalence · Wildlife pathogens

Introduction

Infectious diseases are widely recognized as a threat to biodiversity conservation. The introduction of new pathogens into naïve populations, so called pathogen pollution, is often due to anthropogenic environmental disturbance such as habitat loss and fragmentation, genetic isolation of wild populations, the ever-increasing proximity of humans and their domestic animals, animal movements or translocations, and climate change (Daszak et al. 2000, 2001; Deem et al. 2001; Harvell et al. 2002; Cunningham et al. 2003). Infectious diseases may cause low fertility and increased mortality, and jeopardize, to different degrees, the wild populations' viability and the health of the whole ecosystem.

No environment remains unaffected by emerging or re-emerging wildlife pathogens (Dobson and Foufopoulos 2001), and as shown throughout the world (e.g. Africa, Europe and North America) (Gascoyne et al. 1993; Damien et al. 2002; Mech et al. 2008). There is therefore an urgent need to understand threats, dynamics and impacts, as well as prevalence and distribution of infectious diseases also in South American wildlife. Some studies have already been conducted in countries like Bolivia, Argentina and Brazil (e.g. Uhart et al. 2003; Fiorello et al. 2004; Martino et al. 2004; Deem and Emmons 2005; Curi et al. 2006; Filoni et al. 2006), however, in most cases, the health status of wild populations remains uncertain in the Neotropics. Disease screening is, therefore, among the basic needs for Neotropical species conservation and research.

Carnivora are one of the most endangered taxa amongst mammals worldwide. Large carnivores are threatened due to 'edge effects' rather than stochastic processes, with reserve size and human-induced mortality rates as the crucial drivers towards extinction of carnivores in protected areas (Woodroffe and Ginsberg 1998). However, high mortality rates have been frequently attributed to epidemics in this order (Young 1994; Funk et al. 2001). Features of canid ecology including foraging behaviour, close social contacts, scent communication with infectious material (urine and faeces), and the genetic proximity to domestic dogs (*Canis lupus familiaris*) make them particularly susceptible to disease (Woodroffe et al. 2004). A wide array of pathogens has been reported to affect wild canids (see Murray et al. 1999), some of which have been responsible for important declines of endangered species. Examples include rabies in African wild dogs *Lycaon pictus* (Gascoyne et al. 1993) and Ethiopian wolves *Canis simensis* (Randall



et al. 2004), canine parvovirus in wolves *Canis lupus* from the USA (Mech and Goyal 1993; Mech et al. 2008) and Italy (Martinello et al. 1997), canine distemper virus in African wild dogs (Alexander and Appel 1994; van de Bildt et al. 2002), European red foxes *Vulpes vulpes* (Damien et al. 2002) and Santa Catalina Island foxes *Urocyon littoralis catalinae* (Timm et al. 2009). Furthermore, in South America some studies have confirmed the presence of disease threats such as distemper and parvovirus to wild canids (e.g. Fiorello et al. 2004; Martino et al. 2004; Deem and Emmons 2005; Megid et al. 2009, 2010).

Contact with domestic dogs affects wild canids through competition, predation and disease outbreaks through 'spill over' infection (Butler et al. 2004). Dogs have been frequently implicated as a source of infection for wild canids (Alexander and Appel 1994; Johnson et al. 1994; Laurenson et al. 1998; Cleaveland et al. 2000; Randall et al. 2004; Woodroffe et al. 2004; Megid et al. 2009, 2010). Disease transmission from domestic dogs may constitute an important anthropogenic 'edge effect' (Woodroffe and Ginsberg 1998; Woodroffe et al. 2004). Sympatric wild species may also transmit pathogens to other wild canid populations, causing disease-induced mortality. For example, African wild dogs experienced rabies outbreaks, which were probably transmitted by jackals *Canis mesomelas* in South Africa (Hofmeyr et al. 2004).

Some species of the family Canidae are listed on the International Union for Conservation of Nature—IUCN Red List 2010. The list includes three species from the Brazilian Cerrado (a savannah-like grassland): the hoary fox (*Lycalopex vetulus*) (Lund 1842) and the crab-eating fox (*Cerdocyon thous*) (Linnaeus 1766), both classified as least concern, and the maned wolf (*Chrysocyon brachyurus*) (Illiger 1815) classified as near threatened (Courtenay and Maffei 2008; Dalponte and Courtenay 2008; Rodden et al. 2008). The Brazilian Institute of Natural Resources and Environment (IBAMA) also cites the maned wolf as vulnerable, with parasites featured among the main threats (Machado et al. 2005). These endangered low-density species occur sympatrically in some parts of their distribution (Jácomo et al. 2004), and have been recorded in the Serra do Cipó National Park and the Morro da Pedreira Environmental Protection Area, the Park's buffer zone (Curi and Talamoni 2006).

Given the relevance of disease for carnivore conservation, disease surveillance and infectious disease research are of critical importance. Serological surveys, necropsies and isolated case reports may be of value for the development of management plans because they provide baseline information on pathogen presence and prevalence. Wild and domestic species that might act as reservoir hosts should also be assessed (Woodroffe 1999; Bengis et al. 2002). Despite its drawbacks (possible cross-reactions with non-pathogenic parasite strains and taxonomically related agents, differences in cut-off point determination between laboratories, technical problems and the relatively high costs), cross-sectional serologic analysis reveals evidence of the exposure history of individuals (Bengis et al. 2002; Fiorello et al. 2004) and can indicate the presence and prevalence of disease agents in wild populations.

This study aimed to collect data on the presence and prevalence of a range of multi-host pathogens, including canine parvovirus (CPV), canine distemper virus (CDV), canine adenovirus (CAV), canine coronavirus (CCV), *Toxoplasma gondii*, *Babesia* spp., *Neospora caninum*, helminths and ectoparasites in wild and domestic canid populations around the Serra do Cipó National Park. As this is the first investigation into wild canid pathogens in the region, we selected canine disease agents by their occurrence in the state and by the availability of test protocols and materials.



Methods

Study site

The Serra do Cipó National Park (SCNP) (19°12′–19°20′S, 43°30′–43°40′W; altitude of 1,095–1,485 m, area of 33,800 ha, 154 km perimeter, 21.2°C mean temperature and 1.622 mm rainfall) as well as the Morro da Pedreira Environmental Protection Area, the buffer zone that encircles the park, are situated in the Southern Espinhaço Range, Minas Gerais State, Southeast Brazil (Fig. 1). Human populations and their domestic animals are allowed to reside within the protected areas. Moreover, there are reports of feral dogs hunting inside the park. Many villages and small towns border the SCNP, and dogs are frequently seen inside its boundaries. Other carnivores, such as Procyonids, Mustelids and Felids also occur in the area (Câmara and Murta 2003), and may be involved in some multi-host disease cycles jointly with wild and domestic canids.

Data set

During the dry seasons of May-October 2004 and June-September 2005, we conducted captures of wild canids using cage traps under license number 016/2004 issued by IBAMA, and sampled non-vaccinated adult (>1 year) dogs with access to the protected areas (under the owners' signed permission) inside and around SCNP. Dogs were selected by distance from trapping points of less than 5 km, negative vaccination status (except against rabies), and the accessibility to wild areas. Rabies vaccination campaigns are conducted in the area, but most dog owners do not vaccinate their dogs against other diseases. However, owners were questioned about this to ensure sampling of non-vaccinated dogs only. Accidentally trapped dogs were not sampled because of the lack of information on their vaccination status. We trapped 21 wild canids comprising 12 crab-eating foxes, 7 maned wolves and 2 hoary foxes. They were healthy and clinically normal, and were classified as adults, based on weight, genital development and assessment of tooth wear. Six of the 21 wild canids (6 of 7 maned wolves) were trapped inside the SCNP boundaries. The other 15 animals were caught at points up to 5 km from villages and towns in the Park's buffer zone. The same capture success was observed for domestic dogs (n = 21), unintentionally captured at the same points as the wild canids (capture ratio wild canids/domestic dogs of 1:1). These were only photographed, recorded and released immediately. Wild canids were

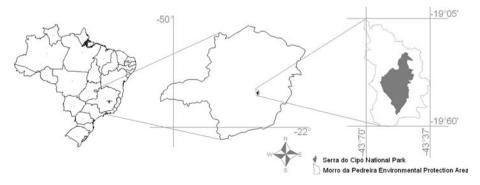


Fig. 1 Map of the Serra do Cipó National Park and the buffer zone, Minas Gerais State, Brazil



chemically restrained with blowguns and home made darts charged with 2 mg/kg of xilazine chlorhydrate and 8 mg/kg of ketamine chlorhydrate, and marked with atoxic black hair dye to avoid re-sampling. Domestic dogs (n = 70) were manually restrained for sample collection and clinical examination. For more data on trapping effort and success, restraint and clinical aspects, see Curi and Talamoni (2006).

Blood from the cephalic or femoral veins was collected in vacuum tubes without anticoagulant and left at room temperature for 4 h. Serum was separated by centrifugation and
frozen at -20°C, before the serological testing described below. Ear point blood smears
were prepared and fixed in methanol, and stored before staining with methylene blue and
a microscopic search for hemoparasites. For the identification of macroparasitic species,
urine samples were only collected from male wild canids (to avoid damage to the females'
urinary/reproductive tract) to search for parasite eggs, such as *Dioctophyma renale* and
Capillariidae. Urine samples were centrifuged and the microscopic analysis was performed
on the sediment. Faecal samples were collected from the rectum of animals or from the traps
and cooled for up to 5 days before parasitological analysis, which included flotation, sedimentation and Baermann routine tests. Ectoparasites were manually collected and stored in
70% ethanol for identification, and 10 specimens of *Amblyomma* were analysed for the
presence of hemoparasites in their haemolymph fluid. Necropsies were performed on two
road-killed *C. thous* around the SCNP. Domestic dogs were only serologically tested.

Serum samples of wild canids were tested for antibodies to CPV using the haemag-glutination inhibition test (1:80; Senda et al. 1986), to CDV (1:2; Appel and Robson 1973), CAV (1:16; Appel et al. 1975) and CCV (1:4; Mochizuki et al. 1987) using the serum neutralization test, to *Babesia* spp. and to *N. caninum* using the indirect immunofluorescence antibody test (1:40; Dell'Porto et al. Dell'Porto et al. 1990, 1:50; Cáñon-Franco et al. 2004), and to *T. gondii* using the modified agglutination test (1:25; Dubey and Desmonts 1987; Dubey et al. 2007). All tests were performed in duplicates, negative and positive serum controls were used. Discrepancies between duplicates were resolved by selecting the lowest titres. Samples from domestic dogs were tested for antibodies against CPV, CDV and *Babesia* spp. only.

Data from serological tests were analyzed as apparent prevalence (n positive/n examined \times 100, see Gardner et al. 1996), since information about specificity and sensitivity of the tests are unavailable for wild canid species. We analyzed the proportion of positives with the chi-square test (χ^2) or Fisher's exact test (Zar 1996), and utilized SPSS 9.0 for Windows (SPSS base 9.0 User's guide, USA) and Epiinfo 6.04d (Center for Diseases Control and Prevention, USA) for frequency and 95% confidence interval calculation. In order to analyze the proportion of positives for pathogen exposure, wild canids were grouped due to the small number of *per* species cases. Proportional analysis of positive faecal samples was performed for maned wolves and crab-eating foxes.

Results

Every wild canid sample showed evidence of exposure to at least one pathogen, and each of the three species showed seropositivity for at least two canine pathogens. Maned wolves and crab-eating foxes had antibodies for three of seven pathogens surveyed (CPV, CAV and *T. gondii*), hoary foxes had antibodies for two diseases (CPV and CCV), and domestic dogs showed antibody titres for all three pathogens surveyed (Tables 1, 2).

Seroprevalence varied by pathogen and canid species (Tables 1, 2). Significant differences between wild and domestic canids' exposure were detected for the three agents



Table 1 Number of positives/sampled animals for selected pathogens in domestic and wild canids from Serra do Cipó National Park, Southeast Brazil, sampled in 2004 and 2005

Positives/sampled					
Serologic tests	Canis l. familiaris	Chrysocyon brachyurus	Cerdocyon thous	Lycalopex vetulus	
CDV	46/70	0/3	0/9	0/2	
CPV	41/70	7/7	12/12	2/2	
CAV	NT	6/7	1/12	0/2	
CCV	NT	0/7	0/10	1/2	
Babesia spp.	30/70	0/3	0/9	0/2	
T. gondii	NT	6/7	7/10	0/2	
N. caninum	NT	0/7	0/10	0/2	

CDV canine distemper virus, CPV canine parvovirus, CAV canine adenovirus, CCV canine coronavirus, NT not tested

Table 2 Number of positive/sampled animals and proportion of positives and Confidence Interval (95% CI) for selected pathogens in canids from Serra do Cipó National Park, Southeast Brazil, sampled in 2004 and 2005

	Domestic dogs	Domestic dogs		Wild canids		
Serologic tests	No of positives/ sampled	Proportion (95% CI)	No of positives/ sampled	Proportion (95% CI)		
CDV*	46/70	66% (53–77%)	0/14	0% (0–23%)		
CPV*	41/70	59% (46–70%)	21/21	100% (84–100%)		
CAV	NT	_	7/21	33% (15–57%)		
CCV	NT	_	1/19	5% (0-26%)		
Babesia spp.*	30/70	42% (31–55%)	0/14	0% (0-23%)		
T. gondii	NT	_	13/19	68% (43-87%)		
N. caninum	NT	_	0/19	0% (0–17%)		

CDV canine distemper virus, CPV canine parvovirus, CAV canine adenovirus, CCV canine coronavirus, NT not tested

tested in both groups: CPV, for which seroprevalence was higher in wild canids [100% (21/21) vs. 59% (41/70) in domestic dogs, $\chi^2 = 12.7$, 1 df, P < 0.001], CDV, for which seroprevalence was higher in domestic dogs [66% (46/70) vs. 0% (0/14) in wild canids, $\chi^2 = 20.3$, 1 df, P < 0.001), and *Babesia* spp., for which seroprevalence was higher in domestic dogs [42% (30/70) vs. 0% (0/14) in wild canids, $\chi^2 = 9.1$, 1 df, P = 0.002) (Table 2). Titre range for CPV was 160–640, for CDV was 8–128, and for *Babesia* spp. was 40–1280. Antibodies for CAV were detected in maned wolves and crab-eating foxes (titres ranged from 32–256). Antibodies for CCV were only detected in one hoary fox (titre of four). Maned wolves and crab-eating foxes showed high prevalence of antibodies to *T. gondii* (titres 25–500). All wild canids were seronegative for *N. caninum* (Tables 1, 2).

The helminth eggs found in faecal samples were: Trichuridae, Ancylostomidae, *Physaloptera* sp., *Toxocara* sp., Trematoda, Acantocephala, *Platynossomun* sp., *Spirometra* sp.



^{*} Chi-Square test and Fisher's Exact Test: statistically significant differences (P < 0.05)

Table 3 Number of examined faecal samples and prevalence of gastrointestinal helminths in wild canids from Serra do Cipó National Park, in 2004–2005

Parasites	Chrysocyon brachyurus Positives/sampled	Cerdocyon thous Positives/sampled	
Trichuridae	6/10	0/10	
Ancylostomidae	8/10	2/10	
Physaloptera sp.	2/10	0/10	
Toxocara sp.	0/10	1/10	
Trematoda	0/10	1/10	
Acantocephala	3/10	1/10	
Platynossomun sp.	1/10	2/10	
Spirometra sp.	1/10	3/10	
Hymenolepidae	5/10	1/10	

Faecal samples were collected from captured animals (n = 17), and from the traps (n = 3)

Table 4 Number of wild canids [captured (n = 21) and necropsied (n = 2)] infested with ectoparasites in Serra do Cipó National Park region, 2004–2005

	Cerdocyon thous		Chrysocyon brachyurus		Lycalopex vetulus	
Ectoparasites	Positives/ sampled	Proportion (95% CI)	Positives/ sampled	Proportion (95% CI)	Positives/ sampled	Proportion (95% CI)
Amblyomma sp.a	10/14	71% (42–92%)	3/7	43% (10–82%)	2/2	100% (16–100%)
A. cajennense	4/14	29% (8–58%)	3/7	43% (10–82%)	1/2	50% (1-99%)
A. tigrinum	2/14	14% (2–43%)	1/7	14% (0-58%)	0/2	0% (0-84%)
A. ovale	2/14	14% (2–43%)	0/7	0% (0-41%)	0/2	0% (0-84%)
Pulex irritans	1/14	7% (0–34%)	1/7	14% (0-58%)	0/2	0% (0-84%)
Ctenocephalides felis	1/14	7% (0–34%)	0/7	0% (0-41%)	0/2	0% (0–84%)

^a Ticks in early stages (larvae, nymph)

and Hymenolepidae. High prevalence was found for Trichuridae and Ancylostomidae in maned wolves (Table 3). All urine samples were negative for parasite eggs or larvae. Two necropsied crab-eating foxes were in high autolysis stage, and in the intestinal tubes were found *Rictularia* sp., Hymenolepididae (proglottids), *Ancylostoma* sp. (direct examination of intestinal content) and *Capillaria* sp., *Trichuris vulpis*, *Ancylostoma* sp., *Spirocerca* sp., Hymenolepididae, and Acari eggs (faecal material examination).

Ectoparasites collected from captured and necropsied animals were *Amblyomma* cajennense, A. tigrinum and A. ovale ticks and Pulex irritans and Ctenocephalides felis felis fleas (Table 4). No hemoparasite or parasitized cell was found in either blood smears or tick haemolymph fluid.

Discussion

Based on equal ratios of captures of wild and domestic canids at the same locations, we believe that contact rates, hence disease transmission opportunities between wild and domestic canids, may be considerably high in this region.



Our analysis indicates that domestic dogs are significantly more exposed to CDV and Babesia spp. infection than wild canids, whereas wild canids are more exposed to CPV. High prevalence and titres for CPV in wild canids and dogs indicate that the virus actively circulates in this area. This is expected since CPV has great environmental resistance, its transmission occurs mainly by contact with faeces from infected animals, and direct contact is not required for efficient transmission (Steinel et al. 2001). The main reported population impacts of CPV are decreased fecundity because of early mortality, high pup mortality and reduced population turnover (Mech and Goyal 1993; Steinel et al. 2001; Mech et al. 2008), which would pose a major threat to local wild canid populations. Other surveys from different regions worldwide have found evidence for CPV infection and mortality in wild canids (e.g. Johnson et al. 1994; Martinello et al. 1997; Laurenson et al. 1998; Arjo et al. 2003; Martino et al. 2004; Deem and Emmons 2005). Fiorello et al. (2007) found antibodies to CPV in crab-eating foxes from Bolivia. Antibody titres for CPV above 80 are considered protective for captive maned wolves, based on titres considered desirable for domestic dogs (Maia and Gouveia 2001). Despite this, we cannot guarantee that the CPV antibody titres found in this study (all samples of wild canids above 80) are indeed protective for crab-eating foxes, hoary foxes, and maned wolves in field conditions. This question warrants further investigation.

The lack of antibodies against CDV in wild canids and the high prevalence in domestic dogs highlight that the introduction of CDV from domestic dogs into naïve wild canid populations could have devastating consequences (with generalized viral spread and severe clinical signs a likely outcome of infection) (Harder and Osterhaus 1997; Deem et al. 2000). Close monitoring of mortality and improved knowledge of species-specific sensitivity and specificity of the tests used would help evaluate these results further. The local domestic dog population showed evidence of exposure and may act as a source of infection to naïve wild canid populations. Direct contact is required for efficient transmission of CDV through aerosols of respiratory and other body secretions, although the virus can survive in the environment for 2 days at 25°C or longer at lower temperatures (Deem et al. 2000). Therefore, high contact rates of wild canids and domestic dogs as inferred from our study area might cause CDV spread and perpetuation. Studies in other areas reported antibodies for CDV and mortality caused by distemper in wild canids (e.g. Alexander and Appel 1994; Laurenson et al. 1998; Damien et al. 2002; van de Bildt et al. 2002; Martino et al. 2004; Deem and Emmons 2005; Timm et al. 2009), and recent case reports confirmed natural infection of CDV in crab-eating foxes and hoary foxes in Brazil, with phylogenetic analyses of the viruses implicating domestic dogs as the source of infection (Megid et al. 2009, 2010).

The high prevalence of CAV in maned wolves (Table 1) indicates widespread circulation in this species inside SCNP (all six seropositive individuals were caught within the Park's boundaries). Unfortunately, we did not test domestic dogs for CAV antibodies. Seropositivity for CAV infection has also been reported in African and South American wild canids (Laurenson et al. 1998; Martino et al. 2004; Deem and Emmons 2005, Fiorello et al. 2007), and it may cause respiratory disease and high mortality in carnivores (Murray et al. 1999), highlighting the potential threat for wild canids in our study area.

In Bolivia, a survey on domestic dogs revealed that CDV, CPV and CAV may represent disease risks for wild carnivores (Fiorello et al. 2004). In Northern Brazil, Courtenay et al. (2001) found no evidence for CDV and CPV infection in crab-eating foxes, despite the high likelihood of intra-specific contacts. Low seroprevalence in domestic dogs may explain the lack of seropositivity in local foxes.



Low prevalence of CCV antibodies indicates that the virus may not be the most significant threat for wild canids in this area, which would be supported by the reported low mortality levels due to gastrointestinal signs (Murray et al. 1999). However, we did not test domestic dogs for this agent. To our knowledge, this is the first report of CCV antibodies in free-ranging hoary foxes. Serological evidence for exposure to CCV has also been reported in maned wolves in Argentina (Deem and Emmons 2005) and wolves in Alaska (Zarnke et al. 2001).

Ruas et al. (2003a) confirmed *Babesia* spp. infection in foxes (*L. gymnocercus*) in Southern Brazil. In our study, the absence of antibodies for *Babesia* spp. in wild canids is consistent with the fact that ticks from the genus *Rhipicephalus* (the main vector of *Babesia* spp.) were not found in trapped wild canids, no hemoparasites or parasitized cells were observed in the blood smears, and none of the *Amblyomma* ticks analysed were infected with *Babesia* spp. In contrast, seroprevalence in dogs from SCNP area was considerably higher.

High prevalence of *T. gondii* antibodies in maned wolves and crab-eating foxes indicates that this agent may be common in the SCNP area. There was no evidence of exposure in hoary foxes, which may be due to the limited sample size. Wild canids from Brazil (Gennari et al. 2004) and Argentina (Martino et al. 2004) have also been reported to be exposed to *T. gondii*. We did not detect antibodies for *N. caninum* in wild canids from our study area, but Cáñon-Franco et al. (2004) reported evidence of infection in Brazilian wild canids, and significant seroprevalence was found in foxes from Argentina (Martino et al. 2004).

Regarding helminth parasites, this is the first report of *Platynossomun* infection in freeranging maned wolves. The genus *Capillaria* was already described in crab-eating foxes and pampas foxes *L. gymnocercus* (Ruas et al. 2003b) and in maned wolves (Vicente et al. 1997) from Brazil. Capillariidae eggs were found in the urine of maned wolves from Argentina (Beldomenico et al. 2002). Our results indicate that the maned wolf is more exposed to helminths than the crab-eating fox, with higher prevalence of Trichuridae and Ancylostomidae infection in SCNP (Table 3).

Some of the ectoparasite taxa reported here are often found in domestic carnivores. *Amblyomma cajennense* is common in the study region as well as in wild canids from the border region of São Paulo and Mato Grosso states, and other areas in Brazil (Labruna et al. 2002; 2005). *A. tigrinum* is a frequent tick of wild carnivores in Brazil and *A. ovale* has been recorded in crab-eating foxes and maned wolves (Labruna et al. 2005). The two flea species were previously described in crab-eating foxes (Cerqueira et al. 2000).

Population size and structure estimates have never been performed on carnivore species in the SCNP, as is the case in most parts of the Cerrado biome. As a result, there is a lack of demographic data, which would be crucial for epidemiological control planning in this area. Even so, efforts should be directed towards the prevention of contact between wildlife, humans and domestic animals especially near the reserve's boundaries and in the buffer zone, with the aim of a reduction of disease prevalence and incidence. Population control by sterilization, and vaccination of dogs against CDV, CPV and other diseases may be effective (see Woodroffe et al. 2004), and increased awareness of local inhabitants may help prevent the access of dogs to wild areas, hence reduce infection risks for wildlife.

This is the first multiple sero-parasitological study conducted on wild canids from Brazil, and the data presented here indicate that wild canids are threatened by canine pathogens in the area. This baseline data should alert wildlife managers about disease threats, especially in Brazil, and can be used in wild canid population viability analysis, to assess long-term changes, e.g. after ecological disturbances, as well as for comparative



purposes (Deem et al. 2001). This sort of research contributes towards identifying which pathogens are present in each wild population, and revealing the biogeography of hosts and their parasites. An ambitious, but not impossible, goal would be to have such data continually updated and available for management plans and translocation programmes in Neotropical species.

More investigation and understanding of parasite and disease distribution and dynamics in wildlife/domestic animal interface areas, as well as more interaction amongst wildlife veterinarians, conservation biologists and epidemiologists are necessary for the design of effective management plans aimed at Neotropical species conservation.

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References

- Alexander KA, Appel MJG (1994) African wild dogs (*Lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. J Wildl Dis 30:481–485
- Appel M, Robson DS (1973) A microneutralization test for canine distemper virus. Am J Vet Res 34:1459–1463
- Appel M, Carmichael LE, Robson DS (1975) Canine adenovirus type-2 induced immunity to two canine adenoviruses in pups with maternal antibody. Am J Vet Res 36:1199–1202
- Arjo WM, Gese EM, Bromley C et al (2003) Serologic survey for diseases in free-ranging coyotes (*Canis latrans*) from two ecologically distinct areas of Utah. J Wildl Dis 39:449–455
- Beldomenico PM, Hunzicker D, Taverna JL et al (2002) Capillariidae eggs found in the urine of a freeranging maned wolf from Argentina. Mem Inst Oswaldo Cruz 97:509–510
- Bengis RG, Kock RA, Fischer J (2002) Infectious animal diseases: the wildlife/livestock interface. Rev Sci Tech OIE 21:53–65
- Butler JRA, du Toit JT, Bingham J (2004) Free-ranging domestic dogs (*Canis familiaris*) as predators and prey in rural Zimbabwe: threats of competition and disease to large wild carnivores. Biol Conserv 115:369–378
- Câmara EMV, Murta R (2003) Mamíferos da Serra do Cipó. Editora PUC Minas, Belo Horizonte
- Cáñon-Franco WA, Yai LEO, Souza SLP et al (2004) Detection of antibodies to Neospora caninum in two species of wild canids, Lycalopex gymnocercus and Cerdocyon thous from Brazil. Vet Parasitol 123:275–277
- Cerqueira EJL, Silva EM, Monte-Alegre EF et al (2000) Considerações sobre pulgas (Siphonaptera) da raposa *Cerdocyon thous* (Canidae) da área endêmica de leishmaniose visceral de Jacobina, Bahia, Brasil. Rev Soc Bras Med Trop 33:91–93
- Cleaveland S, Appel MGJ, Chalmers WSK et al (2000) Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. Vet Microbiol 72:217–227
- Courtenay O, Maffei L (2008) *Cerdocyon thous*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1. Available at www.iucnredlist.org. Cited 27 April 2010
- Courtenay O, Quinnel RJ, Chalmers WSK (2001) Contact rates between wild and domestic canids: no evidence of parvovirus or canine distemper virus in crab-eating foxes. Vet Microbiol 81:9–19
- Cunningham AA, Daszak P, Rodríguez JP (2003) Pathogen pollution: defining a parasitological threat to biodiversity conservation. J Parasitol 89:878–883
- Curi NHA, Talamoni SA (2006) Trapping, restraint and clinical-morphological traits of wild canids (Carnivora, Mammalia) from the Brazilian Cerrado. Rev Bras Zool 23:1148–1152
- Curi NHA, Miranda I, Talamoni SA (2006) Serologic evidence of *Leishmania* infection in free-ranging wild and domestic canids around a Brazilian National Park. Mem Inst Oswaldo Cruz 101:99–101
- Dalponte J, Courtenay O (2008) Pseudalopex vetulus. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1. Available at www.iucnredlist.org. Cited 27 Apr 2010



- Damien BC, Martina BEE, Losch S et al (2002) Prevalence of antibodies against canine distemper virus among red foxes in Luxemburg. J Wildl Dis 38:856–859
- Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science 287:443–449
- Daszak P, Cunningham AA, Hyatt AD (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop 78:103–116
- Deem SL, Emmons LH (2005) Exposure of free-ranging maned wolves (Chrysocyon brachyurus) to infectious and parasitic disease agents in Noël Kempff Mercado National Park, Bolivia. J Zoo Wildl Med 36:192–197
- Deem SL, Spelman LH, Yates RA et al (2000) Canine distemper in terrestrial carnivores: a review. J Zoo Wildl Med 31:441–451
- Deem SL, Karesh WB, Weisman W (2001) Putting theory into practice: wildlife health in conservation. Conserv Biol 15:1224–1233
- Dell'Porto A, Oliveira MR, Omar M (1990) Babesia canis in stray dogs of S\u00e3o Paulo city. I. Comparative study of methods for diagnosis. Braz J Vet Res Anim Sci 27:41-45
- Dobson A, Foufopoulos J (2001) Emerging infectious pathogens of wildlife. Phil Trans R Soc Lond B 356:1001-1012
- Dubey JP, Desmonts G (1987) Serologic responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet J 19:337–339
- Dubey JP, Cortés-Vecino JA, Vargas-Duarte JJ et al (2007) Prevalence of *Toxoplasma gondii* in dogs from Colombia, South America and genetic characterization on *T. gondii* isolates. Vet Parasitol 145:45–50
- Filoni C, Catão-Dias JJ, Bay G et al (2006) First evidence of feline herpesvirus, calicivirus, parvovirus, and *Ehrlichia* exposure in Brazilian free-ranging felids. J Wildl Dis 42:470–477
- Fiorello CV, Deem SL, Gompper ME et al (2004) Seroprevalence of pathogens in domestic carnivores on the border of Madidi National Park, Bolivia. Anim Conserv 7:45–54
- Fiorello CV, Noss AJ, Deem SL et al (2007) Serosurvey of small carnivores in the Bolivian Chaco. J Wildl Dis 43:551–557
- Funk SM, Fiorello CV, Cleaveland S et al (2001) The role of disease in carnivore ecology and conservation. In: Gittleman JL, Funk SM, Macdonald D et al (eds) Carnivore conservation. Cambridge University Press, Cambridge
- Gardner IA, Hietala S, Boyce WM (1996) Validity of using serological tests for diagnosis of diseases in wild animals. Rev Sci Tech OIE 15:323–335
- Gascoyne SC, Laurenson MK, Lelo S et al (1993) Rabies in African wild dogs (*Lycaon pictus*) in the Serengeti region, Tanzania. J Wildl Dis 29:396–402
- Gennari SM, Cáñon-Franco WA, Yai LEO et al (2004) Seroprevalence of *Toxoplasma gondii* antibodies from wild canids from Brazil. Vet Parasitol 121:337–340
- Harder TC, Osterhaus ADME (1997) Canine distemper virus—a Morbilivirus in search of new hosts? Trends Microbiol 5:120–124
- Harvell CD, Mitchell CE, Ward JR et al (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296:2158–2162
- Hofmeyr M, Hofmeyr D, Nel L et al (2004) A second outbreak of rabies in African wild dogs (*Lycaon pictus*) in Madikwe Game Reserve, South Africa, demonstrating the efficacy of vaccination against natural rabies challenge. Anim Conserv 7:193–198
- Jácomo ATA, Silveira L, Diniz-Filho JAF (2004) Niche separation between the maned wolf (Chrysocyon brachyurus), the crab-eating fox (Dusicyon thous) and the hoary fox (Dusicyon vetulus) in central Brazil. J Zool Lond 262:99–106
- Johnson MR, Boyd DK, Pletscher DH (1994) Serologic investigations of canine parvovirus and canine distemper in relation to wolf (*Canis lupus*) pup mortality. J Wildl Dis 30:270–273
- Labruna MB, Paula CD, Lima TF et al (2002) Ticks (Acari: Ixodidae) on wild animals from the Porto-Primavera hydroelectric power station area, Brazil. Mem Inst Oswaldo Cruz 97:1133–1136
- Labruna MB, Jorge RSP, Sana DA et al (2005) Ticks (Acari: Ixodida) on wild carnivores in Brazil. Exp Appl Acarol 36:149–163
- Laurenson MK, Sillero-Zubiri C, Thompson H et al (1998) Disease as a threat to endangered species: Ethiopian wolves, domestic dogs and canine pathogens. Anim Conserv 1:273–280
- Machado ABM, Martins CS, Drummond GM (2005) Lista da fauna brasileira ameaçada de extinção: incluindo as espécies quase ameaçadas e deficientes em dados. Fundação Biodiversitas, Belo Horizonte
- Maia OB, Gouveia AMG (2001) Serologic response of maned wolves *Chrysocyon brachyurus* to canine distemper virus and canine parvovirus vaccination. J Zoo Wildl Med 32:78–80
- Martinello F, Gallupo F, Ostanello F et al (1997) Detection of canine parvovirus in wolves from Italy. J Wildl Dis 33:628–631



- Martino PE, Montenegro JL, Preziosi JA et al (2004) Serological survey of selected pathogens of freeranging foxes in southern Argentina, 1998–2001. Rev Sci Tech OIE 23:801–806
- Mech LD, Goyal SM (1993) Canine parvovirus effect on wolf population change and pup survival. J Wildl Dis 29:330–333
- Mech LD, Goyal SM, Paul WJ et al (2008) Demographic effects of canine parvovirus on a free-ranging wolf population over 30 years. J Wildl Dis 44:824–836
- Megid J, Souza VAF, Teixeira CR et al (2009) Canine distemper virus in a crab-eating fox (*Cerdocyon thous*) in Brazil. Case report and plylogenetic analyses. J Wildl Dis 45:527–530
- Megid J, Teixeira CR, Amorin RL et al (2010) First identification of canine distemper virus in hoary fox (*Lycalopex vetulus*): pathologic aspects and virus phylogeny. J Wildl Dis 46:303–305
- Mochizuki M, Sugiura R, Akuzawa M (1987) Micro-neutralization test with canine coronavirus for detection of coronavirus antibodies in dogs and cats. Jpn J Vet Sci 49:563–565
- Murray DL, Kapke CA, Evermann JF et al (1999) Infectious disease and the conservation of free-ranging large carnivores. Anim Conserv 2:241–254
- Randall DA, Williams SD, Kuzmin IV et al (2004) Rabies in endangered Ethiopian wolves. Emerg Infect Dis 10:2214–2217
- Rodden M, Rodrigues F, Bestelmeyer S (2008) Chrysocyon brachyurus. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1. Available at www.iucnredlist.org. Cited 27 Apr 2010
- Ruas JL, Farias NAL, Soares MP et al (2003a) *Babesia* sp. em graxaim do campo (*Lycalopex gymnocercus*) no sul do Brasil. Arq Inst Biol (São Paulo) 70:113–114
- Ruas JL, Soares MP, Farias NAL et al (2003b) Infecção por *Capillaria hepatica* em carnívoros silvestres (*Lycalopex gymnocercus* e *Cerdocyon thous*) na região sul do Rio Grande do Sul. Arq Inst Biol (São Paulo) 70:147–150
- Senda M, Hirayama N, Yamamoto H et al (1986) An improved hemagglutination test for study of canine parvovirus. Vet Microbiol 12:1-6
- Steinel A, Parrish CR, Bloom ME et al (2001) Parvovirus infections in wild carnivores. J Wildl Dis 37:594-607
- Timm SF, Munson L, Summers BA et al (2009) A suspected canine distemper epidemic as the cause of a catastrophic decline in Santa Catalina Island foxes (*Urocyon littoralis catalinae*). J Wildl Dis 45:333–343
- Uhart MM, Vila AR, Beade MS et al (2003) Health evaluation of pampas deer (*Ozotocerus bezoarticus* celer) at Campos del Tuyú Wildlife Reserve, Argentina. J Wildl Dis 39:887–893
- van de Bildt MWG, Kuiken T, Visee AM et al (2002) Distemper outbreak and its effect on African wild dog conservation. Emerg Infect Dis 8:211–213
- Vicente JJ, Rodrigues HO, Gomes DC et al (1997) Nematóides do Brasil. Parte V: Nematóides de mamíferos. Rev Bras Zool 14:1-452
- Woodroffe R (1999) Managing disease threats to wild mammals. Anim Conserv 2:185-193
- Woodroffe R, Ginsberg JR (1998) Edge effects and the extinction of populations inside protected areas. Science 280:2126–2128
- Woodroffe R, Cleaveland S, Courtenay O et al (2004) Infectious disease. In: Macdonald DW, Sillero-Zubiri C (eds) Biology and conservation of wild canids. Oxford University Press, Oxford
- Young TP (1994) Natural die-offs of large mammals: implications for conservation. Conserv Biol 8:410–418
- Zar JH (1996) Bioestatistical analysis. Prentice-Hall, New Jersey
- Zarnke RL, Evermann J, Ver Hoef JM et al (2001) Serologic survey for canine coronavirus in wolves from Alaska. J Wildl Dis 37:740–745

