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 *Platynosomum* 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) ($19^{\circ}12'–19^{\circ}20'S$, $43^{\circ}30'–43^{\circ}40'W$; altitude of 1,095–1,485 m, area of 33,800 ha, 154 km perimeter, $21.2^{\circ}C$ 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 haemagglutination 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; Cañon-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	<i>Canis l. familiaris</i>	<i>Chrysocyon brachyurus</i>	<i>Cerdocyon thous</i>	<i>Lycalopex vetulus</i>
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
<i>Babesia</i> spp.	30/70	0/3	0/9	0/2
<i>T. gondii</i>	NT	6/7	7/10	0/2
<i>N. caninum</i>	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

Serologic tests	Domestic dogs		Wild canids	
	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%)
<i>Babesia</i> spp.*	30/70	42% (31–55%)	0/14	0% (0–23%)
<i>T. gondii</i>	NT	–	13/19	68% (43–87%)
<i>N. caninum</i>	NT	–	0/19	0% (0–17%)

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

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

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, *Phylosaloptera* sp., *Toxocara* sp., Trematoda, Acantocephala, *Platynosomum* sp., *Spirometra* sp.

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	<i>Chrysocyon brachyurus</i> Positives/sampled	<i>Cerdocyon thous</i> Positives/sampled
Trichuridae	6/10	0/10
Ancylostomidae	8/10	2/10
<i>Physaloptera</i> sp.	2/10	0/10
<i>Toxocara</i> sp.	0/10	1/10
Trematoda	0/10	1/10
Acantocephala	3/10	1/10
<i>Platynosomun</i> sp.	1/10	2/10
<i>Spirometra</i> 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

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

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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