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SEROLOGIC EVIDENCE OF CANINE PARVOVIRUS IN DOMESTIC DOGS, WILD CARNIVORES, AND MARSUPIALS IN THE ARGENTINEAN CHACO

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?1 *Abstract:* The transmission of pathogens between domestic dogs and generalist wildlife species may be modified by environmental degradation, biodiversity losses, host densities, and increased contact rates in remnant forest patches. A serologic survey of canine parvovirus (CPV) in rural domestic dogs and wild mammals was conducted in two neighboring rural areas (disturbed and protected) from Pampa del Indio, northeastern Argentina, between 2008 and 2011. A total of 174 domestic dogs and 26 wild mammals—4 crab-eating foxes (*Cerdocyon thous*), 3 crab-eating raccoons (*Procyon cancrivorus*), 17 white-eared opossums (*Didelphis albiventris*), and 2 gray four-eyed opossums (*Philander opossum*)—were examined for antibodies to CPV using a hemagglutination inhibition assay. Domestic dogs were numerous and their movements unrestricted. The main function of dogs differed significantly between areas, with more dogs used for herding or hunting around the protected area. The seroprevalence of antibodies to CPV in dogs from both areas was very high (93.9–94.6%) and increased steeply with age. Nearly all carnivores and marsupials showed high exposure to CPV. Although a higher exposure to CPV was expected in wild mammals from disturbed areas as a result of enhanced contact between dogs and wildlife, no significant differences were found between areas. To the authors' knowledge, this study is the first to document exposure to CPV of free-ranging *Pr. cancrivorus, D. albiventris,* and *Ph. opossum*, and the first to include a detailed demographic study of the domestic dog populations living in the area. This study highlights that dogs and wildlife have potential opportunities for contact and shows that the edges of the protected area may be as suitable as other fragmented areas for the transmission of CPV. Rural domestic dogs may pose serious threats to the health and conservation of wild carnivores in both disturbed and protected areas, especially in the Gran
?2 Chaco, where habitat fragmentation is severely increasing.
Key words: *Cerdocyon thous, Didelphis albiventris,* dogs, parvovirus, *Philander opossum, Procyon cancrivorus.*

INTRODUCTION

Environmental changes caused by anthropogenic influences have generated multiple impacts on the ecology of infectious diseases[21] and may result in new threats for human health and wildlife conservation efforts.[10,21,23] Human populations continue to increase in size and expand into wildlife habitats with their domestic animals and pathogens.[1,23] Domestic dogs interact frequently with wildlife as predators, prey, or competitors,[6,22] thus modifying the patterns of wildlife activity, habitat use, and reproduction.[44] Moreover, rural domestic dogs may function as a bridge between domestic and sylvatic environments and allow the transmission of parasites among humans, livestock, and wildlife.[23,37]

The densities of rural dog populations are very high around the world, and, therefore, introduced canine pathogens can potentially result in epidemics.[28] Dog populations are able to maintain multiple infectious agents[10,41] and have caused epidemic outbreaks in many species of wild carnivores, especially in the vicinities of protected areas.[10,36,39]

Dogs can act as a source of canine parvovirus (CPV) for other species.[43] CPV is transmitted through fecal contamination and may survive in the environment for 5 mo or longer.[25] There are two types of CPV: canine parvovirus type-1 (CPV1)[5] and canine parvovirus type-2 (CPV2).[29] The pathogenicity of CPV1 is undetermined, and CPV1 may cause unapparent to severe illness. CPV2 shows three antigenic variants (CPV2a, CPV2b, and CPV2c)[16,29] and causes severe clinical symptoms with hemorrhagic enteritis and myocarditis.[4] Domestic dogs infected with CPV may exert substantial mortality on wild canids (e.g., *Lycaon pictus* pups)[45] and have limited the popu-

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lation growth of *Canis lupus*.[26] In addition, CPV antibodies have been found in *Didelphis virginiana*,[41] *Cerdocyon thous*,[13,18] and *Procyon lotor*[35] in the Americas.

The Gran Chaco, the second most biodiverse ecoregion (after the Amazon) in the Americas, has suffered drastic fragmentation and habitat loss during recent decades.[42] The municipality of Pampa del Indio in the Argentinean Chaco has undergone significant rates of deforestation and land use change, which led to a substantial decline in suitable habitats for wildlife species. There is no local disease surveillance system for domestic canine and feline populations in rural areas, where domestic dogs are typically numerous, free ranging, rarely vaccinated, and are used for various purposes, including herding and hunting.[7,8,12] The very few rural dog populations examined for CPV in the Gran Chaco differed widely in seroprevalence between areas, ranging from 2% in Santiago del Estero in Argentina[27] to >95% in the Isoso of Bolivia.[12] Wild mammals from the Gran Chaco apparently have rarely been examined for the occurrence of CPV antibodies, and, therefore, the co-occurrence of CPV antibodies in domestic dogs and wild mammals has not been investigated.

This study evaluated the demographic characteristics of two dog populations and assessed the occurrence of antibodies to CPV in domestic dogs, wild carnivores, and marsupials in a well-defined rural area in the Argentinean Chaco to test whether there was any difference in host exposure to CPV between disturbed and protected areas. Previous studies suggested that the fragmentation and consequent shrinkage of ecosystems could concentrate individuals into restricted areas, increase edge, and allow the arrival of new species (i.e., parasites) into the disturbed habitats, promoting the transmission of diseases.[17] Therefore, wild mammals from disturbed areas would face higher risks of exposure to CPV than would those from protected areas, and one expects to find no difference in seroprevalence of CPV between dogs living on the edges of the protected area and in the disturbed area.

MATERIALS AND METHODS

Study area

Field work was conducted in two well-defined rural areas in the municipality of Pampa del Indio (25°55′S, 56°58′W), Chaco Province, Argentina: a disturbed area (450 km^2) and a protected area (Parque Provincial Pampa del Indio) and its vicinities (Fig. 1). The disturbed area comprised five rural villages with 173 inhabited houses. Most of the houses were made of mud and thatch and were surrounded by small patches of native dry forest affected by selective logging, intentional fires, and subsistence hunting. The protected area (8,633 ha) comprised a well-preserved primary hardwood forest and was surrounded by four small rural villages (including 55 inhabited houses) similar to those found in the disturbed area. The study area is on the transition between the dry (western) and humid (eastern) Chaco.

Dog and wildlife surveys

Dogs were enumerated in three cross-sectional surveys conducted house to house in the disturbed area and in one survey carried out on the edges of the protected area between August 2008 and July 2010. The head of each household was informed of the study objectives, and oral informed consent was requested in all cases. A standard demographic questionnaire was completed for each dog,[7] including name, sex, age, birthplace, history of vaccination and deworming, movement in the forest, and the dog's main function.

Dogs were handled with the owners' help. A physical examination was carried out to detect pathognomonic signs of illness, and then a blood sample of up to 7 ml was drawn from the antebrachial cephalic, external saphenous, or jugular vein from all available dogs aged 4 mo or more for serologic studies. One year later, on a subsequent visit to the study houses, information on survival or apparent cause of death of each dog during the intervening period was requested.

Wild mammals were sampled during three 3-wk surveys conducted between August 2008 and August 2009 in forest patches close to houses in the disturbed area and within the protected area. Captures were carried out in two randomly selected square areas (quadrats) using National and homemade traps deployed every 50 m along two to six transect lines (1–4 km in length) in each survey. *Procyon cancrivorus* were caught through direct chemical immobilization using a blowgun and anesthetic darts (Dan-Inject ApS Sellerup, 7080 Børkop, Denmark). Trap locations were georeferenced (Garmin Legend C, Garmin Ltd., Olathe, Kansas 66062-3426, USA). Traps were baited with beef or chicken scraps, checked every morning, and re-baited when needed.

Wild carnivores and marsupials were subjected to a visual examination and were then given parenteral anesthesia using 5–10 mg/kg of tiletamine clorhydrate and zolazepan clorhydrate

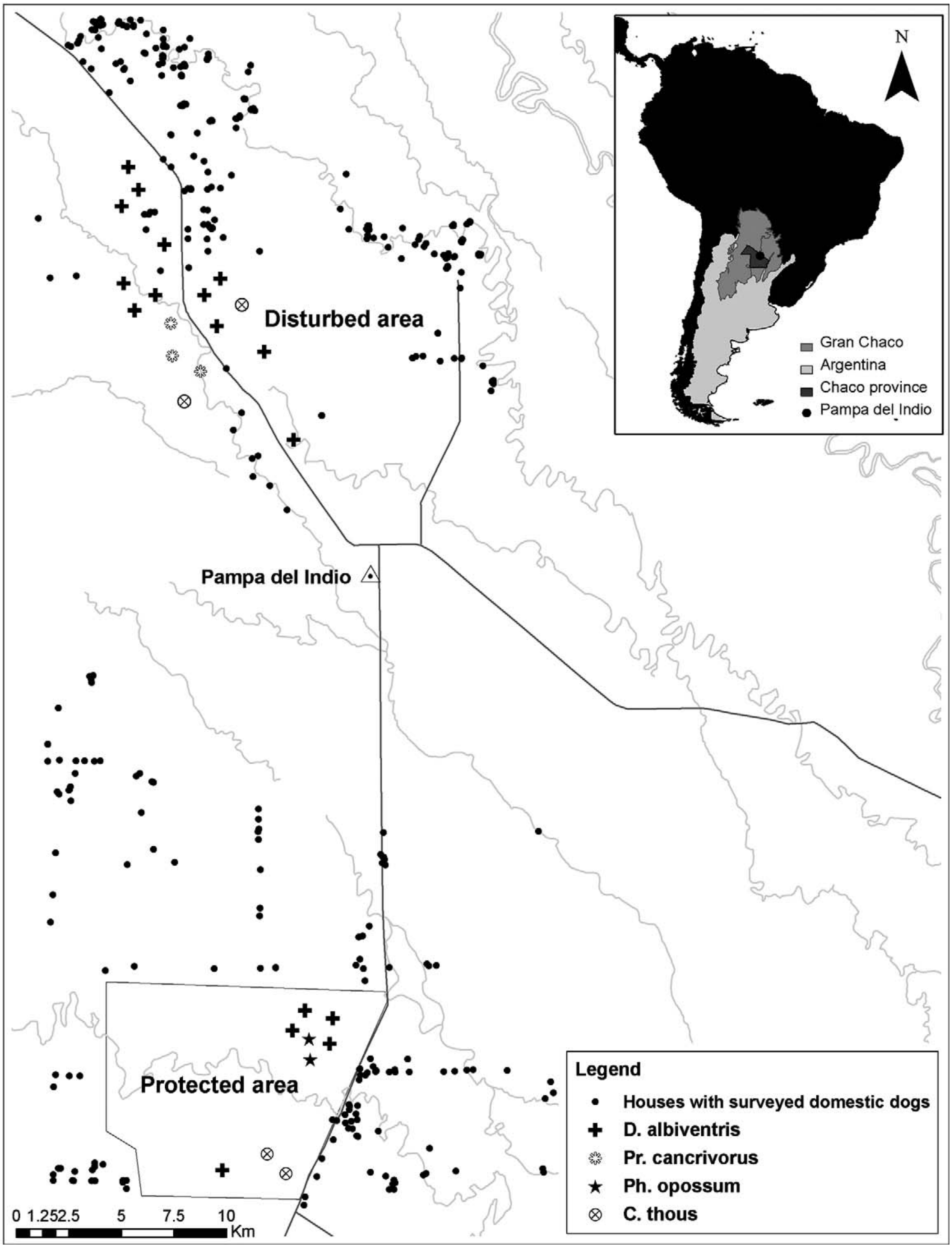

Figure 1. Geographic location of surveyed domestic dogs and wild mammals in Pampa del Indio, Chaco, Argentina.

(Zelazol®, Fort Dodge, La Vall De Bianya, Girona 17813, Spain)[20] injected intramuscularly. Maintenance was carried out by inhalatory anesthesia with Isoflurane® delivered with a vaporizer (Iso-Tec®, Datex-Ohmeda GE Healthcare, Little Chalfont Bucks, HP7 9NA United Kingdom) and medicinal O_2 (0.25–3 L/min). Mammals were maintained on thermic cushioned surfaces in a quiet environment. All animals were sexed, measured, and weighed with a scale (Pesola® AG, Baar, CH-6340, Switzerland); marked with numeric metal tags (National Band & Tag Company, Newport, Kentucky 41071, USA); and bled by venipuncture from the antebrachial cephalic, saphenous, coccygeal, or jugular vein. All animals were released at the capture site upon full recovery from anesthesia.

Between 2 and 4 hr after collection, blood samples from dogs and wild mammals were centrifuged for 15 min at 3,000 rpm and the serum separated in three aliquots. Serum samples were stored for 1 to 20 days in cryovials at –20°C and then kept at –70°C at the laboratory until analysis.

Processing of dogs and wildlife was conducted according to the protocol approved by the "Dr. Carlos Barclay" Independent Ethical Committee for Clinical Research from Buenos Aires, Argentina (TW-01-004; Review 670-04-2008 and 787-06-2010). Capture and transit permits for wild mammals were obtained from the provincial government.

Laboratory analysis

Antibodies against CPV were detected by a hemagglutination inhibition (HI) assay conducted at the Institute Rosenbusch Argentina, as described elsewhere.[9] In order to remove any natural agglutinins, 50 μl of serum was mixed with 50 μl of 25% kaolin in phosphate-buffered saline (PBS) (1 : 5 final concentration) and 100 μl of packed pig erythrocytes 0.8% in PBS, and the mixture was incubated at 36 ± 1°C for 1 hr. The kaolin and the erythrocytes were removed by centrifugation at 10,000 *g* over the course of 15 min. For the HI test, 50 μl of 4 hemagglutination units of CPV2a were added to 50 μl of the treated serum. After incubation for 1 hr at room temperature, 50 μl of 0.8% pig erythrocytes was added, and the microplates were incubated at room temperature for 1 hr. The HI titer was expressed as the reciprocal of the highest dilution of serum showing complete inhibition of hemagglutination. Serum samples showing HI titers $\geq$10 were considered positive or indicative of exposure to CPV. The diagnostic test did not differentiate between CPV1 and the antigenic variants of CPV2.

Data analysis

Mann–Whitney tests were used for comparisons of the mean number of dogs per household and the median age of dog populations between areas. Fisher exact tests were used to examine the association between type of area and the proportions of dog-owning households, sex ratios, native dogs (born in the study area), vaccine coverage against canine rabies and other viral agents, and whether the dog had ever been dewormed during the previous 2 yr. Exact binomial confidence intervals for proportions were estimated using Stata 10.0 (Stata Press, College Station, Texas 77845, USA).

To evaluate the relationship between potential risk factors and the antibody seroprevalence of CPV in dogs from both areas, adjusted odds ratios and 95% confidence intervals were estimated from maximum-likelihood logistic multiple regression analysis clustered on dog's village to provide robust standard errors (Stata 10.0). For these analyses only native and unvaccinated dogs were included. The variables considered were area, age (in months), sex, and primary function of the dog (three levels: household-associated, hunting, and herding). Reference levels were residing in the disturbed area, females, and household-associated function. Interaction terms were then added to both models and tested for significance at $\alpha = 0.05$.

RESULTS

Dog demography

A total of 525 and 237 dogs were enumerated in the disturbed and protected areas, respectively (Table 1; Fig. 1). Nearly all households had dogs, and the mean number of dogs per household was approximately three in both areas. In comparison with the disturbed area, the dog population from the edge of the protected area was significantly older (median age, 3.0 yr), had more males (84.8%), had a better mean body condition, and was more frequently vaccinated against rabies (14.7%) and dewormed (13.4%) (Table 1). In the disturbed area, dogs were mainly associated with the household (82%) or used for hunting (8%), whereas in the edge of the protected area the most common functions were household-associated (32%), herding (32%), and hunting (22%), with significant differences in dog function between areas ($P < 0.001$). Annual survival rates varied

Table 1. Demographic characteristics of dog populations from the disturbed area[8] and edge of the protected area, Pampa del Indio, Chaco, Argentina.

Attribute	Disturbed area	Edge of protected area	P
Total number of dogs enumerated (No.)	525	237	
Dog-owning households (%)	96.0	97.5	>0.5[a]
Mean number of dogs per household (No. [range])	3.1 [0–9]	3.0 [0–8]	>0.5[b]
Median age (yr [first–third quartiles])	2.0 [1–4]	3.0 [1–5]	0.01[b]
Sex ratio (male : female)	2.9 : 1.0	5.5 : 1.0	0.001[a]
Males (%)	74.4	84.8	0.001[a]
Native to the study area (%)[c]	68.0	72.6	>0.15[a]
Vaccinated against canine rabies (%)[d]	4.0	14.7	0.001[a]
Vaccinated against other viral agents(%)[e]	1.3	1.3	>0.2[a]
Dewormed (%)[f]	7.3	13.4	0.009[a]

[a] Data missing for 82 and 22 dogs inhabiting the disturbed and edge of protected areas, respectively.
[b] Data missing for 71 and 6 dogs inhabiting the disturbed and edge of protected areas, respectively.
[c] Data missing for 69 and 6 dogs inhabiting the disturbed and edge of protected areas, respectively.
[d] Data missing for 71 and 6 dogs inhabiting the disturbed and edge of protected areas, respectively.
[e] Fisher test.
[f] Mann–Whitney test.

little between the disturbed (90%) and protected (85%) areas. The most common apparent cause of death reported by dog owners from both areas was disease (including infectious and noninfectious diseases).

Wild mammals

A total of 26 individuals from four mammalian species were captured using 4,698 trap-nights (3,259 and 1,439 trap-nights in disturbed and protected areas, respectively) (Fig. 1). All animals were clinically normal by physical examination. The examined mammals were 2 crab-eating foxes (*C. thous*), 2 gray four-eyed opossums (*Philander opossum*), and 5 white-eared opossums (*Didelphis albiventris*) from the protected area and 3 crab-eating raccoons (*Pr. cancrivorus*), 2 crab-eating foxes (*C. thous*), and 12 white-eared opossums (*D. albiventris*) from the disturbed area.

Canine parvovirus

A total of 174 dogs selected at random (82 from the disturbed area and 92 dogs living on the edges of the protected area) were examined for CPV exposure (Table 2). Both dog populations had very high seroprevalence to CPV (93.9–94.6%). Exposure to CPV increased significantly with increasing age but did not vary with type of area, sex, and function (Table 2). Antibody titers to CPV were high in both types of area, being ≥80 in 93.9% of the dogs from the disturbed area and in 82.6% of the dogs from the protected area.

Exposure to CPV was detected in *C. thous, Pr. cancrivorus, Ph. opossum,* and *D. albiventris* (Table 3). Opossums had the largest seroprevalence of CPV both in the disturbed (91.7%) and protected areas (100.0%). All wild mammals had positive titers for CPV, except for one opossum (with a titer lower than 10). *Procyon cancrivorus* had higher titers than other wild species, reaching 640 in one of the specimens. *Didelphis albiventris* showed a wide range of titers in both areas (20 to 320), whereas *Ph. opossum* had relatively lower titers (10 and 40). Three of four studied *C. thous* had titers of 80, the titer most frequently found in domestic dogs (Table 3).

DISCUSSION

Domestic dog populations showed high seroprevalence to CPV, indicating that CPV is endemic in the area, with intense circulation in domestic and peridomestic habitats from disturbed areas and in the edges of the protected area. This present study also documents a high exposure to CPV in specimens of four species of wild carnivores and marsupials living in disturbed and protected areas of the Argentinean Chaco.

CPV has previously been isolated in *Cerdocyon* and *Procyon* genera,[19,24] but the virus in Didelphidae has not been isolated yet, and its pathogenicity remains unknown.[41] CPV antibodies were detected in *C. thous* in Bolivia and Brazil[13,18] and in *D. virginiana* in Mexico.[41] Published records of CPV in *Ph. opossum* were not found. *Didelphis* opossums are adapted to a large diversity of disturbed or undisturbed habitats and apparently

Table 2. Seroprevalence of canine parvovirus in dogs according to potential risk factors.

Factor	% Seropositive (No. tested)	Odds ratio (95% confidence interval)	*P*
Area			
Disturbed area	93.9 (82)	—	—
Edge of protected area	94.6 (92)	0.72 (0.15–3.30)	>0.68
Age class (mo)		1.10 (1.01–1.15)	<0.02
≤12	84.1 (37)	—	—
14–34	95.7 (44)	—	—
36–60	97.9 (48)	—	—
>60	100 (30)	—	—
Sex			
Female	93.1 (27)	—	—
Male	94.5 (137)	0.69 (0.12–3.89)	>0.68
Main function			
Household associated	93.5 (115)		
Hunting	94.4 (21)	2.03 (0.17–23.14)	>0.57
Herding	96.5 (28)	3.10 (0.29–33.55)	>0.35

are not affected by several infectious agents. To the best of the authors' knowledge, this is the first report of exposure to CPV in free-ranging *Pr. cancrivorus, D. albiventris,* and *Ph. opossum*.

Although a higher exposure to CPV in wild mammals was expected from disturbed areas as a result of enhanced opportunities for contact between dogs and wildlife, no significant differences were found between areas. A variety of closely related parvoviruses can be found in different species of wild mammals.[3] Although wild species can be infected and develop an immune response, the severity of clinical signs varies widely according to the type of parvovirus.[40] One limitation of this study's assessment of parvovirus in wild mammals is that this study was unable to evaluate the differences between related parvoviruses, and the researchers have not attempted to isolate the pathogen from either host. Therefore, this study cannot rule out the possibility of serologic cross-reactions with antibodies elicited by related virus.

CPV2 emerged in the canine population likely by mutation from feline parvovirus or derived from one of the closely related viruses of wild carnivores.[15] Later, raccoon parvoviruses may have played a central role in the transition between CPV2 and their variants.[2] CPV resulted from a successful cross-species transmission leading to a pandemic in the new host.[2,31] Although it is highly likely that transmission between populations of wild and domestic carnivores occurs in both directions, little is known about the epidemiology and evolution of parvovirus in wild carnivore populations.[15] CPV may continue increasing its ability to spread in other species (i.e., carnivores) as a result of their fast evolutionary dynamics (including sequential molecular events of adaptation and a high intrinsic rate of mutation without recombination).[38]

While cross-species transmission was well documented in CPV, the evidence of transmission in nondomestic species is scarce,[3] even more so in the Gran Chaco, where populations of domestic dogs have different characteristics from those in

Table 3. Seroprevalence and antibody titers of canine parvovirus in wild mammals and domestic dogs from
?8 disturbed and protected areas, Pampa del Indio, Chaco, Argentina.

	% Seropositive (No. examined)		Antibody titer								
Species	Disturbed area	Protected area	<10	10	20	40	80	160	320	640	1,280
Didelphis albiventris	91.7 (12)	100 (5)	1	—	2	4	6	—	4	—	—
Philander opossum	ne	100 (2)	—	1	—	1	—	—	—	—	—
Cerdocyon thous	100 (2)	100 (2)	—	—	—	1	3	—	—	—	—
Procyon cancrivorus	100 (3)	ne	—	—	—	—	—	2	—	1	—
Domestic dogs	93.9 (82)	94.6 (92)	—	10	2	9	93	18	19	17	6

other areas and where environmental degradation is severely increasing. There, dogs and wildlife may make direct or indirect contact on the edges of or within protected areas and in peridomestic areas because movements of dogs are not restricted and because the dogs usually hunt in the forest. In addition, the dogs living on the edges of the protected area were more frequently used for hunting than were the dogs in the disturbed area. The protected area was surrounded by small villages and had no barriers to the entrance of humans and domestic animals. Dogs gained free access to the protected area, either individually or with their owners, during illegal hunting trips or during traditional celebrations. A 2-day religious festival celebrated in the protected area every year involves a massive movement of people, with horses and dogs coming from neighboring and distant towns. Many dogs are abandoned after the celebrations, which increases the chance of transmission of infectious disease agents to local dog and wildlife populations. Clearly, several optimal conditions for disease spillover are in place. In this specific context, the edges of the protected area may be as suitable as other fragmented areas for the transmission of CPV.

The seroprevalence of CPV in the study dogs was as high as in the Bolivian Chaco, but strikingly different from the 2% found in a remote marshland area in southern Santiago del Estero.[27] The age–seroprevalence curve in dogs showed that exposure to CPV occurred very early in life, as in Bolivia,[11,12] and exceeded 90% in dogs older than 1 yr of age. High seroprevalence of CVP over all age classes may indicate the occurrence of previous outbreaks or subclinical circulation of the virus in the study area.

The demography of the study dog populations differed in some respects between areas. Both populations had a similar density per household and frequency of native individuals, but the dogs from the disturbed area were younger and the sex ratio was less skewed. In contrast, dogs from the edges of the protected area were in better condition, and vaccination and deworming coverage were substantially larger. This pattern was likely associated with a recent rabies vaccination campaign carried out in response to a local outbreak of rabies. Most of the dogs assisted their owners' activities, especially in the edge of the protected area, where more than 50% of dogs were used for herding or hunting. In the disturbed area, the larger dog population was in a poorer body condition and lacked vaccination and deworming coverage. These characteristics may favor outbreaks of infectious agents that could spread quickly to other dog populations and wildlife species.

Rural domestic dogs may pose serious threats for the health and conservation of wild carnivores in both disturbed and preserved areas. The high population abundance and fast turnover rates of rural domestic dogs make them ideal reservoir hosts for multiple pathogens. Rural dog populations in the Gran Chaco pose serious risks to wildlife for two reasons: first, the poor health status of dogs[27,33] could increase the duration of viral shedding and the maintenance of subclinical infections, and second, the frequent co-occurrence of multiple pathogens may increase disease severity in dogs[34] and enhance the release of viral agents.

The results described in this study have major epidemiologic significance and suggest that infection and transmission of canine parvovirus in dogs and wild mammals should be studied in more detail to assess the real impacts of disease on wild carnivore populations in the Gran Chaco. The occurrence of species-specific CPV strains with antigenic differences remains to be investigated through other more specific methods, such as monoclonal antibodies and molecular techniques.[2,30]

Supervised canine vaccination programs could decrease the exposure of wildlife to infectious agents, as they have done in Tanzania.[1,14] The entrance of domestic animals into protected areas should be prevented to reduce their contact with wildlife. The increasing trend of environmental degradation in the Gran Chaco may increase the risk of spillover of canine pathogens to wild carnivore populations, and, therefore, local conservation programs should include a disease surveillance strategy.

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