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Exposure of pampas fox (*Pseudalopex gymnocercus*) and crab-eating fox (*Cerdocyon thous*) from the Southern region of Brazil to Canine distemper virus (CDV), Canine parvovirus (CPV) and Canine coronavirus (CCoV)

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ABSTRACT

The exposure of 13 Brazilian free-ranging nondomestic canids (five pampas fox - Pseudalopex gymnocercus *and eight crab-eating fox -*Cerdocyon thous*) from Southern region of Brazil, to Canine distemper virus (CDV), canine parvovirus (CPV) and Canine coronavirus (CCoV) was investigated. Antibodies against CDV were detected in 38.5% (5/13) of the samples. There were anti-CDV antibodies in 60% (3/5) of* P. gymnocercus *and in 25% (2/8) of* C. thous*. The frequency was higher among the adults and males. Eleven canids (84.6%) presented antibodies against CPV, 80% (4/5) were from* P. gymnocercus *and 87.5% (7/8) were from* C. thous*. There was no difference in positivity rate against CPV between gender and age. Antibodies against CCoV were detected in 38.5% (5/13) of the samples, with 60% (3/5) of positivity in* P. gymnocercus *and 25% (2/8) in* C. thous*. The frequency of antibodies against CCoV was higher among the adults and males. The study showed that these canids were exposed to CDV, CPV and CCoV.*

Key words: *Pseudalopex gymnocercus*, *Cerdocyon thous,* Canine distemper virus (CDV), Canine parvovirus (CPV), Canine coronavirus (CCoV), Serology

INTRODUCTION

In South America, wild canids are represented by seven genus and 11 species. Three wild canid species are natural in Rio Grande do Sul State (RS): pampas fox (*Pseudalopex gymnocercus*), crab-eating fox (*Cerdocyon thous*) and maned wolf (*Chrysocyon brachyurus*) (Freire, 1990; Macdonald, 1993; González, 2001). *P. gymnocercus* and *C. thous* are widely found in the farms of the southern region of RS, on the border

regions of Uruguay and Argentina. These canids are not yet included in the endangered species risk group, but are considered vulnerable (Macdonald, 1993). These animal's vulnerability are in part due to the destruction of their natural environment, either by deforestation for extending the agricultural borders, or spreading of urban communities on the natural environment, as well as the habitat fragmentation caused by the roads (Sinkoc et al., 2009; Furtado et al., 2003; Deplazes et al., 2004). Besides, diseases caused by the

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pathogens common to domestic animals and to other wild species can result in alteration in these animal populations (Mech et al., 1997; Steinel et al., 2001; Curi et al., 2006). Nevertheless, to our knowledge, there is no report about sanitary conditions of these animals.

Infections caused by Canine distemper virus (CDV), Canine parvovirus (CPV) and Canine coronavirus CCoV) cause clinically important contagious diseases in domestic dogs. The CDV is responsible for the development of distemper, a neurological disease associated to high levels of mortality in pups domestic dogs (Krakowka et al.., 1985; Headley and Graça, 2000). The CPV and the CCoV are causative agents of gastroenteritis (Carmichael and Binn,1981; Tennant et al., 1993). These agents have been reported in several wild canid species around the world. Exposure to CDV and CPV have already been detected in wolves (*C. lupus*) (Goyal et al., 1986; Mech et al., 1986; Mech et al., 1997) as well as in some fox species such as *Vulpes vulpes* and *Urocyon cinereoargenteus* (Damien et al.., 2002). Infection and clinical signs of disease due to CPV were found in jackals (*Canis aureus, Canis adustus, Canis mesomelas*), grey foxes (*Vulpes macrotis mutica*), Asiatic raccoon dogs (*Nyctereutes procyonoides*; Steinel et al.., 2001), and wild African hunting dogs (*Lycaon pictus;* Steinel et al., 2001). There are reports of coyote (*Canis latrans*) infections by the CDV, CPV and CCoV (Evermann et al.,1980; Green et al.., 1984; Thomas et al.., 1984; Guo et al., 1986; Gese et al., 1991; Cypher et al., 1998). To the best of our knowledge, there are no reports on exposure of pampas fox (*P. gymnocercus)* and crab-eating fox (*C. thous)* to viral agents.

Although some infections can be preoccupying to wildlife, data regarding the pathogens infecting the wild animals are scarce. The understanding about the viral infections that infect wild canids is highly important for the establishment of monitoring programs. This work was carried out aiming to detect the evidences of infections of wild canids found in RS, by viruses that commonly affect the domestic dogs (*Canis familiaris*). In this context, the presence of antibodies against CDV, CPV and CCoV in free-ranging pampas fox (*P. gymnocercus*) and crab-eating fox (*C. thous*) was investigated.

MATERIAL AND METHODS

In the present study, a total of 13 free-ranging canids, including five pampas fox (*P. gymnocercus*) and eight crab-eating fox (*C. thous*) specimens were analyzed. They were captured in rural areas near the Laguna dos Patos coast and in Cerro Alegre, a district from Pelotas municipality, between 2002 and 2003. The captures were performed using the traps, with authorization from the National Environmental Agency - IBAMA (numbers 112/1999 and 022/2002). The age of the animals was determined by their body size, fur coloring and dental aspect (Macdonald, 1993). Five animals were classified as juveniles (<12 months) and eight as adults. Five animals were male and eight were female. Blood samples were collected from the jugular or brachial veins and after coagulation, the blood was centrifuged at 400x g for 10 min to obtain the serum. The serum was inactivated at 56 °C for 30 minutes and then stored at -20 °C until further use.

Antibodies to CDV and CCoV were determined by serum neutralization assays as described by Appel and Robson (1973) and Pratelli et al. (2002). For CDV, serial two-fold serum dilutions starting from 1:10 were prepared in Eagle medium (MEM; Gibco BRL, UK), and incubated for one hour at 37°C with 100 $TCID_{50}$ of the Lederle strain of CDV. For the CCoV, serum samples were initially diluted in 1:5 and then two-fold until 320, and then were added 100 $TCID_{50}$ of the strain Mav 795 of CCoV. Following incubation, a suspension containing 40,000 MDCK cells was added to each well. The microplates were incubated at 37 °C in atmosphere containing 5 % of CO_2 for five days. In all the plates, negative and positive control sera were included. Reading of microplates were carried out when reverse titering confirmed the 100 $TCID_{50}$. The antibody titer was defined as the reciprocal of the higher dilution capable of totally inhibiting the virus cytopathic effect. Serum samples with titer < 10 were considered negative for CDV and those with titer < 5 were considered negative for CCoV.

Antibodies against CPV were evaluated through hemagglutination inhibition test (HI) as described by Carmichael and Binn (1981). Initially, the serum samples were diluted 1:5 with borate buffer saline (BBS; 1.5 M NaCl, 0.5 M H_3BO_3, 1.0 M NaOH, pH 9.0). Then, they were treated to remove

the inhibitors as follows: to the amount of diluted serum a suspension of 25% caulim in phosphate buffered saline (PBS; pH 7.2) was added and incubated for 30 min at room temperature, homogenizing periodically. After centrifugation at 400xg for 10 min, the supernatant was adsorbed in equal volume (50 µl) of swine red blood cells diluted at 50% in VAD buffer pH 6.0 (0.15 M NaCl, 0.3 M Na$_2$HPO$_4$, 0.15 M NaH$_2$PO$_4$), for 1 h at 4 °C. After centrifugation, the supernatant was collected and stored at -20 °C until further use. Treated samples were diluted from 1:20 to 1:2560 in microplates with a "V" bottom with BABS buffer (BBS with 0.2% of bovine fetal serum), incubated with CPV Cornell strain (ATCC - VR2017; 4 hemagglutination units/25 µl) and after 2 h at 37 °C, a volume of 50 µl of a swine red blood cells suspension at 0.5 % (in VAD, pH 6.0) was added. The reading was made after overnight incubation at 4 °C. The HI titre was indicated as the highest serum dilution completely inhibiting the viral hemagglutination. In all the microplates, negative serum samples and samples with known antibody titers were included as controls. Serum samples with titer < 20 were considered negative (Carmichael and Binn, 1981).

RESULTS

The results are presented in Table 1. From the 13 tested serum samples by serum neutralization assay, five (38.5%) presented antibodies against CDV. The frequency of anti-CDV antibodies was 60% (3/5) in pampas fox (*P. gymnocercus*) and 25% (2/8) in crab-eating fox (*C. thous*). A higher number of males (3/5) presented antibodies against CDV, compared to the number of females (2/8). The frequency of antibodies was higher among the adults (50%; 4/8) than among the juvenile animals (20%; 1/5).

From the 13 tested sera by HI for CPV, 11 (84.6%) were positive and two negative (15.4%). The frequency of anti-CPV antibodies in pampas fox (*P. gymnocercus*) was 80% (4/5) and in crab-eating fox (*C. thous*) was 87.5% (7/8). The frequency of antibodies for CPV did not differ between the sex and age groups.

Table 1 - Antibodies to Canine distemper virus (CDV), Canine parvovirus (CPV) and Canine coronavirus (CcoV) in serum samples of pampas fox (*Pseudalopex gymnocercus*) and crab-eating fox (*Cerdocyon thous*) from the Southern region of Brazil. Rio Grande do Sul, 2006.

	CDV		CPV		CCoV	
	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)
P. gymnocercus (n=5)	2 (40)	3 (60)	1 (20)	4 (80)	2 (40)	3 (60)
C. thous (n= 8)	6 (75)	2 (25)	1 (12,5)	7 (87,5)	6 (75)	2 (25)

n= number of animals examined

Neutralizing antibodies against CCoV were detected in 38.5% (5/13) of canids. The frequency of antibodies against CCoV in pampas fox (*P. gymnocercus*) was 60% (3/5) and 25% (2/8) in crab-eating fox (*C. thous*). The frequency of antibodies against CCoV was higher in the males (80%; 4/5) than in females (12,5%; 1/8) and higher in the adults (50%; 4/8) than in juveniles (20%; 1/5).

DISCUSSION

The present study reports for the first time the presence of antibodies to CDV, CPV and CCoV in pampas fox (*P. gymnocercus*) and crab-eating fox (*C. thous*) from Southern region of Brazil. The contact of the specimens evaluated in the present study with CDV, CPV and CCoV could be associated with agricultural production in the areas of natural distribution of free-ranging wild canid populations. Such activity has increased the possibility of contact between the wild and domestic canids, favoring the transposition of infectious agents from one host to another. Pampas foxes (*P. gymnocercus*) and crab-eating foxes (*C. thous)* usually approaches rural houses and camping sites to find food favoring to exposition to viral agents found in domestic dogs. The seropositivity level to CDV and CCoV was higher

among the adults, comparing with juvenile animals, probably reflecting a higher exposition to these agents. Male animals also presented a higher tendency to positivity, which reflect the behavior differences. Generally, males have a higher migratory activity, and can have contact with other animals with higher frequency. Males normally mark their territory with feces, for intra-species communication, moving around a large area for this activity (González, 2001).

The number of animals positive to CDV (38.5%) was similar of the serological studies realized in coyotes (Gese et al., 1991) and in domestic dogs from the Southern region of Brazil (Dezengrini et al., 2007). CDV do not persist well in the environment and require contact to be transmitted (Krakowka et al., 1985) and this may explain the lower frequency compared with CPV that was detected in this study.

The frequency of antibodies to CPV was 84.6%, with 80% in pampas fox (*P. gymnocercus*) and 87.5% in crab-eating fox (*C. thous*), indicating that a large proportion of these animals was exposed to this virus. The high prevalence observed in the wild canids is also reported in the domestic dogs (Thomas et al., 1984; Caetano et al., 2006; Dezengrini et al., 2007) and it is characteristic of highly contagious infections. This could be explained by the persistence of CPV in the environment, which could contribute for a continuous and intense exposition. This could also explain the fact that there was no correlation between the seropositivity and factors such as sex and age of the animal.

The frequency of antibodies against CCoV found in the wild canids of this study (38.5%) was in agreement with observed prevalence among the domestic canids (Pratelli et al., 2002; Caetano et al 2006; Dezengrini et al., 2007). The lower frequency of CCoV compared with CPV, was probably due to the biological characteristics of this virus. Although for both the viruses the transmission occurs mainly by exposition of susceptible animals to the virus eliminated through feces of infected animals (Pratelli, 2006), CCoV is less persistent in the environment and can be excreted in feces in smaller quantities than CPV.

This study showed the serologic evidence that free-ranging pampas foxes (*P. gymnocercus*) and crab-eating foxes (*C. thous*) from Southern region of Brazil were exposed to Canine distemper virus (CDV), Canine parvovirus (CPV) and Canine coronavirus (CCoV). At present, the significance of these pathogens to the overall health of these wild canid populations is unknown. Further studies should be conducted to evaluate the impact of the infections caused by these virus in the populations of these animals specimens, as well as evaluating and proposing preventive measures.

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RESUMO

Foi investigada a ocorrência de exposição em 13 canídeos não domésticos de vida livre (cinco graxains-do-campo - *Pseudalopex gymnocercus* e oito graxains-do-mato - *Cerdocyon thous*) da região sul do Brasil ao vírus da cinomose canina (CDV), parvovírus canino (CPV) e coronavírus canino (CCoV). Anticorpos contra o CDV foram detectados em 38,5% (5/13) das amostras. Haviam anticorpos anti-CDV em 60% (3/5) dos *P. gymnocercus* e em 25% (2/8) dos *C. thous*. A freqüência foi maior entre machos e adultos. Para CPV, 11 canídeos (84,6%) apresentaram anticorpos, 80% (4/5) eram da espécie *P. gymnocercus* e 87,5% (7/8) eram *C. thous*. Não houve diferença de positividade para o CPV entre sexos e idades. Anticorpos contra o CCoV foram detectados em 38,5% (5/13) das amostras, sendo 60% (3/5) de positividade entre os *P. gymnocercus* e 25% (2/8) entre os *C. thous*. A freqüência de anticorpos para CCoV foi maior entre os machos e adultos. O estudo revelou que estes canídeos foram expostos ao CDV, CPV e CCoV.

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