PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research

ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 38(8):1681-1684, agosto 2018
DOI: 10.1590/1678-5150-PVB-4989

Original Article
Animais Selvagens/*Wildlife Medicine*

Antibodies against canine distemper virus, parvovirus and *Ehrlichia* spp. in wild captive carnivores in midwestern Brazil[1]

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ABSTRACT.- Taques I.I.G.G., Morgado T.O., Braga I.A., Paz R.C.R., Corrêa S.H.R., Fritzen J.T.T., Alfieri A.A. & Aguiar D.M. 2018. **Antibodies against canine distemper virus, parvovirus and *Ehrlichia* spp. in wild captive carnivores in midwestern Brazil**. *Pesquisa Veterinária Brasileira 38(8):1681-1684*. Laboratório de Virologia e Rickettsioses, Hospital Veterinário, Universidade Federal de Mato Grosso, Av. Fernando Correa da Costa 2637, Boa Esperança, Cuiabá, Mato Grosso, MT 78090-900, Brazil. E-mail: danmoura@ufmt.br

The occurrence of antibodies against canine distemper virus (CDV), parvovirus and *Ehrlichia* spp. in wild captive carnivores was evaluated in a zoological park in midwestern Brazil. Serum samples were collected between 2007 and 2014 from 45 carnivores. Antibodies were evaluated by virus neutralization assay for CDV, hemagglutination inhibition test for parvovirus, indirect immunofluorescent and Enzyme-linked immunosorbent assay for *Ehrlichia* spp. Antibodies against CDV and parvovirus were detected in 75% of *Canidae* and *Felidae*. *Procyonidae* were negative for CDV, although one *Mustelidae* was positive. Two *Canidae* presented antibodies reactive to *E. canis* antigens. The high antibodies rates to CDV and parvovirus suggest the contact with both pathogens, however since no clinical history of disease are registered in the Zoo-UFMT, we can presume that carnivores have responded satisfactorily against the antigens. The low serological rates observed against *Ehrlichia* spp. may be resulted to the low occurrence of ticks among carnivores.

INDEX TERMS: Antibodies, canine distemper virus, parvovirus, *Ehrlichia* spp., carnivora, ehrlichiosis, serology, virology, zoological, wild mammals, viroses, parasitoses.

RESUMO.- [**Anticorpos contra o vírus da cinomose canina, parvovírus e *Ehrlichia* spp. em carnívoros selvagens cativos no centro-oeste do Brasil.**] A ocorrência de anticorpos contra o vírus da cinomose canina (CDV), parvovírus e *Ehrlichia* spp. em carnívoros selvagens em cativeiro foi avaliada em um parque zoológico do centro oeste do Brasil. As amostras de soro foram coletadas entre 2007 e 2014 de 45 carnívoros. Os anticorpos foram avaliados por ensaio de neutralização de vírus para CDV, teste de inibição de hemaglutinação para parvovírus, imunofluorescência indireta e ensaio imunoenzimático ligado à enzima para *Ehrlichia* spp. Anticorpos contra CDV e parvovírus foram detectados em 75% de canídeos e felídeos. Procionídeos foram negativos para CDV, embora um mustelídeo fora positivo. Dois canídeos apresentaram anticorpos reativos aos antígenos de *E. canis*. As altas taxas de anticorpos para CDV e parvovírus sugerem o contato com ambos os patógenos, entretanto desde que nenhuma história clínica de doença está registrada no Zoo-UFMT, podemos presumir que os carnívoros têm respondido satisfatoriamente contra os antígenos. As baixas taxas serológicas observadas contra *Ehrlichia* spp. pode ser resultado da baixa ocorrência de carrapatos entre os carnívoros.

TERMOS DE INDEXAÇÃO: Anticorpos, cinomose canina, parvovírus, *Ehrlichia* spp., carnívoros selvagens, erliquiose, sorologia, virologia, zoológico, mamíferos selvagens, viroses, parasitoses.

INTRODUCTION

Canine distemper virus (CDV) and parvovirus are two of the most important canine viral infectious diseases in Brazil today (Decaro & Buonavoglia 2012, Greene & Vandeveld 2012).

[1] Received on September 19, 2017.
Accepted for publication on March 22, 2018.

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Moreover, both viruses can infect wild carnivores, particularly Canidae and Felidae. CDV, a relatively large (150-250nm) enveloped and single-stranded RNA genome of the family *Paramyxoviridae*, has been reported in terrestrial carnivores (Deem & Emmons 2005, Greene & Vandeveld 2012). In Brazil, infection or serological evidence of CDV were reported in free-ranging and captive *Procyonidae*, *Felidae*, and *Canidae* (Jorge et al. 2010, Rego et al. 1997).

Canine parvovirus and feline panleukopenia virus were recently reclassified in the genus *Protoparvovirus* (Cotmore et al. 2014). The virus is the etiological agent of severe gastroenteritis in dogs and is often lethal for *Felidae* (Decaro & Buonavoglia 2012). Serologic surveys have detected antibodies against parvovirus in free-ranging wild carnivores, and the disease has led to the death of captive *Canidae* in Brazil (Jorge et al. 2010).

The genus *Ehrlichia* consists of tick-transmitted obligate intracellular bacteria that infect primarily leukocytes of mammals (Harrus et al. 2012). Canine Monocytotropic Ehrlichiosis (CME) is caused by *Ehrlichia canis* and vectored by the brown dog tick, *Rhipicephalus sanguineus*. Vertebrate hosts for CME include members of the family *Canidae*, but domestic dogs are considered the main reservoir host (Harrus et al. 2012). In Brazil molecular detection and anti-*Ehrlichia* spp. antibodies have been identified in captive and free-ranging wild carnivores (André et al. 2010, Almeida et al. 2013).

The Zoological Park of the Federal University of Mato Grosso (UFMT Zoo) is the only one in Brazil located on a university campus and currently has overcrowded and with several structural problems. This zoo has various captive carnivore species such as puma (*Puma concolor*), ocelot (*Leopardus pardalis*), maned wolf (*Chrysocyon brachyurus*), bush dog (*Speothos venaticus*), hoary fox (*Lycalopex vetulus*), crab-eating fox (*Cerdocyon thous*), and other species from different Brazilian biomes. Given the importance of the three aforementioned agents for captive carnivores, this study focused on an evaluation of antibodies against canine distemper virus CDV, parvovirus and *Ehrlichia* spp. in these species at the UFMT Zoo, and the findings suggest that these animals have been exposed to these pathogens.

MATERIALS AND METHODS

Serum samples were collected from 45 carnivores: ten *Canidae* (*Cerdocyon thous*, *Chrysocyon brachyurus*, *Speothos venaticus*, and *Lycalopex vetulus*), six *Felidae* (*Puma concolor* and *Leopardus pardalis*), 23 *Procyonidae* (*Nasua nasua* and *Procyon cancrivorus*), and six *Mustelidae* (*Galictis cuja* and *Eira barbara*) between 2007 and 2014. The sample collection was authorized by the Chico Mendes Institute for Biodiversity Conservation - ICMBio (Permit no. 42303-1) and performed in accordance with the Ethical Guidelines for Animal Research of UFMT (Protocol no. 23108.029695/14-8).

Antibodies against CDV were evaluated by the virus neutralization (VN) assay using as antigen the CDV Lederle strain in Vero cells. *Canidae* and *Felidae* serum samples were tested against CPV (CPV-2b) antigen by the hemagglutination inhibition (HI) test and *Ehrlichia* sp. by indirect immunofluorescent assay (IFA), using the Cuiabá #16 strain of *E. canis* (Appel & Robson 1973, Carmichael et al. 1980, Aguiar et al. 2016). Samples testing positive in IFA were subsequently evaluated by ELISA using a specific *E. canis* peptide (TRP19) (Aguiar et al. 2016). The samples were tested in duplicate for CDV and CPV and in triplicate in the enzyme immunoassay for *E. canis*. Cut-off values were 8 for CDV and 80 for parvovirus, while *E. canis* titers of ≥40 were considered positive (Carmichael et al. 1980, Deem & Emmons 2005, Aguiar et al. 2016). The optical density threshold was set at >0.300 OD units above the negative control absorbance (Aguiar et al. 2016).

RESULTS

Table 1 lists the number of each carnivore species evaluated in this study. All the *Canidae* species were seropositive for CDV. Two *Chrysocyon brachyurus* had titers of 64, one *Speothos venaticus* presented a titer of 16 and another titer of 64, and one *Lycalopex vetulus* presented a titer of 64. Serum samples from *Cerdocyon thous* were collected twice (Table 2) and different CDV antibody titers were detected. The first sample collected from *C. thous* #3 was positive and the second sample was negative (titers of 32 and 4, respectively), and the other four samples remained positive in the second sample (Table 2). Seropositive *Felidae* were represented by one *Puma concolor* and one *Leopardus pardalis*, with CDV antibody titers of 8 and

Table 1. Antibodies against canine distemper virus, canine parvovirus, and *Ehrlichia* spp. in wild captive carnivores in midwestern Brazil

Species	Common name	Year of collection	Antigens (positive/tested)		
			CDV (VN) Cut-off: 8	Parvovírus (HI) Cut-off: 80	*Ehrlichia* spp. (IFA) Cut-off: titer ≥40
Cerdocyon thous	crab-eating fox	2007, 2008, 2012, 2014	5/5	2/5	1/5[a]
Chrysocyon brachyurus	maned wolf	2013	2/2	2/2	1/2[b]
Speothos venaticus	bush dog	2008	2/2	2/2	0/2
Lycalopex vetulus	hoary fox	2013	1/1	1/1	0/1
Puma concolor	puma	2013, 2014	1/2	2/2	0/2
Leopardus pardalis	ocelot	2011, 2014	1/4	3/4	0/4
Procyon cancrivorus	crab-eating raccoon	2013	0/3	NT	NT
Nasua nasua	coati	2012, 2015	0/20	NT	NT
Galictis cuja	lesser grison	2014	1/4	NT	NT
Eira Barbara	tayra	2007, 2015	0/2	NT	NT

[a] Positive for *Ehrlichia canis* TRP19 antigen in ELISA, [b] Negative for *Ehrlichia canis* TRP19 antigen in ELISA; CDV = canine distemper virus, NT = not tested.

Table 2. Antibodies against canine distemper virus, canine parvovirus, and *Ehrlichia* spp. in *Cerdocyon thous* sampled at different moments

Cerdocyon thous	Antibody titers											
	Canine distemper virus (VN)				Parvovirus (HI)				*Ehrlichia* spp. (IFA)			
	2007	2008	2012	2014	2007	2008	2012	2014	2007	2008	2012	2014
#1	64	—	—	64	10	—	—	80[b]	Neg	—	—	Neg
#2	32	—	—	8	20	—	—	40	Neg	—	—	Neg
#3	—	32	—	4[a]	—	Neg	—	Neg	—	10,240[a]	—	Neg
#4	64	—	32	—	10	—	80[b]	—	Neg	—	Neg	—
#5	—	64	16	—	—	40	20	—	—	Neg	Neg	—

Cut-off CDV-VN = 8, cut-off parvovirus-HI = 80, cut-off *Ehrlichia* spp.-IFA = titer ≥40; [a] positive in the first sample and negative in the second sample, [b] seroconversion in the second sample.

32, respectively. *Procyonidae* were negative, although one *Mustelidae* (*G. cuja*) was positive, with a titer of 8.

CDV antibodies were found in 75% (12/16) of the samples (Table 1). *C. brachyurus* was positive with a titer of 160 and 320, two *S. venaticus* had titers of 1280, one *L. vetulus* had a titer of 160, and two *P. concolor* had titers of 160 and 1280, respectively. One *L. pardalis* presented a titer of 40 and three others had titers of 1280. Two samples from *C. thous* were positive, with a titer of 80 in the second sample (Table 2).

One *C. brachyurus* was positive for *Ehrlichia* spp. antigen by IFA (IFA titer of 320). *Ehrlichia* sp. antibodies were also detected in a sample from one *C. thous* collected at the beginning of the survey (Table 2) (IFA titer of 10,240). This sample was also reactive to TRP19 antigen in the ELISA, considering a detectable absorbance of 2,536 for TRP19 peptide and 0,176 for the negative control peptide.

DISCUSSION

In this study, antibodies against canine distemper virus CDV, parvovirus, and *Ehrlichia* spp. were detected in captive carnivores in a zoological park in midwestern Brazil. The occurrence rate of anti-CDV and anti-parvovirus antibodies in canids and felids was high indicating that both families were exposed to these pathogens. Serological surveys have shown a high incidence of anti-CDV and anti-CPV in Brazil (Jorge et al. 2010, Furtado et al. 2013), and these etiological agents are potentially fatal to captive and free-ranging carnivores. Several documented outbreaks of CDV have decimated carnivore populations in ecological and zoological parks around the world (Appel et al. 1994, Roelke-Parker et al. 1996). Maia & Gouveia (2002) demonstrated that the major pathogens causing mortality among captive canids in Brazil are CPV and CDV. These reports highlight our findings and demonstrate the need for the adoption of prophylactic measures to prevent the exposure of captive carnivores and consequent disease, which may, in some circumstances, be fatal.

Unfortunately, due to the scanty data available on these animals, we do not know whether seroconversion in some of them occurred prior to their capture or in the zoo. On the other hand, we identified different antibody titers occurring in samples from *Cerdocyon thous* collected at different times between 2007 and 2014 (Table 2). Two animals showed parvovirus antibodies, suggesting seroconversion occurred in the zoo, probably due to *in loco* exposure. Unfortunately, due to the long period elapsed between collected samples it is difficult to determine if these animals were in the ascending or descending phase of the antibody titer. Except for one sample whose CDV antibody titer remained unchanged, the other animals showed a decrease in titers, suggesting that exposure occurred before capture. This same dynamic was observed by Furtado et al. (2013) in free-ranging jaguars infected with CDV in the Pantanal. Another hypothesis that explains the occurrence of anti-CDV antibodies is vaccination (Wagner & Bhardwaj 2012), however, no data concerning vaccination procedures were available in the zoo.

The zoological park is located in an urban area and is surrounded by neighborhoods where many households have domestic dogs and cats. Although domestic pets are not allowed into the zoo, they are often observed in its vicinity. Therefore, in addition to the potential for CDV and parvovirus transmission among captive mammals, the presence of susceptible animals' close to the zoological park should and must be considered in the prophylaxis of these infections. In Germany, feral domestic cats were responsib le for parvovirus transmission for large captive felids (Wasieri et al. 2009). For CDV, interaction with domestic dogs has been determinant for transmission to wild carnivores (Roelke-Parker et al. 1996). In Brazil, CDV-related mortality has been documented in bush dogs and crab-eating foxes in the peri-urban environment, suggesting that contact with domestic animals enables infection (Megid et al. 2009, 2010).

Although the death of *Lycalopex vetulus* by canine distemper virus CDV infection has been molecularly characterized in Brazil, we report for the first time antibodies against CDV in captive *L. vetulus* (Megid et al. 2010). In addition, this study describes the first identification of antibodies against parvovirus in *Speothos venaticus* in Brazil.

Two canids had antibodies anti-*Ehrlichia* spp. A maned wolf reacted with titers of 320, but was negative against specific antigens of *E. canis*, suggesting that species other than *E. canis* may have been responsible for stimulating the serological response. Moreover, one *C. thous* had high antibody titers (10,240) and was also positive in ELISA using a specific antigen of *E. canis*. Interestingly, six years later, this animal tested negative without having undergone any kind of treatment. Most dogs usually become negative 6 to 9 months after treatment. On the other hand, chronic canine ehrlichiosis has been attributed to generate high antibody titers that persist for a long time (Harrus et al. 2012). Hence, in our case, in the absence of treatment, it is reasonable to assume that infection by *E. canis* was not sustainable and that this

animal subsequently eliminated the organism. In Brazil, captive and free-ranging *C. thous* have been found to be positive for *Ehrlichia* spp. in PCR analysis; hence, this animal was presumably infected before it was captured (André et al. 2010, Almeida et al. 2013). The lower infection rates of *Ehrlichia* than of CDV and parvovirus in animals that have been studied can be justified by the form of transmission that requires parasitism by ticks, while the other pathogens can be transmitted by direct contact or even indirect with viral agents. No ticks were observed parasitizing carnivores during sampling.

CONCLUSIONS

Our findings reveal the high occurrence of animals seropositive to canine distemper virus (CDV) and parvovirus in the UFMT Zoo, although deaths resulting from these pathogens have not been reported.

Given the favorable conditions for propagation of these agents, prophylactic measures should be established and adopted to prevent possible future outbreaks.

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