Canine distemper virus infection in a lesser grison (*Galictis cuja*): first report and virus phylogeny[1]

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Infectious diseases in wild animals have been increasing as a result of their habitat alterations and closer contact with domestic animals. Canine distemper virus (CDV) has been reported in several species of wild carnivores, presenting a threat to wildlife conservation. We described the first case of canine distemper virus infection in lesser grison (*Galictis cuja*). A free-ranging individual, with no visible clinical sigs, presented sudden death after one day in captivity. Molecular diagnosis for CDV infection was performed using whole blood collected by postmortem intracardiac puncture, which resulted positive. The virus phylogeny indicated that domestic dogs were the probable source of infection.

INDEX TERMS: Canine distemper virus, *Galictis cuja*, Lesser grison, nucleoprotein, phylogeny.

RESUMO.- [Infecção pelo vírus da cinomose canina em um furão (*Galictis cuja*): primeiro relato e filogenia viral.] Doenças infecciosas em animais selvagens têm aumentado devido às alterações em seu habitat e ao maior contato com animais domésticos. A cinomose já foi descrita em diversas espécies de carnívoros selvagens, representando uma ameaça à conservação da vida selvagem. Nesse estudo é descrito o primeiro caso de infecção pelo vírus da cinomose em um furão (*Galictis cuja*). Um indivíduo de vida livre, sem sinais clínicos aparentes, apresentou morte súbita após um dia em cativeiro. Foi realizado o diagnóstico molecular para detecção do vírus da cinomose canina, sendo o resultado positivo. A filogenia do vírus indicou que cães domésticos foram a provável fonte de infecção.

TERMOS DE INDEXAÇÃO: Vírus da cinomose canina, *Galictis cuja*, furão, nucleoproteína, filogenia.

INTRODUCTION

Infectious diseases are responsible for impacting biodiversity, decreasing the population growth rate and increasing vulnerability to extinction (Chauvenet et al. 2011). In many parts of the world domestic animals are the likely maintenance host and source of virulent pathogens to wildlife. Canine distemper virus (CDV), a single strand RNA virus, belonging to the genus *Morbillivirus*, family *Paramyxoviridae*, was first isolated in 1905 from domestics dogs (*Canis familiaris*) (Appel et al. 1981). Some wild species have been infected by CDV, such as badgers (*Taxidea taxus*), mink (*Mustela vison*), black-footed ferrets (*Mustela nigripes*), wild Taiwan ferret-badgers (*Melogale moschata subauantiaca*), greater grison (*Galictis vitatta*), stone martens (*Martes foina*), polecats (*Mustela putorius*), and badgers (*Meles meles*) (Appel et al. 1981, Rego et al. 1997, Williams et al. 1998,

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Chen et al. 2008, Megid et al. 2010). As occur in domestic dogs, many of these wild species get sick and show severe clinical pictures of CDV infection, leading to death of the animals. In ferrets, domesticated mammal of the type *Mustela putoris furo* belonging to the family Mustelidae, distemper is the most important infectious disease, the mortality rates are closer 100% and unvaccinated dogs may serve as reservoirs of CDV (Fox et al. 1998, Megid et al. 2009).

The lesser grison (*Galictis cuja*) is a mammal of the South America fauna that may be found in Argentina, Bolivia, Brazil, Chile, Paraguay, Peru, and Uruguay. Similar to ferrets lesser grisson (*Galictis cuja*) belongs to the Mustelidae family. Because of the degradation of its natural habitat, this species can be found close to rural or urban areas, searching for food and shelter. Also, the proximity of human habitations with natural areas allows domestic animals to live near to wildlife. Studies related to diseases that occur in lesser grison are extremely scarce, and only a few cases involving parasitological findings have been described (Ferriolli Filho & Barreto 1969, Barros et al. 1990, Wisnivesky-Colli et al. 1992, Labruna et al. 2005). Our objective was to report the first case of CDV infection in a lesser grison; in addition the virus strain phylogeny was performed and discussed.

MATERIALS AND METHODS

A free-ranging young male lesser grison (*Galictis cuja*) was found in Votorantim city (São Paulo State, 23.32 S; 47.26 W, Brazil). The animal was sent to Quinzinho de Barros Zoological Park (Sorocaba city, São Paulo State, 23.06 S, 47.27 W, Brazil), where clinical examination was performed. Clinical signs were not noted, and behavior was considered normal. The animal was placed in a transportation box for its accommodation, and was found dead in the next morning. Necropsy was performed and intracardiac blood was collected, and submitted for CDV molecular diagnosis by reverse transcription-polymerase chain reaction (RT-PCR) for the detection of specific conserved CDV gene of nucleocapsid protein (NP). The positive control used was from the Laboratory of Molecular Biology of Infectious Diseases of Animals, Faculty of Veterinary Medicine and Animal Science (FMVZ), Universidade Estadual Paulista-UNESP, Botucatu, Brazil. Total RNA was extracted with the Ilustra RNAspin Mini RNA isolation kit (GE Healthcare- Little Chalfont- Buckinghamshire, HP7 9N, UK) according to the manufacture's instruction. Purified viral RNA was reverse transcribed (RT) using SuperScript® Reverse Transcriptase (Invitrogen, Carlsbad, CA), and semi-nested PCR amplification with both forward (5ATCCCCAGGRAACAAGCCTACAA3') and reverse primers (5'CCTTGGTGATGCCAAGCTCG3') was performed using Platinum Taq DNA polymerase® (Invitrogen, CA) according to Amaral, (2007). Amplified products with the expected molecular weight (331 bp) were purified using Illustra GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare- Little Chalfont- Buckinghamshire, HP7 9N, UK) and sequenced with both forward (5'ATCCCCAGGRAACAAGCCTAGAA3') and reverse primers (5'CG AAATTTAACCCTCCCATG3') using the BigDye™ Terminator Kit (Applied Biosystems Inc,850 Lincoln Centre Drive Foster City, CA 94404 USA) with an automated sequencer (ABI model 377, Applied Biosystems Inc,850 Lincoln Centre Drive Foster City, CA 94404 USA) according to the manufacturer's instructions. The complete sequences assemblies were created with the PHRED/PHRAP (Ewing & Greene 1998), and CAP3 (Huang & Mada 1999) programs using nucleotide data with quality higher than 20. The five derived CDV sequences from blood were aligned using BIOEDIT v. 7.0.5 (Hall 1999). Phylogenetic analysis was performed on the aligned data set, and a rooted tree was constructed using the distance-based Neighbor-joining method in the Mega v.4.0 package (Tamura et al. 2007) with the rinderpest virus sequence as an out-group. Bootstrap values were calculated from 1000 replicates using the heuristic method. Accession numbers for sequences acquired from the public databases (GenBank) were: Brazilian CDV strains isolated from dogs (GenBank:DQ005130, DQ005131, DQ005132, DQ005133, DQ005134); European CDV strain isolated from dogs (AF166268, AF166269); North American CDV strain isolated from dog (EU716337); North American CDV strains isolated from raccoons (AY443350, AY466011, AY542312); Vaccine CDV strain (Onderstepoort, AY684629, Lederle, EF418783): and Rinderpest virus (out-group: EF186058, EF186062), AY738625, and from recent research (Fox, Megid et al. 2010).

RESULTS

The necropsy was performed and did not revealed macroscopic abnormalities; unfortunately the histopathological examination could not be performed because clinical signs were not observed. The RT-PCR for CDV was positive, and phylogenetic analysis of the CDV NP gene indicated that the strain was compatible to the ones isolated from Brazilian domestic dogs (Fig.1).

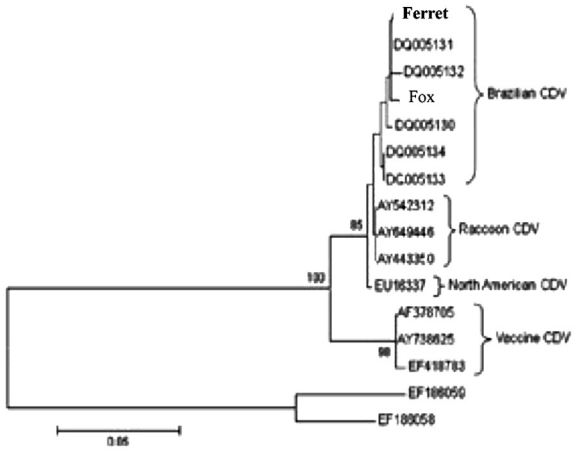

Fig.1. Phylogenetic trees based on a fragment of 234 bp from CDV NP gene. Neighbor-joining tree was generated by using the MEGA 4.0 program with 1,000 bootstrap replication. Brazilian CDV strains isolated from dogs (GenBank: DQ005130, DQ005131, GenBank: DQ005132, GenBank: DQ005133, GenBank: DQ005134), North American CDV strain isolated from dog (GenBank: EU716337), North American CDV strains isolated from raccoons (GenBank: AY443350, GenBank: AY542312, GenBank: AY649446), Vaccine CDV strain (GenBank: AY738625, GenBank: AY684629 (Onderstepoort), GenBank: EF418783 (Lederle), and Rinderpest virus (out-group: GenBank: EF186058, GenBank: EF186059)., Fox (Megid et al. 2010). Ferret: lesser grison (*Galictis cuja*) described in this study.

DISCUSSION

The presence of canine distemper virus in blood without clinical signs can suggest that the animal was in incubation period for the disease or it´s responsible for the sudden death. According to Fox et al (1998) distemper may cause

viremia in blood before the clinical signs normally characterized by respiratory and nervous signs, however these researchers also reported that in a CDV-infected animals, clinical signs of distemper could not be observed. Moreover, in an outbreak of canine distemper in black-footed ferrets (*Mustela nigipes*), neurologic disorders were also not observed (Williams et al. 1998) in accordance to the absence of clinical signs in this animal and can be justified by the considerable biological variation between CDV strains, which may influence incubation periods and clinical signs (Appel et al. 1981). CDV was detected in blood samples and was phylogenetically similar to the viruses that affects domestic dogs and foxes in Brazil (Fig.1). The same results were obtained by Rego et al. (1997), which reported a greater grison infected by CDV. In the same state (São Paulo) of this study, CDV infection on a crab-eating fox (*Cerdocyon thous*) and a hoary fox (*Lycalopex vetulus*) were described (Megid et al. 2009, 2010). All these cases were phylogenetically evaluated and characterized as a domestic dog's CDV strains, as the one described in our study. Cases of dogs with symptoms characteristic of CDV infection were observed in vaccinated population worldwide, suggesting that there is antigenic differences between a sample wild geographically different from the vaccine strain (Harder & Osterhaus 1997). As described in this case report, Gamiz et al. (2011) reported that there are differences between the field samples and vaccine samples of CDV in Mexico.

Fragmentation of wild species habitat has placed wildlife in contact with domestic animals, which are potential reservoirs of pathogens (Medina-Vogel 2010). The CDV found in wild carnivores have been resulted from transmission from local domestic dogs (Ferreyra et al. 2009, Megid et al. 2009, 2010, Müller et al. 2011). For example, in Africa, the population of stray dogs has changed the ecology of diseases, allowing CDV to be transmitted to wild animals (Cleaveland et al. 2002). In a study realized by Campos (2004) the lesser grison was identified as part of dog's diet, indicating that these species can occupy the same areas. Therefore, the occurrence of infectious diseases in wild animals could be a consequence of deficient vaccination in domestic dogs, as reported by Vos et al. (2009), who demonstrated the importance of domestic dog's rabies vaccination to prevent the rabies virus transmission to wild animals.

CONCLUSIONS

The changes in ecological systems may lead wild carnivores to have contact with areas occupied by men and domestic animals.

Domestic dogs were the probable source of CDV infection in the present case, and the vaccination against CDV in these animals could contribute to wildlife conservation, avoiding the transmission of the virus from domestic to wild animals.

The strains isolated in Brazil and the sample described in this report are not clustered together within the vaccine samples (Fig.1) putting into question the effectiveness of vaccines against canine distemper.

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