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Canine Distemper Virus in a Crab-eating Fox (*Cerdocyon thous*) in Brazil: Case Report and Phylogenetic Analyses

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ABSTRACT: Although canine distemper is enzootic worldwide and has a wide host range, there are no reports of canine distemper virus in crab-eating foxes (*Cerdocyon thous*) that provide information on virus phylogeny and histopathologic lesions. The objective of this study is report and describe canine distemper in a crab-eating fox (*C. thous*), with a focus on the phylogeny of the virus strain and the histopathologic lesions in the animal.

Key words: *Cerdocyon thous*, clinical signs, histopathology, nervous system, virus phylogeny.

Canine distemper virus (CDV) is a RNA virus belonging to the family *Paramyxoviridae*, genus *Morbillivirus*. Most terrestrial carnivores are susceptible to CDV infection, and recently, the host range has expanded to include large cats (*Panthera leo*, *Panthera pardus*, and *Panthera tigris* in California in 1992 and *P. leo* in Tanzania in 1994) and collared peccaries (*Tayassu tajacu*; Appel and Summers, 1999). Canine distemper is endemic in Brazil, being one of the most frequent and important infectious diseases of small animals. In many parts of the world, dogs are the likely maintenance host and represent a source of CDV to wildlife. Canine distemper originating from dogs has been reported in lions in Serengeti (Roelke-Parker et al., 1996), Baikal seals (*Phoca sibirica*) in Siberia (Mamaev et al., 1995), crabeater seals (*Lobodon carcinophaga*; Bengston et al., 1991), and African wild dogs (*Lycaon pictus*; Van de Bildt et al., 2002).

Increased global urbanization potentially increases contact between wild and domestic animals and increased transmission of infectious diseases. Wild carnivores are of particular concern as they are phylogenetically similar to common domestic species (Cleaveland et al., 2000).

In Germany, a higher prevalence of CDV antibodies was detected in wild canids in urban and suburban areas and no positive samples were detected in animals from rural areas (Frölich et al., 2000). Phylogenetic analyses showed that CDV found in these wild carnivores was genetically related to the virus found in domestic dogs (Frölich et al., 2000). This genetic similarity was also demonstrated in island foxes (*Urocyon littoralis*; Timm et al., 2000) and in lions from Serengeti Park (Cleaveland et al., 2000).

Antibodies to CDV have been previously reported from crab-eating foxes (*Cerdocyon thous*; Fiorello et al., 2007). In this study we report a case of canine distemper virus disease in a crab-eating fox and information on the phylogeny of the virus strain.

A young female crab-eating fox that had generalized myoclonus compatible with the neurologic phase of canine distemper was found in a suburban area. The animal was submitted for supportive treatment but died 1 day after. Samples from brain, lung, liver, kidney, and urinary bladder were collected, and each was processed for histopathology (Prohet et al., 1992),

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fluorescent antibody assay with the canine distemper fluorescent conjugate (VMRD, Pullman, Washington) according to the instructions of manufacturer, and reverse transcriptase polymerase chain reaction (RT-PCR).

Briefly, total RNA was extracted with the Illustra RNAspin Mini RNA Isolation Kit (GE Healthcare, Little Chalfont–Buckinghamshire, UK) according to the manufacturer's instructions. Reverse transcription and RT-PCR was performed with the use of the protocol of Amaral (2007).

Amplified products with the expected molecular masses were purified with the use of a commercial kit (Illustra GFX™ PCR DNA and Gel Band Purification Kit, GE Healthcare) and sequenced with both forward and reverse primers with the use of the BigDye™ Terminator Kit (Applied Biosystems, Foster City, California) with an automated sequencer (ABI model 377, Applied Biosystems) according to the manufacturer's instructions. The complete sequence assemblies were created with the PHRED/PHRAP (Ewing and Green, 1998) and CAP3 (Huang and Madan, 1999) programs with the use of nucleotide data with quality higher than 20. The derived CDV sequences were aligned with the use of BIOEDIT v. 7.0.5 (Hall, 1999).

Phylogenetic analysis was performed on the aligned data set and a rooted tree was constructed with the use of the distance-based neighbor-joining method in the Mega v.4.0 package (Tamura et al., 2007) with the rinderpest virus sequence as an outgroup. Bootstrap values were calculated from 1,000 replicates with the use of the heuristic method.

Accession numbers for sequences acquired from the public databases were Brazilian CDV strains isolated from dogs (AY738653, DQ005128, 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 (AY684629 [Onderstepoort], EF418783 [Lederle]), and rinderpest virus (outgroup: EF186058, EF186062)

Clinical signs presented by the crab-eating fox were identical to those observed in dogs and compatible with the neurologic phase of canine distemper. The presence of virus was detected by fluorescent antibody testing and RT-PCR in all samples analyzed, demonstrating virus dissemination similar to that observed in dogs and suggesting absence of immunity in this animal (Greene and Appel, 2006).

Histopathologic lesions similar to those observed in domestic dogs were observed and characterized by interstitial pneumonia with congestion and pulmonary edema, focal emphysema, atelectasis, and hemorrhagic focci. Multifocally distributed areas of mixed inflammatory cells and multinucleated cells were associated with pneumocyte proliferation. Multifocal areas of coagulation necrosis were present. The spleen had lymphoid necrosis. In the central nervous system, moderated demyelination of white matter and focal areas having Gitter cells associated with malacia in the cerebellum were observed. The spinal cord had demyelination and Wallerian degeneration. The frontal cortex had discrete edema and the pons had demyelination and discrete gliosis (Greene and Appel, 2006; Zachary, 2007)

The susceptibility of free-ranging crab-eating fox to canine distemper is demonstrated in this report. Although this species was not suspected to be highly susceptible to CDV (Fiorello et al., 2007), the case is consistent with results from Rego et al. (1997), who reported five cases of canine distemper in wild zoo animals in Brazil; one of these was a crab-eating fox.

Transmission of canine distemper virus in the Serengeti was associated with higher density of domestic dogs that provided a source of CDV infection for wildlife (Cleaveland et al., 2000). Van de Bildt et al. (2002) observed the same results with CDV in African wild dog in

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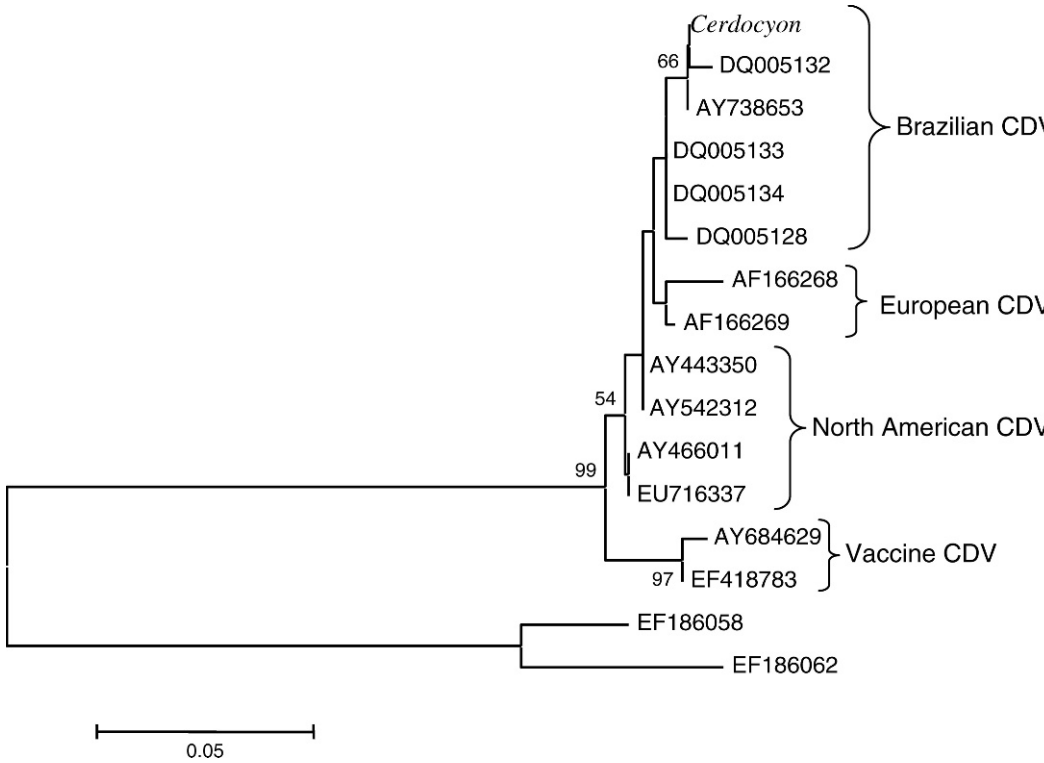


FIGURE 1. Phylogenetic trees based on a 192–base pair canine distemper virus (CDV) nucleoprotein gene fragment. The neighbor-joining tree was generated by using the MEGA program with 1,000 bootstrap replication. Brazilian CDV strains isolated from dogs (AY738653, DQ005128, DQ005132, DQ005133, DQ005134), 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 (AY684629 [Onderstepoort], EF418783 [Lederle]), and rinderpest virus (outgroup: EF186058, EF186062).

Tanzania. Canine distemper is a very common disease in suburban and farm dogs in Brazil because of the absence of vaccination. The phylogeny of the CDV virus from the crab-eating fox (Fig. 1) supports a domestic dog source. Similar results are reported by Hirama et al. (2004) and Frölich et al. (2000), in Japan and Germany, respectively, where CDVs from wild animals were closely related to CDVs from domestic dogs. Phylogenetic results also demonstrate that the CDV strains from domestic dogs in Brazil differed from the vaccinal strain (especially Onderstepoort strains; Negrão et al., 2006). Similar results have been reported from Japan and Italy (Uema et al., 2005; Martella et al., 2007) and are consistent with our study, where the vaccinal strain grouped in a different cluster from CDV strains derived from dogs or the crab-eating fox.

This is the first report of canine distemper in a free-living crab-eating fox in Brazil, and our phylogenetic analysis suggests that potential CDV transmission between domestic dogs and wild carnivores such as the crab-eating fox should be further evaluated and considered.

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