SHORT COMMUNICATIONS

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Canine Distemper Virus in Wild Felids of Costa Rica

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ABSTRACT: Several highly infectious diseases can be transmitted through feces and cause elevated mortality among carnivore species. One such infectious agent, canine distemper virus (CDV; *Paramyxoviridae*: *Morbillivirus*), has been reported to affect wild carnivores, among them several felid species. We screened free-ranging and captive wild carnivores in Costa Rica for CDV. Between 2006 and 2012, we collected 306 fecal samples from 70 jaguars (*Panther onca*), 71 ocelots (*Leopardus pardalis*), five jaguarundis (*Puma yaguaroundi*), 105 pumas (*Puma concolor*), five margays (*Leopardus wiedii*), 23 coyotes (*Canis latrans*), and 27 undetermined *Leopardus* spp. We found CDV in six individuals: one captive jaguarundi (rescued in 2009), three free-ranging ocelots (samples collected in 2012), and two free-ranging pumas (samples collected in 2007). Phylogenetic analyses were performed using sequences of the phosphoprotein (P) gene. We provide evidence of CDV in wild carnivores in Costa Rica and sequence data from a Costa Rican CDV isolate, adding to the very few sequence data available for CDV isolates from wild Central American carnivores.

Key Words: Canine distemper virus, Costa Rica, phosphoprotein gene, sequence analysis, wild felids.

Over the last two decades, infectious diseases have been responsible for population reductions of many wildlife species (Daszak et al. 2000; Tompkins et al. 2015). Canine distemper virus (CDV; *Paramyxoviridae*: *Morbillivirus*), for example, infects terrestrial and marine carnivores (Deem et al. 2000), has a worldwide distribution, and can cause high morbidity and mortality in immunologically naive populations (Kapil and Yeary 2011). The virus is transmitted by direct contact; infection thus occurs primarily upon inhalation of infectious aerosols or through ingestion, via oral fluids and feces (Sakai et al. 2013). It is believed that the virus originated in Peru and was then brought to Spain in the 17th century, from where it spread throughout Europe (Blancou 2004). In addition, the highest genetic diversity of CDV strains is in South America (Panzera and Aldaz 2014).

The spread of CDV in natural environments appears to be mainly associated with ecologic dynamics and anthropogenic factors that allow contact of susceptible wildlife species with multiple hosts, including human companion animals, such as the domestic dog (*Canis lupus familiaris*), which is known for its capacity to propagate CDV (Almberg et al. 2010; Viana et al. 2015). For example, in Brazil, Nava et al. (2008) found antibodies to CDV in wild felids, including pumas (*Puma concolor*), jaguars (*Panther onca*), and ocelots (*Leopardus pardalis*), and Rosa et al. (2012) found CDV in domestic dogs in Brazilian cities.

We surveyed captive and free-ranging wild carnivores in Costa Rica for CDV by examining preexisting and newly collected fecal samples from several localities (Fig. 1). Field sampling was opportunistic and noninvasive; however, in some cases a trained dog was used to locate felid scat. Scat samples from captive individuals were also collected from rescue centers. Samples were kept at –80 C until analysis.

We examined 306 fecal samples, collected between 2006 and 2012, from 35 captive and 271 wild animals. Experts in carnivore feces identification made preliminary identification of the species from which the samples were collected and identifications were confirmed using molecular methods (Soto-Fournier 2014). For this, DNA was extracted using the QIAamp DNA Stool Mini Kit® (Qiagen, Hilden, Germany), with posterior modifica-

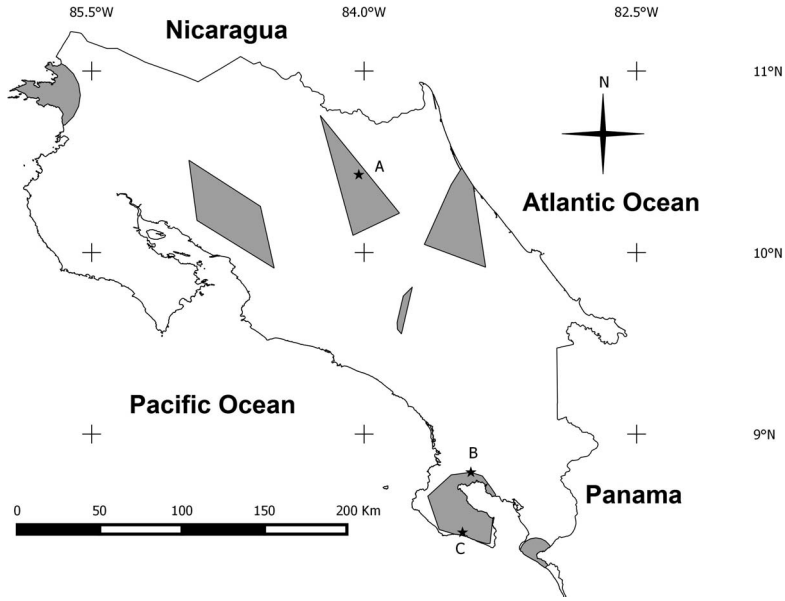

FIGURE 1. Shaded polygons indicate areas of Costa Rica where feces of wild carnivores were collected. Asterisks indicate locations of positive samples from free-ranging felids: A=La Selva, Sarapiquí; B and C=Osa Peninsula.

tions (Chaves et al. 2010). Six gene regions from four mitochondrial genes (385 bp of cytochrome b [cytb], 178 bp of 12S rRNA [12S], 134 bp of ATPase-6 [ATP6] and 443 bp of 16S rRNA [16S]) were amplified for molecular identification of samples (Soto-Fournier 2014). Sequences were compared with mtDNA sequences of captive carnivore species as reference and with the NCBI Nucleotide Database (Pruitt et al. 2014). Sequence similarities among species were assessed by constructing a phylogenetic tree to infer the origin of the samples.

For the detection of CDV, we extracted total RNA from samples using the QIAamp Viral RNA Mini Kit® (Qiagen), according to the manufacturer's instructions. Paramyxoviruses were detected using reverse-transcriptase PCR (Tong et al. 2008) and positive samples were tested for CDV.

A fragment of the phosphoprotein (P) gene (632 bp) was amplified by PCR and sequenced as described by Pardo et al. (2005). Samples were sequenced using the BigDye® Terminator v3.1 cycle sequencing kit and cleaned with the BigDye XTerminator® Purification kit (Applied Biosystems, Foster City, California, USA). Products were analyzed on a 3130xl Genetic Analyzer (Applied Biosystems). Sequence alignments were performed using Clustal W (Larkin et al. 2007). Phylogenetic and molecular evolutionary analyses were conducted using the distance matrix method (maximum likelihood algorithm) and the nucleotide distance measure (Kimura 2-parameter model); the complete deletion option within MEGA 6 (Tamura et al. 2013) was also used. Data sets were bootstrapped based on 1,000 resamplings of the original data set to produce a majority rule consensus tree. Sequences were deposited in GenBank (accessions KP711845–KP711850). The CDV P-gene sequences used for comparison were also obtained from GenBank.

The DNA analysis confirmed that the scat came from 70 jaguars, 71 ocelots, five jaguarundis (*Puma yaguaroundi*), 105 pumas, five margays (*Leopardus wiedii*), 23 coyotes (*Canis latrans*), and 27 undetermined *Leopardus* spp. individuals. We found CDV in six samples (2%). One positive sample collected in 2011 was from a jaguarundi brought into

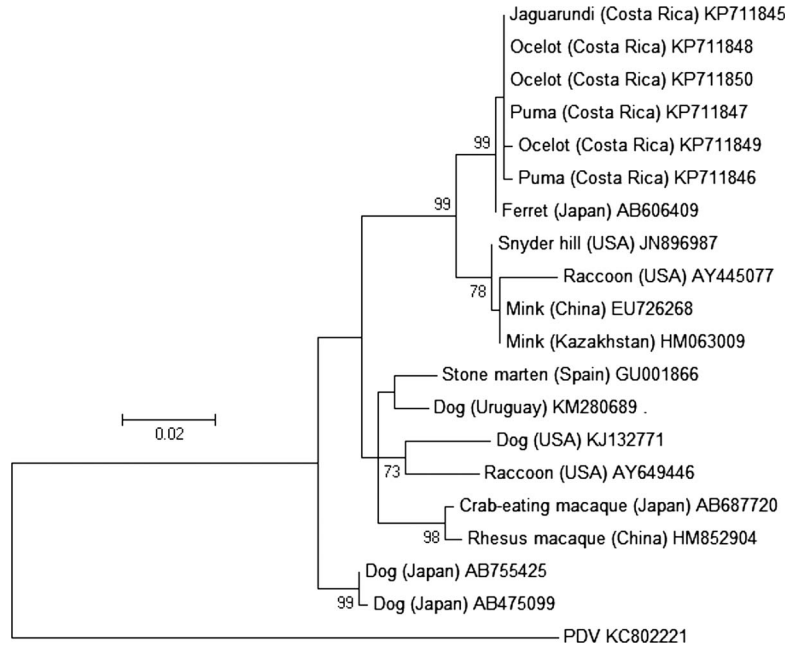

FIGURE 2. Bootstrap consensus tree of canine distemper viruses based on P-gene sequences; values <70 are not shown. PDV=phocine distemper virus.

captivity in 2009; the other positive samples came from free-ranging felids (three ocelots collected in 2012 and two pumas collected in 2007). The positive ocelot samples were collected in the northern limit of the Osa Peninsula and the positive puma samples were collected in the southern portion of the Osa Peninsula and in La Selva Biological Station, Sarapiquí (Fig. 1).

The sequences of the P-gene fragment (573 bp) were 99.65% to 100% identical when compared pairwise. When compared to sequences in Genbank, our sequences had the highest similarity to CDV isolated from ferrets (*Mustela putorius furo*) collected in Japan in 2010, with differences in only one or two nucleotides (Fig. 2).

In the Mesoamerican region, the majority of investigations have been directed towards the detection of CDV in domestic animals, with a few attempts on free-ranging felines. In Costa Rica, one case of a domestic dog with CDV was reported, in which microscopic examination revealed bronchointerstitial lung pneumonia by morbillivirus infection (Berrocal and Lopez 2003). In Guatemala domestic cats (*Felis catus*) and margays were sampled to determine the prevalence of antibody to several pathogens (including CDV) that pose a potential risk to native wild felids; however, results for CDV were negative for all samples (Lickey et al. 2005). In Panama, ocelots have been screened for CDV with negative results (Franklin et al. 2008).

Our detection of CDV in wild carnivores is an initial step in improving our knowledge of the transmission cycles of CDV in tropical wildlife. This basic information can help us recognize the routes of infection of this multihost pathogen and identify situations in which human activities enable interaction between domestic animals and susceptible, at-risk, wild populations (Acosta-Jamett et al. 2015).

Finally, but not less importantly, scat sampling is a proven noninvasive tool for the study of infectious agents excreted through feces. Capturing animals would not likely yield the same number of samples we obtained and scat analysis permits a higher likelihood of detection. Moreover, the fact that the hosts can shed CDV within 60–90 d of

infection may facilitate its detection (Greene and Appel 1990; Riley et al. 2004). However, even though no serologic study of infection by CDV has been conducted in Costa Rica, this would likely show a higher background population exposure than the 2% prevalence we detected. Thus, additional research is still needed to understand the CDV transmission cycles in wild felids in the neotropical region.

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