Canine distemper outbreak by natural infection in a group of vaccinated maned wolves in captivity

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Abstract

Canine morbillivirus, also known as canine distemper virus (CDV) is one of the most important infectious diseases threat to the health and conservation of free ranging and captive wild carnivores. CDV vaccination using recombinant vaccines has been recommended for maned wolf (Chrisosyon brachyurus) after the failure of modified live vaccines that induced disease in vaccinated animals. However, there has been a lack of systematic evaluation about the response of this preventive protocol in zoo carnivores due to ethical reasons that do not approve vaccination trials with challenge in that species. Here we report a CDV outbreak in a captive population of maned wolf with an index case that was previously vaccinated with a recombinant vaccine. Five juveniles and one adult from a group of seven maned wolves housed in an outdoor exhibit died in April-May 2013 in a zoo in the Metropolitan Region, Chile. Clinical signs ranged from lethargy to digestive and respiratory signs. Diagnosis of CDV was confirmed by histopathology, antibody assays and viral molecular detection and characterization. The phylogenetic analyses of the nucleotide sequence of H gene of the CDV genome identified in the two positive samples suggest a close relation with the lineage Europe 1, commonly found South America and Chile. CDV infections in maned wolf have not been previously characterized. To the authors best knowledge is the first report of the clinical presentation of CDV in a canine species previously inmmunized with a recombinant vaccine. Further research will be necessary to understand the impact of CDV in wild maned wolf populations and new protocols (with boosters) that could improve the effectiveness of the recombinant vaccine against CDV in wild carnivores.

Transboundary and Emerging Diseases

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ABSTRACT

Canine morbillivirus, also known as canine distemper virus (CDV) is one of the most important infectious diseases threat to the health and conservation of free ranging and captive wild carnivores. CDV vaccination using recombinant vaccines has been recommended for maned wolf (*Chrisosyon brachyurus*) after the failure of modified live vaccines that induced disease in vaccinated animals. However, there has been a lack of systematic evaluation about the response of this preventive protocol in zoo carnivores due to ethical reasons that do not approve vaccination trials with challenge in that species. Here we report a CDV outbreak in a captive population of maned wolf with an index case that was previously vaccinated with a recombinant vaccine. Five juveniles and one adult from a group of seven maned wolves housed in an outdoor exhibit died in April-May 2013 in a zoo in the Metropolitan Region, Chile. Clinical signs ranged from lethargy to digestive and respiratory signs. Diagnosis of CDV was confirmed by histopathology, antibody assays and viral molecular detection and characterization. The phylogenetic analyses of the nucleotide sequence of H gene of the CDV genome identified in the two positive samples suggest a close relation with the lineage Europe 1, commonly found South America and Chile. CDV infections in maned wolf have not been previously characterized. To the authors best knowledge is the first report of the clinical presentation of CDV in a canine species previously inmmunized with a recombinant vaccine. Further research will be necessary to understand the impact of CDV in wild maned wolf populations and new protocols (with boosters) that could improve the effectiveness of the recombinant vaccine against CDV in wild carnivores.

KEYWORDS: Canine distemper virus, outbreak, *Chrysocyon brachyurus* , zoo, vaccination.

INTRODUCTION

Canine morbillivirus, also known as canine distemper virus (CDV) is the etiological agent of canine distemper (CD). It is a highly contagious disease in which elevated mortality and morbidity in dogs and is, along with rabies, the most important infectious diseases threat to the health and conservation of free living and captive wild carnivores. This virus is present in most countries and several *Carnivora* families are susceptible, including the *Canidae, Procyonidae, Mustelidae, Hyaenidae, Ursidae, Viverridae, Felidae, Ailuridae, Phocidae, and Otariidae.* It can also affect other mammal orders like*Cetartiodactyla* , *Primates* , *Rodentia* and*Pilosa* . The maned wolf (*Chrysocyon brachyurus*) is the largest member of the *Canidae* family endemic/native of South America. According to the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species, the maned wolf is considered to be near threatened. The four main threats affecting wild maned wolf populations throughout their distribution range are: habitat loss and alteration; human persecution due to livestock losses and cultural beliefs; increasing vehicular traffic in highways resulting in road kills; and pathogens contracted from domestic animals due to increased contact in disturbed environments.

Canine distemper virus vaccination using recombinant dog commercial vaccines has been recommended as a preventive protocol for captive maned wolf and other wild carnivore species. When modified live vaccines (MLV) were used in wild animals some of them induced disease . However, there has been a lack of systematic evaluation about the response of this protocol in zoo carnivores by ethical reasons that do not approve vaccination trials with challenge in these species. Although CDV has been historically recognized as a threat for the maned wolf conservation, little is known of the pathological effects in the species . To the knowledge of the authors, there are no reports documenting the clinical disease by natural CDV infection in maned wolves. Furthermore, this is the first report of recombinant CDV vaccine failure to protect a wild canid

species against the clinical infection produced by this virus and the first CDV outbreak documented in maned wolves (*Chrysocyon brachyurus*).

Case Presentation

A 9-year-old female maned wolf part of the collection of a zoological institution in Chile, was presented with unspecific clinical signs in April 2013; these signs included lethargy, sialorrhea and diarrhea. This female was kept in the same enclosure with a 7-year-old male maned wolf and their five 1-year-old pups. Unfortunately, after 6 days of supportive treatment that included intravenous fluid therapy, antibiotics, and Vitamin K, the female died. Seven days later four of the pups started showing progressive clinicals signs, except from one female that died suddenly, without any clinical sign, 9 days after the death of the 9-year-old female (figure 1). The remaining 4 cubs clinical signs range from lethargy to digestive and respiratory signs and died in a lapse of 16 days despite supportive treatment (figure 1). The 7-year-old male was observed clinically healthy during the outbreak.

In addition to the family group where the outbreak took place, there were two additional 2-year-old maned wolves housed at a different area, approximately 1 Km away. These animals were clinically healthy during the outbreak, but were tested as a control population.

Bloodwork (Complete blood count and chemistry panel) were performed in all animals (but the pup that suddenly died), along with a coagulation panel, haemoparasites, fecal parasitology, distemper serology and Distemper PCR. A seroneutralization tests was performed in 6 animals after the outbreak with stored samples.

All five pups had been vaccinated with a single dosage, 9 months prior to the outbreak (July 2012), and both adults had their annual booster 5 months prior to the outbreak (November 2012). The vaccine used in all cases were single doses of 1 ml of Recombitek C6 vaccine, (Merial LLC, imported and distributed by Sanofi Pasteur S.A. Andres Bello 2711, Las Condes, Santiago, 832000, Chile) subcutaneously (SC) in the interscapular space. All the animals were in apparent good health status and had showed adequate body weight and normal appetite prior the outbreak. It is important to mention that the vaccine used is the only CDV recombinant vaccine available in South America.

A complete necropsy was performed soon after the death of 5 of the 6 animals. In all cases the most relevant macroscopic findings were moderate pulmonary edema and mild, multifocal intestinal erosions. Due to financial constraints tissue samples from only 2 pups/individuals were collected for histologic evaluation. A full set of tissues from 2 pups were submitted in 10% buffered formalin for histologic evaluation.

figure1

Immunofluorescence assay (IFA)

Serum from 6 animals was tested for CDV IgG and IgM using MegaScreen®. A fluorescent intensity [?]1:40 was determined to be positive (Megacor, 2006).

Seroneutralization assay

Serum from 6 animals was tested for CDV Ab. Serum was diluted and challenged to a fix concentration of a native viral strain of CDV, previously isolated at the Virology Laboratory of the Agricultural and Livestock Service (SAG) of Chile. The cellular substrate for the test was base in MDCK cells, American Type Collection, from renal cortex. After 72 hrs. of incubation on an atmosphere of 5% CO2, 37degC and 95% humidity, the samples were titled. Titers of [?]1:16 of neutralizing antibodies were consider positive .

Nucleic acid extraction and PCR assays (qPCR and RT-PCR)

qPCR: Viral RNA was extracted using the TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using the Lig RNAse Inhibitor Solution and Bio Transcriptase Solution, part of VetqPCR-realtime CDV Real Time Kit, according to manufacturer's instructions (Bioingentech Ltd. Chile).

RT-PCR: Viral RNA extraction was performed using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase kit plus oligonucleotide primers CDV1 (forward) and CDV2 (reverse), according to manufacturer's instructions (Invitrogen, Carlsbad, CA, USA).

Every fragment RT-PCR positive were then sent to a local sequencing center (Genytec Ltda.) for sequencing in triplicate. The resulting nucleotide sequence were aligned using the ClustalW program, and then compared with all sequences deposited in GenBank using the BLAST program. Pairwise and multiple sequence alignments at the nucleotide and amino acid levels and sequence similarities were calculated using the MEGA v6 software.

RESULTS (Table 1)

Histopathology

The histological evaluation in both cases showed severe diffuse (bronchointerstitial) lymphocytic and histiocytic pneumonia, with the presence of intracytoplasmic eosinophilic inclusion bodies. Similar inclusion bodies were found in kidney, digestive epithelium, trachea, liver, lymph nodes and urinary bladder. Other minor findings include diffuse congestion of the liver, atrophy and moderate to severe diffuse lymphoid depopulation on lymph nodes and spleen. In one of the 2 animals a moderate focal ulcerative gastritis was found. The remaining samples did not show pathological changes.

Antibody detection assays

The six individuals from the outbreak that were tested with the IFA yielded positive to the presence of IgM and IgG, and the two animals from the control population tested negative for IgM and IgG.

Samples from those six animals that yield positive to IFA were then tested with SN. Four out of those six animals had titers of [?]1:16, with the remaining two animals showing titers below 1:16, our predetermined threshold ; the second population was not challenge with the seroneutralization test.

Virus detection and characterization

Positive results were also obtained via qPCR in the 6 samples from the outbreak population. From the positive, four samples were run thru a RT-PCR for the detection of the gen H of CDV. Two samples were positive for the detection of the gen H. Thru nucleotide alignment two consensus sequences were obtained: "Chile/lobo crin/1" (4517) and "Chile/lobo crin/2" (7490). The BLAST program was then used to identify the origin of these amplified DNA fragments.

table1

DISCUSSION

Infectious diseases are an important threat for the health and conservation of free ranging wildlife and under human care; in the latter scenario it can even put in risk the success and the viability of breading programs. The canine distemper virus (CDV) has been identified as one of the most significant diseases for wild carnivores in zoos , making the implementation of vaccination protocols strongly recommended . This manuscript describes the first report of this naturally acquired disease in maned wolves, that culminated with the death of 6 vaccinated animals. As such this represents a new challenge in the CDV vaccination protocols for wild canid species.

The pathological, immunohistochemical and molecular findings observed in maned wolves are consistent with those previously described in several species infected with CDV . The phylogenetic analyses of the nucleotide sequence of H gene of the CDV genome identified in the two positive samples suggest a close relation with the lineage Europe 1, commonly found South America and Chile (unpublished).

CDV has been described as one of the most important infectious diseases affecting the maned wolf in captivity, however this conclusion has been solely based in serological studies or clinical sings , without

having a complete correlation of clinical, pathological and molecular evidence in every report. Without this triad is challenging to rule in clinical causality of a pathogen over a host .

The current recommendation by Infectious Diseases Manual of using a recombinant canarypox-vectored is based on the evidence that commercially available canine MLV can cause reversion of vaccine strains to virulent virus and vaccine-induced CDV in non-domestic carnivores. Inactivated CDV whole-virus vaccines do not produce sufficient immunity to prevent infection after virus challenge . The six maned wolves that ultimately died on this outbreak were vaccinated with one dosage of Recombitek within the previous year. Despite this inmunization with the recommended single dosage, six out of seven maned wolves died within 3 weeks since the beginning of the outbreak.

Although this is the first report of recombinant CDV vaccine failure to protect a wild canid species against the clinical infection, it has been previously documented in snow leopard (*Panthera uncia*) despite prior vaccination with the monovalent canine rCDV vaccine 3 months prior .

A recently published paper supports our findings by showing that a single dosage protocol, of Recombitek CDV vaccine, did not elicit a measurable humoral immune response and produced low nonprotective titers in maned wolves as evidenced by the fatal outcome

To the best of our knowledge, this is the first report of the clinical presentation of CDV in a canine species previously inmmunized with a recombinant vaccine. The effectiveness of protection against CDV with a single dose of this type of vaccine (canarypox vectored) has been recently challenged by different studies where the humoral and cellular immune response has been measured in red fox (*Vulpes Vulpes*) and giant panda (*Ailuropoda melanoleuca*) respectively, and in both studies the expected effect was not observed. Facing this new body of evidence it's clear the need for new studies of cellular and humoral immune response in this and other species of wild carnivores, taking in consideration that as wild species it's not ethically viable to perform challenge studies.

The present study confirms the lethal nature of CDV in maned wolf and reinforces the necessity for new protocols of preventive medicine that includes the vaccination for this species in captivity. It is imperative the development of new studies on wild populations to understand the role of this pathogen in the maned wolf conservation and population dynamics. Finally, the protection failure of the vaccine against CDV shown in this report makes necessary new studies that could present new protocols that improve the effectiveness of its use in this and other species, perhaps contemplating additional periodical boost dosages.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABLE STATEMENT

Derived data supporting the findings of this study are available from the corresponding author upon request.

Hosted file

`Table 1.pdf` available at https://authorea.com/users/373880/articles/491492-canine-distemper-outbreak-by-natural-infection-in-a-group-of-vaccinated-maned-wolves-in-captivity