Canine Distemper Virus (CDV) in Grey Wolf (Canis lupus) in Fars Province of IRAN

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Summary

Emerging and re-emerging viral diseases shared between wildlife and domestic animals are continually spreading to new geographic locations, influenced by human activities and environmental change. Canine distemper virus (CDV) is probably one of the best examples of a disease that has been proved to be capable of compromising the conservation of several wild carnivore species. In this article, we describe a case report of CDV in a grey wolf (*Canis lupus*) in Iran. A grey wolf was found in Fars Province close to Bamou national park. Clinical signs were characterized by neurologic signs, muscle twitching, hyperkeratosis of the footpads and nose and keratoconjunctivitis sicca. After the death of the animal, samples were taken from different organs and sent to collaborator laboratory of Fars Provincial Office of Veterinary Organization. RT-PCR assays confirmed canine distemper virus in the grey wolf. This is the first documented report of canine distemper virus in wild species from Fars Province of Iran.

Introduction

Canine distemper virus (CDV) is an enveloped, negative-sense, single-stranded RNA virus belonging to the family Paramyxoviridae that comprises four recognized subfamilies (Avulavirinae, Metaparamyxovirinae, Orthoparamyxovirinae and Rubulavirinae). The subfamily Orthoparamyxovirinae includes eight genera which contain Morbillivirus, Respirovirus and Henipavirus genera that collectively show a wide host range of vertebrates from fish to mammals, with transmission primarily occurring via the respiratory tract (Afonso *et al.* 2016). Viruses of the genus morbillivirus have caused devastating outbreaks in humans and animals (Oleaga et al.2021). Morbillivirus is a causative agent of canine distemper virus, which is related antigenically to measles (in humans), rinderpest (in cattle and buffalo), and peste des petits ruminants (in sheep and goats) (Sykes 2014).

Canine distemper virus (CDV) was first described by H. Carré in 1905 and with passing years, the host range of this disease appears to have widened as interspecies transmissions and viral recombination events have occurred, leading to epizootics with high mortality. The host range of CDV is wide; species in all families in the order Carnivora (Canidae fox Vulpes spp., grey wolf Canis lupus, raccoon dog Nyctereutes procyonoides and …), Mustelidae (ferret, mink, skunk, wolverine, marten, badger, otter), most Procyonidae (raccoon, coatirnundi), Hyaenidae, some Viveridae (binturong, palm civet), Ailuridae (red panda), Ursidae (bear), and large Felidae are susceptible to CDV. Reports of new hosts continue to appear (Williams 2001). Despite the wide host range, dogs are the principal reservoir host for CDV and they likely act as reservoirs of infection for wildlife (Greene 2014).

Transmission mainly occurs by direct animal-to-animal contact or by exposure to respiratory,

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oral, ocular fluids and exudates, while other body excretions and secretions (e.g., urine and faeces) could also contribute to viral shedding during the acute phase of infection. Transplacental transmission has been documented in dogs (Alfano *et al.* 2022). CDV main targets are mucous membranes and lymphoid tissue.

This virus enters in body through upper respiratory system and there in lymph nodes it primarily replicates and causes immunosuppression then diffuse to epithelium and CNS at around 10 days after transmission.

Mostly cause lymphopenia in primary step. When it reaches to lower respiratory tract, gastrointestinal tract and CNS can lead to form of lesions on these organs and causes appearance of systemic, cutaneous and nervous signs (Tariq *et al.* 2013). Signs of CDV vary depending on species, viral strain, environmental conditions, host age and immune status. In canids and mink, and probably other species, juveniles appear to be most susceptible. Incubation period can be ranges from about 1 week to 1 month or more (Williams 2001).

The most definitive procedure for diagnosis of this virus is the amplification of its different genes through molecular methods. Since the nucleoprotein (NP) gene is the most conserved gene of CDV; amplification of the NP gene is the best way to identify various strains of CDV in biological specimens (Namroodi *et al.* 2015).

Situated in southern Iran, the Fars Province is the fourth largest province of the country.

Besides its idiosyncratic zoogeographic position, a broad range of geographic and physiographic situations, accompanied by climatologically diverse environments in this province, have provided enormous diversity.

According to the checklist and conservation status of mammals of Fars Province which published in 2019, the confirmed mammals in this province, comprise of 72 species in 53 genera, 28 families and seven orders. 25% of total confirmed mammal in Fars province that means 18 species are belong to order of Carnivora (Zarei *et al.* 2019).

In the present paper, described a case of infected to Canine Distemper Virus in a grey wolf (*Canis lupus*) that had found by rangers in Fars Province of Iran, near the Bamou national park.

Case History

On February 9, 2021, in one of the towns at the entrance of Shiraz city and approximately 10 km distance from the Bamou national park, an immature grey wolf (*Canis lupus*) with lethargy and general weakness was observed by local people (Fig 1).

After people informed the Fars environmental organization, rangers transferred it to wildlife rehabilitation center in Bamou National Park.

Figure 1. *The location where the Infected Wolf was found (Google Map. 2021)*

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Grey wolf was male and based on the principles of age detection from teeth examination he was about 8-month-old. Clinical examination revealed Severe dehydration, neurologic signs of depression and ataxia, hyperkeratosis of the footpads and nose, mucopurulent nasal and ocular discharge and muscle twitching in left leg and flank. During physical examination other clinical sign such as fever (40.1 °C) abnormal respiratory sounds and keratoconjunctivitis sicca were observed (Fig 2).

Figure 2. *Immature Grey wolf infected with Canine Distemper Virus*

Since all the mentioned signs were part of typical symptoms of CDV in dogs and wild canids, the initial diagnosis was based on canine distemper virus. To confirm CDV, Rapid immunochromatographic antigen test kits (BIONOTE Rapid Test, Canine Distemper Virus Antigen Test, Cat, no: RG1103DD, Korea, relative sensitivity: 98.8%, relative specificity: 97.7%) was applied according to the manufacturer's instructions (Fig 3).

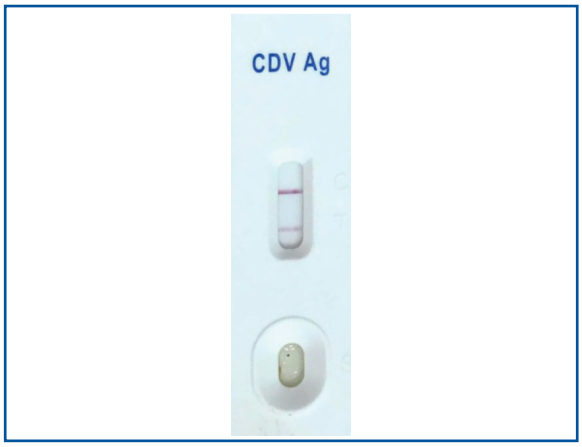

Figure 3. *Positive rapid immunochromatographic antigen test of grey wolf*

The results were checked up to 5–10 min later and were positive. Complete blood count and biochemical analysis were performed and presented in Table I, Table II respectively.

Table I. *Complete Blood Count Findings in grey wolf infected with Canine Distemper Virus*

Variable	Unit	Grey wolf with CDV	Reference Range
Hemoglobin	g/dL	10.9	13.3-19.7
PCV	%	28.1	39.7-56.5
MCV	fL	56.96	63.4-78
MCH	g/dL	21.72	22-27
MCHC	/µL	32.65	30.5-37.3
Neutrophils	/µL	5933	4353-9719
Lymphocytes	/µL	695	737-2403
Eosinophilis	/µL	255	0-661
Monocytes	/µL	119	0-468
Basophils	/µL	36	0-55
Platelets	$^{x}10^{3}$/µL	157	166-336

Table II. *Serum Biochemistry Findings in grey wolf infected with Canine Distemper Virus*

Variable	Unit	Grey wolf with CDV	Reference Range
Glucose	mg/dL	103	91-157
Albumin	g/dL	2.8	3.0-3.8
Globulin	g/dL	1.6	2.1-3.3
Calcium	mg/dL	11.4	9.2-10.8
Phosphorus	mg/dL	5.2	2.2-5.4
Total bilirubin	mg/dL	0.4	0-0.2
Cholesterol	mg/dL	123	118-248
Creatine	mg/dL	1.5	0.7-1.7
Urea nitrogen	mg/dL	25	14-32
ALT	U/L	30	19-110
ALP	U/L	48	8-112

Unfortunately, less than 24 hours after admission grey wolf died. Despite the positive rapid distemper test, tissue samples from various organs (lung, urinary bladder, kidney, brain, spleen, lymph nodes, pancreas, intestine, stomach and skin) were taken at systemic necropsies, fixed in 70% ethanol and samples were sent to collaborator laboratory of Fars Provincial Office of Veterinary Organization to analyze by RT-PCR for the detection of CDV. RNA extraction kit (Bioneer Co., Daejon, Korea) was used for extraction of RNA from all specimens. The extracted RNA was reverse-transcribed into cDNA using two-step reverse transcription polymerase chain reaction (RT-PCR) kit (Vivantis,

Selangor, Malaysia) following the manufacturer's recommended reaction conditions.

Different kinds of samples (kidney, lung and kidney) were positive for RT-PCR assays.

Discussion

Increased global urbanization potentially increases conflict among wild and domestic animals and transmission of infectious diseases. Wild carnivores are special concern as they are phylogenetically similar to common domestic species (Megid *et al.* 2009).

Domestic dogs (including feral populations) are considered to be the reservoir species for canine distemper virus in most, if not all, locations (Allen *et al.* 2016). In addition to wolves, canine distemper virus originated from dogs has been reported in lions (*Panthera leo*) in Serengeti (Roelke-Parker et al.1996), Baikal seals (*Phoca sibirica*) in Siberia (Mamaev *et al.* 1995), Caspian seals (*Phoca capsica*) in the Caspian Sea (Kuiken *et al.* 2006) and African wild dogs (*Lycaon pictus*) in Tanzania (Van de Bildt *et al.* 2002). In Europe, the virus has been linked to mortality of Eurasian badger (*Meles meles*), stone marten (*Marten foina*) or red fox (*Vulpes vulpes*) in Denmark, Switzerland and Italy (Oleaga *et al.* 2021).

Since CDV in excised tissues or secretions survives for around an hour at 37 ° C and for 3 hours at 20°C (room temperature) and in warm climates, it does not persist in kennels after infected dogs have been removed and on the other hand, storage and survival time of CDV are longer at colder temperatures and at near-freezing (0 ° C to 4 ° C) it survives in the environment for weeks (Greene 2012). In result, it is not surprisingly that Disease prevalence exhibits temporal fluctuations and increases during the cold season. (Alfano *et al.* 2022). In another study was shown that 77.7% of infected dogs were referred in the cold seasons of the year. (Sarchahi *et al.* 2022). Moreover, virus can be excreted up to 60 to 90 days after infection. Although shorter periods of shedding are more typical and most recovered dogs clear the virus completely, some may harbor virus in their CNS (Greene 2012). The dogs' origin can be a risk factor because 50% of suspected dogs were referred from shelters or were stray dogs (Sarchahi *et al.* 2022). In another study on different impacts of free ranging dogs on wildlife species in Tehran, Iran, has been shown Almost all free-roaming dogs have been tested positive for canine distemper virus (Memarian *et al.* 2022).

Not only the genetic determinants in viral RNA are involved in CDV occurrence in wild species, but also various factors are effective in establishing contact between the animal reservoir of the disease and the susceptible wildlife population and should be studied, in particular everywhere the anthropogenic pressure is more continuous, must be considered. The size and spatial structure of different population (packs home range, relations between packs, movement patterns), any potential overlap with domestic species and human activities, the co-existence of multiple hosts and pathogens require a multidisciplinary approach to study the dynamic of infections (Di Francesco *et al.* 2019).

In conclusion, the occurrence of canine distemper virus has been reported both in domestic dog and variety species of the wild carnivores around the world. On the other hand, the domestic animals/ wildlife interface is a significant universal problem and solving this involves wide studies on different aspect of interplays between wild and domestic animals. The transmission of infectious diseases like canine distemper virus due to frequent contact between domestic and wild animals is becoming an issue of major interest. Implementation of management programs for reducing and eliminating the direct and indirect contact of domestic animals with wildlife species such as principled management of human waste, managing the stray dog population as a disease reservoir to prevent maintaining the disease between unowned dogs' populations and also disease transmission to wildlife species, is probably the most effective and important way to prevent the transmission of infectious diseases. In addition, more studies are needed to understand more precisely the epidemiology of distemper virus in wild species.

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