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Assessing Seroprevalence of Major Viral Infections in Big Cats

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ABSTRACT

Background: Big cats are widespread across the globe and India is also the host of this group of animals. Canine distemper virus (CDV), a *morbillivirus* that causes one of the most contagious and lethal viral diseases known in canids, has an expanding host range, including wild animals and its cases have also been observed in big cats. In such a situation, serological analysis of the biological samples may give significant information on the presence of diseases in a particular area. Sampling in big cats is often challenging hence in the present study, serum samples of big cats available in the laboratory were subjected to detection of antibodies against major viral infections including canine distemper.

Methods: Serum samples collected from Royal Bengal tigers and Indian leopards irrespective of age and sex of the animals were subjected to the serological analysis of canine distemper virus (CDV), canine *parvovirus* (CPV) and canine *adenovirus* (CAV) infections using commercially available dot-ELISA kit.

Result: Seroprevalence of antibodies against CDV and CPV infections in tigers and leopards was observed which indicated an exposure of these species to canine distemper and canine *parvovirus* infection in the ecosystem. Results also revealed constant seroprevalence of CPV infection throughout the study period whereas CDV seroprevalence was observed variable during this period. These findings also suggested significant presence of the viral pathogens in big cats.

Key words: Big cats, Canine distemper, Canine *parvovirus* infection, Seroprevalence.

INTRODUCTION

Canine distemper is a naturally and worldwide prevalent, highly contagious systemic viral infection of dogs as well as various other carnivores. It has emerged as a significant disease of wildlife and now recognized as a global multi-host pathogen, infecting a wide range of carnivore species (Deem *et al*., 2000). Reports of CDV outbreaks in big cats such as tigers (*Panthera tigris*), lions (*Panthera leo*) and leopards (*Panthera pardus*) have challenged the belief that the felidae group of animals is resistant to CDV infection (Gilbert *et al*., 2015). However, according to Martinez-Gutierrez and Ruiz-Saenz (2016), only 49% of the reported cases of CDV infections in the big cats present clinical signs of the disease. Some animals can acquire the infection, suffer a subclinical and/or asymptomatic disease and remain seropositive for years. Similarly, canine *parvovirus* infection may develop as a clinical or sub-clinical infection in wild cats and severity of the infection may depend on various factors including maternal derived antibodies, age, immune status of the individual and underlying diseases (Dissanayake *et al.,* 2016). In such a situation, it is speculated that these seropositive animals will have the potential to pose a risk of infection to other seronegative animals (Ramanathan *et al*., 2007). With a limited population density of such endangered species, transmission of the pathogen could be fairly rapid. Therefore, it is important to develop a disease surveillance strategy by using biological samples of tigers and leopards to test for the presence of viral infections (Sidhu *et al*., 2019; Nayak *et al*., 2020).

"Big cat" is the common name given to the members of the genus *Panthera, Acinonyx* as well as few ones from the genus *Felis*. These include tiger, leopard, lion, cheetah, cougar, jaguar *etc.* (Davis *et al*., 2010). In India, Royal Bengal tigers and Indian leopards are spread wide across the country and form the major part of apex predators in the forest ecosystem. Focal cases of canine distemper and *parvovirus* infection have been reported in Indian big cats (Nayak *et al.,* 2020) but related studies in India are limited. Ramanathan *et al.* (2007) observed seroprevalence of CDV (87.5%) and CPV (100%) in captive Asiatic lions whereas studies on one of the wild carnivores - Indian foxes revealed seroprevalence of CDV (18%), CPV (48%) and CAV (52%) infections (Belsare *et al*., 2014). This raises a concern regarding prevalence of major viral infections in big cats and associated species in Indian subcontinent. Biological sample collection from big cats is often challenging on field hence assessing seroprevalence of viral infection in already available samples may provide

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significant information. Considering these facts, the present study was conducted to assess the seroprevalence of important viral infections such as canine distemper, canine *parvovirus* infection as well as canine *adenovirus* infection in big cats.

MATERIALS AND METHODS

The present study was conducted for the period of year 2018 to year 2021 at School of Wildlife Forensic and Health, N.D.V.S.U., Jabalpur (Madhya Pradesh). Serum samples from major species of big cats; Royal Bengal tiger (*Panthera tigris tigris*) and Indian leopard (*Panthera pardus fusca*) irrespective of age and sex collected during the veterinary interventions at various tiger reserves and zoological parks of Madhya Pradesh were included in the present study.

Total 20 serum samples collected from both the big cats from captivity as well as from free-range were analyzed for the presence of IgG antibodies against CDV, CPV and CAV infections using commercially available dot-ELISA kits (BioGal's Immunocomb kit, Bio Galed, kibbutz Galed, Israel, 192400). These antibody test kits were based on solid phase immunoassay technology and antibody concentrations were measured based on the intensity of the colour development which corresponded directly to the antibody level in the test specimens. Results were scored based on the positive reference spot using the calibrated CombScale and documented by titre of virus neutralization test (V.N.T) for CDV and CAV infections whereas for CPV infection, it was documented by the titre of haemagglutination inhibition (H.I.) test.

The results were in the form of 'S' units from S0 to S6, where S0 indicated no antibodies were detected; S1 and S2 suggested that antibodies were detected in low titers and S3 to S6 suggested that antibodies were present with minimum 1:32 titre by virus neutralization test for CDV, 1:80 titre by hemagglutination inhibition test for CPV and 1:16 titre of virus neutralization for CAV. The results were further categorized as seronegative (S0) and seropositive (S1 and above; specifically, S1 and S2 = 'low titers', S3 = 'medium titers' and S4+ = 'high titers') as earlier done by other researchers (Belsare *et al*., 2014; Chaudhary *et al*., 2018; Nayak *et al*., 2020).

RESULTS AND DISCUSSION

Total 20 serum samples of big cats were tested for the presence of IgG antibodies against canine distemper virus (CDV), canine *parvovirus* (CPV) and canine *adenovirus* (CAV) infections, out of which, 12 serum samples (60%) were found seropositive for IgG antibodies against CDV, 19 serum samples (95%) were found seropositive for IgG antibodies against CPV and 1 serum sample (5%) was found seropositive for IgG antibodies against CAV infection. Mixed response for IgG antibodies was observed against CDV+CPV in 11 serum samples (55%) and against CDV+CAV as well as CDV+CPV+CAV in 1 serum sample (5%) (Table 1).

Presence of IgG antibodies against CPV infection in the serum samples of captive big cats was due to the practice of routine vaccination against feline panleukopaenia disease in zoological parks of Madhya Pradesh whereas in free-ranging/rescued animals it might be due to post-exposure immunity against this viral pathogen. Although, presence of IgG antibodies in both captive as well as free-ranging/ rescued big cats against CDV was the indication of past exposure to these viral diseases. In a similar study conducted by Nayak *et al*. (2020), serum samples of feral dogs, feral cats and big cats from Panna tiger reserve, Madhya Pradesh (India) were analyzed to assess the seroprevalence for CPV, CDV and CAV infections which revealed the presence of antibodies against CPV and CDV in all the three species however antibodies against CAV infection were not observed in domestic cats and tigers. Their study also indicated exposure of wild population to *parvo* and distemper virus infections.

Seroprevalence of viral infections in Royal Bengal tigers

Out of total 20 samples, 10 serum samples were collected from Royal Bengal tigers in which 7 samples (70%) were found seropositive for IgG antibodies against CDV infection, 9 samples (90%) were found seropositive for IgG antibodies against CPV infection whereas none of the samples were found seropositive for IgG antibodies against CAV infection. Mixed response for IgG antibodies against CDV+CPV infection was observed in 6 samples (60%) (Table 1). Antibody titre against these infections was also observed using CombScale provided with the kit which revealed high to low titre of antibodies in both captive as well as free-ranging animals (Table 2, Fig 1).

Seroprevalence of viral infections in Indian leopards

In the present study, 10 serum samples were collected from Indian leopards. Out of these, 5 serum samples (50%) were found seropositive for IgG antibodies against CDV infection, all the serum samples (100%) were found seropositive for IgG antibodies against CPV infection and 1 serum sample (10%) was found seropositive for IgG antibodies against CAV infection. Mixed response for IgG antibodies against CDV+CPV was observed in 5 serum samples (50%) and against CDV+CAV as well as CDV+CPV+CAV in 1 serum sample (10%) (Table 1). Antibody titre against these infections was also observed using CombScale which revealed high to low titre of antibodies in both captive as well as free-ranging animals (Table 3, Fig 2).

The longevity of circulating antibodies in the big cats exposed and recovered from CDV and CPV infection is not known but the immunity developed in dogs that survive an infection with virulent CDV and CPV is generally believed to be long-lived (Coyne *et al*., 2001; Greene and Appel, 2006).

Antibody titre against CDV infection was low to high in both captive and free-ranging/rescued animals. However, there was no history of vaccination against CDV in all these tested animals which indicated natural exposure and recovery from CDV infection. These samples were collected during the veterinary interventions such as immobilization for radiocollaring/rescue operations or for routine health

Table 1: Seropositivity against canine distemper virus (CDV), canine *parvovirus* (CPV), canine *adenovirus* (CAV) infections in big cats.

Particular	Royal Bengal Tiger		Indian Leopard		Total number of positive cases	Per cent sero-positive
	Free-ranging/ rescued	Captive	Free-ranging/ rescued	Captive		
Seropositive for CDV	2/2	5/8	2/4	3/6	12/20	60.00
Seropositive for CPV	1/2	8/8	4/4	6/6	19/20	95.00
Seropositive for CAV	0/2	0/8	1/4	0/6	1/20	5.00
Mixed response to CDV+CPV	5/2	1/8	2/4	3/6	11/20	55.00
Mixed response to CDV+CAV	0/2	0/8	1/4	0/6	1/20	5.00
Mixed response to CDV+CPV+CAV	0/2	0/8	1/4	0/6	1/20	5.00

Table 2: Sero-prevalence of antibodies against canine distemper virus (CDV), canine *parvovirus* (CPV) and canine *adenovirus* (CAV) infections in Royal Bengal tigers.

Particular		Royal Bengal Tiger		Total
		Free-ranging/rescued	Captive	
Serum samples tested		4	6	10
Seropositive for CDV	High titre of antibodies (>1:32 V.N.T.)	0	2	2
	Medium titre of antibodies (=1:32 V.N.T.)	0	1	1
	Low titre of antibodies (<1:32 V.N.T.)	2	2	4
Seropositive for CPV	High titre of antibodies (>1:80 titers H.I.)	3	6	9
	Medium titre of antibodies (=1:80 titers H.I.)	0	0	0
	Low titre of antibodies (<1:80 titers H.I.)	0	0	0

Table 3: Sero-prevalence of antibodies against canine distemper virus (CDV), canine parvovirus (CPV) and canine adenovirus (CAV) infections in Indian leopards.

Particular		Indian Leopard		Total
		Free-ranging/rescued	Captive	
Serum samples tested		2	8	10
Seropositive for CDV	High titre of antibodies (>1: 32 V.N.T.)	1	1	2
	Medium titre of antibodies (=1: 32 V.N.T.)	0	0	0
	Low titre of antibodies (<1: 32 V.N.T.)	1	2	3
Seropositive for CPV	High titre of antibodies (>1: 80 titers H.I.)	2	8	10
	Medium titre of antibodies (=1: 80 titers H.I.)	0	0	0
	Low titre of antibodies (<1: 80 titers H.I.)	0	0	0
Seropositive for CAV	High titre of antibodies (>1: 16 titer of V.N.T.)	1	0	1
	Medium titre of antibodies (=1: 16 titer of V.N.T.)	0	0	0
	Low titre of antibodies (<1: 16 titer of V.N.T.)	0	0	0

investigations under captivity, hence, history provided with the samples showed none of the animals, on the day of sample collection, had clinical signs related to CDV infection. In the present study, results indicated that the big cats both in the wild as well as under captivity had been exposed to CDV at certain point in their lifetime.

Regarding CPV infection, high titre of antibodies were observed in all the tested samples of captive as well as free-ranging/rescued animals except one free-ranging tiger which was rescued nearby human habitation. In captive animals, high titre of protective antibodies was the result of routine vaccination practice against *parvovirus* infection (Feline panleukopaenia) however high titre of IgG antibodies against CPV in the free-ranging/rescued animals indicated that *parvovirus* is widely prevalent amongst big cats in natural habitat and immunity due to natural exposure is very high to protect these animals from virulent infections.

Belsare *et al.* (2014) also used the same method to observe prevalence of antibodies to three viral pathogens, canine *parvovirus* (CPV), canine distemper virus (CDV) and canine *adenovirus* (CAV), in Indian foxes (*Vulpes bengalensis*) at the Great Indian Bustard Wildlife Sanctuary (Maharashtra) and observed exposure to one or more of the three pathogens in foxes. Results of the present study indicated sero-prevalence of antibodies against CPV and CDV infections in tigers and leopards which were also in agreement with the findings of Ramanathan *et al.* (2007), Santos *et al.* (2009) and Beineke *et al.* (2015) in wild felids.

Detection of IgG antibodies against CAV infection in only one sample of Indian leopard was intriguing. CAV-1 is

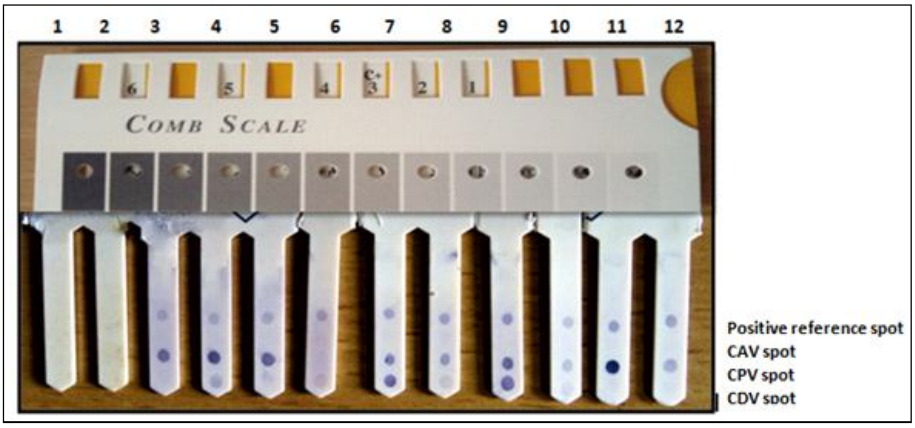

Fig 1: Evaluation of dot-ELISA assay for detection of IgG antibodies against CDV, CPV and CAV infections in serum samples of Royal Bengal tigers.
Comb 1: Non-specific serum sample. Comb 2: PBS solution (1X). Comb 3-12: Test serum samples of Royal Bengal tigers.

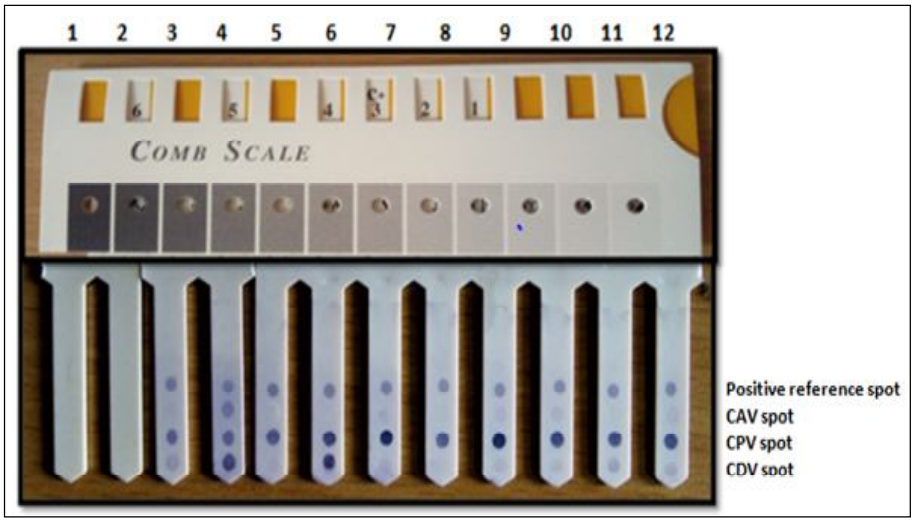

Fig 2: Evaluation of dot-ELISA assay for detection of IgG antibodies against CDV, CPV and CAV infections in serum samples of Indian leopards.
Comb 1: Non-specific serum sample. Comb 2: PBS solution (1X). Comb 3-12: Test serum samples of Indian leopards.

the only adenovirus known to infect carnivores which is the causative agent of infectious canine hepatitis and has been observed commonly in domestic dogs (Chethan *et al.*, 2021). There is no report of this infection in felidae, however, there are some research work which reported the cases of inclusion-body-hepatitis-like condition in felids (Benko, 2015). Krishna and Gupta (1993) also reported a solitary case of inclusion body hepatitis of possible viral aetiology in a leopard at Renuka Safari (Himachal Pradesh, India) died of jaundice and its tissue samples also showed the presence of intranuclear basophilic inclusion bodies in the hepatocytes. Additionally, Shrivastav *et al.* (2003) reported a case of inclusion body hepatitis in a free-ranging toddy cat (*Paradoxurus hermaphrodites*) by observing intranuclear basophilic inclusion bodies in hepatocytes. Although this disease has not been recognized elsewhere and its aetiology has not been established. However results of the present study in which anti-CAV antibodies were observed in a leopard might indicate the exposure of this individual to inclusion body hepatitis virus or cross-reactivity to related adenoviruses like human adenoviruses as reported in domestic cats by Benko (2015).

Additionally, out of total 20 serum samples of big cats, 3 samples were collected in the year 2018, 7 samples were collected in the year 2019 and 10 samples were collected in the year 2021 (Table 4). Sero-prevalence of CPV was observed constant throughout the study period however; CDV sero-prevalence was numerically variable for the mentioned timeline. Our results regarding changing dynamics of CDV in big cats were in agreement with the findings of Viana *et al.* (2015) who analyzed CDV serology records of lions and domestic dogs to characterize the annual probability of CDV infection and observed distinct changes in the pattern of the estimated annual probability over the time. However in the present study, due to field constraints, we could not analyze antibody titre in the same

Table 4: Year-wise seropositivity (%) against canine distemper virus (CDV), canine parvovirus (CPV), canine adenovirus (CAV) infections in big cats.

Particular	Year 2018	Year 2019	Year 2021
Seropositive for CDV (Per cent positive)	66.66 (2/3)	100.00 (7/7)	30.00 (3/10)
Seropositive for CPV (Per cent positive)	100.00 (3/3)	85.71(6/7)	100 (10/10)
Seropositive for CAV (Per cent positive)	0.00 (0/3)	14.28 (1/7)	0.00 (0/10)

individual repeatedly for consecutive years hence our results could not speculate the exact timing of CDV emergence in these individuals. However, sero-prevalence of these diseases observed over the years suggested constant presence of pathogens in the ecosystem.

Seroprevalence studies give an insight to the prevalence of pathogens in an area (Jain *et al*., 2019; Farooq *et al*., 2021). However, it is significantly important to understand that seropositivity means detection of an antibody level for a specific infectious pathogen in an individual (WHO, 2013) and it does not necessarily indicate active clinical infection. It also indicates exposure and recovery of that particular animal from the infection. High seroprevalence may indicate high transmission rates of the pathogens and/or high post exposure survival rates as observed in CPV seroprevalence (95%) in the present study. Similarly, low seroprevalence may indicate low transmission rates of the pathogen or high mortality induced by the pathogen as observed in CDV seroprevalence (60%). Big cats are mostly solitary animals; hence low transmission rates can justify low seroprevalence of CDV antibodies in big cats in the present study.

In such a situation, commercially available dot-ELISA kits were used as facilities for serological analysis because traditional methodologies such as virus neutralization test (V.N.T.) is not properly established in the country. These commercial kits are used widely in veterinary medicine for diagnosis of the infectious diseases and have been recommended as a standard tool for population-based serological studies (Wright *et al*., 1993). The dot-ELISA kits have also been validated for their use in the serological analysis by earlier researchers in the serum samples of wild carnivores (Belsare *et al*., 2014; Nayak *et al*., 2020). We also tested these kits for non-specific serum sample from a herbivore species as well as by using 1X PBS to rule out the possibilities of false positive results which revealed no colour development on positive reference spot/test spots (Fig 1 and 2). Hence, results of the present study suggested usefulness of commercially available dot-ELISA kits as facilities for serological analysis where traditional methodologies such as virus neutralization test (V.N.T.) or haemaggutination-inhibition (H.I.) have not been established.

CONCLUSION

Seroprevalence of antibodies against CPV and CDV in tigers and leopards confirmed the exposure of these species to the important viral infections in the ecosystem. Results also suggested that CPV in big cats is endemic whereas CDV in big cats might be a risk factor to unexposed individuals. Commercially available dot-ELISA kits used in the present study showed satisfactory results with minimum resources. Although we suggest further comparison of these kits with traditional methods such as V.N.T. for sero-surveillance of major carnivore pathogens in big cats so that it can be utilized widely with minimum resources in wildlife epidemiology laboratories to cover a large population of wild animals across the country.

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