

**SHORT COMMUNICATIONS**

**Serosurvey for selected viral pathogens among sympatric species of the African large predator guild in northern Botswana**

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## Abstract

The recent increase in the creation of trans-boundary protected areas and wildlife corridors between them lend importance to information on pathogen prevalence and transmission among wildlife species that will become connected. One such initiative is the Kavango Zambezi Transfrontier Conservation Area (KAZA/TFCA) of which Botswana's Okavango Delta constitute a major contribution in terms of wildlife and ecosystems. Between 2008 and 2011, we collected serum samples from 14 lions (*Panthera leo*), four leopards (*P. pardus*), 19 spotted hyenas (*Crocuta crocuta*), and six cheetahs (*Acinonyx jubatus*) in the Okavango. Samples were tested for antibodies against: canine distemper virus (CDV), feline panleukopenia virus, enteric coronavirus, feline calicivirus, feline herpesvirus (FHV-1), and feline immunodeficiency virus (FIV). Evidence of exposure to all of these pathogens was found to varying degrees in at least one of the species sampled. High seroprevalence (> 90%) was only found for FHV-1 and FIV in lions. Only hyenas (26%, 5/19) were seropositive against CDV. Apart from one case, all individuals displayed physical conditions consistent with normal health for a minimum of 12 months following sampling. Our results emphasize the need for a comprehensive multi-species approach to disease monitoring and the development of coordinated management strategies for sub-populations likely to be connected in trans-boundary initiatives.

**Key words:** Carnivores, conservation and management, KAZA/TFCA, Okavango Delta, pathogen prevalence, trans-boundary wildlife areas.

In large carnivore conservation, disease ecology has mainly focused on clinical host-pathogen relationships, disease-mediated extinction, and the consequences that human activities and domesticated animals have on the introduction and spread of diseases into wildlife

populations (Woodroffe 1999, Cleaveland et al. 2007, Alexander and McNutt 2010). More recently, studies have focused on cross-species transmission, multi-host pathogens, and infection reservoir dynamics (Lembo et al. 2008, Alexander et al. 2010). Our knowledge remains, however, relatively limited on the ecology of pathogen prevalence and transmission in complex, large trans-boundary ecosystems, where differential ecological and climatic conditions may further confound the epidemiological scenario.

In recent years, the creation of large trans-boundary parks and wildlife corridors between ecosystems has become an integral part of conservation action plans (Silveira et al. 2014). A comprehensive understanding of the health status of sub-populations that will become connected through such initiatives is fundamental for the management of species nationally and internationally. One such initiative is the Kavango Zambezi Trans-Frontier Conservation Area (KAZA/TFCA) in southern Africa. Despite its unique wildlife and ecosystems and the central role that Botswana's Okavango Delta plays within the KAZA/TFCA scenario, relatively little is known about pathogen transmission and prevalence among its large carnivore species.

Our aim is to investigate the seroprevalence of various viral pathogens among four co-occurring large carnivore species: lion (*Panthera leo*), spotted hyena (*Crocuta crocuta*), leopard (*P. pardus*) and cheetah (*Acinonyx jubatus*). Furthermore, we emphasise differences in seroprevalence between individuals that did or did not come into contact with human activities and individuals that did not.

This study was conducted in the Okavango Delta in northern Botswana, between 2008 and 2011, over an area of 2,000 km<sup>2</sup> (Fig. 1). The only permitted human activities were photographic and trophy-hunting tourism. With the exception of Sankuyo village, no settlements were located within the study area (Fig 1). The southern boundary of the study

area was delimited by the Southern Buffalo Fence (SBF). Subsistence pastoralism was common practice on the southern side of the fence (Fig. 1). Most households south of the fence and in Sankuyo had domestic dogs and occasionally cats. Farmers and domestic dogs rarely accompanied free-ranging livestock and contact between dogs and wildlife far from settlements is therefore limited (Alexander and McNutt 2010).

Animals were anesthetized by a qualified veterinarian as part of an ongoing carnivore research. During immobilization, animals were clinically examined and thoroughly checked for symptoms related to viral infections. Samples were collected from a total of 14 lions, 19 hyenas, four leopards, six cheetahs. Blood samples were obtained from the medial saphenous vein and collected in dry tubes (BD Vacutainer®). Samples were centrifuged at 5,000 rpm for 10 min within 6 hours of collection. Serum was collected and stored at  $-18^{\circ}\text{C}$  until serology was performed.

Serum samples were tested for antibodies at the Department of Veterinary Tropical Diseases, University of Pretoria against six pathogens of concern to the species examined in this study: canine distemper virus (CDV; Onderstepoort strain), feline panleukopenia virus (FPV), feline enteric coronavirus (FCoV), feline calicivirus (FCV), feline herpesvirus (FHV-1) and feline immunodeficiency virus (FIV). An enzyme-linked immuno-absorbent assay (ELISA) using a puma-lentivirus-derived synthetic peptide as coating antigen was used as a diagnostic test for infection with FIV (Van Vuuren M et al. 2003). For the three felid species, antibody titers for CDV, FPV, FCoV, FCV, FHV-1 were assessed via standard Indirect Fluorescent Antibody assay (IFA) using in-house prepared IFA slides. Serum samples were tested at a 1:20 screening dilution. The conjugate used was fluorescein-labelled anti-feline IgG antibody diluted in 0.05% Evans blue counter stain. Slides were viewed using a microscope with fluorescence function and examined for cytoplasmic, nuclear, whole cell and inclusion body

fluorescence. For hyenas, CDV analyses were carried out using a serum neutralisation test with the Onderstepoort virus strain. Subjects whose serum samples showed evidence of virus neutralization at dilution levels superior or equal to 1:8 were considered likely exposed to CDV (Appel and Robson 1973). We tested hyenas only for CDV because the other tests used anti feline conjugates.

The majority of the sampled individuals were fitted with GPS radio collars, which enabled constant monitoring of their movements (Cozzi et al. 2013). Where no collar was deployed, the possibility that an animal would have crossed the SBF and moved into pastoral land (Fig. 1 & Tab.1) was estimated based on the long-term knowledge of its movements and the dynamics of the group it belonged to.

All six pathogens tested were present in the study population (Table 1). Cheetahs, leopards and lions tested negative against CDV, while 26% (5/19) of the hyenas tested positive. None of the seropositive hyenas showed, however, obvious signs of disease at the time of capture, nor did any of the individuals that were regularly monitored. FPV and FCoV exposures were detected in only one (17%), and two (33%), respectively, of the six sampled cheetahs. In contrast, antibodies for FCV were found in lions (21%, 3/14) and leopards (75%, 3/4), but not in cheetahs. Only one leopard tested positive for FIV, and the same individual also tested positive for FCV. All 14 lions tested positive for FIV and all but one individual (92%) were positive for FHV-1. All lions were in good condition at the time of capture and during the entire study period.

Our results identified the presence of multi-host pathogens across four species of the African large carnivore guild in the Okavango Delta, Botswana. Although to varying degrees, all

127 pathogens tested for were present within the study population. Nevertheless, individuals were  
128 in healthy condition when sampled and throughout the entire study period.

129 Of possible concern is the detection of CDV positive individuals, only hyenas, within the  
130 ecosystem. The five CDV seropositive hyenas belonged to three different clans whose  
131 collective territories spanned from the SBF well into Moremi Game Reserve (Fig. 1).  
132 Members of these clans have been known to interact on occasions. Hyenas in the study  
133 population regularly cross the SBF (Cozzi et al. 2013) thereby increasing their chances of  
134 exposure to CDV due to interactions with domestic dogs (Alexander and McNutt 2010).  
135 However, recent studies suggested that domestic dogs are not the sole driver of CDV  
136 infection in wildlife populations (Harrison et al. 2004). As yet it is unknown whether CDV is  
137 persistently present in the Okavango ecosystem, whether hyenas act as a potential reservoir  
138 species for the virus, or whether they encounter the virus periodically from other wild and  
139 domestic sources (Harrison et al. 2004). Because all positive samples were collected in 2009  
140 and the fact that two (out of five) positive individuals were approximately 18 months old may  
141 suggest an episodic CDV exposure.

142 We found high pathogen prevalence only for FIV and FHV-1 in lions. High level of  
143 seropositivity of both pathogens have been reported in other free-ranging lion populations, but  
144 negative demographic impacts or manifestations of diseases directly linked to such exposure  
145 are rare or non-existent (Packer et al. 1999, Ramsauer et al. 2007). Epidemiological models  
146 predict that a high contact rate within social groups increases the prevalence of directly  
147 transmitted infections (May and Anderson 1979). The highly cohesive social structure of lions  
148 may explain the observed FIV seroconversion rate of 100%. FIV transmission between lions  
149 and leopards is theoretically possible but recent study demonstrated that most species for  
150 which FIV is endemic harbour monophyletic, genetically distinct species-specific FIV strains,  
151 suggesting that FIV transfer between felid species is infrequent (Troyer et al. 2008). The

individual leopard that tested positive to FIV was also seropositive to FCV. It was found dead seven months after sample collection following a constant decline in condition. All cheetahs were FIV seronegative.

The cheetah positive to parvovirus was the only cheetah that frequently travelled across the SBF (Cozzi et al. 2013) where it may have come into contact with unvaccinated domestic cats and dogs, which can transfer viral antigens to cheetahs (Thalwitzer et al. 2010, Avendaño et al. 2016). Cross-reactions are possible with related viruses that share group-specific antigens including canine parvoviruses that can also infect felids. The solitary nature of the cheetah, however, provides limited opportunity for viral transmission between wild cheetahs during active infection thus reducing contamination within the population (Munson et al. 2004). Both FCoV and FHV-1 are assumed to have minimal impact on the general health of wild felids (Packer et al. 1999, Ramsauer et al. 2007), and are therefore of minor concern.

Due to the limited sample size, we could not test for differences between genders, age, social status and group membership and we therefore suggest that additional samples should be collected in the future. Because tests on feline species were based on antibodies detection with possible cross-reactivity with some other antigens, results should ideally be validated by non species-specific tests. Nevertheless, this study lays the groundwork for future studies. In general, the wide-ranging behaviour of these large carnivore species increases exposure to, and likely transmission rates of pathogens within and between them. The current trend emphasizing large landscape management of wildlife species therefore lends importance to a more holistic, community wide approach to wildlife disease management.

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270 **Table 1:** Serological results conducted on samples collected between 2008 and 2011 in the  
271 Okavango Delta, Botswana. 0 = negative, grey cells = positive (titer values are indicated).

Species	Sex	Age	Sampled	Group	Crossed	CDV	FPV	FEC	FCV	FHV	FIV
Cheetah ( <i>Acinonyx jubatus</i> )	F	Adult	10/2009	NA	N	0	0	0	0	0	0
	F	Adult	11/2010	NA	N	0	0	0	0	0	0
	F	Adult	07/2010	NA	N	0	0	≥ 1:20	0	0	0
	F	Adult	05/2011	NA	Y	0	≥ 1:20	0	0	0	0
	M	Adult	12/2010	NA	N	0	0	0	0	0	0
	M	Adult	07/2010	NA	Possible	0	0	≥ 1:20	0	0	0
Lion ( <i>Panthera leo</i> )	F	Adult	06/2009	Mogoge	Unlikely	0	0	0	0	≥ 1:20	≥ 1:20
	M	Adult	11/2008	Gomoti	Y	0	0	0	≥ 1:20	≥ 1:20	≥ 1:20
	M	Adult	01/2009	Flycamp	Unlikely	0	0	0	0	≥ 1:20	≥ 1:20
	F	Adult	10/2010	Flycamp	Y	0	0	0	0	≥ 1:20	≥ 1:20
	M	Adult	03/2009	Xini	N	0	0	0	≥ 1:20	≥ 1:20	≥ 1:20
	F	Adult	05/2011	Xini	N	0	0	0	0	≥ 1:20	≥ 1:20
	F	Adult	05/2011	Xini	N	0	0	0	0	≥ 1:20	≥ 1:20
	F	Adult	11/2008	Clare	N	0	0	0	0	≥ 1:20	≥ 1:20
	F	Adult	01/2010	Clare	N	0	0	0	0	≥ 1:20	≥ 1:20
	F	Adult	11/2010	Kazikini	Possible	0	0	0	0	≥ 1:20	≥ 1:20
	M	Adult	05/2011	Chitabe	Possible	0	0	0	0	0	≥ 1:20
	F	Adult	09/2009	Santaw	N	0	0	0	≥ 1:20	≥ 1:20	≥ 1:20
	F	Adult	09/2010	Santaw	N	0	0	0	0	≥ 1:20	≥ 1:20
	M	Adult	05/2011	Santaw	N	0	0	0	0	≥ 1:20	≥ 1:20
Leopard ( <i>Panthera pardus</i> )	M	Adult	08/2009	NA	N	0	0	0	≥ 1:20	0	1
	M	Adult	02/2009	NA	N	0	0	0	≥ 1:20	0	0
	F	Adult	10/2009	NA	N	0	0	0	0	0	0
	F	Adult	09/2009	NA	N	0	0	0	≥ 1:20	≥ 1:20	0
Spotted hyena ( <i>Crocuta crocuta</i> )	F	Adult	06/2009	Ginger	N	0	NA	NA	NA	NA	NA
	F	Adult	08/2009	Ginger	N	0	NA	NA	NA	NA	NA
	F	Adult	08/2009	Giner	N	0	NA	NA	NA	NA	NA
	NA	12-18 months	08/2009	Ginger	N	0	NA	NA	NA	NA	NA
	NA	12 months	08/2009	Ginger	N	0	NA	NA	NA	NA	NA
	F	Adult	10/2010	Ginger	Unlikely	0	NA	NA	NA	NA	NA
	F	Adult	11/2010	Fly	Unlikely	0	NA	NA	NA	NA	NA
	NA	NA	08/2009	Fly	Possible	1:20	NA	NA	NA	NA	NA
	M	12-18 months	08/2009	Fly	Unlikely	1:10	NA	NA	NA	NA	NA
	M	18-24 months	08/2009	Fly	Unlikely	1:28	NA	NA	NA	NA	NA
	F	12-18 months	08/2009	Tori	Likely	0	NA	NA	NA	NA	NA
	M	Adult	08/2009	Tori	Likely	0	NA	NA	NA	NA	NA
	M	NA	08/2009	Tori	Likely	0	NA	NA	NA	NA	NA
	F	Adult	08/2009	Tori	Y	0	NA	NA	NA	NA	NA
	M	18 months	08/2009	Tori	Likely	0	NA	NA	NA	NA	NA
	F	Adult	01/2009	Vera	Likely	1:10	NA	NA	NA	NA	NA
	F	Adult	08/2009	Xini	N	1:10	NA	NA	NA	NA	NA
	F	Adult	09/2009	Athena	Possible	0	NA	NA	NA	NA	NA
	M	Adult	05/2011	Athena	Likely	0	NA	NA	NA	NA	NA

**Figure 1:** The study area in the Okavango Delta, northern Botswana. Dashed lines: rivers. Sampling locations are shown for the four species. Black symbols: CDV positive; dark grey: FCV positive; light grey: FPV or FEC positive.

