Host Range and Prevalence of Canine Parvovirus CPV-2a and 2b Strains in Wild Carnivores of the Serengeti-Maasai Mara Ecosystem in Tanzania

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ABSTRACT

Canine parvovirus is an emerging fatal virus that causes disease in wild carnivore populations. This study was conducted to determine the host range and prevalence of canine parvovirus in wild carnivore populations of the Serengeti–Maasai Mara ecosystem in Tanzania. 154 wildlife blood and tissue samples from 11 species were collected between 2002 and 2009 from Serengeti National Park and the surrounding villages. The samples were analyzed by deploying convectional polymerase chain reaction. A total of 14 samples (9.1%) were positive for CPV (type-2a and 2b), with hosts being jackals (Canis aureus), African wild dogs (Lycaon pictus), lions (Panthera leo), and mongoose (Helogale parvula). More significantly, this study is the first to report CPV infection in dwarf mongoose and lions. The detection of CPV in wild carnivores suggests that there is an inter-species transmission between wild and domestic carnivores.

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1. Introduction

Canine parvovirus (CPV) is a single stranded DNA virus from the family *Parvoviridae* (Decaro & Buonavoglia, 2012; Knipe & Howley, 2007). The viruscauses fatal disease in domestic dogs and several wild carnivorespecies (Driciru et al., 2006; Gese et al., 1991; Stei nel et al., 2001). CPV presents with hemorrhagic

enteritis and myocarditis both in dogs and cats (Burtonboy et al., 1979; Decaro et al., 2005; Parrish et al., 1982). Felids and canids of the ages about one to four months are more susceptible to CPV infections(Hoelzer and Parrish, 2010), though cases have been reported in older animals (Mason et al., 1987). The disease tends to be more acute in dogs than in cats(Mochizuki et al., 1993).
Canine parvovirus is reported to have emerged from feline panleukopenia virus (FPV) during the 1970safter which it spread rapidly around the world(Decaro et al., 2007; Shackelton et al., 2005). The CPV type-2 (CPV-2) emerged first, following cross species transmission from wild felids to domestic canids (Allison et al., 2012; Parrish et al., 2008), and was only able to infect domestic dogs. Later further antigenic variants emerged (CPV-2a, CPV-2b and CPV-2c(Decaro & Buonavoglia, 2012) with the ability to infect and replicate in wild carnivore species.

The host range of CPV is mainly determined by the VP2 gene which is responsible for expression of the capsid proteins which act as epitopes for the specific binding of transferrin receptor type- 1 (Hueffer et al., 2003; Hueffer & Parrish, 2003). The ability of the CPV to bind, replicate and infect lymphoid, intestinal (crypts of Lieberkuln) and bone marrow cells through transferrin type-1 receptors has resulted from the mutation and adaptation of the VP2 gene (Truyen and Parrish, 2013). Consequently, mutations of this gene can result in the virusbeing able to infect new host species.

Canine parvovirus is able to infect several wild and domestic canid and felid species (Alexander et al., 1994; Steinel et al., 2000; Steinel et al., 2001; Truyen et al., 1998), including leopards, cheetah, mongoose, jackals, wolves, foxes, lions, tigers and wild dogs. Large cats are reported to be more susceptible to new CPV strains, CPV-2a, CPV-2b and CPV-2c, than small cats(Steinel et al., 2000)**.** Several research findings have reported the presence of CPV-2a/2b in wild carnivores; CPV-2a in stone marten (*Martens foina*) (Duarte et al., 2013), CPV-2b in cheetah, bat- eared fox, honey badger and Siberian tiger (Steinel et al., 2000),Vietnamese leopard cats (Ikeda et al., 2000). Currently, little is known on the impacts caused by CPV infections and its contribution to the decline of the wild carnivore populations.

The horizontal mode of transmission of CPV is achieved either by oral contact with infected faecesor, since the virus can remain viable in the environment for long periods about five months or more (Acosta-Jamett, 2010), by contact with contaminated surfaces such as soil(Nandi & Kumar, 2010; Steinel et al., 2001).The interspecies transmission is possible through prey-predator relationship or by contact with contaminated surfaces. The high mobility of some wildcarnivores such as African wild dogs (*Lycaonpictus*) increases the chances of spreading CPV to different areas.

Canine parvovirus can be diagnosed by several methods including: haemaglutination, immune chromatographic test, microscopy, ELISA, and polymerase chain reaction (PCR). PCR is highly specific, sensitive and rapid and is capable of detecting the virus infaeces, blood or tissues even when present in very small quantities(Decaro & Buonavoglia, 2012; Desario et al., 2005).
Domestic dogs have been implicated as the source of several pathogenic infections to susceptible wild carnivore species (Butler et al., 2004; Cleaveland et al., 2000; Woodroffe, 1999). Examples being canine distemper in lions, rabies in wild dogs and CPV in leopards, cheetah, foxes, mongooses, bush dogs, coyotes and wolves.

The aim of the study was to determine the host range and prevalence of canine parvovirus in wild carnivores of the Serengeti National Park ecosystem using PCR assay to type the Canine parvovirus strains.

2. Materials and Methods

2.1 Ethics Statement

Animals were captured and handled in accordance with animal ethics under the permission given from Tanzania Wildlife Research Institute (TAWIRI-Ministry of Natural resources and tourism) and according to guidelines of the International Union for Conservation of Nature (IUCN)(CPSG. 2020).

2.2 Biological Samples

Wild carnivore samples used in this study (74 whole blood samples and 80 tissue samples (liver, lymph node, spleen, heart, intestine, and gland)) were collected opportunistically from chemically immobilized wild animalsand postmortem examinations performed in the Serengeti National Park between 2002 and 2009. The samples came from the following species: lions (*Panthera leo*), jackals (*Canis aureus*), African wild dogs (*Lycaon pictus*), dwarf mongooses (*Helogale parvula*), cheetah (*Acinonyxj ubatus*), hyenas (*Crocuta crocuta*), leopards (*Panthera pardus*), civets (*Civettictis civetta*), and wolf (*Proteles cristata*), bat-eared foxes (*Otocyon megalotis*) and servals (*Leptailurus serval*). The samples were collected by Carnivore Disease Project (CDP) under the permission from TAWIRI. Following collection all samples were stored at minus 20ºC until used.

2.3 Viral DNA Extraction

Viral DNA from whole blood and tissue samples was extracted using ZR Viral DNA kit (Zymo Research Corp., Irvine CA 92614,California, USA) and ZR Genomic DNA^{TM} -Tissue Kit (Zymo Research Corp., Irvine CA 92614,California, USA) respectively, according to the manufacturer's instructions. Both kits use *Fast-Spin* column purification technology. A multivalent vaccine DHLPP (Vanguard Group Inc., Manhattan, New York NY 10017,Pfizer-USA) containing attenuated canine parvovirus strain was used as a positive control.

2.4 PCR Assay

Two specific primer pairs and a universal primer used in convectional PCR were designed to amplify VP1/VP2 of the capsid genes; The primer pairsPbs/Pbas detect CPV type-2b and Pabs/Pabas detect CPV type-2a/2b and the primer pair H for/Hrev were designed to amplify a larger fragment of the VP2 gene from any of the canine parvovirus strains for sequencing purposes. The primer pairs Pb andPab were designed byPereira et al. (2000) and the primer pair Hfor/Hrev was designed by Buonavoglia et al. (2001). The primer sequences are shown in table 1.

The primer pairs Pb and Pab yielded amplicons of the same size; both of the primer sets were used in separate reactions. The samples that gave positive result to primer Pab would then be amplified with primer Pb to obtain type-specific results. The Oligonucleotides were synthesized by Inqaba biotechnical Industries Ltd-South Africa.

Tab 1
Sequences of the Primers Used in Convectional PCR

CPV type	Primer	Primer sequence	Location	Amplicon size
CPV-2a	Pabs	5'-GAAGAGTGGTTGTAAATAATT-3'	3025-3045	427
	Pabas	5'-CCTATATAACCAAAGTTAGTAC-3'	3685-3706	
CPV-2b	Pbs	5'-CTTTAACCTTCCTGTAACAG-3'	4043-4062	427
	Pbas	5'-CATAGTTAAATTGGTTATCTAC-3'	4449-4470	
CPV	H *for*	CAGGTGATGAATTTGCTACA	3556-3575	610
	H *rev*	CATTTGGATAAACTGGTGGT	4185-4166	

The PCR assay was performed as per Pereira et al. (2000) with some modifications, The reaction mixture consisted of 25µl of PCR master mix, DreamTaq Green Master Mix (2x) containing: DreamTaq DNA polymerase, 2x DreamTaq Green buffer, dATP, dCTP, dGTP and dTTP,0.4 mM each, and 4mM MgCl2. The primerpairs used in amplification were Pbs/Pbs, Pabs/Pabs and H *for*/H *rev*, each 1µl (0.4µM), 6.5µl of nuclease free water and3µlof template DNA.The convectional PCR thermal cycling conditions were set as: activation of DreamTaq DNA polymerase at 94ºC for 3minutes, 30cycles of denaturation at 94ºC for 30 seconds, primer annealing at 55ºC for 1minute, extension at 72 ºC for 1 minute, final extension at 72 ºC for 5 minutes and 4 ºC final hold.

2.5 Gel-electrophoresis

The PCR products were analyzed using 1.5% agarose gels stained with Gel-Green nucleic acid stain to view the 610bp and 427bp bands to verify the presence of the CPV strains. The PCR products were run on gel along with a DNA ladder of 100bp in 1X TBE electrophoresis buffer. Then the results from the gels were visualized by ultraviolet illumination using Gel DocTMEZImager (Bio-Rad Laboratories, Inc. California, USA).

2.6 Statistical Analysis

The data were analyzed using Mintab16 (Mintab16, Inc, state college, Pennsylvania) to estimate the CPV DNA prevalence in 11 surveyed wild carnivore species. The 95% confidence intervals for CPV DNA prevalence were estimated. For this study one sample proportional statistical analysis was performed on Mintab16 platform.

3. Results

The summarized PCR results are shown in Table 2. The general prevalence of CPV type 2a and type 2b DNA in the eleven surveyed wild carnivore species of the Serengeti National Park ecosystem was 14/154 (9.1%) [95% CI = 4.6%–13.6%]. The prevalence of CPV in each species tested was: African wild dogs 4/16 (25%), mongoose 1/4 (25%), lions 3/89 (3.4%), jackals 6/11 (54.5%). CPV was not detected in leopards (n=1), civets (n=6), servals (n=2), hyenas (n=15), foxes (n=2), and wolves (n=1) and cheetah (n=7). CPV-2b, which was detected in four carnivore species, had a prevalence of 7.8%, whilst CPV-2a, which was detected in two species, had a prevalence of 1.3%. Both CPV-2a and 2b were found in lions and jackals, but only the CPV-2b variant was found in African wild dogs and mongoose.

Tab 2

PCR Results Showing the Number of Positive Samples over Total Number of Tested Samples for the Wild Carnivore Species Recruited in the Study

Species (N)	CPV-2a	CPV-2b	Total
1. Lion (89)	1/89	2/89	3
2. Jackals (11)	1/11	5/11	6
3. African wild dogs (16)	0/16	4/16	4
4. Dwarf mongoose (4)	0/4	1/4	1
5. Cheetah (7)	0/7	0/7	0
6. Leopards (1)	0/1	0/1	0
7. Hyena (15)	0/15	0/15	0
8. Civets (6)	0/6	0/6	0
9. Aardwolf (1)	0/1	0/1	0
10. Foxes (2)	0/2	0/2	0
11. Servals (2)	0/2	0/2	0
Prevalence	1.3%	7.8%	9.1 %

The oldest sample that tested positive was collected in 2002 from a dwarf mongoose (*H.parvula*). More cases of canine parvovirus were detected in the samples collected during the years: 2003, 2004, 2005 and 2008, specifically in jackals, African wild dogs and lions(Figure 2).The positive CPV cases reported in this study were from both adults and juveniles wild carnivores.

Fig 1

Agarose Gel Electrophoresis and Gel Green-Florescence of PCR Amplified Samples Using Primer Pb. The Samples 96, 97, 98, 99, and 100 Were Positive for CPV-2b

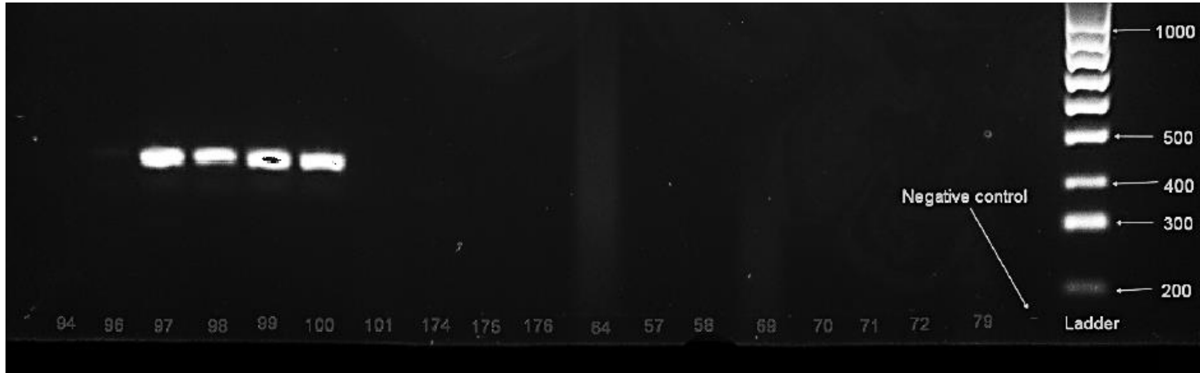
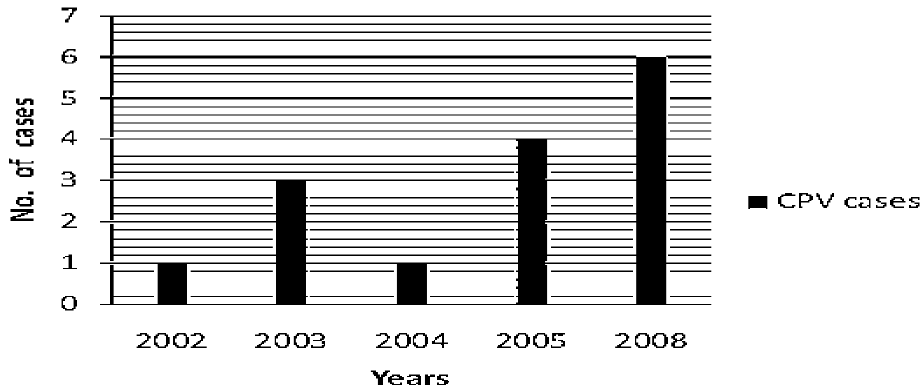


Fig 2

Number of Samples from the Serengeti National Park that were CPV Positive in each of the Years 2002, 2003, 2004, 2005 and 2008



4. Discussion

This study has found the first global evidence of CPV infection in lions *(P. leo*) and dwarf mongoose (*H.parvula*) with prevalence of 3.4% and 25%, respectively. In addition, we have shown that CPV infects jackals and African wild dogs. The highest prevalence was found in jackals with half of the samples testing positive (6 positive out of 12 samples tested). A quarter of the African wild dogs (4/16) and dwarf mongoose (1/4) samples were positive, whereas, a small proportion of lion samples (3/89) were positive for CPV -2a and 2b. All four positive species belong to three families in the order carnivora: *felidae*

(lions-*P.leo*), *canidae*(jackals-*C. aureus*, African wild dogs-*L.pictus*) and *herpstidae*(mongoose-*H.parvula*). These findings suggest thatCPV circulates in the Serengeti-Maasai Mara ecosystem.

The higher prevalence of CPV in jackals can probably be explained both by their ranging behaviour, dispersing up to 842km from their native areas(Alexander et al., 1994), and their sympatric life where they often scavenge on domestic refuse in the vicinity of human settlements and share carcasses with other wild carnivore species. These behaviours likely result in higher chances of interaction with domestic dogs and exposure to pathogens of canine (Alexander et al., 1994; Butler et al., 2004).

Despite previous reports of infection (Ikeda et al., 2000; Laurenson et al., 1998; Mech & Goyal, 1995; Steinel et al., 2000; Steinel et al., 2001), CPV wasn't detected in leopards, cheetah, civets, servals, bat-eared fox and aardwolf. Possible explanations could be the small number of samples assayed, however the relatively solitary nature of most of these species, which would make transmission less likely, could also be a factor.

More species were positive for CPV-2b (*C. aureus, P.leo*, *L. pictus, and H. parvula*) than CPV-2a (*C. aureus* and *P. leo*only) which is in accordance with reports thatindicate that most CPV cases in both domestic and wild carnivores (<70%) are of CPV type-2b; furthermore this result conforms to situation that CPV-2b is a more prevalent strain in southern Africa(66%)(Steinel et al., 1998). This finding suggests that CPV-2b is a more prevalent strain in wild carnivore populations of the Serengeti ecosystem.

More than half of the jackal samples in this study were positive, which is higher than a previous report from Kenya (34%) in 1994(Alexander et al., 1994), A possible explanation for CPV infection being common in this species is that jackals, being visitors to human settlements(Alexander et al., 1994), frequently come in to contact with domestic dogs (Butler et al., 2004), which increases the chances of transmission of pathogens.

In mongoose, the CPV prevalence of 25% as per this study differs from the seroprevalence that was reported in a seroprevalence study of parvoviruses in Egyptian mongoose (58%) from Portugal conducted in 2013(Duarte et al., 2013). Wild felids have been reported to be susceptible to CPV infections (Decaro & Buonavoglia, 2012;Parrish, 1999; Truyen, 2006), consequently the lowprevalence of CPV found in the wild cats (lions3.4%, servals 0%,leopards0%,and cheetah 0%)sampled in this study was unexpected. This might suggest that wild cats/felid species are more resistant to canine parvovirus infections compared to canid species such as jackals and wild dogs. Alternatively, demographic, behavioral or social characteristics of wild felids might make them less likely to be infected than dogs.

The results from this study show that canine parvovirus has been circulating in wild carnivores of the Serengeti ecosystem before 2002, as evidenced by the detection of CPV DNA in dwarf mongoose (*H.parvula*) samples collected in 2002; whereas, a seroprevalence study done in Kenya in 1994 using samples collected between 1978 and 1988 from free-ranging black-backed jackals (*C. mesomelas*) tested positive for CPV, showing that the virus was soon after its emergence in 1978 (Alexander et al., 1994).

Despite the fact that CPV affects mostly puppies(Nandi & Kumar, 2010), but in this study all samples that tested positive for CPV DNA were from juvenile and adult animals, this suggests that even adult canids and felids may still be susceptible to canine parvovirus.

All wild carnivore species in Tanzania are considered native (currently there are no reports regarding the import of wildlife carnivore species from other countries), which suggests that the possible source of CPV infections in wild carnivores might be either imported domestic carnivore species (domestic dogs and cats) or human visitors from other countries (carrying contaminated items such as shoes, clothes, and instruments). Alternatively, transmission may occur across national borders from natural wild animal species interactions.

5. Conclusion

The current study reports the prevalence of CPV-2a and 2b (9.1%) in wild carnivores of the Serengeti-Maasai Mara ecosystem in Tanzania, this shows that CPV might be an important pathogen in wild carnivores and probably it contributes to the mortality of such wild populations. On the other hand, the detection of CPV-2a and 2b in lions (*P.leo*) and dwarf mongoose (*H.parvula*) suggests an expansion of the host range of the virus, a sort of cross species transmission, though pathological studies in these species are required so as to give proof to the ability of the virus to infect and replicate in such species.

6. Recommendations

The extent and rate of wild life-domestic animals' interface that results into successful inter-species transmission of pathogens from either sides' domestic to wildlife and vice versa, and the impacts of vaccination of domestic carnivores as a control to protect wildlife species are not well explored.

In this study, we suggest, first, an assessment of the extent of sympatry between wild life species and domesticated animals to ascertain the contribution of the domestic carnivores in transmitting the pathogens to wild life species. Second, the assessment of the impact of vaccination of domestic dogs against canine parvovirus in places surrounding wildlife reserved areas(places of wild life-domestic species interface) in controlling the transmission of CPV into susceptible wild carnivore species.

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