determine the prevalence and types CPV strains in asymptomatic domestic dogs in villages surrounding the Serengeti National Park, Tanzania.

2. Materials and Methods

2.1 Study site

The study was carried out in the Mara, Simiyu and Arusha regions of northern Tanzania.

2.2 Samples Collection

A total of 77 domestic dog blood samples were used for analysis in this study. The samples were simple randomly selected (in terms of age and location) from an archive that contained frozen samples which had been collected from healthy domestic dogs (both puppies and older dogs had equal chance to be selected for blood sampling) attending rabies vaccination clinics in villages bordering the Serengeti National Park, a zone of 10 Km from the park, between 2007 and 2009 years. The samples used came from 12 villages from Serengeti, Tarime and Loliondo districts found in Mara, Simiyu and Arusha regions, northern Tanzania.

2.3 DNA extraction

The viral DNA was extracted from whole blood samples using a ZR Viral DNA Kit (Zymo Research Corp. USA) according to the manufacturer's instructions. A multivalent vaccine DHLPP (Vanguard, Pfizer-USA) containing attenuated canine parvovirus strain was used as a positive control.

2.4 Primers for convectional PCR

The primers used in convectional PCR were designed to amplify VP1/VP2 of the capsid genes. The specific primer pairs; Pbs/Pbas detect CPV type-2b, Pabs/Pabas detect both CPV type-2a and type-2b, and the primer pair H for/Hrev was designed to amplify a larger fragment of DNA for sequencing purposes. The primers Pb and Pab were designed by Pereira et al. (2000) [40] and H was designed by Buonavoglia et al. (2001) [41].The primer pairs Pb and Pab yielded amplicons of the same size, so they were used in separate reactions. All primers used were synthesized by Inqaba biotechnical Industries Ltd (Republic of South Africa) (The primer sequences are shown in Table 1).

Table 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	

s-sense and as-antisense

2.5 PCR assay

The PCR assay was performed as per Pereira et al. (2000) [40] with some modifications. The PCR reaction mixture(25µl) consisted of DreamTaq Green Master Mix(2x) containing: Dream Taq DNA polymerase, 2x Dream Taq Green buffer, dATP, dCTP, dGTP and dTTP,0.4 mM each, and 4 mM $MgCl_2$. The primer pairs used in amplification were Pbs/Pbs, Pabs/Pabs and H *for*/H *rev*, each 1µl (0.4µM), 6.5 µl of water free nuclease and 3µl of 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 55 ºC for 1minute, extension at 72 ºC for 1 minute, final extension at 72 ºC for 5 minutes and 4 ºC final hold.

2.6 Gel-Electrophoresis

The Gel-electrophoresis of the PCR products were analyzed using 1.5% agarose gels stained by Gel-Green nucleic acid stain to view the 610bp and 427bp bands so as 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 Doc ^{TM} EZ Imager (Bio- Rad Laboratories, Inc) (Figure 1 & 2).

2.7 Statistical Analysis

The summarized data were analyzed using Mintab16 (Mintab16, Inc, state college-Pennsylvania) to estimate the CPV DNA prevalence in domestic dogs. 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 prevalence of CPV DNA in apparently healthy domestic dogs was 10.4 % (8/77) [95% CI=3.58% – 17.22%]. The prevalence of CPV-2a was 6.5% (5/77) and CPV-2b 3.9% (3/77).The CPV positive cases were detected in samples collected from six different villages, namely Merenga (MR), Losoito (LS), Nyamburi(NY), Nyambwaga (NM), Itiryo (IT), and Kitaramanka (KT), in different districts (Serengeti and Tarime). Among the 12 sampled villages, 4 positive cases were from 2 villages in Tarime district (two cases from each, Nyamwaga and Itiryo), also, 4 positive cases were from four villages (one case from each, Bisarara, Merenga, Losoito, and Nyamburi) in Serengeti district, whereas, none of case was detected from among the three sampled villages (Pinyinyi, Engakaseko, and Malambo) in Loliondo district. In summary, Serengeti district-4 cases, Tarime district-4 cases, and none in Loliondo district, therefore, six out of 12 sampled villages had positive cases for CPV-2a and 2b.

The positive samples were from the domestic dogs with the ages of 6, 8, 9 and 10 months, but also 1 and 2 years old [Four cases (50%) were from below one year and the other

four cases (50%) were from one year and above]. In terms of sex, six out of eight of the positive samples were females, furthermore, all the positive CPV cases were from the samples collected in 2008 and 2009 years, and none of the samples from 2007 tested positive.

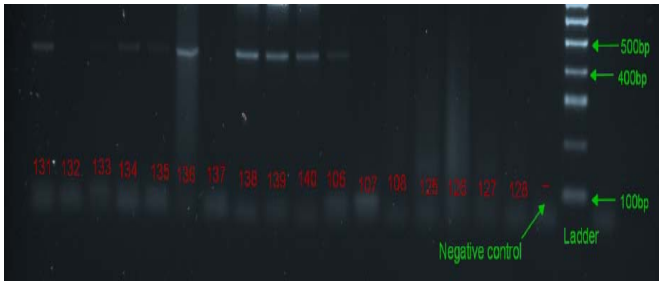

Figure 1: Agarose gel-electrophoresis and GelGreen-florescence of PCR amplified samples using primer Pab. The samples 131,134,135,136,138,139,140 and 106 are positive for CPV-2a/b.

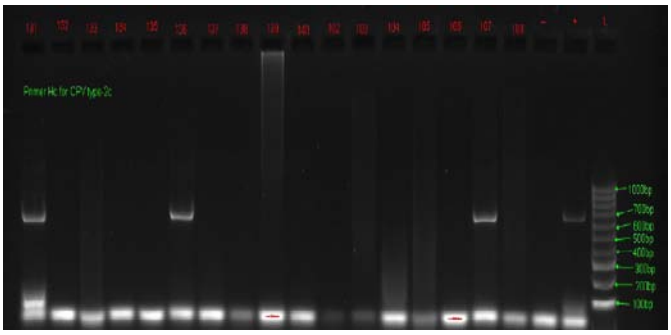

Figure 2: Agarose gel-electrophoresis and GelGreen-florescence of PCR amplified samples using primer H. The samples 13, 136, and 107 are positive for CPV.

4. Discussion

This study provides the first data on the prevalence of CPV antigenic strains type-2a and 2b in asymptomatic domestic dogs in Tanzania. Approximately one in ten healthy dogs was positive (8 out 77) which suggests that dogs can be potential carriers of the virus.

In this study, the prevalence of antigenic strains CPV-2a was almost twice as high as CPV-2b. This finding is in agreement with studies in countries such as Italy, German, Korea, China, French and Taiwan where CPV-2a has been reported to be more predominant [18, 41-43]. In contrast, CPV-2b has been reported to be the predominant strain in other parts of the world. E.g. Southern Africa, USA, Japan and Turkey [39, 44].The reason why the CPV stains vary in frequencies in different localities is unknown [45]. Since the samples used in this study were collected from only one geographical region (Northern Tanzania including Musoma, Simiyu and Arusha regions), consequently the reported prevalence of CPV-2a is being higher than CPV-b in Tanzania might change if domestic dogs had been sampled from the entire country.

This study reported a prevalence of 10.4% (8/77), but in some other places, there are some reports showing even higher prevalence. For instance, analyses of the faeces of stray dogs in South Korea and Cape Verde (Africa) detected a seroprevalence of CPV-2a of 93.8%, and a prevalence of CPV DNA of 43.3% (23/53) respectively [31, 32]. The reason for variation in proportions of CPV strains in different countries is currently unknown. However, probably this can be associated to poor animal husbandry which normally results in free-ranging/stray dogs and also, ineffective policies for controlling the disease, for example, lack of routine vaccination campaigns.

Though, 12 villages were sampled, but the CPV positive cases were detected in samples from only six villages in two districts. In Serengeti district, there were two times the number of villages 4/12 (33%) with positive cases of CPV compared to the number of villages 2/12 (17%) with such cases in Tarime district, and surprisingly, none of the villages in Loliondo district had a positive case. So, Serengeti district had the largest proportion (33%) of the CPV cases detected in domestic dogs followed by Tarime district which had a proportion of 17%. The proportion of the villages without positive CPV-2a and 2b cases from all three districts was 6\12 (50%). Although, the proportion of the villages with positive cases in Serengeti district was half of the number to that of Serengeti, but there were equal number of the positive cases of the CPV-2a and 2b in such districts. Therefore, from these results we suggest that CPV is localized to some places, since the positive cases were detected in some villages.

Normally CPV affects puppies [22, 23], though in some cases it has been reported to affect older dogs [24], but in this study two positive cases were from old domestic dogs with ages above one year. There are two possibilities of explaining this result, in the first case, there is a probability that the positive adult dogs could have been infected when puppies and then remained carriers of the virus, alternatively, the dogs could have been infected at their adult age, supporting the view that the adult dogs are susceptible to canine parvovirus.

The detection of canine parvovirus DNA in samples dating back to 2007 shows that CPV has been circulating in domestic dogs for at least seven years. Indeed, 34% (14/55) of sera samples collected from free ranging black-backed jackals (*Canis mesomelas*) between 1987 and 1988 in Kenya were found to be positive for antibodies against CPV [46] indicating that the virus has been circulating in East Africa since at least 1987. Since only emerged in 1978 in Ontario [47] these findings indicate how fast CPV has spread.

The presence of CPV in domestic dogs from the villages around the Serengeti national park, and reports of domestic dogs, which live in villages on the periphery of the Serengeti ecosystem, roaming some distance into park areas in search for food [48] suggests that transmission to wild carnivores is a possibility. Furthermore, transmission might occur when wild carnivores enter human settlement areas to scavenge for food or predate upon domestic dogs [46, 49]. Sick dogs are likely to be easier targets and more frequently eaten which may further potentiate transmission.

Canine parvovirus causes high mortality in puppies dramatically impacting reproductive success [50]. In small and endangered wildlife populations, therefore, the

implications of a CPV outbreak could be catastrophic [51]. Indeed, CPV has been implicated as a barrier to the recovery of wolf populations in North America [52].

The asymptomatic dogs can continue shedding the virus in the environment where it stays viable for longer periods, up to 52 days [16],Therefore, the transmission and spread of the virus can occur in absence of direct contact between the animals, since they can contract it from the contaminated environment [53], this has an implication in making the disease endemic in dogs because other susceptible dogs may contract the virus either from the dog shelter such as kernels or from the contaminated environment.

5. Conclusion

This current study report the detection of CPV-2a and 2b in apparently healthy domestic dogs in regions surrounding the Serengeti ecosystem, Tanzania. The detection of the CPV in asymptomatic dogs shows that the CPV can remain unnoticed among the dog population, hence creates a potential risk in terms of transmission and persistence of the disease, since they can continue shedding the virus in the environment. Furthermore, it was found that CPV-2a is a predominant circulating strain in domestic dogs in Tanzania.

Despite of the safe and effective vaccines in place, CPV remains to be an important pathogen which causes mortality and morbidity in domestic dogs in Tanzania. Since the communities living in proximity to the Serengeti ecosystem own domestic dogs which are seldom vaccinated because people are ignorant on the importance of vaccination, poor infrastructures and high costs of the vaccines, these limit the availability of vaccines, consequently, this may result in a large number of unvaccinated dogs of which make the CPV to continue persisting among the dog population, hence serve as reservoirs and source of infections both to other domestic dogs and susceptible wild carnivore species. So vaccination of domestic dog pathogens could probably be an appropriate practice in controlling the disease both in wild and domestic carnivores, so to ensure successes, the practice should be compulsory and enforced by laws.

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