academicJournals

Vol. 7(21), pp. 2510-2518, 21 May, 2013
DOI: 10.5897/AJMR12.1391
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

The epidemiology of *cryptosporidium* in cats and dogs in the Thohoyandou region, South Africa

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Accepted 30 April, 2013

Cryptosporidium **spp have emerged over the last decade as important diarrheal causing agents particularly among HIV patients. Zoonotic transmission has been reported and their occurrence in domestic animals is of potential significance from both clinical and public health perspectives, yet the occurrence of these organisms among animals in South Africa, particularly the Vhembe district, has not been described. The objective of this study was to determine the prevalence and potential risk factors of** ***Cryptosporidium*** **spp in domestic animals, particularly cats and dogs in the Thohoyandou region. Fresh stool samples were collected from cats (25 samples) and dogs (25 samples) including stray and home based animals in different areas of the Thohoyandou region and Polymerase Chain Reaction (PCR) was used to amplify the** ***Cryptosporidium*** **genetic material using specific primers to the 18S rRNA gene. From the 50 samples tested, 8 (32.0%) of the cats samples had** ***Cryptosporidium*** **and 11 (44%) of the dogs samples had** ***Cryptosporidium*** **with no significant difference (χ^2=0.764, p=0.280).** ***Cryptosporidium*** **was more common in diarrheal samples in cats and dogs, 3/4 (42.9%) and 7/14 (50%) respectively. The infection rate was higher in stray animals particularly in the stray cats [3/4 (75%)] compared to home based cats [5 (23.8%)] (χ^2=4.046, p=0.044). Stray dogs were also more infected [6 (46.2%)] than home based dogs [5 (41.7%)], but the difference was not statistically significant (p=0.821). Animals from more rural parts of the region were more infected. The results of this study for the first time demonstrated the high prevalence of** ***Cryptosporidium*** **infections among cats and dogs in the Thohoyandou region and its implications in causing diarrhea and that stray animals poses a threat to the community and other animals.**

Key words: *Cryptosporidium*, cats, dogs, Venda, South Africa, epidemiology.

INTRODUCTION

Cryptosporidium is an intestinal coccidian that has emerged as an important parasite in a number of host species worldwide. These intracellular parasites of the genus *Cryptosporidium* (Apicomplexa) infect vertebrates, including humans, companion and farm animals, wild animals, birds and reptiles (de Oliveira et al., 2012; Fayer et al., 2000). Recently, the major concerns about the public health dangers of pet ownership have increased considerably, and while many potentially zoonotic organisms are associated with cats, enteric pathogens are of particular concern (Hill et al., 2000; Abarca et al., 2011). The majority of humans infections are caused by *C. hominis* and *C. parvum*. However, they are also known to be infected by other species of *Cryptosporidium*, namely, *C.*

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(Akiyoshi et al., 2003; Gatei et al., 2003; Guyot et al., 2001). *meleagridis*, *C. canis*, *C. felis*, *C. andersoni*, and *C. muris C. parvum* bovine genotype (genotype II) is known to be infectious to many mammalian hosts worldwide (Fayer et al., 2000); however, several distinct genotypes exist within *C. parvum*, and their relationships and host specificities are still largely unknown (Morgan et al., 1999; Xiao et al., 2002). Among the companion animals, the first report of *Cryptosporidium* infection in the cat was in 1979 in Japan (Iseki, 1979) and ever since, this parasite was described in asymptomatic and symptomatic cats having persistent diarrhea, anorexia and weight loss (Monticello et al., 1987; Fayer et al., 2006; Queen et al., 2012). The potential for zoonotic transmission among both cats and humans was demonstrated following the infection of 6-year old cats, orally inoculated with *Cryptosporidium* oocysts obtained from an immunosuppressed person (Current et al., 1983). The agent was also found in an 8-year old child who was infected with *Cryptosporidium* after being in contact with an infected cat (Egger et al., 1990).

The first report of *Cryptosporidium* in dogs was in 1983 in England UK (Wilson et al., 1983). Since then several studies have documented the occurrence, prevalence and risk factors of Cryptosporidium in dogs (Bajer et al., 2012). In Florida, USA, the prevalence of Cryptosporidium was 12% among dogs found in an animal shelter, while in Brazil Cryptosporidium was found in 10.7% of the 28 dogs tested (Dado et al., 2012; Tupler et al., 2012). However, there is little or no data on the occurrence of Cryptosporidium among animals in the African continent (Berrilli et al., 2012).

In Vhembe region, domestic animals are commonly seen lingering in the streets, searching for food and water. This suggests that the owners might not have the resources (food, money) to adequately care for the animals. This makes it highly possible for these animals to get infected as they move around looking for food in the rubbish and drinking untreated water in the streets. This also increases the chances of home based animals getting infected from the stray animals that they intermingle with in the streets and when they come back home, they can pass on their infection to other domestic animals in the neighborhood and allowing the increase of the infection to one or many different species. Previous studies in the Vhembe district have demonstrated high prevalence of *Cryptosporidium* spp in humans (Samie et al., 2006).

However, the sources of infections were not identified. Furthermore, the occurrence of *Cryptosporidium* in other animals in the Limpopo Province is not known. It is thus important to understand the epidemiology of *Cryptosporidium* in domestic animals that will help to take further measures in the protection of the environment and improve the living conditions of the residents. The present study determined the epidemiology of *Cryptosporidium* spp among pet animals including cats and dogs in the Thohoyandou region in Vhembe District.

MATERIALS AND METHODS

Study site and sample collection

The study was conducted in the Thohoyandou region which is a town in Vhembe district in the far north region of South Africa in the Limpopo Province. All the fecal samples used in the present study were collected in different areas of Thohoyandou including P-west, P-east, Maungani, Tshisahulu and Sibasa. Thohoyandou P-west is a semi-urban area closer to the town centre and separated from the University of Venda by a stream called Mvundi stream. Maungani and Tshisahulu are rural areas situated within 15 kilometers from the Centre of Thohoyandou town. Sibasa is urban location 20 kilometers away from the Thohoyandou centre. It is more urbanized and cleaner than the other regions. P-east is situated north of Thohoyandou and is positioned on the eastern side of the main road to Sibasa. The samples were collected from the northern part of P-east which is more rural. The samples were collected between April and August 2007 by secretly following the animals and only fresh samples were collected. The sample collection was done by taking the feces using a sterile spatula into a sterile collection tubes which were then kept in the cooler box for transportation to the laboratory. In the laboratory the collected samples were stored at -20°C until analyzed.

A total of 50 fecal samples were collected of which 25 samples were from dogs and the other 25 were from cats. Of the 25 samples from the dogs, 13 were from stray dogs and 12 of the fecal sample were from home based animals. Fourteen of the 25 dog's fecal samples were diarrheal (Soft) and 11 were non diarrheal (Hard). Out of the 25 fecal samples collected from cats, 3 were from stray cats and 22 were from home based animals. Four (4) of the 25 cats' fecal samples were diarrheal and 21 were non diarrheal. The demographic information of the animals from which the stool samples were collected are shown in Table 1.

Extraction of genomic DNA from stools samples

Approximately 200 µl of stool was added into a 2.0 ml Eppendoff tube. The samples were pretreated by the freeze and thaw method to break down the oocysts. Briefly, tubes were placed into floating microfuge tube holder which was then placed into the freezer (-20^{o}C) for 30 min. Afterward the tube holder was placed in a 95°C water bath for another 5 min. These steps were repeated for a total of 7 times. After the initial freeze and thaw pretreatment, the genomic DNA was purified from the stools using the QIAamp mini stool DNA (Qiagen, California, USA) according to the manufacturer's instruction with slight modifications. Briefly, 1 ml of Buffer ASL was added to each sample tubes and the tubes were vortexed at full speed for 1 min to homogenize the stool sample. Then the tubes were returned to floating tube rack and incubated at 95°C for 15 min. After the incubation, the tubes were centrifuged at 13400 rpm for 1 min. One InhibitEX tablet was added to one of the remaining 2.0 ml tubes then supernatant was added to InhibitEX tablet and vortexed for 1 min at full speed, then centrifuged at 13400 rpm for 3 min. The supernatant was pipetted into 1.5ml microfuge tube and centrifuged at 13400rpm for 3 min.

Detection of *Cryptosporidium* by Polymerase Chain Reaction (PCR)

The PCR protocol based on the amplification of a specific sequence of the 18S rRNA gene was used to detect and quantify

Table 1. Demographic information of the animals.

Number of animals	Dog	Cat	Total
	25	**25**	**50**
Age			
Puppies	6	2	8
Adult animals	19	23	42
Sex of the animals			
Females	12	12	24
Males	13	13	26
Diarrheal characteristics of the stools			
Diarrheal	14	7	21
Non diarrheal	11	18	29
Life style of the animals			
Stray (Outdoors)	12	4	16
Home based (indoors)	13	21	34
Location of the animals (area)			
P-west	6	4	10
P-east	5	4	9
Maungani	5	4	9
Tshisaulu	4	6	10
Sibasa	5	7	12

Cryptosporidium spp. (Samie et al., 2006). The primers used were: Crypt PF: 5′-CTG CGA ATG GCT CAT TAT AAC A-3′ and Crypt PR: 5′- AGG CCA ATA CCC TAC CGT CT-3′. These primers were designed to detect the 18S rRNA gene of as many *Cryptosporidium* species as possible, with sequences matching *C. hominis* (AF093491), *C. parvum* (AF164102), *C. meleagridis* (AF112574), *C. canis* (AB210854), and *C. suis* (AF108861) completely and with a single base pair (bp) mismatch at forward primer position 5 of 22 for *C. felis* (AF112575) and position 18 of 22 for *C. muris* (X64343). The reaction was run in a total volume of 25 µl made of 12.5 µl of the iQ^{TM} SYBR® Green Supermix (Bio-Rad, CA), 1 µl of each primer (concentration of primer), 5.5 µl of Nuclease Free (DNase, RNase, and Proteinase) water (Fisher Biotech, NJ) and 5 µl of genomic DNA extract. The cycling conditions were four cycles with cycle 1 for 13.5 min at 95°C, cycle 2 was repeated 50 times with 45 s denaturation at 95°C, 45 s annealing at 60°C and 60 s chain extension at 72°C with data collection enabled during the last two steps. Cycle 3 was 72°C for 10 s with set point temperature increase after cycle 2. The last cycle was held at 4°C. Each run included at least two positive controls (Genomic DNA extracted from pure *Cryptosporidium* oocysts) and one negative control (water). All these cycle conditions of PCR were performed in an end-point PCR thermocycler (BIORAD).

The amplified products from PCR and RFLP were detected and verified for size, specificity and purity by running a 1% agarose gel. The gel was viewed under a UV transilluminator (G-BOX), from Vacutec and the band sizes were determined by comparing with the 100 bp ladder (Brody et al., 2004).

Statistical analysis

The results of the study were analyzed using the SPSS software Version 10.1. The χ^2 test was used to determine the relationship between *Cryptosporidium* results of the patients who provided the stool samples and other parameters such as diarrheal symptoms, sex, age, origin, or lactoferrin test results. The differences were considered significant when the *p* value was less than 0.05.

RESULTS

Detection of *Cryptosporidium* spp in animal stool samples

The stool samples collected from the different animals were analyzed by PCR and clear bands were observed after agarose gel electrophoresis as indicated on Figure 1. The primers used are able to detect any type of *Cryptosporidium* as previously demonstrated by Parr et al. (2007) since it targets a conserved region of the 16SrRNA. *Cryptosporidium parvum* (Iowa isolate) was used as positive control while PCR water was used as negative control. This served to verify the specificity and sensitivity of the test making sure that all the bands that were observed actually indicated the presence of *Cryptosporidium* spp.

Prevalence of *Cryptosporidium* among cats and dogs in the Thohoyandou region

Out of the 50 samples collected, *Cryptosporidium* spp was found in 19 (38%) animals including cats and dogs. When the animals were considered separately, dogs

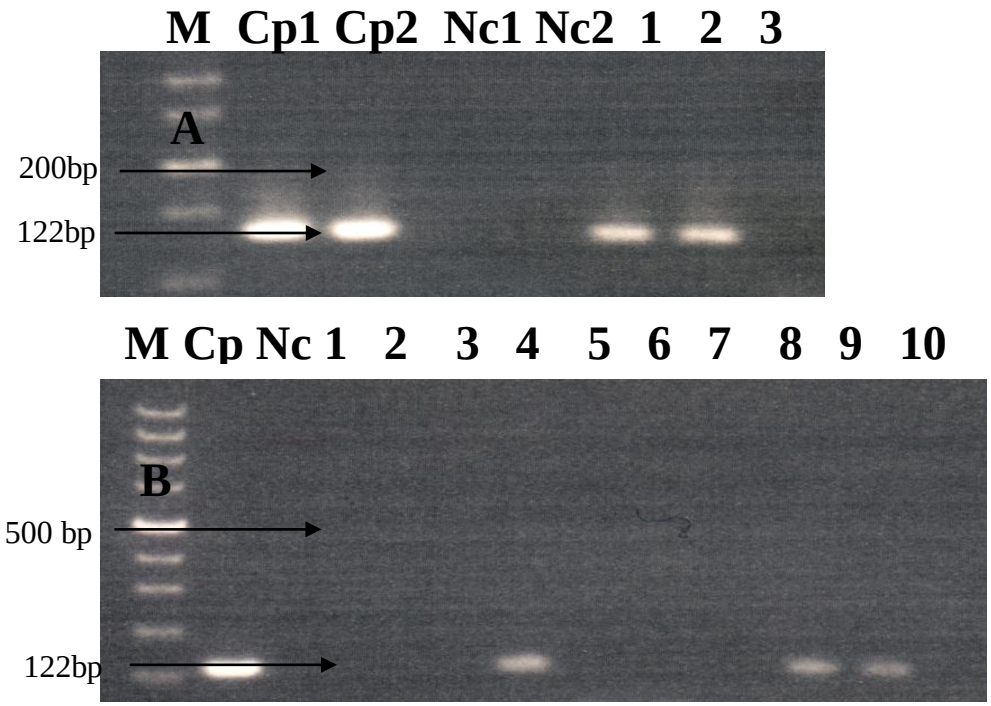

Figure 1. Pictures of agarose gel electrophoresis showing in (A) the PCR products of the first reaction testing for the specificity and sensitivity of the method: The reaction comprised two positive controls (Cp1 and Cp2), two negative controls (Nc1 and NC2) and three samples of which one positive was from the cat (1) and one positive from a dog (2) and another one negative (3) from the dog as well. In **B** we have a positive control (Cp) and a negative control (Nc) and the rest of the wells were samples from cats (1 – 5) and from dogs (6 – 10). M represents the molecular ladder.

were more infected than the cats, but the difference was not statistically significant (p=0.280). Among the cats 8 (32%) samples contained *Cryptosporidium* genomic DNA while 17 (68%) were negative. Among the dogs 11 (44%) samples were positive for *Cryptosporidium* DNA and 14 (56%) of the dogs fecal samples were negative. Table 2 shows the prevalence of Cryptosporidium among the two groups of animals.

Distribution of *Cryptosporidium* according to the gender of the animals

Of the 25 samples collected from the cats in the Thohoyandou region, 12 (48%) were females while 13 (52%) were males. The female cats were more infected than the males although the difference was not statistically significant (χ^2=0.019, p=0.891). Of the 12 female cats, 4 (33.3%) were positive for *Cryptosporidium* spp while 8 (66.7%) were negative. Of the 13 male cats, 4 (30.8%) tested positive while 9 (69.2%) were negative.

Out the 25 samples collected from the dogs, 12 (48%) fecal samples were from female dogs while 13 (52%) fecal samples were from male dogs. However, male dogs appeared to be more infected than the females and the difference was close to be significant (χ^2=3.381, p=0.066) and the Odd Ratio 4.800, 95% CI: 0.860 – 26.785. Out of the 12 female dogs 3 (25%) were infected with *Cryptosporidium* while 9 (75%) were negative. Among the 13 male dogs 8 (61.5%) were positive for *Cryptosporidium* while 5 (38.5%) were negative. Table 3 shows the distribution of *Cryptosporidium* according to the gender of the animal.

Distribution of *Cryptosporidium* according to the age of the animals

In total, 8 (16%) of the 50 samples collected were from young animals (age less than 6 months) while 42 (84%) were adults. Among the cats only 2 (8%) fecal samples were from kittens while 23 (92%) fecal samples were from adult cats. All 2 samples from young cats were negative and 8 (34.8%) from the 23 adult cats samples tested positive for *Cryptosporidium* DNA and 15 (65.2%) were negative (Table 4). Among the dogs, 6 (24%) samples were from puppies and 19 (76%) were from adult dogs. From the 6 samples collected from puppies, 3 (50%) were positive for *Cryptosporidium* DNA and 3 (50%) were negative. In adult dogs, 8 (42.2%) were positive and 11 (57.8%) were negative (Table 4).

Table 2. Prevalence of Cryptosporidium among cats and dogs in the Thohoyandou region.

Animal type	Cryptosporidium (PCR) Number (%)		Total
	Negative	Positive	
Cat	17 (68.0%)	8 (32.0%)	25
Dog	14 (56.0%)	11 (44.0%)	25
Total	31 (62.0%)	19 (38.0%)	50
χ^2=0.764, p=0.280			
OR=1.670, 95% CI: 0.527 – 5.290			

Table 3. The distribution of cryptosporidium in different genders of animal type.

Animal type	Sex	Cryptosporidium PCR		Total	Statistic
		Negative	Positive		
Cats	Female	8 (66.7%)	4 (33.3%)	12 (48%)	-
	Male	9 (69.2%)	4 (30.8%)	13 (52%)	χ^2=0.019, p=0.891
	Total	17 (68.0%)	8 (32.0%)	25	OR=0.889, 95% CI: 0.165 – 4.777
Dogs	Female	9 (75.0%)	3 (25.0%)	12 (48%)	-
	Male	5 (38.5%)	8 (61.5%)	13 (52%)	χ^2=3.381, p=0.066
	Total	14 (56.0%)	11 (44.0%)	25	OR=4.800, 95% CI: 0.860 – 26.785

Table 4. The distribution of *Cryptosporidium* according to the age of the animals.

Animal type	Age	Cryptosporidium PCR		Total	Statistic
		Negative	Positive		
Cat	young	2 (100.0%)		2	-
	adult	15 (65.2%)	8 (34.8%)	23	χ^2=1.023, p=0.312
	Total	17 (68.0%)	8 (32.0%)	25	
Dog	young	3 (50%)	3 (50%)	6	-
	adult	11 (57.8%)	8 (42.2%)	19	χ^2=0.982, p=0.546
		14 (56.0%)	11 (44.0%)	25	OR=1.800, 95% CI: 0.264 – 2.573

Distribution of *Cryptosporidium* in the different areas of the Thohoyandou region

The samples were collected from five different areas of the Thohoyandou region namely P-west, P-east, Maungani, Tshisahulu and Sibasa. Among the cats, 4 samples were from P- west, of which 1 (25%) was positive and the other 3 (75%) were negative. Four samples were from P-east of which 3 (75%) were positive for *Cryptosporidium* and 1 (25%) was negative. Four samples were from Maungani of which 1 (25%) was positive and the other 3 (75%) were negative. Six of the samples were from Tshisahulu of which 2 (33.3%) samples were positive for *Cryptosporidium* and 4 (66.7%) were negative. Seven samples were from Sibasa and out of the 7 samples only 1 (14.3%) sample was positive and 6 (85.7%) were negative. Among the dogs, 6 samples were from P- west. From these 6 samples, 3(50%) were positive for *Cryptosporidium* while the other 3 (50%) were negative. Five samples were collected from P-east and all of the samples were negative. Five samples were collected from Maungani and out of these 5 samples, 4 (80%) were positive for *Cryptosporidium* and only 1 (20%) was negative. Four samples were collected from Tshisahulu and all of the samples 4 (100%) were positive for *Cryptosporidium* DNA. Five samples were collected from Sibasa and all the 5 samples were

Table 5. The distribution of *Cryptosporidium* in different origin of the samples of the animal type.

Animal type	Origin of the sample	*Cryptosporidium PCR*		Total
		Negative	Positive	
Cat	P-west	3 (75%)	1 (25%)	4
	P-east	1 (25%)	3 (75%)	4
	Maungani	3 (75%)	1 (25%)	4
	Tshisaulu	4 (66.7%)	2 (33.3%)	6
	Sibasa	6 (85.7%)	1 (14.3%)	7
	Total	17 (68%)	8 (32%)	25
Dog	P-west	3 (50%)	3 (50%)	6
	P-east	5 (100%)		5
	Maungani	1 (20%)	4 (80%)	5
	Tshisaulu		4 (100%)	4
	Sibassa	5 (100.0%)		5
	Total	14 (56.0%)	11 (56.0%)	25

negative for *Cryptosporidium*. Table 5 shows the distribution of *Cryptosporidium* in different areas of the Thohoyandou region.

Distribution of *Cryptosporidium* in animal stools according to the diarrheal characteristic of the sample

Of all the samples collected 21 were diarrheal (according to the softness of the sample) while the other 29 were hard (Non diarrheal). Among the cats, 18 samples were non diarrheal while 7 were diarrheal. Of the 18 non diarrheal fecal samples 5 (27.8%) were positive for *Cryptosporidium* and 13 (42.9%) were negative. Of the 7 diarrheal samples, 3 (42.9%) were positive for *Cryptosporidium* and 4 (57.1%) were negative.

Among the dogs, 11 of the fecal sample collected from dogs were non diarrheal and 14 of the fecal sample were diarrheal. Out of the 11 non diarrheal fecal samples, 4 (36.4%) were *Cryptosporidium* positive and 7 (63.6%) were negative. Seven (50%) of the 14 diarrheal fecal samples were C*ryptosporidium* positive and 7 (50%) were negative. Table 6 shows the distribution of *Cryptosporidium* in animal stools according to the diarrheal characteristic of the sample.

The distribution of *Cryptosporidium* among cats and dogs in the Thohoyandou region according to their life style (Stray or Home based)

Of the 50 samples collected, 17 were from stray animals and 33 were collected from home based animals. Among the cats, 21 were home based (living in a house with their owner) while 4 were stray animals living in the bush on their own. Out of the 21 sample from home based cats, 5 (23.8%) were positive for *Cryptosporidium* and 16 (76.2%) were negative. Of the 4 samples from stray cats, 3 (75%) were *Cryptosporidium* positive and 1 (25%) was negative. Stray cats were thus more infected than home based animals and the difference was statistically significant (χ^2=4.046, p=0.044). Among the dogs, 13 of the fecal samples were from stray dogs and 12 were from home based animals. Out of the 13 fecal samples from stray dogs 6 (46.2%) were positive for *Cryptosporidium* DNA and 7 (53.8%) were negative while among the home based animals, 5 (41.7%) were positive for *Cryptosporidium* and 7 (58.3%) were negative. Stray dogs were more infected than home based animals even though the difference was not significant (χ^2=0.051, p=0.821). Table 7 shows the distribution of *Cryptosporidium* according to their life style.

DISCUSSION

The present study investigated the prevalence of *Cryptosporidium* spp among cats and dogs in the Thohoyandou region as well as their distribution according to the demographics of the animals and geographic location. In general, dogs had higher *Cryptosporidium* infection rate than cats even though the difference was not significant. More urbanized areas had lower infection rate as compared to rural areas and stray animal were also more infected. This could be due to the presence of better sanitation and/or the presence of the veterinary center in this urban area, thus people in these urban areas may be aware of the parasitic infections of these companion animals and they take good care (medication and food wise) of the animals. Previous studies in Venda have also indicated poor level of hygiene in Venda (Potgieter et al., 2005). However, more detailed studies need to be conducted

Table 6. The distribution of *Cryptosporidium* in different sample characteristics.

Animal type	Samples characteristic	*Cryptosporidium PCR*		Total	Statistics
		Negative	Positive		
Cat	Non Diarrheal	13 (72.2%)	5 (27.8)	18	-
	Diarrheal	4 (57.1%)	3 (42.9%)	7	χ^2=0.527, p=0.468
	Total	17 (68.0%)	8 (32.0%)	25	OR=1.950, 95% CI: 0.317-12.009
Dog	Non Diarrheal	7 (63.6%)	4 (36.4%)	11	
	Diarrheal	7 (50.0%)	7 (50.0%)	14	χ^2=0.465,p=0.495
		14 (56.0%)	11 (44.0%)	25	OR=1.750, 95% CI: 0.348 –8.795

Table 7. Distribution of *Cryptosporidium* in different life style of animals.

Animal type	Life style	*Cryptosporidium PCR*		Total	Statistics
		Negative	Positive		
Cat	Home based	16 (76.2%)	5 (23.8%)	21	-
	Stray	1 (25%)	3 (75%)	4	χ^2=4.046, p=0.044
	Total	17 (68%)	8 (32%)	25	OR=9.600, 95% CI: 0.807 – 114.173
Dog	Home based	7 (58.3%)	5 (41.7%)	12	-
	Stray	7 (53.8%)	6 (46.2%)	13	χ^2=0.051, p=0.821
	Total	14 (56%)	11 (44%)	25	OR=1.200, 95% CI: 0.246 – 5.844

in order to clarify the role of hygienic habits in the transmission of *Cryptosporidium* as well as other parasitic organisms in the Venda region. With respect to the hygienic–sanitary conditions, it was noted that properties with poor hygienic conditions were more affected by *Giardia* sp. and *Cryptosporidium* sp., confirming that poor sanitation is a predisposing factor for the infection with these protozoans, as previously described (El-Sherbini et al., 2012; FitzGerald et al., 2011; Mohammed et al., 1999; Teixeira et al., 2007).

In our study, the prevalence of *Cryptosporidium* in cats and dogs (about 40%) was higher compared to the prevalence in human (16%) previously described by our group in the Vhembe district (Samie et al., 2006). However, similar distribution of *Cryptosporidium* has been described in cats and dogs elsewhere. For example parasitological study of stool samples from cats and dogs in Ontario, Canada, showed a high overall positivity rate in samples from both dogs (40%) and cats (36.6%) (Shukla et al., 2006) while in a study in Costa Rica 75% of dogs and 67% of cats were found to be infected (Scorza et al., 2011). In the present study we also found that cats were less infected than dogs (32 and 44% respectively). This might be due to the fact that most of the cats' fecal samples were from home based cats. Also, owned cats live mostly indoors unlike home based dogs which also spend most of their time outdoors where they interact with other animals. Stray or outdoors animals were more infected than home based animals with a significant difference among stray and home based cats ($p<0.05$). This might be explained by the fact that outdoors animals will easily get in contact with contaminated food and water as well as other infected animals with which they interact. Studies by other authors, also reported that outdoor cats were approximately five times more likely than indoor cats to be infected with *Cryptosporidium* species, which is consistent with greater opportunity for the cats to prey on infected hosts or become infected through contaminated soil or water (McReynolds et al., 1999; Rambozzi et al., 2007). In the present study, all stray cats and dogs had higher infections than the home based animals. The determination of the specific genotypes in these animals will be useful in further understanding the risk of these animals for zoonotic infections by those *Cryptosporidium* spp that are able to infect humans.

Among the home based dogs only 5 (41.7%) out of the 12 owned dogs fecal samples were infected. Whereas in cats only 5 (23.8%) out of the 21 owned cat fecal samples. Both the stray dogs 6 (46%) and cats 3 (75%) had a higher number of *cryptosporidium* infections; these could be due to social (pack) behaviors of dogs which help in

the spread or transmission of these parasite through contact of the infected animals. In the city of Campos dos Goytacazes, in Brazil, 45% of the 200 dogs stool analyzed were positive for Cryptosporidim and the identified risk factors included the social level of the owner, presence of cats and sporadic stage of vomiting and diarrhea (Ederli et al., 2008).

Bugg et al. (1999) in their study of parasitism in dogs also demonstrated a significant influence of the presence of multiple dogs can affect the prevalence of parasites in dogs in Australia. The current study also found that the degree of contact that cats and dogs had with other cats and dogs significantly influenced the prevalence of parasitic infection. It is also possible, that among the stray dogs and cats especially those living in the P-west, Maungani and Tshisahulu are infected due to the river water contaminated with *cryptosporidium* spp. as these animals are generally seen during the day drinking from the river. In P-west rain water collects at Mvundi River and fecal waste of infected animals can be washed away by the rain water to the river, which is already contaminated by other waste products (plastics, papers, tins, etc.). However, more detailed research need to be conducted in order to prove the presence of these parasites in the river water. This could be the same way some stray animals in Maungani and Tshisahulu get their infections as the animals drink from Dzindi River which flows through these areas. Previous studies have demonstrated that juvenile domestic animals such as goats, calves, puppies, and kittens were significantly more parasitized by *Giardia* sp. and *Cryptosporidium* sp. than adult animals (Cardoso et al., 2012; Matos-Fernandez et al. 1993). Noordeen et al. (2001) found a strong correlation between the age and presence of the protozoan in goat stools, with young animals being more susceptible than adult ones. In the present study we found similar results, particularly with young dogs (puppies) in which half [3 (50%)] of the 6 individuals were infected with *Cryptosporidium* spp. compared to 42.2% out of the 19 adult dogs samples collected. These juvenile animals might be more venerable to the infection by these parasites because of their immature immune systems. Among the cats, stools were collected only from 2 kittens and both were negative for *Cryptosporidium*. This could be due to the fact that the 2 young cats fecal samples were collected in Sibasa, which is an urban location with better sanitation and those young cats were home based. More studies are thus needed with higher numbers of samples to clarify the role of young age on the infectivity of *Cryptosporidium* among young cats. The age of the cat could be an important risk factor associated with parasitic infection, with cats less than 6 months old being more likely to be parasitized than older cats. These findings were obtained in previous studies (Hill et al., 2000; Spain et al., 2001).

In the present study, *Cryptosporidium* was commonly found in diarrheal samples, even though the difference with non diarrheal samples was not significant. From this research one can hypothesise that there is correlation between *Cryptosporidium* infection and diarrheal stools among cats and dogs. These could be because of compromised immunity due to poor diet, depression or stress, seasonal changes, etc. Further investigations are needed to be done to precisely determine this correlation (de Oliveira Lemos et al., 2012). In a study in Colorado, dogs attending Dog park were more likely to be positive for *Giardia* or *Cryptosporidium* than non-dog park-atten-ding dogs (p=0.0279) (Wang et al., 2012). This therefore indicates that other risk factors such as socializing might also increase the chances of animals to be infected.

In conclusion, this study has for the first time demonstrated a high prevalence of *Cryptosporidium* among cats and dogs. Animals from more rural parts of the region were found to be more infected than those living in more urbanized areas indicating that poor sanitation might constitute a risk factor for these animals to be infected by enteric parasites such as *Cryptosporidium*. Although this study was not able to demonstrate any possible zoonotic transmission of *Cryptosporidium* from these animals to human, it can be argued that the high level of *Cryptosporidium* in these animals constitutes a great risk for humans living with these animals and to the environment and deserves the attention of policy makers to take more stringent measures for the control of these infections in human communities. It is particularly important to control domestic animals and limit their movement to avoid them getting contaminated.

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