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Prevalence and risk factors of *Cryptosporidium* species among domestic animals in rural communities in Northern South Africa

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Abstract. To determine the prevalence and possible risk factors for the transmission of *Cryptosporidium* species among animals in rural Limpopo Province, South Africa. A total of 314 stool samples from 64 households were collected from animals in three villages situated in the Vhembe and Mopani Districts, South Africa and examined for *Cryptosporidium*, using the modified Ziehl Neelsen technique and confirmed by the real time PCR method. A questionnaire was developed to capture demographic data as well as other household information from the owners of the animals. Positive samples were further sequenced for the identification of the species present in the samples. The overall prevalence of *Cryptosporidium* among the animals was 31.2%. Of all the animal types tested goats (47.7%) appeared to be the most infected followed by cattle (26.8%) and chicken (7.4%). From the 64 households surveyed 43 (67.2%) had at least one or more infected animals. Adult animals were more infected (32%) compared to young animals (29%) but the difference was not statistically significant (p=0.793). The gender of the animal as well as the consistency of the stool did not affect the occurrence of *Cryptosporidium*; however, the level of education as well as the gender of the owners significantly affected the prevalence of *Cryptosporidium* among the animals they kept. *C. parvum* was the most commonly isolated organism while *C. andersoni* was identified in our region for the first time as well and occurred in both goats and cattle. This study showed a high prevalence of *Cryptosporidium* in domestic animals, which could constitute a health threat to both animals and humans in the region. The gender of the head of the Household and level of education were very significant factors in the prevalence of *Cryptosporidium* among the animals. Community education will be useful in helping reduce the impact of these infections.

INTRODUCTION

Cryptosporidium species are coccidian protozoan parasites that can live in the intestine of humans and animals. They are found worldwide, where infection occurs most commonly in individuals who are immunocompromised (Ettinger & Feldman, 1995, Helmy *et al*., 2015). The development of *Cryptosporidium* infections in immunocompromised individuals is a key concern because of its association with malnutrition and severe disease among these individuals (Abdou *et al*., 2013). Outbreaks of *Cryptosporidium* associated diarrhea have been associated with fecally-contaminated recreational waters and day care centers, and infected farm animals have also been recorded (Giratto *et al*., 2003; Levallois *et al*., 2013).

Humans, wildlife and domestic livestock potentially contribute *Cryptosporidium* to surface waters (Ryan & Power, 2002). On farms, transmission of *Cryptosporidium* spp. can result from ingestion of contaminated food or water, by direct transmission from

host to host, or through insect vectors (Follet-Dumoulin *et al.*, 2001). The disease is readily transmissible since oocysts persist for long periods in suitable environment and low numbers of oocysts may produce infection in susceptible hosts (Castro-Hermida *et al.*, 2002; Ramirez *et al.*, 2004). In animals, cattle are most commonly affected by *Cryptosporidium*, and their feces are often assumed to be a source of infection for other mammals including humans.

Cryptosporidium oocysts excreted with faeces of infected farm animals, particularly calves, can be a source of human infection and may have a great influence on public health (Majewska *et al.*, 1999). The proportion of human cryptosporidiosis of zoonotic origin is unknown in South Africa, but infection in domestic and wild ruminants provide the greatest source of environmental contamination for human infection either by direct contact or indirectly through faecal contamination of food or water for human consumption (Tzipori & Griffiths, 1999; Smith *et al.*, 2006). Most data on the incidence of *Cryptosporidium* infection in animals are related to cattle; however, very little data exist on the occurrence of *Cryptosporidium* among farm animals in South Africa. Also ovine cryptosporidiosis seems to be widespread and epidemiological studies have indicated that this protozoan is common in sheep and goat herds, although its prevalence is not as well documented as in cattle (Causapé *et al.*, 2002), and that the infection often causes death of diarrhoeic lambs and kids (Kaminjolo *et al.*, 1993; Olson *et al.*, 1997).

The Vhembe and Mopani Districts are situated in the Northern part of South Africa and is mostly rural with a high number of cattle and other animals being kept by the population. In this region most people keep different domestic animals and are very often in contact with them, and even those who do not have animals frequently go and collect dry stools to fertilize garden soil; whereas some women in the villages go and collect fresh cattle dung to decorate their yards and huts. Therefore, the present study determined the occurrence and distribution of *Cryptosporidium* species among household animals in rural areas in the Mopani District situated in the Northern region of South Africa as well as the potential risk factors that could contribute to the transmission of these organisms among animals living in these communities.

MATERIALS AND METHODS

Study area and survey

The selected study areas were three villages located in Malamulele and Giyani regions and included Makuleke village, Ngove village, and Nkomo village. Makuleke is situated on the eastern side, about 35 kilometers away from the small town, Malamulele, but very close to the fence of Kruger National Park near the Punda Maria gate. This rural area is part of Vhembe District in the Limpopo Province. Although there was no sheep herd during the survey, Makuleke is one of the wide villages where cattle, goats and chickens are the most common domestic animals. Apart from the rivers around, animals depend on the water from Makuleke dam for drinking. Ngove and Nkomo are also big villages very close to each other and situated near Giyani town under Mopani District in the Limpopo Province. These two villages have similar types of animals as those found in Makuleke village and sheep are rare. The areas of drinking water by animals are commonly rivers.

Before sample collection, a survey was conducted to collect demographic as well as other data concerning the animals. Questionnaires were distributed to the participating households asking the owner of the animals about the following: Education level; Occupation; Age; Sex; Marital status; Household income; Number of people in the household; children less than 5 years, children with diarrhea, household diarrhea over the last three months, Age of the person(s); Type of treatment sought by household in case of diarrhea, for example, traditional, public or private clinic/hospital; possible cause of diarrhea, Number of animals in compound; where do they sleep? How do they give them food? General health of the animals, geophagia habit of the animal

owners, as well as primary water source. Other information collected concerned Household water treatment, by the owners, water storage in the household and hygiene and sanitation. The survey questionnaires were collected through interview by a trained researcher and the data was entered into an excel sheet for further processing.

The study was approved by the University of Venda research committee. The objectives of the study were explained to the owners and their consent obtained before the beginning of the study.

Sample collection

Fresh stool samples were randomly collected immediately after they were dropped by the animals, and put in a tightly closed collection containers and marked properly, then transported to the laboratory and stored in the fridge at 4ºC until they were tested. The total number of samples examined was 313, (of which 187 samples were obtained from cattle, 93 samples from goats, 28 sample from chicken, 4 samples from sheep and 1 sample from human). From all the samples, 166 were collected in Ngove village, 141 samples were collected in Nkomo village, whereas only 6 samples were collected in Makuleke village. Many of the tested animals were adult although samples from young animals were also collected. None of the animals was aged less than two weeks of age at the time of samples collection.

Sample analysis

A total of 313 stool samples were collected from cattle, goats, sheep, and chicken compounded in the total number of 63 households in three villages in the Limpopo Province. The modified Ziehl Neelsen technique was used to detect *Cryptosporidium* oocysts from each sample (Morgan *et al*., 1998). Briefly, approximately 10 µl of sample was smeared on a slide and left to air dry. The smear was fixed using 100% methanol. The smear was flooded with Carbol-fuchsin and rinsed with tap water after one minute. The slide was decolorized with 5% sulphuric acid and then counter stained with methylene blue for 1-2 minutes. Finally the slides were rinsed with water and allowed to air dry. The slides were then observed under the microscope at 100 x magnifications using immersion oil for the presence or absence of the oocysts.

Detection of Cryptosporidium by quantitative Real time PCR (qPCR)

The real time PCR technique targeting specific sequence of the 18s rRNA gene was used with few modifications (Samie *et al*., 2006), to confirm the microscopic detection of *Cryptosporidium* spp. The primers used were Crypt F: 5' – CTG CGA ATG GCT CAT TAT ACCA-3' and Crypt R: 5' – AGG CCA ATA CCC TAC CGT CT-3'. The reaction was run in a total volume of 25 µl made of 12.5 µl of the Maxima^{tm} SYBR® Green Supermix (Fermentas) (2X), 0.4 µl of each primer (20 pmol/µl), 6.7 µl of Nuclease Free (DNase, RNase, and Proteinase) water (Fisher Biotech, NJ) and 5 µl of genomic DNA extract. The real-time PCR conditions were four steps with step 1 for 13.50 min at 95ºC, step 2 was repeated 50 times with 15s denaturation at 95ºC, 15s annealing at 60ºC and 20s chain extension at 72ºC with data collection enabled during the last two steps. The third step was repeated 50 times with 0.5ºC set point temperature increase after step 2. The last step was held at 4ºC. Each run included at least two positive controls (Genomic DNA extracted from pure *Cryptosporidium* oocysts) and one negative control (Distilled water).

Sequence analysis for the identification of the infecting species

Positive samples were sent to Inqaba Biotech (Pretoria, South Africa) for sequencing. Once the sequences were received from the commercial company, these were edited using Staden package software, Bioedit and MEGA6 software were used to align sequences and draw phylogenetic trees.

Data analysis

The results of the study were analyzed using the SPSS software Version 20.1. The chi-square (χ^2) test was used to determine the relationship between *Cryptosporidium*

results of the animals and other parameters. The differences were considered significant when the p value was less than 0.05.

RESULTS

Demographic characteristics of the study population

A total of 314 samples were collected from 64 households from three different villages in the Vhembe and Mopani Districts. The number of samples collected from each household varied between 1 and 16. Most of the samples came from cattle (62%) followed by goats (28%), chicken (8.6%) and 4 samples (1.3%) were collected from sheep. Most of the samples were from adult animals while only 21% were from young animals. Most of the stools samples were hard while only about 12% were soft. The animals were kept in an open shade in the compound and were scavenging for food around the compound or in the vicinity of the villages. All the households indicated that their animals were healthy except two that indicated that some animals were sick. However, the type of sickness was not indicated but was not related to diarrhea.

Demographic characteristics of the participating owners

Among the owners of the animals, most were males (64%) and most of the owners (61%) were aged above 45 years. The majority of the owners were married (72%) and many did not have formal education (34%) while 33% had some secondary education and 9% had had some tertiary education mostly at the level of Diploma. About 54% of the households had more than 5 people and 41% had a child of less than 5 years old. About 15% of the households indicated that a child had had diarrhea over the past three months while a total 24% indicated that at least one person in that household had had diarrhea over the past three months. Most of the household (28%) had an income less than 1000Rands per month while 8.7% had an income above 12000Rands.

Animal ownership among the population

According to the survey conducted, households were keeping up to 5 different types of animals at the same time. In fact most households (30%) kept three different types of animals while 3% kept up to 5 types of animals at a time. The most common animals kept were cattle (79% of the household) while about 10% kept cats and 5% kept the donkey.

Water sources and other hygiene habits among the owners

Most of the households (54%) used water from the communal taps, while 31% used water from the borehole and 9% used rain water when available. About 25% of the studied population indicated that they treat their water before drinking the most common form of treatment of boiling followed by the use of chlorination (mostly jik). All households stored their water due to the fact that the water is not always available in the taps or that the taps were far from the compound and 22% of the households stored their water for more than 7 days. Many households collected the water from the containers by pouring it directly while 70% used a cup with handle. About 12% of the households cleaned their containers after a week while 19% cleaned their container after a month or more. Most of the participants used pit latrines while 8% used flush toilet.

Prevalence of cryptosporidium in the animal population

From a total of 64 households surveyed, 21 (32.8%) did not have infected animals. The prevalence of infected animals in the households varied from 11 to 100% of all the animals kept in that specific household. Of the 314 samples 98 (31.2%) tested positive for *Cryptosporidium*. Of all the animals tested, goat (47.7%) appeared to be the most infected of followed by cattle (26.8%) and chicken (7.4%) (Table 1). Only 4 sheep were tested and 2 were positive.

Prevalence of cryptosporidium among the animals according to the characteristics of the animals

There was no difference in the prevalence of *Cryptosporidium* among male and female animals, and adult animals were more infected (32%) compared to young animals (29%) but the difference was not statistically significant (p=0.793). The prevalence of *Cryptosporidium* in the stool of the animals varied between the villages. Only 6 samples were collected from Makuleke and all of them were found to be infected with *Cryptosporidium*. In Ngove Village 35% of the animals were infected while in Nkomo 23% were infected. The occurrence of *Cryptosporidium* did not vary with the consistency of the stools and was 32% and 31% in soft and hard stools respectively (Table 1).

Prevalence of cryptosporidium among the animals according to the characteristic of the owner

The prevalence of *Cryptosporidium* varied according to the level of education of the owners and was more common among animals that were kept by persons with no formal education (40%) compared to those that were kept by individuals that had a tertiary education (15%) (Table 3). The prevalence of *Cryptosporidium* did not vary according to the age of the owners however; there was a statistically significant difference between the prevalence of *Cryptosporidium* in the animals according to the sex of the owner and was 35% among animals owned by females compared to 28% for animals kept by male owners. The prevalence of *Cryptosporidium* was also higher among animals that were kept by individuals with lower income although animals from household with income above 12000 had a higher prevalence compared to those that had an income between 6001 and 12000Rands. The prevalence of *Cryptosporidium* was higher among animals that were kept by widows (67%) while the prevalence of Cryptosporidium was lower among animals owned by individuals that were married (26%).

Prevalence of Cryptosporidium in relation to some household characteristics

There was no difference between the occurrences of *Cryptosporidium* among animals kept by households with or without

Table 1. Prevalence of *Cryptosporidium* among the animals according to the characteristics of the animals

Parameter	Target	Crypto positive	Total	Statistics (Chi square and p value)
Sex of the animal	Female	74 (32.5%)	228	χ^2=2.775; p=0.250
	Male	22 (31.4%)	70	
Age of the animal	Adult	78 (31.6%)	247	χ^2=0.463; p=0.793
	Young	19 (29.2%)	65	
Consistency of the stool	Soft	12 (32.4%)	37	χ^2=0.029; p=0.864
	Hard	86 (31%)	277	
Village	Makuleke	6 (100%)	6	χ^2=18.54; p=0.001
	Ngove	59 (35.3%)	167	
	Nkomo	33 (23.4%)	141	
Animal type	Cattle	52 (26.8%)	194	χ^2=4.590; p=0.032
	Goat	42 (47.7%)	88	χ^2=15.540; p=0.0001
	Chicken	2 (7.4%)	27	χ^2=7.796; p=0.005
	Sheep	2 (50%)	4	χ^2=0.666; p=0.414
Total		98 (31.2%)	314	

diarrhea particularly in children. However, there was association with the occurrence of diarrhea in the household in general. The prevalence of *Cryptosporidium* was lower among animals that were kept in households that had less than 5 people compared to those that were kept in households with more than 6 people (35% and 27% respectively) (Table 2).

Prevalence of Cryptosporidium among the animals in relation to the presence of other animals in the household

The prevalence of *Cryptosporidium* among the animals tended to increase with the number of species of animals kept by the household (Table 4). For example the prevalence among animals kept in household with a single species of animal was 29% and

Table 2. Prevalence of *Cryptosporidium* in relation to some household characteristics

Parameter	Target	Positive	Total	Statistics
No. of people in the household	Five people or less	50 (35%)	143	χ^2=2.331; p=0.127
	More than five people	45 (26.9%)	167	
Children less than 5 years old	No	55 (30.6%)	180	χ^2=6.676; p=0.036
	Yes	40 (30.5%)	131	
Diarrhoea in household	No	59 (36.9%)	160	χ^2=11.260; p=0.004
	Yes	5 (10.9%)	46	
Eating soil	No	88 (31.5%)	279	χ^2=7.925; p=0.019
	Yes	7 (21.9%)	32	

Table 3. Prevalence of *Cryptosporidium* among the animals according to the characteristic of the owner

Parameter	Target	Crypto positive	Total	Statistics
Education level	No formal education	42 (39.6%)	106	χ^2=11.031; p=0.012
	Primary education	28 (36.4%)	77	
	Secondary education	24 (23.1%)	104	
	Tertiary education	4 (14.8%)	27	
Age of owner	Less than 45	20 (33.9%)	59	χ^2=0.262; p=0.609
	Above 45	58 (30.4%)	191	
Sex of owner	Female	38 (34.9%)	109	χ^2=8.132; p=0.017
	Male	57 (28.2%)	202	
Marital status of owner	Married	58 (25.6%)	227	
	Single	33 (42.3%)	78	
	Widow	4 (66.7%)	6	
Household income	R1–R500	19 (22.1%)	86	
	R501–R1000	27 (41.5%)	65	
	R1001–R3000	30 (39%)	77	
	R3001–R6000	5 (29.4%)	17	
	R6001–R12000	7 (17.9%)	39	
	More than 12000	7 (25.9%)	27	

Table 4. Prevalence of *Cryptosporidium* among the animals in relation to the presence of other animals in the household

Parameter	Target	Positive *Cryptosporidium*	Total	Statistics
Number of animal type	1	25 (28.7%)	87	χ^2=10.406; p=0.034
	2	38 (42.2%)	90	
	3	20 (21.1%)	95	
	4	11 (34.4%)	32	
	5	4 (40%)	10	
Cattle	No	27 (41.5%)	67	χ^2=4.073; p=0.044
	Yes	71 (28.5%)	249	
Goat	No	53 (27.5%)	193	χ^2=3.279; p=0.070
	Yes	45 (37.2%)	121	
Chicken	No	39 (37.9%)	103	χ^2=3.161; p=0.075
	Yes	59 (28%)	211	
Cats	No	85 (30%)	283	χ^2=1.843; p=0.175
	Yes	13 (41.9%)	31	
Dog	No	79 (29.9%)	264	χ^2=1.277; p=0.258
	Yes	19 (38%)	50	
Donkey	No	97 (32.7%)	297	χ^2=5.370; p=0.020
	Yes	1 (5.9%)	17	

42% among animals kept in households with two species and 40% for animals kept in households with five species. In general, there was a high prevalence among animals that were kept in household with cats and dogs in comparison to the other animals while the prevalence was lower among animals that were kept together with cattle, chicken and donkey. There was however a statistically significant association between the prevalence of *Cryptosporidium* among animals that were kept in households where there were goats.

Prevalence of Cryptosporidium among the animals in relation to hygiene and other feeding habits adopted in the household

There was no association between the prevalence of *Cryptosporidium* among animals and the type of place in the compound where they were kept whether it was open shade or closed shade. However, animals kept by households that sometimes used containers to feed the animals had a lower prevalence (27%) compared to those in the households where animals always scavenged (32%). There was no association between the prevalence of *Cryptosporidium* among animals according to water sources used by the household but there was a slight relationship between the prevalence of *Cryptosporidium* among the animals and the level of hygiene in the households in terms of water storage, cleaning of containers and the way they collected water from the containers (Table 5).

Cryptosporidium *genotyping*

The type of *Cryptosporidium* present in the stool samples was determined after sequence analysis of the PCR amplicons. Of all the samples that were positive by real time PCR from the animals, 12 were successfully sequenced and two species (*C. parvum* and *C. andersoni*) were identified. Of these 6 were from cattle and the other 6 were from goats. Out of the 12 samples 10 (83%) were

Table 5. Prevalence of *Cryptosporidium* among the animals in relation to hygiene and other feeding habits adopted in the household

Parameter	Target	Positive	Total	Statistics
Open shade	Closed shade	21 (32.3%)	65	χ^2=0.120; p=0.729
	Open shade	74 (30.1%)	246	
Animal feeding	Always scavenging	86 (32%)	269	χ^2=0.505; p=0.477
	Use container	12 (26.7%)	45	
General health of your animals	Good health	91 (31%)	294	χ^2=0.417; p=0.518
	Some sickness	4 (23.5%)	17	
Rain water usage	No	88 (30.7%)	287	χ^2=0.467; p=0.494
	Yes	10 (37%)	27	
Communal tap	No	39 (27.1%)	144	χ^2=2.110; p=0.146
	Yes	59 (34.7%)	170	
Tap in the yard	No	75 (30.7%)	244	χ^2=0.114; p=0.736
	Yes	23 (32.9%)	70	
Borehole	No	75 (34.4%)	218	χ^2=3.387; p=0.066
	Yes	23 (24%)	96	
Water storage	Less than 7 days	74 (30.2%)	245	χ^2=0.526; p=0.468
	More than 7 days	24 (34.8%)	69	
Water collection	Cup with handle	61 (27.9%)	219	χ^2=3.798; p=0.051
	Pour directly	37 (38.9%)	95	
How often do you clean the container	7 days or less	63 (28.9%)	218	χ^2=2.228; p=0.328
	>1 week <1 month	12 (32.4%)	37	
	More than a month	23 (39%)	59	
Open pit	No	14 (26.9%)	52	χ^2=0.534; p=0.465
	Yes	84 (32.1%)	262	
Use the bush	No	95 (32.2%)	295	χ^2=2.240; p=0.134
	Yes	3 (15.8%)	19	
Flush toilet	No	90 (31.3%)	288	χ^2=0.003; p=0.960
	Yes	8 (30.8%)	26	

C. parvum while 2 (17%) were *C. andersoni*. Of the two *C. andersoni*, one was from a goat and one was from a cow. Of the 10 *C. parvum*, 5 were from goats and 5 were from cattle.

DISCUSSION

The present study showed that there was a high prevalence of *Cryptosporidium* spp. (31.0%) in our study areas. Studies in other parts of the African Continent have reported infection rates as low as 7.5% in Ethiopia (Wegayehu *et al.*, 2013). Although, only 6 samples were collected from one of the study sites, known as Makuleke village, all were found to have *Cryptosporidium*. This could be due to the fact that the village is very close to the fence of the Kruger National Park (KNP). Wildlife, especially elephants, exiting the KNP may be potentially important disseminators of *Cryptosporidium* oocysts within the environment, exposing domestic

animals and humans to possible infection (Samra *et al.*, 2010). In a previous study *Cryptosporidium* was found to be common in sheep and goat herds (Causapé *et al.*, 2002), and that was confirmed in the present study with high infection in sheep and goats (48.0% and 45.0% respectively) compared to that of cattle (25.0%) and chicken (5.0%). The low percentage of infection in chicken may be due to the fact that this parasite tends to cause respiratory infection in chickens instead of intestinal infection (De Graaf *et al.*, 1999), therefore *Cryptosporidium* oocysts may be rare in their stool samples. In some instances there may be no *Cryptosporidium* infection in chickens on examination of fecal samples (Chen & Qiu, 2011). In general, the average rate of infection in Giyani region was higher (30.0%), and ranged from 6.0% of chickens to 48.0% of sheep, compared to the average rate found in China that was about 19.0%, and ranged from 9 to 23.0% on different farms (Chen & Huang, 2012).

Amongst all animals that tested positive, 30.1% were kept in the open shade, whereas high infection 32.3% was in those kept in the closed shade. The the high infection rate in animals that were kept in the closed shade might indicate that these areas could have been dirtier than the open shade environment. A high risk of exposure to *Cryptosporidium* infection in animals could be associated with dirty floors where animals sleep (Swai & Schoeman, 2010). The differences in *Cryptosporidium* infection rates among communities may reflect variation in water quality (Salyer *et al.*, 2012). In the present study, high percentage of *Cryptosporidium* infection was observed in animals kept in households that stored water for more than seven days 34.8%, compared to those from households that stored water from zero to seven days 30.2%. Therefore, the high infection in households that stored water for more than seven days may be indicating poor quality of water used that lead to infection in household members, and then possibly transmitted to animals. The high rate of infection in households where they cleaned containers after a month could be due to unhygienic condition of water in which the containers keep in contact with their hands on a daily basis and possibly with dirty-floor houses (Swai & Schoeman, 2010) where the water containers are kept. There was no difference in the prevalence of *Cryptosporidium* among male 31.4% and female animals 32.5%. Education was recorded as one of the factors that have a more significant impact on the incidence of *Cryptosporidium* infection and similar findings have been described elsewhere (Jarmey-Swan *et al.*, 2010). In the present study, the prevalence of *Cryptosporidium* among household animals varied according to the level of education of the owners and was more common among animals that were kept by persons with no formal education compared to those that were kept by individuals that had a tertiary education, indicating the decreasing rate of animal infection according to the level of education from those with higher education 14.8% to those with no formal education 39.6%. These could possibly mean that people with better education are aware of the possible infections of their animals, therefore reduce all the risks of infection and probably afford the medications of these animals. Better education and increased awareness of cryptosporidiosis by the general public could potentially reduce case numbers (Robertson *et al.*, 2002).

Our results showed that the prevalence of *Cryptosporidium* among the animals tended to increase with the number of different animal types kept by the household. For example the prevalence among animals kept in household with a single species of animal was 29.0%; and 40% for animals kept in households with five different types of animals, and the difference was statistically significant (χ^2=10.406; p=0.034). Therefore the higher infection rate may be due to the overcrowding and the hygienic conditions of the area that encourages the spread of *Cryptosporidium* infection among the animals (Causapé *et al.*, 2002) and must be considered as potential risk factors. If the number of animal species can be reduced from each household, the *Cryptosporidium* infection rate will decrease in the communities. Among all the animals that were tested positive with *Cryptosporidium*, only 23.5% were found having some

sicknesses but none of them had diarrhoea, whereas the rest (31.0%) were healthy. A study in China demonstrated the higher infection rate among cattle with diarrhea than that among asymptomatic animals (Chen & Huang, 2012). Therefore, in the present study we can hypothesize that the asymptomatic animals that were tested positive with *Cryptosporidium* had low infection rate than they could cause diarrhea. The present study demonstrated no variation in *Cryptosporidium* infection with the consistency of the stool samples, 32.4% and 31.0% for soft and hard stools respectively. Similar results were recorded in the study from West Uganda, and the reason could be that, the consistency of all the samples collected was normal (Salyer *et al*., 2012) soft and hard, there were no watery or diarrheal stool samples.

There was no statistical significance between *Cryptosporidium* infection in animals and the types of latrine used in households. This might not have a direct influence on the animals but for the household inhabitants. Different results were obtained from a study in Peruvian children that demonstrated that cryptosporidiosis was more frequent in children from houses without a latrine or toilet (Bern *et al*., 2002). There was a statistically significant difference in the prevalence of *Cryptosporidium* among animals according to the sex of the owner. The highest infection rate 34.9% was among animals owned by females compared to 28.2% for animals kept by male owners. According to the Tsonga customs, it is forbidden for a female to enter in the animal compound because of the myth that it causes miscarriage particularly in cattle, goats and sheep. This may be discouraging women from reducing excess stools for their own animals in the compounds, therefore, promoting high prevalence of *Cryptosporidium* infection in animals own by females.

Cryptosporidium parvum was the most commonly detected organism among both the cow and the goats. This is in accord with other studies that had shown that cattle and goats are infected with *C. parvum* (Taylan-Ozkan *et al*., 2016). Different types of *Cryptosporidium* have been described among animals. Danišová *et al*. (2017) identified a number of *Cryptosporidium* spp. among livestock including *C. parvum*, *C. andersoni* and *C. muris* among others. There was a high prevalence of *Cryptosporidium* infection in sheep and goats in the Giyani region in Mopani District, whereas higher prevalence in cattle was recorded in the Malamulele region in Vhembe District. Education is required to raise awareness in the community particularly those with domestic animals to make a clean environment for their animals and also control the number of animal species in their households in order to reduce the transmission of *Cryptosporidium* infection. Further studies are needed using molecular methods in order to identify the species of *Cryptosporidium* infecting the animals.

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