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Original Article

Cryptosporidium Infection in Sheep and Goats in Southern Botswana and Its Public Health Significance

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ABSTRACT

Fecal samples of 166 sheep and 222 goats collected from 14 farms in southern Botswana were screened for the presence of *Cryptosporidium* infection using enzyme-linked immunosorbent assay (ELISA) and Modified Ziehl-Neelsen (MZN) staining technique. An overall prevalence rate of 13.3% (22/166) in sheep and 12.2% (27/222) in goats were detected. ELISA proved to be more sensitive in detecting *Cryptosporidium* infection than MZN (P = 0.04). Lambs and goat kids aged ≤ 4 weeks showed highest infection rates of 19.4% (13/67) and 16.5% (15/91), respectively. The infection rate of 16.7% (42/251) was recorded in small ruminants ≤ 12 weeks compared to 5.1% (7/137) in adults (P= 0.002). Diarrheic animals demonstrated 20.7% (12/58) prevalence in comparison to 11.2% (37/330) in animals excreting normal solid feces (P= 0.07). The significance levels in the infection rates in sheep versus goats and males versus females were P=0.87 and P= 0.76, respectively. Animals reared under traditional communal management system exhibited more susceptibility to cryptosporidiosis than those under semi-intensive husbandry system (P= 0.04). Of the 62 environmental samples taken, *Cryptosporidium* oocysts were detected in 2 of 27 (7.4%) soil and 1 of 22 (4.5%) water samples. None of 14 manure specimens derived from every sampled small stock farm was found positive. The results of the present study revealed the occurrence of *Cryptosporidium* infection in small stock population in southern Botswana. It suggested potential role of young lambs and goat kids for transmission of human cryptosporidiosis via environmental contamination. Application of molecular techniques for characterization of *Cryptosporidium* field isolates including zoonotic species and genotypes is urgently required. Creation of farmers' awareness through extension education program on good animal husbandry practices will help in devising appropriate strategies to control animal and human cryptosporidiosis.

Key words: *Cryptosporidium*, enzyme-linked immunosorbent assay, feces, goats, oocysts, sheep.

INTRODUCTION

Livestock plays a crucial role both in national economies and livelihood of rural communities of Sub-Saharan African countries (Sibanda *et al*., 2014). Cryptosporidiosis caused by a protozoan parasite of genus *Cryptosporidium* with a cosmopolitan distribution is considered to be one of the principal enteric pathogens producing severe gastroenteritis in new-born ruminants and children. According to Constable (2014) *Cryptosporidium* infection may be associated with severe outbreaks of diarrhea with high case fatality rates in 4 to10 days old lambs and 5 to 21 days old goat kids. *Cryptosporidium* species identified in sheep are *C. parvum, C. ubquitum, C. xiaoi, C. hominis, C. fayeri* and *C. andersoni*, whereas *C. parvum, C. xiaoi* and *C. hominis* may infect goats (Xiao, 2010). Transmission of cryptosporidiosis is through ingestion of oocysts from the infected individuals via contaminated food, water and pasture. Close proximity of humans and livestock as well as the ability of the runoff from livestock production operations contaminating ground and surface water supplies represents an ever present public health risk of transmission of *Cryptosporidium* infection. Several outbreaks of human cryptosporidiosis reported world-wide have been associated with contamination of food and water by cow dung and human stools (Fayer *et al*., 2000; Rose *et al*., 2002). A combined *Cryptosporidium* and *Escherichia coli* infection resulted into deaths of more than 500 children during a water-borne outbreak in 2006 in Botswana. Majority of the infected children were enrolled under HIV/AIDS's prevention of mother to child transmission program (Anonymous, 2007). In a preliminary study carried out by Sharma and Machete (2009) in Gaborone area of Botswana, *C. parvum* infection rates of 13, 16.2, 8.2 and 12.5% were recorded in goat kids, lambs, adult goats and sheep, respectively. The present cross-sectional survey was undertaken to determine *Cryptosporidium* infection in sheep and goats of six semi-intensively and eight extensively managed small stock farms. Water, soil and manure samples collected from the premises of the selected farms were tested for the presence of *Cryptosporidium* organisms and to assess their possible role in contamination of the environment.

MATERIALS AND METHODS

Sample Collection and Farming System

During October 2010 to March 2012, fecal samples were collected from 388 animals belonging to 14 small stock farms located in South East, Kweneng and Kgtaleng districts of southern Botswana. Of 14 farms, six were comprised of both sheep and goats, while the remaining six and two kraals were keeping goats and sheep, respectively. The animals included 91 goat kids and 67 lambs aged ≤ 4 weeks, 55 kids and 38 lambs > 4 weeks to ≤ 12 weeks, 76 adult goats and 61 adult sheep. All animals were sampled once during the study period. Semi-intensive animal management system was being practiced on five farms with 209 animals where grazing was allowed on the farm premises. On nine kraals with 179 animals, extensive/traditional husbandry system was being followed, where communal native pastures were used for grazing and watering. Twenty to fifty grams fecal samples either directly from the rectum or a portion of freshly deposited feces that did not have contact with the floor were collected from each animal into sterile plastic containers without any preservative, placed on ice packs and transported to Parasitology Laboratory. Fecal consistency was recorded at the time of their collection. These were categorized as either diarrheic passing soft to liquid feces or /non-diarrheic with normal formed stools. Borehole water was used for drinking on all the farms. A total of 62 environmental samples consisting of 27 soils, 22 water and 13 manure samples were collected from each farm. Soil samples were collected from different sites of a farm by using a soil augur, mixed them well and approximately one kilogram was brought to Laboratory. Similarly manure samples were taken from the backyard manure dumping sites of each farm. Two-liter water samples were

collected in sterile glass bottles from the borehole available at each semi-intensively managed farm, whereas water samples were taken from the communal boreholes. Fecal, soil and water samples were brought on ice packs in cooler boxes and kept in the refrigerator till their processing within a week.

Microscopic Examination of Fecal Smears

Cryptosporidium species oocysts were detected in the stained fecal smears microscopically following Modified Ziehl-Neelsen (MZN) technique as described by O.I.E (2004). Malachite green was used as counterstain in place of Methylene blue. Examination of the slides was carried out using calibrated light microscope at x1000 magnification under oil immersion objective. *Cryptosporidium* oocysts appeared light to bright red spherules with refractive walls measuring 4-5 µm in diameter on a green background in MZN stained fecal smears. Soil and liquid manure samples were processed by Centrifugal flotation technique using Sodium chloride solution. Smears were made by taking a drop of supernatant, fixed them with methanol and stained with MZN stain. Water samples were filtered through a 47 mm diameter, 0.45±0.02 µm pore size membrane filter. Material retained by filters were examined microscopically as a 0.9% saline smear at magnification of 400x for *Cryptosporidium* oocysts following the technique adopted by Bakir *et al*., (2003).

Detection of Cryptosporidium Coproantigen

A commercial RIDASCREEN Cryptosporidium (C1201) Enzyme-linked immunosorbent assay (ELISA) diagnostic kit (R-Biopharm AG, Darmstadt, Germany) was used to detect *C. parvum* antigen in fecal samples. ELISA was carried out following the technique described by the manufacturer of the kit. Multiskan microplate reader (Labsystems Oy, Helsinki, Finland) was used for photometric measurements at 450nm wavelength.

Data Analysis

Chi square values were calculated using 2×2 contingency table. P values (right-tailed probability of chi-squared distribution) were determined using CHIDIST function in Microsoft Excel. The results were considered significant at P = 0.05.

RESULTS

The summary of the results is presented in Tables 1 and 2. The overall prevalence rates of *Cryptosporidium* infection in small ruminants were 8.5% (33/388) and 12.6% (49/388) by MZN and ELISA, respectively. The infection rates in respect of sheep and goats of all age groups were 13.3% and 12.2% (P=0.87) by ELISA and 9% and 7.2% by MZN (P =0.64). On examination of fecal smears stained by MZN stain, light to bright red sub-spherical to spherical oocysts measured between 4 to 5µm and appeared very similar to those of *Cryptosporidium parvum* morphologically. ELISA proved to be more sensitive in detecting *Cryptosporidium* coproantigen when compared to MZN (P=0.04). Subsequent to this, the infection rates and the comparisons made between different age groups, genders, fecal consistencies and the management systems in this study will be those tested by ELISA only. The highest *Cryptosporidium* infection rates of 19.4% and 16.5% were recorded in ≤ 4 weeks old lambs and goat kids followed by 15.8% and 14.5% in ≤12 weeks old lambs and kids and 4.9% and 5.3% in adult sheep and goats, respectively. The observed significance level in the infection rates was P = 0.002 between young small ruminants ≤ 4 weeks and adults. The variations in the infection rates between ≤ 4 weeks old lambs and kids versus ≤ 12 weeks were P =0.84 and P =0.94, respectively. Male and female animals demonstrated infection rates of 11.8% and 13.4%, respectively (P = 0.76). As evident from Table 2, majority of the sampled animals (85%) were asymptomatic and passing normal formed feces. A total of 58 animals were found excreting loose to liquid feces with *Cryptosporidium* infection rate of

20.7% in comparison to 11.2% in non-diarrheic animals (P=0.07). Animals under traditional communal management conditions showed higher prevalence rate of 16.8% (30/179) compared to 9.1% (19/209) in those reared under semi-intensive management system (P=0.04).

All but three sampled small stock farms (79%) had at least one animal harboring *Cryptosporidium* species infection at the time of our sampling. The prevalence rate was highest and peaked 16.5% (16/97) on Notwane Farm followed by 15.9% (7/44) and 15.4% (2/13) on Belabela and Kopi Farms, respectively. The infection rates on the remaining eight farms ranged from 0 to 14.3%.

Table 1: *Cryptosporidium* infection in sheep and goats of different age groups using Modified Ziehl-Neelsen staining technique and Enzyme-linked immunosorbent assay

Age groups	Number animals tested	Number animals positive		% Prevalence ± SE		P- value
		ELISA	MZN	ELISA	MZN	
Lambs (≤4 weeks)	67	13	9	19.4 ± 4.8	13.4 ± 4.2	0.03(EIA)
Lambs (5 weeks-≤ 12 weeks)	38	6	5	15.8 ± 5.9	13.2 ± 5.5	(Between <4 weeks-old & adult)
Adult sheep	61	3	0	4.9 ± 2.8	0.00	
Total sheep	166	22	15	13.3 ± 2.6	9 ± 2.2	
Goat kids (≤4 weeks)	91	15	10	16.5 ± 3.9	11 ± 3.3	0.04 (EIA)
Goat kids (5 weeks - ≤12 weeks)	55	8	6	14.5 ± 4.8	10.9 ± 4.2	(Between <4 weeks-old & adult)
Adult goats	76	4	1	5.3 ± 2.6	1.3 ± 1.3	
Total sheep	166	22	15	13.3 ± 2.6	9 ± 2.2	0.87 (EIA)
Total goats	222	27	16	12.2 ± 2.2	7.2 ± 1.7	0.64 (MZN)
Total small stock	388	49	33	12.6 ± 1.7	8.5 ± 1.4	0.04 (EIA : MZ)

Table 2: *Cryptosporidium* infection in genders, diarrheic and non-diarrheic, and semi-intensively and extensively reared sheep and goats using ELISA

Parameters	Number animals tested	Number animals positive	% Prevalence ± SE ELISA	P-value
Male small stock	186	23	11.8 ± 2.4	0.76
Female small stock	202	27	13.4 ± 2.4	
Diarrheic animals	58	12	20.7 ± 5.3	0.07
Non-diarrheic animals	330	37	11.2 ± 1.7	
Small stock under semi-intensive management	209	19	9.1 ± 2	0.04
Small stock under extensive/traditional management	179	30	16.8 ± 2.8	

None of the animals was detected positive on Oodi Lands, Adam Apple and Modipe farms. Of the 62 environmental samples taken, *Cryptosporidium* oocysts were detected in 2 of 27 soil (7.4%) and 1 of 22 water (4.5%) samples only. All the 14 manure specimens collected from each sampled small stock farm were found negative for oocysts. Two soil samples, one each from Malope and Belabela Farms were positive for *Cryptosporidium* organisms. Single water sample that was tested positive belonged to Tsiping kraal.

DISCUSSION

In this study, the diagnosis of *Cryptosporidium* infection was based on detection of oocysts in fecal samples using MZN staining technique and coproantigen by ELISA. MZN technique was less sensitive than ELISA in detecting *Cryptosporidium* infection which may possibly be on account of not employing the concentration step while processing the fecal samples as well as when excretions of oocysts in feces are low and intermittent. Our observations are similar to those of Katanika *et al*., (2001) and Marks *et al*., (2004) who also reported reduced

sensitivity of MZN technique in comparison to commercial ELISA. Morphologically oocysts detected in the fecal smears of nine sheep and six goats were indistinguishable from those of *C. parvum*. Santin *et al*., (2004) suggested that the identification of oocysts solely on morphological characteristics must be reassessed using molecular techniques to validate species of *Cryptosporidium*. Goma *et al*., (2007) from Zambia reported the presence of zoonotic *C. parvum* in sheep and opined that sheep and possibly goats should be considered as reservoirs for human infection. In another study, Imre *et al.*, (2013) identified two zoonotic subtypes IIa and IId of *C. parvum* isolates after molecular typing in 20 of 24 positive fecal samples of diarrheic newborn lambs in Romania. The overall *Cryptosporidium* infection rates of 13.3% and 12.2% in respect of sheep and goats observed in this investigation were similar to those reported earlier in Botswana (Sharma and Machete, 2009), but lower than those of Chartier *et al*. (2002), Watanabe *et al*. (2005). Our observations regarding the increased infection rates among 4 to 12 weeks old lambs and kids in comparison to adult sheep and goats corroborate the findings of Quilez *et al.,* (2008), Paraud and Chartier (2012). Infection rates varied from 2 to 85% in lambs worldwide (Santin *et al*., 2007; Yang *et al*., 2009) and from 5 to 30% in goat kids (Castro-Hermida *et al*., 2005; Delafosse *et al*., 2006; Goma *et al*., 2007). According to Causape *et al*. (2002) the age of the animals has a great influence on the frequency of isolation of *Cryptosporidium* and the highest prevalence was reported in lambs aged between 8 and 14 days old with 76% of them excreting oocysts. Paraud *et al*., (2010) reported excretion of *Cryptosporidium* oocysts at the age of 4 days with a peak at 7 days of age and decline after 3 weeks in naturally infected kids. Lower prevalence rates of 4.9 and 5.3% in adult sheep and goats in this study were almost similar to those of Castro-Hermida *et al*., (2007a, b) from Spain and Wang *et al*., (2010) from China that ranged from 2.1 to 5.3% in adult healthy ewes and 7.7 to 9% in goats. Lower infection rates with advancing age may be associated with acquisition of active immunity (Zu *et al*., 1992; Streter *et al*., 1995). Infection rates were probably underestimated in this study since the animals were sampled once only that may be found negative microscopically and serologically because of intermittent and or lower excretion of oocysts. Van Gool *et al*., (2003) suggested consecutive collection and testing of three fecal specimens per animal. According to Anderson (1985) and Walker *et al*. (2001) the temperature extremes and dry weather conditions also have adverse impact on the viability of *Cryptosporidium* species oocysts. Similar weather conditions prevailed throughout southern Botswana during most of the sampling period in this study which might be responsible for lower prevalence rates.

Our finding showing non-significant differences in the prevalence rates among diarrheic (20.7%) and non-diarrheic (11.2%) small ruminants is contrary to research reports of Causape *et al*., (2002), Noordeen *et al*., (2012) who pointed out the role of *Cryptosporidium* as one of the important enteric pathogens that cause neonatal diarrhea in lambs and goat kids. However, it corroborated the results of the studies conducted by Goma *et al*., (2007), Pritchard *et al*., (2008), Gharekhani *et al*., (2014) who reported asymptomatic nature of cryptosporidiosis. Mild gastroenteritis observed in 58 out of 388 animals (15%) might have caused by concurrent enteric viral (rotavirus, coronavirus), bacterial and parasitic (*Salmonella, Escherichia coli, Haemonchus, Eimeria*) infections. Lower number of diarrheic animals in the present investigation may probably due to the fact that some animals might have either suffered with bouts of diarrhea prior to our sampling or could be recovering from the clinical infection. The differences in the infection rates in sheep versus goats and males versus females were insignificant in our study possibly on account of similar management conditions for both species as well as for males and females. A total of 11 of 14 farms situated in South East, Kweneng and Kgtaleng districts of southern Botswana were found harboring *Cryptosporidium* infection. Poor sanitary conditions, higher stocking density and low nutritional status of animals on most of the infected small stock farms might have influenced the prevalence rates of *Cryptosporidium* infection corresponding with the observations of Mohammed *et al*., (1999), Nydam and Mohammed (2005). Higher infection rates in animals

reared under traditional husbandry system may be on account of their constant grazing on the infected communal lands in conjunction with higher stocking density especially during kraaling of flocks at nights that might have favored transmission and spread of the disease.

The presence of *Cryptosporidium* oocysts in approximately 5% environmental samples belonging to three farms can be considered significant since cryptosporidiosis caused by *C. parvum* organisms is both zoonotic and poses a threat to human health. Geurden *et al*., (2008) reported an average excretion of 6832 oocysts per gram (opg) of feces in lambs and a mean excretion of 231,929 opg in goat kids. Robertson *et al*., (2010), Yoder and Beach (2010) reported that even asymptomatic sheep and goats or animals with cryptosporidial diarrhea have the potential to transmit cryptosporidiosis to humans and other mammals. We could not find any significant association between the presence of *Cryptosporidium* oocysts in the soil and water samples and the magnitude of infection in animals on three farms. According to Barwick *et al*., (2003), this may be possible by taking into consideration the fact that the animal sampling was undertaken from the animals directly and the soil sampling was cumulative sampling. In this report we could not conduct tests to check the viability of oocysts nor the sources of water and soil contamination. Small stock found infected with cryptosporidiosis could be one of the important vehicles through which oocysts may travel into water sources especially during rainy season and can even contaminate public water supplies, but human excreta, livestock and wild animals may also contaminate these sources. The use of communal grazing, watering points and overstocking should be reduced to minimize transmission of gastrointestinal parasitic infections (Bacha and Haftu, 2015). It would be worthwhile to investigate livestock farms in more districts and different regions in order to know the status of *Cryptosporidium* infection and molecular characterization of different isolates prevalent in Botswana. Creation of farmers' awareness through extension education program on good animal husbandry practices will greatly help in devising appropriate strategies to control animal and human cryptosporidiosis.

CONCLUSIONS

The present study demonstrated the occurrence of *Cryptosporidium* infection in small stock population in southern Botswana and suggested a potential role of young lambs and goat kids in particular for transmission of human cryptosporidiosis via environmental contamination. It underlined the importance of creating awareness among animal handlers and farm owners living in close proximity to naturally infected sheep and goats and through contaminated farm environment of acquiring this zoonotic infection. Further studies are warranted to determine the status of this infection in small ruminants from different regions of Botswana and molecular characterization of *Cryptosporidium* species and genotypes.

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