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Prevalence of *Cryptosporidium* species oocysts among small ruminants in Potiskum livestock market, Yobe State, Nigeria

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ABSTRACT: A cross-sectional study was carried out to determine the prevalence of *Cryptosporidium* species oocysts in small ruminants in Potiskum livestock market, Yobe State, Nigeria. Faecal samples from 203 animals; 100 sheep and 103 goats were tested using modified Ziehl-Neelsen staining technique. An overall prevalence of 20.7% (42/203) was recorded in the study with a higher prevalence of 21.4% (22/103) in goats than in sheep 20% (20/100); although the difference was not significant (p>0.05). In goats, the rate of *Cryptosporidium* species oocyst was higher in male (27.8%) than in female (22.4%), in young (30.6%) than in adults (22.4%), in those with loose/watery faeces (45.5%) than those with well-formed faeces (22.8%) but the differences were not significant (p>0.05). In sheep, the rate of infection was higher in female (16.9%) than in male (13.0%), in young ((19.2%) than in adults (14.9%) but the differences were not significant (p>0.05). The prevalence was significantly (p = 0.010) higher in sheep with loose/watery faeces (45.5%) than in those with well-formed faeces (12.4%). Breed of sheep did not significantly (p > 0.05) influence the prevalence rates, although the Uda breed had higher prevalence (25%) than the Balami (14.3%) and Yankasa (12.7%) breeds. A similar trend was observed for the sahelian breed of goats (35.7%) compared to the Red sokoto (23.7%), West African dwarf (16.7%) and mixed breeds (10.0%) (p = 0.367). Animals brought to the livestock market from Ngalda town showed highest prevalence of 36.4% than those from the other areas with prevalence rates of (0 to 33.3%). The finding of *Cryptosporidium* species oocysts in 20.7% of small ruminants in this study underscores the role of this zoonotic parasite diseases in animals and man and loose/watery faeces is a significant factor in the spread among small ruminants.

Keywords: Sheep, goats, market, modified Ziehl-Neelsen, prevalence, Potiskum.

INTRODUCTION

Cryptosporidium is a zoonotic protozoan parasite that causes cryptosporidiosis, an enteric infection in a wide variety of mammals including humans, cattle, sheep, goat, pig and horses worldwide (Quilez et al., 2008; Nasir et al., 2009). Clinically, the disease is characterized by anorexia and diarrhea, which may result in poor growth rate (Taylor et al., 2007). Infection with *Cryptosporidium* in young sheep and goats may lead to morbidity and even mortality due to diarrhea (Thompson et al., 2005). *Cryptosporidium* species infecting sheep and goats have been found to be of public health significance (Robertson et al., 2010; Wang et al., 2014). Oocysts are discharged in the faeces of infected ruminants and are of primary importance for the dispersal and survival of the parasites (Bowman, 2003).

The oocysts are a source of infection for animals and humans (Singla et al., 2013) and transmission occurs by

faeco oral route (OIE, 2016). In humans, infection with *Cryptosporidium* causes diarrheal disease (Checkley et al., 2015), and chronic and fatal disease in immunocompromised individuals (Wilhelm and Yarovinsky, 2014). Additionally, *Cryptosporidium* infection has been linked to cancer in humans (Lendner et al., 2011; Benamrouz et al., 2012).

For effective disease prevention, an understanding of the environmental factors that predispose animals and man to infectious causes of diarrhea like *Cryptosporidium* species is required (Rossle and Latif, 2013; Collinet-Adler et al., 2015). Although several studies aimed at detecting and identifying *Cryptosporidium* species in ruminants have been conducted in Nigeria (Kwaga et al., 1988; Maikai et al., 2011; Pam et al., 2013; Akinkuotu and Fagbemi, 2014; Danladi and Ugbomoiko, 2015), the knowledge of parasite occurrence and worldwide distribution in animals is still not complete (Xiao, 2010).

Potiskum livestock market is one of the largest livestock markets in West Africa and small ruminants from other parts of the state are brought to the market for sale (Ramsar, 2008). These animals are kept in the market until they are sold and then transported to other parts of the country. This practice poses a potential risk of transmission of parasite like *Cryptosporidium* species between animals as well as to humans.

Thus, the aim of the study was to determine the prevalence of *Cryptosporidium* species oocysts among small ruminants in Potiskum livestock market, Yobe State, Nigeria.

MATERIALS AND METHODS

Study area and study design

The study area was Potiskum Local Government Areas of Yobe State, Nigeria (Figure 1). It is located between longitude 11°43′N and latitude 11°04′E. Potiskum livestock market is the largest livestock market in the state and serves as the main market where animals are loaded in trucks and transported to other parts of the country. The mean temperature in Potiskum is about 37^{o}C. The highest temperature of 42^{o}C is normally experienced in April, while minimum temperature (about 30^{o}C) are normally recorded in December (Iloeje, 1977). About 713 mm precipitation falls annually. The profile of the soil is poorly developed and it has a low water retention capacity (Price et al., 1990). Potiskum have an average relative humidity of 39.5% (Eludoyin et al., 2014).

Sampling

A total of 203 faecal samples were randomly collected from sheep and goats in the livestock market, 100 from sheep and 103 from goats of different ages. Convenience sampling technique was employed to select the animals, which was based on the availability of the animals and the willingness of the owners to participate in the study. Age of the animals was determined using their dentition. Sheep and goats between the age range of six (6) months and below were considered to be young, while those up to 7 months and above were considered as adults (Dagnachew et al., 2011). Sampling was done between October and December 2016. Fresh faecal samples were collected from the rectum of each animal using a disposable hand gloves and emptied into a sterile, airtight, plastic tube. Samples were stored in 10% formaldehyde and were transported in icebox to the Parasitic Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for analysis. General information on the animal was recorded which includes species, age, sex, breed, source of animal and faecal consistency. Faeces were observed for either being well formed or loose/watery.

Sample processing and laboratory procedures using Formol-Ether concentration and modified Ziehl-Neelsen Staining (mZN) technique

The modified Ziehl-Neelsen technique was performed as described by WHO (1991). Briefly, 1 gm of faeces was mixed in 10 ml of 10% formaldehyde in a universal bottle using an applicator stick. The homogenized faeces was sieved into a centrifuge tube using a funnel and gauze to which 3 ml of diethyl ether was added. The centrifuge tubes were corked and shaken gently to mix properly. The tube was centrifuged at 2000 rpm for 2 minutes and the supernatant decanted. The sediment was mixed with a spatula from which a thin smear was made on a clean glass slide. After air-drying, the smear was fixed in methanol for 2 to 3 min. The slide was flooded with cold carbolfuschin for 5 to 10 min and then with 1% hydrochloric-acid ethanol until colour ceases to flow out and rinsed in tap water. It was then counterstained with 0.25% methylene blue for 30 seconds, rinsed in tap water again and air-dried. The slide was then examined under the compound microscope at x 10 and x 40 objectives. The appearance of a bright rose-pink spherules on a bluish green background indicate the presence of Cryptosporidial oocysts

Data analyses

Statistical Package for Social Sciences (SPSS, version 20.0) (SPSS Inc. Chicago IL, USA) was used to analyze the data obtained. Chi-square, odds ratio and 95% confidence was used to test the association between prevalence of *Cryptosporidium* species oocysts and

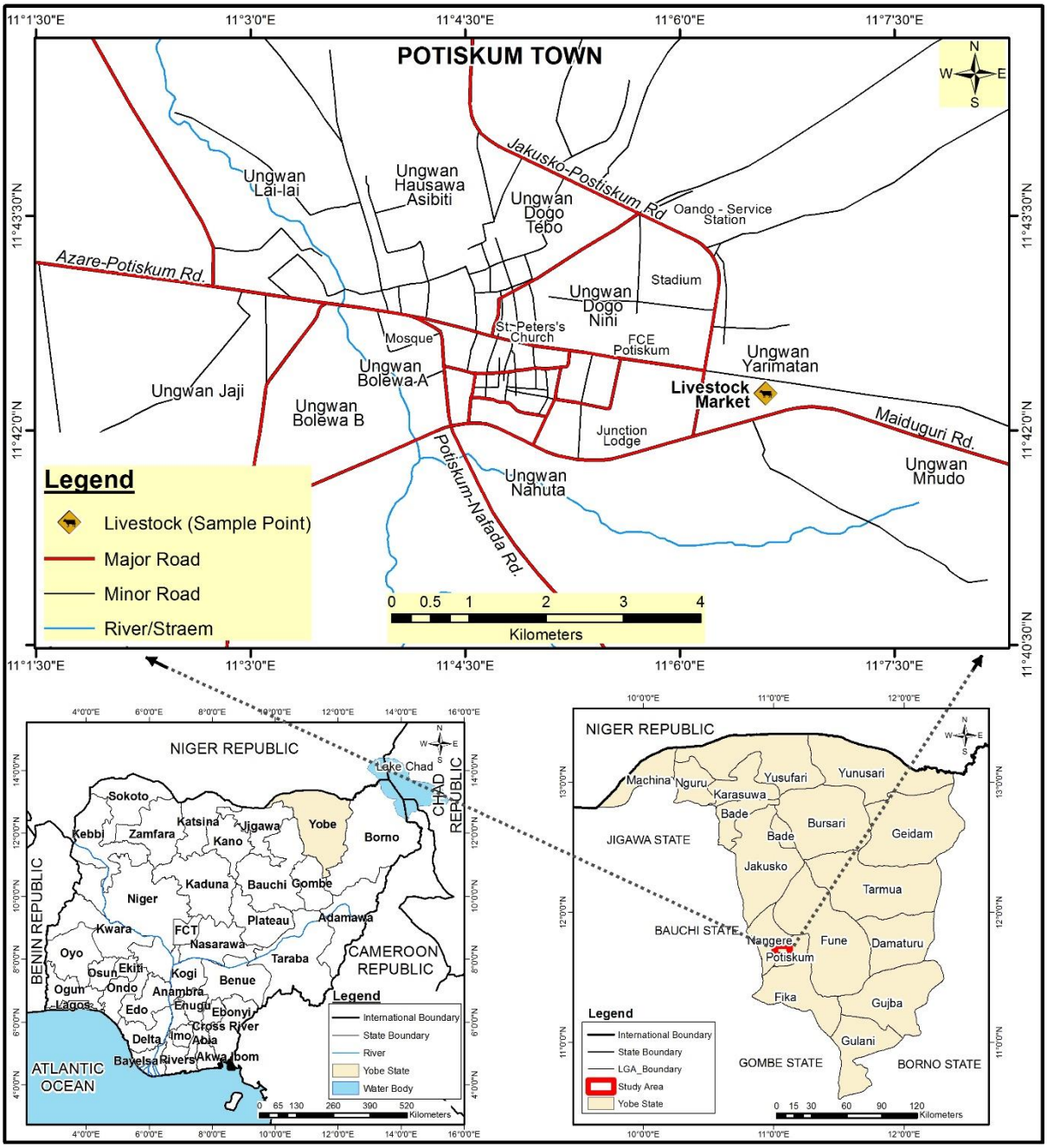

Figure 1. Map of Potiskum Town showing Sampling Points. Modified from the Street Map of Potiskum Town.

species, age, sex, breed, source of animal and faecal consistency of the animals studied. Results were presented in tables. Values of $P \leq 0.05$ were considered statistically significant.

RESULTS

Cryptosporidium species oocysts were detected in the small ruminants examined. A total of 42 (20.7%) of the animals were positive for *Cryptosporidium* species oocysts. The rate of infection was higher in goats (21.4%) than in sheep (20.0%), but the difference was not significant ($p > 0.05$) (Table 1).

Table 2 depicts prevalence with respect to infection in goats. Although infection in male goats was relatively higher than in females, the rates were statistically comparable ($p > 0.05$). Young goats tended to be more infected than the adult ones, However, the distribution was

Table 1. Prevalence of *Cryptosporidium* spp. oocysts and species small ruminants in Potiskum, Yobe State, Nigeria.

Species	No. Samples examined	Positive samples	Specific rate (%)	Odds ratio	95% CI on OR
Sheep	100	20	20.0	0.920	0.466 – 1.817
Goats [ref]	103	22	21.4		
Total	203	42	20.7		

Reference standards (ref).

Table 2. Prevalence of *Cryptosporidium* spp. oocysts and consistency of faeces, sex and age of goats in Potiskum livestock market.

Factors	No. Samples examined	Positive samples	Specific rate (%)	Odds ratio	95% CI on OR	P- value
Sex						
Male	54	15	27.8	1.329	0.542 – 3.259	0.535
Female [ref]	49	11	22.4			
Age (months)						
Young	36	11	30.6	0.656	0.263 – 1.633	0.365
Adults [ref]	67	15	22.4			
Faecal consistency						
Well formed	92	21	22.8	2.817	0.781 – 10.162	0.114
Loose/watery [ref]	11	5	45.5			

Reference standards (ref), CI – Confidence Interval, OR – Odds Ratio.

Table 3. Prevalence of *Cryptosporidium* spp. oocysts and consistency of faeces, sex and age of sheep in Potiskum livestock market.

Factors	No. Samples examined	Positive samples	Specific rate (%)	Odds ratio	95% CI on OR	P-value
Sex						
Male	23	3	13.0	0.738	0.191 – 2.854	0.660
Female [ref]	77	13	16.9			
Age (months)						
Young	26	5	19.2	0.733	0.228 – 2.355	0.602
Adults [ref]	74	11	14.9			
Faecal consistency						
Well formed	89	11	12.4	5.909	1.541 – 22.663	0.010*
Loose/watery [ref]	11	5	45.5			

Reference standards (ref), CI – Confidence Interval, OR – Odds Ratio, *p < 0.05 is significant.

not significant (p>0.05). Goats with loose/watery faeces were 2.817 times prone to infection than those with well-formed faeces, but distribution was not statistically significant (p = 0.114).

The distribution of oocysts in sheep is summarized in Table 3. The occurrence of the oocysts was common in female (16.9%) sheep than male (13.0%), although the distribution was not sex related (p>0.05). Young (19.2%) sheep are slightly more infected than the adult (14.9%), but was not statistically significant (p>0.05). Sheep with loose/watery faeces shedded significantly (p<0.05) higher percentage of oocysts (45.5%) and were more than five

Table 4. Occurrence of *Cryptosporidium* spp. oocysts according to the breed of small ruminants in Potiskum livestock market.

Breed	No. examine	No. positive	Specific rate (%)	df	χ^2	P – value
Sheep Breed						
Yankasa	55	7	12.7			
Balami	21	3	14.3	2	1.931	0.381
Uda	24	6	25.0			
Goat breed						
Sahelian	28	10	35.7			
Red Sokoto	59	14	23.7			
WAD	6	1	16.7	3	3.164	0.367
Mixed breed	10	1	10.0			

WAD – West African dwarf, Df – Degree of freedom, χ^2 –Chi-square.

Table 5. Association between the source of animal and the occurrence of *Cryptosporidium* spp. Oocysts.

Source	Number examined	Number positives	Specific rate (%)	χ^2	df	P-value
Potiskum town	37	8	21.6			
Ngelzarma	39	8	20.5			
Ngalda	11	4	36.4			
Babban gida	23	4	17.4			
Damaturu	42	9	21.4			
Buni yadi	22	5	22.7	4.011	8	0.856
Garga	5	0	0.0			
Gadaka	21	3	14.3			
Garin buba	3	1	33.3			
Total	203	42	21			

df – Degree of freedom, χ^2 –Chi-square.

times likely to be infected than those with well-formed faeces (12.4%).

The rate of infection was higher in Uda (25.0%) breed of sheep, followed by balami (14.3%), and Yankasa (12.7%), but the difference was not significant ($p > 0.05$). Similarly, in breed of goats, the rate of infection was higher in Sahelian (35.7%), followed by Red Sokoto (23.7%), West African dwarf (16.7%) and the least was mixed breed (10.0%), but the difference was not significant ($p > 0.05$) (Table 4).

Table 5 shows the association between the prevalence of *Cryptosporidium* species oocysts in small ruminants and source of the animal in the livestock market. Small ruminants that were brought to livestock market from Ngalda had the highest infection rate of 36.4%, followed by Garin buba (33.3%), Buni yadi (22.7%), Potiskum (21.6%), Damaturu (21.4%), Ngelzarma (20.5%), Babban gida (17.4%) and Gadaka (15.0%). No oocyst was recorded in small ruminants from Garga (0%). but the difference was not significant ($p > 0.05$).

DISCUSSION

The 20% prevalence of *Cryptosporidium* species oocysts in sheep in this study is similar to the report of Regassa et al. (2013) who recorded 22.2% in sheep in Ethiopia. However, it is higher as compared to the 11.7% reported by Danladi and Ugbomoiko, (2015) and 16.0% by Pam et al. (2013) in Nigeria. This indicates that cryptosporidiosis is a global problem affecting a wide range of farm animals. Similarly, the 21.4% prevalence in goats is consistent with the report of Pam et al., 2013, but lower than 72.5% recorded in Veracruz, Mexico (Romero-salas et al., 2016). The prevalence recorded in this study implies that animals in livestock market in Potiskum may serve as reservoirs of infection to other animals and humans in other parts of the country where these animals are been transported to.

A slightly higher rate of detection in male goats than female was observed which is similar to previous reports in goats and other animal species (Maikai et al., 2009; Akinkuotu and Fagbemi, 2014; Danladi and Ugbomoiko,

2015). On the contrary, female sheep were more infected than male as observed by Danladi and Ugbomoiko, (2015). The reason for this observation is not known. Further research is required to elucidate this and other possible reasons for the differences in infection rates between the sexes (Akinkuotu et al., 2014). Previous works have indicated that cryptosporidiosis is significantly associated with young than adult animals (Geurden et al., 2006; Yang et al., 2009; Zhang et al., 2013). But on the contrary, distribution of infection between young and adult animals in this study was not age related (P> 0.05) suggesting a possible interplay of other exposure risks. This was also observed by Ayinmode and Fagbemi (2010).

The higher rate of *Cryptosporidium* infection in sheep with loose/watery faeces recorded in this study, is in line with the reports of Maurya et al. (2013), Caccio et al. (2013) and Danladi and Ugbomoiko (2015) who inferred the presence of diarrhoea in young animals as a significant source of oocyst contamination of the environment. Other factors such as the environment, management practices, genetics and immune status of the animals might have contributed to such outcome.

It has been shown that the breed of small ruminants has no significant influence on the prevalence of *Cryptosporidium* oocysts (Regassa et al., 2013; Kaupke et al., 2017), which is consistent with the findings in this study.

Nine areas were identified as the major sources of these sheep and goats in Potiskum livestock market with small ruminants from Ngalda having the highest prevalence 36.4%. Ngalda is located in Fika Local Government area of Yobe State and it is one of the areas with a very large body of water which encourages several agricultural activities. Sheep and goats in the area are usually seen along the river banks grazing. Wetness and high humidity along the river bank can encourage oocyst survival and increase their viability when present. This may be the reason why small ruminants from Ngalda had the highest rate of occurrence of *Cryptosporidium* oocysts. This report is similar to that of Faleke et al. (2014).

Conclusion

Prevalence of *Cryptosporidium* species oocysts among sheep and goats in Potiskum livestock market is relatively high. Infection was widely distributed among small ruminants regardless of their species, age, sex, or breed. The study also highlights the significance of loose/watery faeces as a key factor in the spread of infection. Efforts should be directed towards improving quarantine systems, especially in livestock markets, in order to ensure healthy production of ruminants and reduce possible zoonotic transmission of the parasite in Nigeria.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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