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Occurrence and factors associated with faecal shedding of Cryptosporidium oocysts in small ruminants in Potiskum local government area, Yobe State, Nigeria

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Abstract

Cryptosporidium is an enteric protozoan pathogen associated with neonatal diarrhoea and mortality in small ruminants. Faecal samples of two hundred small ruminants were examined for *Cryptosporidium* oocysts using formol-ether concentration and modified Ziehl-Neelsen staining technique. Fifty seven (28.5%) out of the 200 faecal samples examined were positive for *Cryptosporidium* oocysts. The occurrence was higher in sheep (33.0%) than goats (24.0%), in male (32.8%) than female (26.6%), in those with loose/watery faeces (74.4%) than those with well-formed faeces (17.4%), in those between age 4-6 months (39.2%) than 0-3 months (22.2%) and those within 7 months and above (21.1%), in those that drank borehole water (28.7%) than those that drank well/stream water (20.0%), in those raised under semi-intensive/extensive (29.9%) than those under intensive system (21.1%). There was significant association between the occurrence of *Cryptosporidium* oocysts and age of small ruminants (OR = 2.368, 95% CI on OR: 1.251 < OR < 4.484). On the other hand, there were no significant associations between the occurrence of *Cryptosporidium* oocysts and sex, breed, faecal consistencies, source of water and management practices of small ruminants (P > 0.05). The species of *Cryptosporidium* infecting small ruminants identified in this study were *C. parvum* (50.9%), *C. ryanae* (26.3%), *C. xiaoi* (12.3%), *C. bovis* (8.8%) and *C. andersonii* (1.8%). The high prevalence of *C. parvum* observed in this study is of public health importance because of it zoonotic nature. This study shows that age influence the occurrence of *Cryptosporidium* oocysts and *C. parvum* is the predominant species in small ruminants in Potiskum, Yobe State.

Keywords: *Cryptosporidium*, Factors, Occurrence, Potiskum, Small ruminants

Introduction

Cryptosporidium is a protozoan parasite that causes cryptosporidiosis, an enteric infection in many species of mammals, including humans (Bhat *et al*., 2013; Paul *et al*., 2014). The disease is characterized by anorexia and diarrhea, often intermittent, which may result in poor growth rate (Santin, 2013) and ruminants are important reservoirs of this parasite (OIE, 2016). The parasite is considered as one of the major enteric pathogens associated with neonatal diarrhoea and mortality (Maurya *et al*., 2013; Bhat *et al*., 2012) and is more severe in young than in adult animals (Akinkuotu & Fagbemi, 2014).

The oocysts are 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 & Yarovinsky, 2014).

Cryptosporidium is one of the causes of diarrhoeal illness in man and animals worldwide (Checkley *et al*., 2015). It has long been a veterinary problem, being predominant in young farm animals (Brar *et al*., 2016). *Cryptosporidium* infection may have an important economic impact on farmers because of high morbidity and sometimes high mortality rates among farm animals (Brar *et al*., 2017). Small ruminants are known as reservoir of *C. hominis* (Connelly *et al*., 2013; Checkley *et al*., 2015) and zoonotic *C. parvum* for humans (Imre *et al*., 2013; Lange *et al*., 2014). Effective disease prevention requires an understanding of the environmental factors (Collinet-Adler *et al*., 2015).

Although several studies aimed at detecting and identifying *Cryptosporidium* in farm animals have been conducted by many authors in Nigeria such as Ayinmode & Fagbemi (2010), Pam *et al.* (2013), Akinkuotu & Fagbemi, 2014 and Danladi & Ugbomoiko, 2015, our knowledge of parasite occurrence in Nigeria and its worldwide distribution in animals is still not complete (Randhawa *et al*., 2012). Sheep and goats are among the most common domestic animals used for economic benefits in Potiskum, Yobe State, Nigeria and there is intense farming of animals especially of small ruminants in the study area and this can pose a risk of easy transmission of parasites like *Cryptosporidium* spp to humans and animals.

The aim of the study was to determine the occurrence and factors associated with faecal shedding of *Cryptosporidium* oocysts in small ruminants in Potiskum Local Government Area of Yobe State, Nigeria which will be useful in the institution of prevention and control measures that will disrupt the transmission cycle between animals and humans.

Materials and Methods

Study area and study design

Potiskum Local Government Area (LGA), Yobe State, Nigeria was the study area. A cross-sectional study was used. Potiskum LGA has ten (10) wards which were all selected for the study, namely: Dodo nini, gwajin alarabe, Bila danchuwa, Mamudo, Yarimaran, Bolewa A, Bolewa B, Dogo tebo, Bare-bari bawuya lele and Hausawa asibiti.

Sample collection

A total of 200 faecal samples were collected from the residential areas. 100 faecal samples each for sheep and goats (10 samples from each species of small ruminants in all the ten wards). Convenience sampling method was employed for the selection of households that keep small ruminants, which was based on the availability of the animal and willingness of the owners to participate in the study. Sampling was done between October to December 2016. Fresh rectal faecal samples were collected from 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 processing.

Administration of questionnaires

Two sections of structured questionnaires was administered prior to sample collection in order to identify the factors associated with faecal shedding of *Cryptosporidium* in small ruminants. *Section A* contains the socio-demograpic information of the respondents and questions relating to the transmission of *Cryptosporidium*. Information on the age, sex, and breed of the animal, and source of water, management practice, species of ruminants kept and faecal consistency were in *Section B*

Sample processing and laboratory procedures using formol-ether concentration and modified Ziehl-Neelsen staining (mZN) technique (WHO)

Approximately, 1 gm of faeces was mixed in 10 mL of 10% formaldehyde in a universal bottle using an applicator stick. The homogenized faeces were sieved into a centrifuge tube using a funnel and gauze to which 3 mL of diethyl-ether was added to extract fat from the filtrate. The centrifuge tube was corked and shaked gently to mix properly. The tube was centrifuged at 2000 rpm for 2 minutes and 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 - 3 min. The slide was flooded with cold carbolfuschin for 5 - 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 examined microscopically using x 10 and x 40 objectives.

The size of the oocyst was measured with the aid of calibrated eyepiece micrometer, and the length and weight of the oocysts were recorded.

Data analyses

Statistical Package for Social Sciences (SPSS, version 17.0) (SPSS Inc. Chicago IL, USA) was used to analyze the data obtained. Chi-square, odds ratio and 95% confidence interval was used to test the association between the occurrence of *Cryptosporidium* oocysts and factors studied. Results were presented in Tables and charts. Values of P < 0.05 were considered statistically significant.

Results

Out of the 200 small ruminants faecal samples examined using modified Ziehl-Neelsen technique, 57 (28.5%) were positive of *Cryptosporidium* oocysts. Of the 100 sheep examined, 33 (33.0%) were positive of *Cryptosporidium* oocysts. Whereas, of the 100 goats studied, 24 (24.0%) were positive for *Cryptosporidium* oocysts (Table 1)

Cryptosporidium infection was higher in male (32.8%) than in female (26.6%), in small ruminants with loose/watery faeces (74.4%) than those with well-formed faeces (17.4%), in the weaner age group (4-6 months) (39.2%) than in the pre-weaned (0-3 months) (22.2%) and adults 7 months and above (21.4%), in small ruminants that drank borehole water (28.7%) than those that drank well/stream water (20.0%), in those managed under semi-intensive/extensive system (29.9%) than those managed under intensive system (21.2%). There were association between the occurrences of *Cryptosporidium* oocysts and species of small ruminants (OR = 1.560; 95% CI on OR: 0.839 < OR < 2.899), sex of small ruminants (OR = 1.374; 95% CI on OR: 0.699 < OR < 2.850), age (OR = 2.368, 95% CI on OR: 1.251 < OR < 4.484), source of drinking water (OR = 1.612; 95% CI on OR: 0.176 – 14.737), and management practices (OR = 1.587; 95% CI on OR: 0.647 < OR < 3.130). However, these associations were not statistically significant (P>0.05) except for the age that showed statistical significance (P<0.05) where the weaner age group (4-6 months) were 2.368 times more likely to occur with *Cryptosporidium* oocysts than the other age groups (Table 2).

Table 1: Association between the occurrence of *Cryptosporidium* oocysts and species of small ruminants in Potiskum, Yobe State, Nigeria

Species	Number examined	Number positive (%)	Odds ratio	95% Confidence interval	P- value
Sheep	100	33 (33.0)	1.560	0.839 - 2.899	0.160
Goats [ref]	100	24 (24.0)	1		
Total	200	57 (28.5)			

Reference standards (ref)

Table 2: Odds ratio (OR) and 95% confidence intervals on factors affecting the prevalence of faecal shedding of *Cryptosporidium* oocysts in Yobe State, Nigeria

Factors	No. Samples examined	Positive samples	Specific rate (%)	Odds ratio	95% CI on OR
Sex					
Male	61	20	32.8	1.374	0.699 - 2.850
Female[ref]	139	37	26.6	1	
Faecal consistency					
Loose/ Watery[ref]	39	29	74.4	1	
Well formed	161	28	17.4	0.073	0.032 – 0.166
Age (months)					
0-3	9	2	22.2	1.048	0.204 – 5.374
4-6	79	31	39.2	2.368*	1.251 – 4.484
7 and above[ref]	112	24	21.4	1	
Source of water					
Borehole	195	56	28.7	1.612	0.176 – 14.737
Well/ Stream[ref]	5	1	20.0		
Management practice					
Intensive	33	7	21.2	1.587	0.647 – 3.130
Semi inte/exten[ref]	167	50	29.9	1	

Reference standards (ref).

*p < 0.05 is significant

There was no statistically significant association (χ^2 = 2.235, p-value = 0.327) between the occurrence of *Cryptosporidium* oocysts and breeds of sheep though it was higher in Uda (50.0%) followed by Yankasa (39.7%), and the last was Balami (23%). Similarly, no significant association (χ^2 = 0.879, p-value = 0.831) was observed between the occurrence of *Cryptosporidium* oocysts and breed of goats with Sahelian, West African Dwarf and crosses 25% each and the least was Red Sokoto (17.2%) (Table 3).

Results of the morphometric measurement of oocysts recorded in this study is presented in Table 4, alongside the standard dimensions of the size of oocysts of *Cryptosporidium* species that corresponded to the morphometric sizes of the oocysts observed. Comparison of the dimensions of oocysts measured in this study with reported standard means/dimensions showed that five different sizes of *Cryptosporidium* oocysts were recorded in this study; 5.04 × 4.49µm, 3.66 × 3.14µm, 3.96 × 3.42µm, 4.72 × 4.16µm and 6.67× 5.90 µm (mean length by mean width of population). These oocysts dimensions/sizes correspond with the size of the oocysts of the following *Cryptosporidium* species; *Cryptosporidium parvum, C. ryanae, C. xiaoi, C. bovis* and *C. andersoni*, respectively.

Discussion

The prevalence of *Cryptosporidium* oocysts in sheep reported here is higher than 11.7% prevalence in Kebbi State, Nigeria (Danladi & Ugbomoiko, 2015), 16% prevalence in Jos, Plateau State, Nigeria (Pam *et al.*, 2013) and 22.2% prevalence in Ethiopia (Regassa *et al.*, 2013), but it was lower than the 40% prevalence reported in University teaching farm in Nigeria (Akinkuotu & Fagbemi 2014). Similarly, the prealence of *Cryptosporidium* infection found in goats in Potiskum is higher than prevalence found

Table 3: Association between the occurrence of *Cryptosporidium* oocysts and breed of sheep and goats in Potiskum, Yobe State, Nigeria

Breed	No Examine	No. positive	Specific rate (%)	df	Chi- square	P – value
Sheep Breed						
Yankasa	73	29	39.7	2	2.235	0.327
Balami	21	5	23.8			
Uda	6	3	50.0			
Goat breed						
Sahelian	12	3	25.0	3	0.879	0.831
Red Sokoto	64	11	17.2			
WAD	4	1	25.0			
Cross	20	5	25.0			

Table 4: Morphometric measurement of oocysts dimensions of 57 *Cryptosporidium* positive faeces in small ruminants in Potiskum, Yobe State, Nigeria

	Corresponding *Cryptosporidium* spp	Measurement observed ±S.D of population		Number of oocysts positive (%)	Standard range of length and width (reference)
		Mean length (µm)	Mean width (µm)		
1	*C. parvum*	5.04 (±0.32)	4.49 (±0.37)	29 (50.9)	3.25 - 5.45 x 3.02 - 5.04, mean = 4.7 x 4.5 [Tilley *et al.* (1991); OIE, 2008; OIE, 2016)
2	*C. ryanae*	3.66 (±0.09)	3.14 (±0.18)	15 (26.3)	3.7 × 3.2 (OIE, 2016)
3	*C. xiaoi*	3.96 (±0.14)	3.42 (±0.23)	7 (12.3)	3.9-3.4 (Fayer & Santin, 2009; OIE, 2016)
4	*C. bovis*	4.72 (±0.11)	4.16 (± 0.05)	5(8.8)	(4.7-5.3 × 4.2-4.8 Mean = 4.6-6.2 OIE, 2016)
5	*C. andersoni*	6.67 (±0.57)	5.90 (±0.17)	1(1.8)	(6.0-8.1 × 5.0-6.5 Mean = 7.4-5.5,(OIE, 2008; OIE, 2016)

in goats in Kebbi State, Nigeria (17.1%) (Danladi & Ugbomoiko, 2015) and 12.2% prevalence in Ethiopia (Regassa *et al*., 2013) but similar prevalence (24%) was recorded in Jos, Plateau State, Nigeria (Pam *et al*., 2013). The prevalence in goats was rather lower than 72.5% recorded in Veracruz, Mexico (Romero-Salas *et al*., 2016). The differences in the prevalence of *Cryptosporidium* infections in sheep and goats in different regions may be due to the differences in the levels of contamination of the environment with oocysts of the parasite. It is also possible that the quality of hygienic conditions of animal husbandry and grazing practices may have influenced the exposure of animals to *Cryptosporidium* infection. Sheep and goats may serve as reservoirs of *Cryptosporidium* for human infections (Koinari *et al*., 2014). There was no significant statistical association between the occurrence of *Cryptosporidium* oocysts and species of small ruminants samples (OR = 1.560; 95% CI on OR: 0.839 < OR < 2.899). The differences in the prevalence of *Cryptosporidium* infections in sheep and goats may be due to the differences in the levels of contamination of the environment with oocysts of the parasite or may be due to differences in the infectivity of different *Cryptosporidium* spp. populations. It is also possible that the hygienic conditions of animal husbandry and grazing practices may have influenced the exposure of animals to *Cryptosporidium* infection.

The higher detection rate in male sheep and goats than in females in this study may be because males are more likely than females to disperse to other colonies or be moved to other pens especially when the females are on heat thereby promoting the dissemination of the oocysts This report is similar with the findings of other researchers (Regassa *et al*., 2013; Akinkuotu & Fagbemi, 2014), in sheep, and in other species by (Maikai *et al*., 2009). Although, *Cryptosporidium* infection is known to be associated with diarrhea (Caccio *et al*., 2013; Danladi & Ugbomoiko, 2015), in this study, small ruminants with well-formed faeces also showed a high rate of infection with *Cryptosporidium* oocysts. This implies that they can serve as reservoirs of infection to humans and other animals (Panousis *et al*., 2008). The finding also support the fact that *Cryptosporidium* oocysts can be detected in non diarrhoeic animals since there can also be an in apparent carriage of the infection in some instances (Maikai *et al*., 2009).

The high prevalence among the younger age group may be because *Cryptosporidium* is considered a problem in newborn farm animals (Ayinmode & Fagbemi, 2010), and could be as a result of undeveloped immune system during this age (Danladi & Ugbomoiko, 2015).

The relatively high rate of occurrence of *Cryptosporidium* recorded in animals that used borehole water as source of drinking water may have originated from other sources such as environmental contamination by other animals in the herds rather than water as also observed by in piglets by Maikai *et al*. (2009). Oocysts may have been known to easily spread among the small ruminants managed under intensive system due to faeco-oral route of transmission.

The higher rates recorded in the Uda breed in sheep may not necessarily mean breed susceptibility but it may be that, that was the most infected breed. This slightly differs from the breeds of goats examined as the prevalence of *Cryptosporidium* oocysts among the breeds were the same except in the Red Sokoto that it varied slightly.

The higher prevalence of *C. parvum* in both sheep and goats in this study is of Veterinary and Public health importance due to its zoonotic nature. *Cryptosporidium parvum* is an important cause of scour in young unweaned livestock (OIE, 2016). The species of *Cryptosporidium* recorded in small ruminants in this study, is similar to the reports of other workers (Li *et al*., 2016; Kaupke *et al*., 2017) who also recorded *Cryptosporidium xiaoi*, *C. parvum*, *C. andersoni, C. bovis*, and *C. ryanae* in small ruminants. Although *C. bovis* is mostly detected in young calves, its finding in this study may be attributed to the fact that, cattle and sheep raised in the same regions have possibility of parasite exchange (Kaupke *et al*., 2017).

In conclusion, the study revealed the occurrence of *Cryptosporidium* oocysts in sheep and goats with higher prevalence in sheep than in goats. Age was found to be an important factor associated with the occurrence of *Cryptosporidium* infection as seen with the higher occurrence of *Cryptosporidium* oocysts among small ruminants of age 4-6 months as compared to the rest of the ages examined. This study has contributed to the increased understanding of *Cryptosporidium* infection in Potiskum Local Government Area of Yobe State, Nigeria.

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