RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v20i2.7

Shallangwa *et al.*/*Sokoto Journal of Veterinary Sciences*, **20**(2): 125 - 133.

Prevalence of *Cryptosporidium* infection in cattle from selected commercial farms and nomadic settlements in Yola, Adamawa State

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Publication History:
Received: 26-01-2022
Revised: 02-05-2022
Accepted: 24-05-2022

Cryptosporidium species are apicomplexan parasites commonly associated with diarrhoea in both men and animals. They are of public health importance. The study aimed to determine the occurrence of *Cryptosporidium* species in cattle in the Yola metropolitan area, Adamawa State, Nigeria. Four hundred and sixteen (416) faecal samples were collected from cattle in commercial farms and nomadic settlements and were analyzed using Modified Ziehl Neelsen (MZN) technique. Twenty-seven (27) positive samples were subjected to nested Polymerase Chain Reaction (PCR) for the amplification of a specific fragment of 18S rRNA gene that was used to detect *Cryptosporidium* spp. Seventy-three (17.5%) out of 416 samples were positive for *Cryptosporidium* oocysts by MZN, and 26 (96.0%) out of 27 isolated oocysts of *Cryptosporidium* detected by MZN were positive by PCR. Commercial farms had a higher prevalence (19.2%) of *Cryptosporidium* oocysts than the nomadic settlements (15.9%). Based on risk factors, there was a significant association ($P<0.05$) between the occurrence of *Cryptosporidium* oocysts and factors such as sex and faecal consistency. At the same time, age, breed, management system, animal source and drinking water source varied insignificantly ($P>0.05$). This study has shown the occurrence of *Cryptosporidium* infection in cattle in the Yola metropolis. There should be an awareness campaign among public health workers on the dangers posed by the organism to humans so that the Government would enforce control and preventive measures.

Keywords: Adamawa State, Cattle, *Cryptosporidium,* Polymerase Chain Reaction, Prevalence

Introduction

Cryptosporidium is an intestinal apicomplexan parasite that infects both man and animals and is important to veterinary and public health. These parasites have been observed in the gastrointestinal tract of many, mostly healthy, animals in all five classes of vertebrates (Tzipori & Widmer, 2008; Bouzid *et al.,* 2013). *Cryptosporidium* causes cryptosporidiosis, a zoonotic disease of global importance and is one of the most common causes of diarrhoea in humans and livestock worldwide (Khair *et al.,* 2014). Cryptosporidiosis is reported to be more severe in newborns, malnourished children, and animals, causing severe diarrhoea accompanied mainly by anorexia, reduced milk intake, dehydration, growth retardation, stiffness, hyperpnoea, slow gait, and depression (Hunter & Nichols, 2002; Fayer, 2004).

The source of infection is the oocyst, and transmission is mainly by ingestion of the oocyst in food, air and water. *Cryptosporidium* spp. constitutes a severe threat to safe water supply as it can be regularly found in raw surface water processed for human consumption (OIE, 2016). Associations between animal contacts and transmission of cryptosporidiosis in humans have been documented (Kiang *et al.,* 2006). Similarly, early childhood infection may lead to impaired physical and cognitive development (Guerrant *et al.,* 2011). The infection may have an economic impact on farmers presenting high morbidity and mortality rates in some instances among farm animals (Brar *et al.,* 2017). Several *Cryptosporidium* species have been identified in cattle, and these include *C. parvum, C. andersoni, C. bovis, C. ryanae, C. hominis, C. felis* and *C. suis* (Wang *et al.*, 2011). *Cryptosporidium parvum* is zoonotic and found in humans (Lange *et al.,* 2014). Control and prevention of the disease require an adequate understanding of the environmental factors that might enhance transmission of the infection (Collinet-Adler *et al.,* 2015).

Cattle is a major source of protein in Nigeria including the study area and common breeds of cattle found in the study area include; Adamawa Gudali, White Fulani, Red Bororo, and imported species of various breeds. Thus, beef is the most widely accepted meat in the area. Besides being bred, fattened, and sold to generate income, cattle rearing in Adamawa State have wider usage, such as the production of beef, hide and skin, agricultural manure, transport of agricultural products from one location to the other. Similarly, it helps to provide power for the tilling of the soil. It also serves as a source of milk and cheese. The bones and blood are also ingredients for the production of chicken feeds among others (Girei *et al.,* 2013). The increased movement of people and their livestock in Adamawa State in the recent past, especially from the Northern part of the State into Yola and its environs as a result of the insurgency, might have posed a high risk for the transmission of cryptosporidiosis and other infectious diseases among the populace.

In Nigeria, some authors have established the occurrence of *Cryptosporidium* infection in different animal species and man (Ayeni *et al*., 1985; Kwaga *et al.*, 1988a; Kwaga *et al.*, 1988b; Maikai *et al*., 2013; Akinkoutu *et al*., 2014; Okojokwu *et al.,* 2016; Abare *et al*., 2018; Chukwu *et al.,* 2019). However, the knowledge of its occurrence in Nigeria and the distribution worldwide is undergoing more study (Randhawa *et al.,* 2012; Khair *et al.,* 2014).

The study aimed to determine the prevalence of *Cryptosporidium* species in cattle in Yola, Adamawa State, Nigeria, which will be useful for the creation of awareness by public health specialists on the dangers posed by the organism so that measures for control and prevention can be instituted.

Materials and Methods

Study area and study design

The study was carried out in Yola which comprises Yola North and Yola South Local Government Areas, Adamawa State, Nigeria. A cross-sectional study was carried out between March and July 2019. Faecal samples were collected from both adult cattle and calves with or without diarrhoea in a nomadic settlement and 7 commercial farms in the study area. It was based on the records obtained from the Ministry of Livestock and Aquaculture Development, Adamawa State Government.

The sampling sites were selected based on convenience sampling technique. Samples were collected from seven (7) identified farms in the Yola metropolis. Four (4) farms were located in Yola North Local Government Area, while three (3) were in Yola South Local Government Area. The faecal samples were proportionally collected. A simple random sampling technique was adopted on the farms, where the faecal samples were collected from cattle restrained inside a crush. The sample collection from the nomadic settlement was also based on convenience sampling method, where faecal samples were collected from cattle that were easily restrained and also permitted by the owners. Faecal samples were collected from 8 herds in the nomadic

settlement which comprised of 26 selected cattle from each herd.

Ethical approval

This work was carried out based on approval by the Ahmadu Bello University Zaria Committee on Animal Use with Approval Number ABUCAUC/2020/75.

Sample size determination

The sample size was calculated as described by Thrusfield (1997), using 22.3% prevalence obtained by Adamu *et al.* (2015) in Maiduguri, North-East Nigeria and 416 faecal samples were obtained.

Sample collection

A total of 416 faecal samples from cattle were collected from the study area. Approximately 20g of faecal specimens was collected directly from the rectum of each of the selected animals using sterile polythene bags. The samples were labelled appropriately, placed on ice in an insulated container in order to maintain a low temperature. All samples were transported to the Parasitic Zoonoses and Helminthology Laboratories of the Department of Veterinary Public Health and Preventive Medicine and the Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria respectively, for processing. Those that were not processed immediately were stored in a refrigerator at 4^{o}C.

Administration of questionnaire

A well-structured and close-ended questionnaire with two sections was designed and administered to owners of the farms visited to ease data processing, minimize variation, and improve the precision of responses. The questionnaires were administered prior to sample collection to enable the identification of the factors that are associated with the faecal shedding of *Cryptosporidium*. Section A comprised of the socio-demographic information of the respondents and questions on *Cryptosporidium* transmission. While the information collected in Section B included the age, breed and sex of the animal. Additional information obtained was the source of water, source of animal, management practice and faecal consistency.

Sample processing and identification of Cryptosporidium oocysts

One gram of faeces was taken and mixed in 10 ml of 10% formalin in a universal bottle using an applicator stick. The homogenized faeces was sieved into a centrifuge tube using a funnel and gauze, after which 3 ml of diethyl ether was added to extract fat from the filtrate. The centrifuge tube was corked and shaken gently to mix properly. The tube was centrifuged at 2000 x g for 2 minutes and the supernatant was decanted. The sediment was mixed with a spatula, from which a thin smear was made on a clean glass slide and air dried. The air dried smear was fixed in methanol for 2 - 3 min. The slide was then flooded with cold carbol fuchsin for 5 - 10 min and then with 1% hydrochloric-acid ethanol until colour ceased 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 X40 magnification. The *Cryptosporidium* oocyst appeared as bright rose-pink spherules on a pale blue background. The positive samples were preserved in 2.5% potassium dichromate pending molecular technique and stored in a refrigerator at 4^{o}C.

Molecular technique

Twenty-seven (37%) of the 73 samples positive by microscopy were randomly and proportionally selected across all the sampling areas for PCR detection. The samples were placed on ice in an insulated container in order to maintain low temperature and transported to Kaduna DNA laboratory for processing.

DNA extraction

Quick-DNA^{TM} Fecal/Soil Microbe Miniprep Kit (Zymo Research) was used in DNA extraction according to the manufacturer's instructions. The pelleted DNA suitable for PCR was then stored in -20°C freezer pending the PCR procedure.

Polymerase chain reaction (PCR)

A nested Polymerase Chain Reaction (PCR) protocol based on the amplification of a specific sequence of 18S rRNA gene was used to detect *Cryptosporidium* spp (Xiao *et al.*, 1999). The method involved the amplification of an approximately 1,325bp long primary product followed by a secondary amplification of an internal fragment with a length of approximately 830bp. The gene fragments were amplified with the primer pairs, including forward (F1) primer (F1: LX0697): 5'-AACCTGGTTGATCCTGCCAGTAGTC-3', reverse (R1) primer (R1: LX0669): 5´-TGATCCTTCTGCAGGTTCACCTACG -3' forward (F2: LX0698) primer: 5'- GGA AGG GTT GTA TTT ATT AGA TAA AG -3', reverse (R2) primer (R2: LX0670): 5´-CTC ATA AGG TGC TGA AGG AGT A -3' for first and second rounds PCR amplifications respectively. The PCR reactions were carried out in a PTC-100^{TM} Programmable Thermal Cycler (MJ Research, Inc.

Watertown, mass. United States of America) using the following PCR protocol; the primary PCR protocol had 35 cycles with an initial hot start at 94^0C for 5min, followed by 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec and a final extension step at 72°C for 5 min. The secondary PCR also has a protocol of 35 cycles with an initial hot start at 94^0C for 5min, followed by 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec and a final extension step at 72°C for 5 min. The positive PCR control was a specific DNA template (*C. ryanae* was used in this case), and a negative PCR control was the master mix without any *Cryptosporidium* DNA.

Gel electrophoresis

The amplified products from PCR were detected and verified for size by running a 1.5% agarose gel at 100 V for 30 mins and stained with Ethidium bromide. The gel was viewed under a UV transilluminator (G-BOX), from vacutec and the band sizes were determined by comparing with the 100bp ladder (Brody *et al.,* 2004)

Data analysis

Data obtained during the study were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS VERSION 20.0). Results obtained were presented in the form of tables. Prevalence rates of *Cryptosporidium* and various risk factors were calculated by dividing the number of positive samples over the number of samples examined for each factor. Chi-square, Fisher's exact test and odds ratio were used to test for associations between the prevalence of *Cryptosporidium* and age, sex, breed, management practice, and water source.

Results

Cryptosporidium oocysts were observed in 73 of the 416 faecal samples examined, with an overall prevalence of 17.5%.

Results obtained from the study showed that 40 (19.2%) of the 208 samples collected from cattle in the commercial farms were positive for *Cryptosporidium* oocysts. Of the 208 samples collected from the nomadic settlement, 33 (15.9%) were positive for *Cryptosporidium* oocysts (Table 1). There was no statistically significant association (P>0.05) (OR= 0.792; 95% CI on OR: 0.477< OR < 1.315; P=0.367).

Out of 114 samples collected from Yola North LGA, 17 (14.9%) were positive for *Cryptosporidium* oocysts, while 56 (18.5%) out of 302 samples collected from Yola South LGA, were positive for *Cryptosporidium* oocysts (Table 1). There was no statistically significant association (P>0.05) between the occurrence of *Cryptosporidium* oocysts and the sampling location (OR=0.770; 95% CI on OR: 0.426<OR<1.391; P=0.385).

Out of 416 samples tested (Table 1), 148 and 268 were males and females respectively. Thirty-four (23%) males and 39 (14.6%) females were infected.

There was a significant statistical association (P<0.05) (OR=0.571; 95%CI=0.342<OR<0.953; P=0.031) between the sex and occurrence of *Cryptosporidium* oocysts.

Out of 68 samples examined in the age group <9 months of age (Table 1), 17 (25.0%) were positive for *Cryptosporidium* oocysts, while 56 (16.1%) of 348 samples that were examined for animals that were $\geq$ 9 months of age were positive. There was no statistically significant association (P>0.05) observed between the age groups and the occurrence of infection (OR=0.575; 95%CI=0.310<OR<1.068; P=0.077)

Animals with loose/watery faeces had the highest infection rate, 27 (35.1%), and well-formed faeces 46 (13.6%) out of 77 and 339 examined samples respectively (Table 1). A highly statistically significant association (P<0.05) was observed between the type of faeces and the occurrence of *Cryptosporidium* oocysts (P<0.05) (OR=0.291; 95%CI=0.166<OR<0.510; P=0.001).

Cattle supplied with borehole/well water had an infection rate of 19.2% (40/208), while those that drank stream water had 15.9% (33/208), respectively (Table 1). There was no statistically significant difference (P>0.05) observed between the type of water supply and occurrence of *Cryptosporidium* oocysts (OR=1.263; 95%CI=0.760<OR<2.097; P=0.367).

The intensive/Semi-intensive system had the highest infection rate (19.2%; 40/208), followed by the extensive system (15.9%; 33/208) (Table 1). There was no statistically significant difference (P>0.05) observed between the management system and the occurrences of *Cryptosporidium* oocysts in the study area (OR=1.263; 95%CI=0.760<OR<2.097; P=0.367).

Out of 295 cattle grown at home, 56 (19%) were infected with *Cryptosporidium* oocyst, while out of 121 bought from the market, 17 (14%) were infected (Table 1). There was no statistically significant association (P>0.05) observed between the source of animal and the occurrence of *Cryptosporidium* oocysts (OR=0.698; 95% CI on OR: 0.387 < OR < 1.258; P=0.231).

Out of 58 samples collected from Holstein-cross, 16 (27.6%) were infected with *Cryptosporidium* oocyst, while 3 (23.1%) out of 13 Sokoto gudali, 7 (19.4%) out

of 36 Red bororo, 40 (15.4%) out of 260 White fulani and 7 (14.3%) out of 49 Adamawa gudali (Table 2). There was no statistically significant association (P>0.05) observed between the type of breed and the occurrence of *Cryptosporidium* oocysts (χ^2= 5.605; P=0.231).

The nested PCR detected species-specific 18S rRNA gene using primers specific for *Cryptosporidium* yielded amplicons corresponding to the positive control (*Cryptosporidium ryanae*) (Figure 1) at 830bp. The expected bands of 830bp were seen for 26 (96%) out of the 27 samples randomly selected from the positive microscopy test.

Discussion

The overall prevalence of *Cryptosporidium* infection in cattle in the study area was 17.5%, which is lower than the prevalence of 22.3% reported by Adamu *et*

Table 1: Odds ratio (OR) and 95% confidence intervals on factors affecting the prevalence of faecal shedding of *Cryptosporidium* oocysts in Yola, Adamawa State, Nigeria

Factors	Number Examined	Number Positive	Specific rate (%)	Odd ratio	95% Confidence interval on OR	P-value
Type of Farm						
Commercial farm[Ref]	208	40	19.2	0.792	0.477-1.315	0.367
Nomadic settlement	208	33	15.9			
Sampling location						
Yola South[Ref]	302	56	18.5	0.770	0.426-1.391	0.385
Yola North	114	17	14.9			
Sex						
Male[Ref]	148	34	23.0	0.571	0.342-0.953	0.031
Female	268	39	14.6			
Age (Months)						
<9[Ref]	68	17	25.0	0.575	0.310-1.068	0.077
≥ 9	348	56	16.1			
Faecal consistency						
Well formed[Ref]	339	46	13.6	0.291	0.166-0.510	<0.001
Loose/watery	77	27	35.1			
Source of water						
Borehole/ well[Ref]	208	40	19.2	1.263	0.760-2.097	0.367
Stream	108	33	15.9			
Management system						
Intensive/Semi intensive[Ref]	208	40	19.2	1.263	0.760-2.097	0.367
Extensive	108	33	15.9			
Animal Source						
Home grown[Ref]	295	56	19.0	0.698	0.387-1.258	0.230
Purchased from livestock market	121	17	14.0			
Total	416	73	17.5			

Note: [Ref]= Reference category

Table 2: Effects of breed on the occurrence of *Cryptosporidium* infection in cattle in Yola, Adamawa State, Nigeria

Breed	Number of samples Examined	Number Positive	Percentage positive (%)	Chi square (χ^2)	P-value
White Fulani	260	40	15.4	5.605	0.231
Red bororo	36	7	19.4		
Adamawa gudali	49	7	14.3		
Sokoto gudali	13	3	23.1		
Holstein-cross	58	16	27.6		
	416	73	17.5		

al. (2015) in Maiduguri, Borno State, in North-East Nigeria. However, the prevalence obtained in the present study is slightly higher than the prevalence of 16.0% reported by Maikai *et al.* (2011) in some breeds of cattle in Kaduna North, Kaduna South, Zaria, Chikun, Lere, and Jema'a LGAs of Kaduna State, North-West Nigeria and prevalence of 7.6% was reported by Ayeni *et al.* (1985) in Ile-Ife, South-West Nigeria. The differences in prevalence could be due to differences in the breed of cattle, age, management systems, geographical location and time of sampling.

A higher infection rate was observed in commercial farms (19.2%) reared under intensive and semi-intensive management systems than in the nomadic settlement (15.9%) raised under an extensive management system. This finding agreed with the report of Abebe *et al.* (2008),

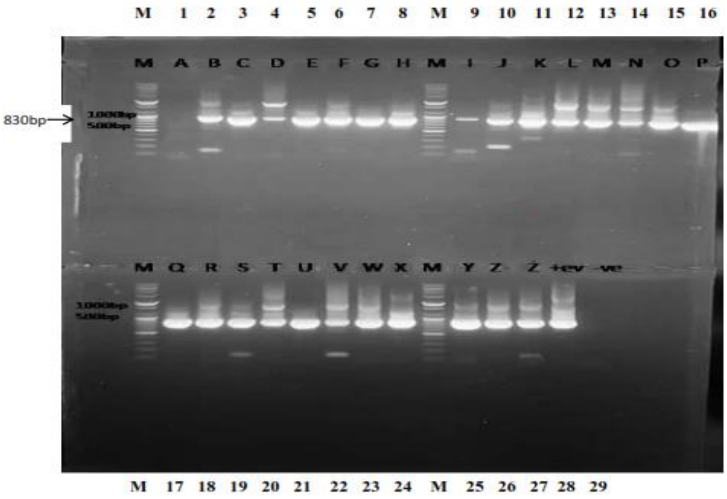

Figure 1: Detection of small subunit (SSU) rRNA gene by nested PCR in *Cryptosporidium* oocysts from bovine faeces in Yola, Adamawa State, Nigeria. Lane M is 100 bp ladder, lane 28 is *C. ryanae,* lane 29 is master mix negative control and test isolates in lane 2-27 respectively. Arrow points to 830-bp amplicons of *Cryptosporidium* specie

Maikai *et al.* (2011) and Anberber *et al.* (2018) reported that animals reared under the intensive and semi-intensive management systems are more at risk of *Cryptosporidium* infection than those in the extensive management system, which was reported to be due to continuous contamination of pasture and their environments. It was observed that the location of sample collection in Yola North and Yola South LGA has no influence on the prevalence of *Cryptosporidium* infection in this study.

A higher infection rate of *Cryptosporidium* oocysts was observed in the male (23%) cattle than in females (14.6%). The reason was not known. More so, other studies reported that the reason why males are more frequently infected than the females cannot be easily deduced without further studies that may provide adequate information (Ibrahim *et al.,* 2007; Maikai *et al.,* 2011; Faleke *et al.,* 2014; Akinkuotu *et al*., 2014; Laatamna *et al.,* 2018).

It was observed in this study, that the infection rate was higher among the animals that were <9 months old compared to those ≥9 months old. Young animals were observed in most of the locations sampled, grazing with adult cattle. A previous study reported that calves grazing together with adult animals easily get infected with the parasite (Ayinmode & Fagbemi, 2010). This finding also agreed with the reports of Fayer *et al.* (1998), Maddox-Hyttel *et al.* (2006), and Ayinmode & Fagbemi (2011), who observed that younger animals are more susceptible to diseases, including *Cryptosporidium* infection. According to Kvac *et al.* (2006), young animals which fall within this age group have deficient and/or undeveloped immune systems which makes them more susceptible to most infections, including *Cryptosporidium* infection.

In the present study, animals with watery faeces had higher occurrence of *Cryptosporidum* infection, than those with loose and well-formed faeces. This is in agreement with previous studies which reported that animals including humans with diarrhoea tended to harbour *Cryptosporidum* species and thus, may present higher rate of infection of the disease than those without diarrhoea (El-Khodery & Osman, 2008; Ghoneim *et al.,* 2013; Helmy *et al.,* 2013). According to Anderson (1998) and De Graaf *et al.* (1999), cryptosporidiosis is commonly associated with diarrhoea especially in calves, though; Chukwu *et al*. (2019) argued that diarrhoea is not always associated with the presence of *Cryptosporidum* species.

According to Mackenzie *et al.* (1994) and Rose *et al.* (2002), the source of water is a risk factor associated with *Cryptosporidium* infection. In the present study, animals whose source of drinking water was a borehole, were more frequently infected with the parasite than those provided with water from a stream or well. However, animals drinking water from boreholes were associated with commercial farms which were intensive/semi-intensively managed, thereby increasing the risk of continuous contamination of their pasture and environment, which have been previously reported to favour transmission of *Cryptosporidium* species (Abebe *et al.,* 2008; Anberber *et al.,* 2018).

Animals sampled at home were significantly more infected than those sampled at the market. This concurs with previous reports of Abebe *et al.* (2008), Maikai *et al.* (2011) and Anberber *et al.* (2018). It is assumed that animals kept close together in one place for a long period may be more susceptible to infections, including cryptosporidiosis.

In this study, Holstein-cross breed had a higher infection rate than other breeds examined. It may be due to their genetic makeup and management system. This breed of cattle was intensively managed, which was previously reported to favour the transmission of cryptosporidiosis (Anberber *et al.,* 2018).

The present study observed expected amplicons from the nested PCR at 830bp. The amplicon was specific for the positive control *Cryptosporidium ryanae*. It was observed that the amplicon was not detected from one sample that was positive by microscopy. The reason for the failure to amplify the fragment could not be readily ascertained, but it may be due to insufficient nucleic acid or purification related to the extraction procedure (Hawash, 2014). A similar finding in a previous study observed that out of 15 *Cryptosporidium* positive stool samples by microscopy and ELISA, six (6) samples were not amplified, which was assumed to be either due to low sensitivity of PCR assay or inhibition of the reaction due to the presence of impurities in the stool samples and co-purified with the target DNA (Hawash, 2014).

The majority of the respondents to whom questionnaires were administered were farmers and most of them had only a primary education level, with a few (14%) having tertiary education. Similarly, the majority of the respondents (63%) were within the ages of 21-39 years. Therefore, the awareness of cryptosporidiosis by respondents in the present study was very poor among all the categories of respondents. This is not unexpected, given the respondents' low-level awareness of the disease. The low-level awareness on the disease in cattle and its zoonotic implication may pose a serious public health concern.

In conclusion, the overall prevalence of *Cryptosporidium* species in cattle was 17.5%. A higher prevalence of *Cryptosporidium* oocysts (19.2%) was registered in the commercial farms than the nomadic settlement (15.9%), and the risk factors associated with the occurrence of *Cryptosporidium* oocysts were sex and faecal consistency. The nested PCR detected 96.0% of *Cryptosporidium* species isolates. There is a need for public enlightenment campaign programs to educate cattle farmers and handlers that cattle could harbour infectious agents such as *Cryptosporidium* species that may infect humans and other animals.

Acknowledgement

The authors wish to acknowledge Prof. JKP Kwaga, and Musa Abare Shehu of the Department of Veterinary Public Health and Preventive Medicine; and Dr Peter Ehizibolo of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for their immense support throughout the duration of the research. Their constructive criticism, encouragement and continued support have made this work a success.

Conflict of Interest

The authors declare that there is no conflict of interest.

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