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Molecular Typing of *Cryptosporidium* in Cattle in Federal Capital Territory, Nigeria

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ABSTRACT

Cryptosporidium is an enteric pathogen with worldwide distribution in humans and animals with cattle as their reservoirs. Molecular tools have been very useful in determining the epidemiology of this zoonotic pathogen of public health importance. Despite the importance of this pathogen to humans and animals, there is a limited published information on the risk factors of its epidemiology in the Federal Capital Territory of Nigeria. Positive faecal samples of 400 cattle in 46 farms located in 6 area councils of the FCT that were earlier screened for *Cryptosporidium* on light microscopy were genotyped by nested PCR using primers that targeted 18S ssUrRNA gene and followed by RFLP. The PCR-RFLP products were further sub genotyped by nested PCR and GP 60 KDa gene amplification. The nucleotides of the amplicons were subsequently sequenced. The overall PCR detection rate was 5.5%. Analysis of the 18S rRNA gene of the PCR-RFLP fragments revealed *Cryptosporidium ryanae* (18.3%), *C. andersoni* (8.5%) and *C. parvum* (4.2%). Analysis of the nucleotide sequence of the GP 60KDa gene of all the *C. parvum* detected showed all to be 100% subtype family IIa and 100% sub genotype IIaA18G3R1. This study in conclusion has established the presence of *Cryptosporidium* in cattle in FCT. The detection of the zoonotic *C. parvum*, its subtype IIa and its allele IIaA18G3R1 is an indication of source of zoonotic transmission of the parasite in the FCT, Nigeria.

Keywords: Cryptosporidium; Characterization; Cattle; PCR; Risk factors

INTRODUCTION

Cryptosporidium is an enteric protozoan parasite in both vertebrates and invertebrates that has worldwide distribution (Xiao and Ryan, 2004; Caccio *et al*., 2005) causing gastrointestinal problems in humans and respiratory problems in birds (Morgan *et al*., 2001). This protozoan has variously been reported to pose significant threat to productivity and survival of the animals underscoring the necessity for the study of their epidemiological risk factors (Tung *et al*., 2012; Jacobson *et al*., 2018). *Cryptosporidium* is transmitted through the ingestion of oocysts found in contaminated food or water from where they infect the gastrointestinal tract (Robertson and Fayer, 2013; Fayer, 2004). Though, severe watery diarrhea and abdominal pain have been associated with cryptosporidiosis, subclinical infections are not any less common (Chen *et al*., 2002; Kotloff *et al*., 2013). The infections could however be chronic, severe and fatal in immunocompromised individuals (Cohen *et al*., 2006; Siwila *et al*., 2007). Infected individuals may excrete large number of *Cryptosporidium* oocysts are shed by infected individuals in their feces leading to contamination of drinking and recreational water, fruits, vegetables and humans and other animals that consume them (Del Coco *et al*., 2008). Calves and lambs are the main source for human cryptosporidiosis (Sari *et al*., 2009) The relative similarity in the morphology and life cycle of the different species of the *Cryptosporidium* in humans and animals made their morphologic and phenotypic differentiation indistinguishable hence the use of molecular tools which has revealed their host specificity. Out of many species differentiated using molecular tools, four (*C. parvum, C. bovis, C. ryanae* and *C. andersoni*) are considered most predominant in cattle worldwide (Xiao, 2010; Santin, 2020). In terms of global distribution, *Cryptosporidium* have been commonly reported in the order *C. parvum, C. bovis* and *C. ryanae* (Feng *et al*., 2018). Unlike *C. parvum*, these species have however, not been associated with diarrhea in cattle (Santin, 2013) and are found mostly in post-weaned calves and heifers (Silverlås *et al*., 2010; Murakoshi *et al*., 2012; Yildirim, 2020). Newborn calves have been suggested to be more susceptible to *Cryptosporidium* infections because of their immature immune system (Fayer *et al*., 2007).

Previous studies have reported various prevalence in cattle in different locations in Nigeria using different detection methods (Maikai *et al*., 2011; Okojokwu *et al*., 2016; Adeiza and Nafarnda, 2020; Ola-Fadunsin *et al*., 2022; Adeiza *et al*., 2023), but to our knowledge, no characterization study has been done on *Cryptosporidium* in cattle in FCT. This study therefore pioneers the molecular characterization of *Cryptosporidium* in cattle in FCT, Nigeria.

MATERIALS AND METHODS

The Study Area

The study area is the Federal Capital Territory (FCT), Nigeria which consists Abaji, Abuja Municipal (AMAC), Bwari, Gwagwalada, Kwali and Kuje area councils. With a population of 2,238,800 (NPC, 2006), FCT covers a landmass of 7,315km² with a human density of 306/km². FCT is located between latitude 8°25′ and 9°20′N of the Equator and longitude 6°45′ and 7°39′E of Greenwich Meridian (Wikipedia, 2017). The territory is bordered in the north by the confluence of Rivers Niger and Benue, west and North by Niger state, northeast by Kaduna, east and south by Nasarawa and to the southwest by Kogi state. The indigenous people are mostly Gbagyl tribe with farming as their major occupation.

The FCT lies in the Guinean forest-savannah mosaic zone of the West African sub-region with an annual total rainfall of 1100 and 1600mm from April to October. The other seasons of the territory are extremely hot dry season and short period of harmattan in between these seasons (www.abujacity.com, 2017).

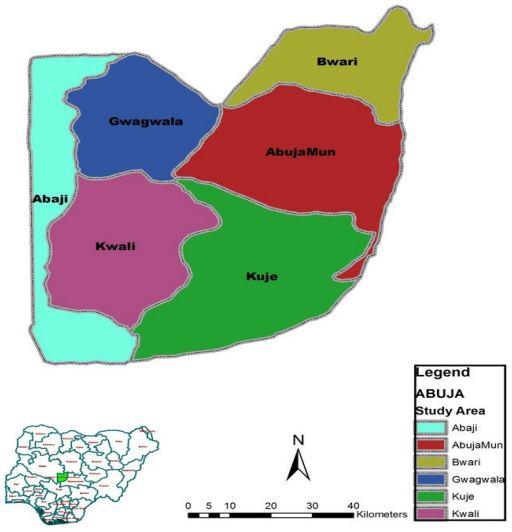

Figure 1: Map of Nigeria showing the location of the sampling points (Area Councils) in FCT, Nigeria. Source: Modified from the Administrative map of Nigeria.

Sample Collection (indicate how many samples were collected from each area council

The 400 sample population in this study was determined using the formula of Mugo (2008) using 13% Cryptosporidium prevalence in cattle in North central, Nigeria. The samples were proportionately sampled from each area council based on the estimated cattle population in each of the area council according to the records at the Naional Bureau of Statistics (2009), resulting in collecting of 76 samples in Abaji, 76 (AMAC), 60 (Bwari), 68 (Gwagwalada), 68 (Kuje) and 52 (Kwali).

Sample Processing

Fresh feces of approximately 2 g each were collected from the rectum of randomly selected 400 cattle aged between 1 day and 720 days old using disposable sterile hand glove into sterile plastic vials by stimulating the areal region of the cattle so that they could defecate. The ages of the cattle were further classified into young age (1- 360 days old) and older cattle (361 – 720 days old) according to cattle age determination using horn ring method (Singh, 2021). The cattle breeds were Sokoto gudali, Bunaji (White Fulani) and the cross between the two breeds. The samples were collected from 46 farms within the Federal Capital Territory between September 2018 and April 2019 using sterile hand gloves. These two breeds are the dominant cattle in Nigeria and usually kept for milk and meat production. While Fulani are known for their drought resistant trait and are widespread in West Africa, Sokoto gudali are heat resistant and more commonly found in the northern part of Nigeria, Benin, Ghana and Mali (Tawah and Rege, 1996). Most of the farms are of little holdings of between 5 to 35 cattle. Each farm owner was administered with questionnaire to obtain risk factor data of sex, age, size of herd, fecal consistency (Visual assessment). The samples were divided into 2 equal portions of 1 g each. One portion was preserved in 2.5% potassium dichromate at a proportion of 1 g of stool in 1 ml of potassium and stored in a refrigerator at 4^{o}C for molecular analysis and the other portion preserved with 10% formalin (1g of feces in 3 ml of formalin) for analysis using Safranin -Methylene Blue staining method using a light microscope at a magnification of x40 and the oocysts confirmed under a higher power (100x).

Genomic DNA Extraction

DNA extraction, gene amplification, and sequencing

The 18S rRNA gene of all the samples that tested positive for *Cryptosporidium* at light microscopy was analyzed to determine the species. The FastDNA SPIN kit for soil (BIO 101, Carlsbad, CA) was used in DNA extraction (Feng *et al*., 2007).

Detection of *Cryptosporidium* using PCR

The 830-bp region of the small-subunit (SSU) rRNA gene was targeted for detection of the *Cryptosporidium* in the DNA of the fecal specimens by nested PCR as described by Feng *et al*., (2007). The DNA of *Cryptosporidium* baileyi was used as the positive control in analyzing each specimen. The PCR digestion products were separated in 1.5% agarose gel electrophoresis after staining with ethidium bromide.

Identification of *Cryptosporidium* species by RFLP

The enzymes SspI and MboII were used to identify the *Cryptosporidium* species in positive specimens through restriction fragment length polymorphism (RFLP) analysis of the secondary PCR products. The products of digestion by these enzymes were separated by 2% agarose gel

electrophoresis. The pattern of bands was used to differentiate the common *Cryptosporidium* species in cattle (Feng *et al*., 2007).

Molecular Typing of *Cryptosporidium* isolates from Cattle in Federal Capital Territory

The secondary PCR products were purified using the Multiscreen PCR Plate (Millipore, Bedford, MA). Representatives of each band patterns of the SSU rRNA gene (*C. andersoni, C. ryanae* and *C. parvum*) were sequenced in both directions to confirm the *Cryptosporidium* species. BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA) was used in performing the sequencing reaction. The sequencing reactions were read on an ABI Prism® 3130 Genetic Analyzer (Applied Biosystems) after being cleaned with Centrisep spin columns (Princeton Separations, Adelphia, NJ). The sequences obtained were aligned with each other and compared with published sequences in the GenBank database using the software ClustalX (ftp://ftp-igbmc.utrasbg.fr/pub/ClustalX/).

Questionnaire analysis

The risk factors of *Cryptosporidium* in cattle in FCT, were analyzed from the results of a close-ended and open-ended (multiple question or dichotomous) well-structured questionnaire with questions targeted on obtaining information on the cattle as the host, and factors of location (Based on Area councils), sex, breed, age and such environmental factors of source and type of waters consumed by the cattle under study, number of cattle in a herd and type of management. The questionnaires were administered through veterinary assistants in this projects who helped interpret to the unlettered cattle owners and sometimes filled in the answers by having a face to face interaction with the farm owners or managers.

Data analysis

Associations between various risk factors and the occurrence of *Cryptosporidium* infection were analyzed using the chi-square test in the EpiInfo (version 3.5.1, Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS

A total of 71 positive samples obtained from 400 fecal samples (17.8%) collected from cattle in the FCT earlier subjected to microscopic examination for *Cryptosporidium* oocysts (Adeiza *et al*., 2023) were characterized in this work. Out of the 71 microscopy positive samples analyzed, 5.5% (22/400) of the SS rRNA PCR products generated gave expected band of 826– 864 bp thereby confirming the presence of *Cryptosporidium*.

Abaji had the highest prevalence of *Cryptosporidium*, 8 (10.5%) in terms of location (Area council). Other locations were Bwari, 6 (10.0%), Kwali 2 (3.9%), Gwagwalada 2 (2.9%), Kuje 3 (4.4%) and AMAC 1 (1.3%). There is no association between prevalence of *Cryptosporidium* and area councils in the FCT (p = 0.079) (Table 1).

Table 1: Distribution of Cryptosporidium positive isolates based on the area councils in FCT, Nigeria

Location	No of specimens examined	Number negative (%)	No of positive samples (%)	Chi-square (X^2)	P-value
Abaji	76	68 (89.5)	8 (10.5)	9.88	0.08
AMAC	76	75(98.7)	1 (1.3)		
Bwari	60	54(90.0)	6 (10.0)		
Gwagwalada	68	66 (97.1)	2 (2.9)		
Kuje	68	65 (95.6)	3 (4.4)		
Kwali	52	50 (96.2)	2 (3.9)		

*% = percentage; Level of significance (Pv) 0.005

Factors facilitating risks of *Cryptosporidium* infection

More than 50% of the risk factors tested in this study showed significant difference in the rate of *Cryptosporidium* infection. Age-*Cryptosporidium* relatedness showed cattle within 1 – 180 days had higher prevalence of *Cryptosporidium* 5 (21.7%) than those of 181-360 days 10 10 (8.2%), 361 -540 days, 2 (1.4%) and 541 - 720 days, 5 (4.6%). The difference in the age of cattle and the rate of *Cryptosporidium* infections is marginally significant (0.05). Sokoto gudali breeds of cattle had higher prevalence of *Cryptosporidium*, 11 (7.8%) than Bunaji 9 (4.2%) and the cross between the two indigenous breeds 2 (4.9%). The difference between the prevalence of *Cryptosporidium* and breeds was not statistically significant (p = 0.337). Prevalence of *Cryptosporidium* in the female cattle, 16 (9.1%) was significantly higher than the males 8 (3.6%). The difference in the prevalence of *Cryptosporidium* and sex was statistically significant (p = 0.020). Regarding texture of feces, diarrheic (watery) feces had higher prevalence of *Cryptosporidium* 6 (21.4%) compared to well-formed 9 (10.6%) and loose feces 7 (7.0%). The difference between prevalence of *Cryptosporidium* in diarrheic feces and other texture of feces was not statistically significant (0.335). Cattle that had access to water bodies as their only means of drinking water had significantly higher rate of *Cryptosporidium* infection 2 (28.6%) compared to those that drank from water bodies and tap, 5 (3.9%), All sources, 12 (7.9%), water bodies and well 2 (9.5%) and water bodies and borehole, 1 (1.1%). The difference in prevalence of *Cryptosporidium* and the water sources was statistically significant (p = 0.008). Type of cattle management practices showed significant impact on the prevalence of *Cryptosporidium*. Cattle raised under intensive method had significantly higher prevalence of *Cryptosporidium*, 13 (19.1%) compared to those raised extensively 8 (3.3%) semi-intensive 1 (1.1%). There was a significant difference in the prevalence of *Cryptosporidium* and the type of management method of rearing cattle (p = 0.001).

Table 2: Distribution of *Cryptosporidium* positive isolates based on potential risk factors of infection in FCT, Nigeria

	Number (n =400) of Samples (%)	Number Negative (%)	Number PCR Positive (%)	Chi-square (X^2)	P-value
Age (Days)					
Young age					
1 – 180	23 (5.6)	18 (78.3)	5 (21.7)	3.83	0.050
181 – 360	122 (30.5)	112(91.8)	10 (8.2)		
Older cattle					
361 – 540	146 (36.5)	144 (98.6)	2 (1.4)	1.53	0.217
541 – 720	109 (27.3)	104 (95.4)	5 (4.6)		
Sex					
Males	224	216 (96.4)	8 (3.6)	5.32	0.020
Females	176	160 (90.9)	16 (9.1)		
Breeds					
Sokoto gudali	142	131 (92.3)	11 (7.8)	2.17	0.337
Bunaji	217	208 (95.9)	9 (4.2)		
Cross	41	39 (95.1)	2 (4.9)		
Fecal texture					
Well-formed	85	17 (20.0)	9 (10.6)	2.18	0.335
Loose	100	10 (10.0)	7 (7.0)		
Diarrhea	215	22 (10.2)	6 (21.4)		
Water consumed					
All sources	150 (38.0)	138 (92.1)	12 (7.9)	13.78	0.008
Water bodies and well	21 (5.3)	19 (90.5)	2 (9.5)		
Water bodies and borehole	93 (23.3)	92 (98.9)	1 (1.1)		
Water bodies and tap	129 (32.3)	124 (96.1)	5 (3.9)		
Water bodies	7 (1.8)	5 (71.4)	2 (28.6)		
Management Practices					
Intensive	68 (17.0)	55 (80.9)	13 (19.1)	29.9	0.001
Semi-intensive	93 (23.3)	92 (98.9)	1 (1.1)		
Extensive	239 (59.8)	231 (96.7)	8 (3.3)		

*% = percentage; Level of significance (Pv) 0.005

Distribution of *Cryptosporidium* species

The SS rRNA gene of the 71 microscopy *Cryptosporidium* positive fecal samples from the 400 cattle in FCT were subjected to digestion using *SspI* and *VspI* restriction enzymes and partial sequencing. The digested products were 3 *Cryptosporidium* genotypes. Of the amplicons, 13 (18.3%) *were Cryptosporidium ryanae (*AB513679 and EU410344), 6 (8.5%) *C. andersoni* (GQ121017 and AB513856) and 3 (4.2%) *C. parvum* (YaK EF6133). Two of these samples (2.8%) had mixed infections of *C. andersoni and C.ryanae* (Figure 2).

Of these genotypes, 14 (9.7%) were detected in the young cattle while 8 (3.13%) were detected in the old. Specifically, young cattle had higher infection of *C. ryanae* (6.3%) than the older ages (1.2%). All the detected *C. parvum* (100%) were found in the young cattle, *C. andersoni* had greater prevalence (1.6%) in the older cattle than the young ones (1.4%). In terms of sex, prevalence of *C. andersoni* was higher in the females (1.7%) than the males (1.3%). *C. ryanae* was higher in females (5.1%) than males (1.8%). The female cattle had higher prevalence of *C. parvum* (1.1%) than males (0.5%). The distribution of genotypes according to the rest risk factors is as presented in Table 3.

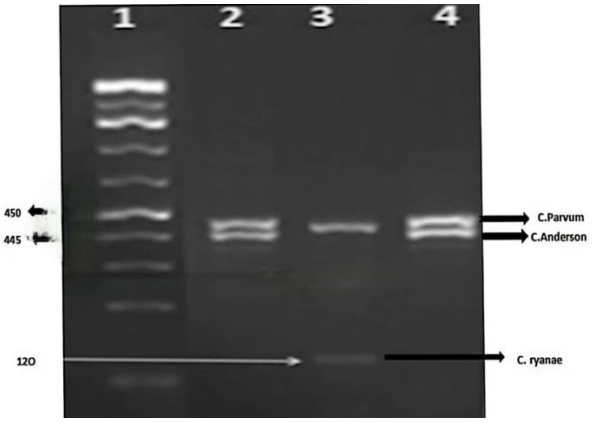

Figure 1: Products of Gel electrophoresis of Cryptosporidium SSU rRNA PCR–RFLP from digested PCR products using *SspI* and *VspI* restriction enzymes. Lane 1: 1000 bp molecular marker; lane 2, 3 and 4*: C.parvum;* lanes 2 and 4: *C. ryanae*; lane 3: *C. Parvum* with trace of *C.andersonni*.

On sequencing, based on *C. parvum* subtype classification using the number of trinucleotides repeats within the 60KDa glycoprotein gene (Jex *et al*., 2007) the 3 *C. parvum* species detected in this study were found to cluster in subtype IIa family (100%). On sub-genotyping, all the members of the subtype IIa family subscribed to allele IIaA15G2R1 (Figure 2).

Table 3: Distribution of Cryptosporidium genotypes based on potential risk factors of infection in FCT, Nigeria

Risk Factors	Number (n) of Samples (%)	Number Positive (%)	*C. andersoni*	*C. parvum*	*C.ryanae*	*C. andersoni/C.ryanae*
Age (Days)						
1 – 180	23 (5.8)	5 (21.7)	0	3	2	0
181 – 360	122 (30.5)	9 (7.4)	1	0	7	1
361 – 540	146 (36.5)	2 (1.4)	1	0	1	0
541 – 720	109(27.3)	4 (3.7)	2	0	1	1
Sex						
Males	224 (56.0)	7 (3.1)	2	1	2	2
Females	176 (44.0)	13 (7.4)	2	2	9	0
Breeds						
Gudali	142 (35.5)	10 (7.0)	1	2	6	1
Bunaji	217 (54.3)	8 (3.7)	3	0	4	1
Cross	41 (10.3)	2 (4.9)	0	1	1	0
Fecal texture						
Loose	85 (21.3)	9 (10.6)	0	2	6	1
Diarrhea	100 (25.0)	6 (6.0)	1	0	5	0
Well-formed	215 (53.8)	5 (2.3)	3	1	0	1
Waters consumed						
Water bodies	152 (380)	12 (7.9)	3	2	5	2
Water bodies and well	21 (5.3)	1 (4.8)	0	0	1	0
Water bodies and borehole	93 (23.3)	1 (1.1)	0	0	1	0
Water bodies and tap	129 (32.3)	4 (3.1)	0	1	3	0
All sources	7 (9.9)	2 (1.8)	1	0	1	0
Management practices						
Intensive	68 (17.0)	12 (17.7)	1	1	8	2
Semi-intensive	93 (23.3)	1 (1.1)	1	0	0	0
Extensive	239 (59.8)	8 (3.3)	3	2	3	0
Total	71 (31.0)	22	6	3	11	2

*% = percentage; C = Cryptosporidium

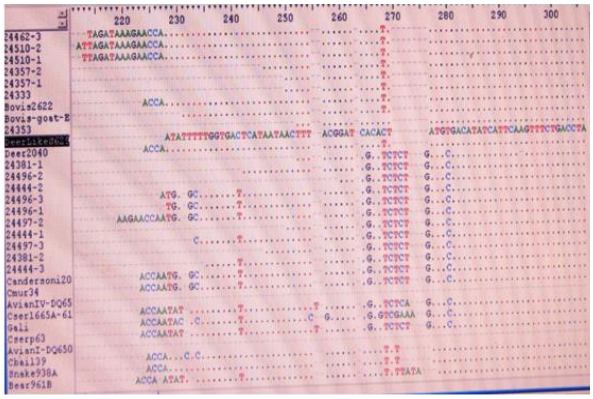

Figure 2: Photomicrograph of sequencing product showing the genetic variation in SSU region of the rRNA gene of *Cryptosporidium* spp. Dots denote sequence identity to Deerlikes629. Dashes denote deletions.

DISCUSSION

Molecular tools have become the contemporary instruments for detection and genotyping of *Cryptosporidium* into species to determine its epidemiology. An overall prevalence of *Cryptosporidium* in cattle in FCT using PCR (5.5%) was established in this study. This figure is like the findings of Faleke *et al*., 2014. The figure is comparatively lower than the 17.8% in FCT (Adeiza *et al*., 2023), 16% in Kaduna state (Maikai *et al*.,2011), 13.0% in Lokoja, North Central, Nigeria (Adeiza and Nafarnda, 2021), 32.3% in Oyo state, Nigeria (Ayimnode and Fagbemi, 2011), and 37.5% (Akinkuotu *et al*., 2014). The differences in prevalence may be due to study area ecology, sampling and detection methods (Adeiza *et al*., 2021). Degree of infectivity of cattle from different area councils vary with Abaji showing the highest prevalence but there is no significant difference in the number of positive samples among the different locations ($p>0.05$).

In terms of age distribution, prevalence was significantly higher (0.050) in young cattle than the older ones. Of the specific age groups, cattle of 1- 180 days of age showed the highest frequency of occurrence of *Cryptosporidium* (21.7%) over the other age groups in the study. The difference was not however significant ($p = 0.217$). The relatively higher vulnerability of the young cattle to *Cryptosporidium* than the old may be due to the immaturity of their immune systems (Fayer *et al*, 2007). Variability in terms of species showed that the number of species prevalent in the young cattle is also more than those in the older cattle with an apparent decline in frequency and number of species occurrence as the age of cattle advances. This decline may be due to the development of a partial protective immune response after multiple infections with the protozoan (Ares-Mazás *et al*., 1999). Many reports from earlier studies (Wang *et al*., 2011; Murakoshi *et al*., 2012; Silva *et al*., 2013; Smith *et al*., 2014) corroborated lower than 10% prevalence of *Cryptosporidium* in healthy cattle advancing towards the age of 2 years. A significantly high sex/parasite discrimination ratio was apparent in this study. Female cattle showed significantly higher (0.020) prevalence of *Cryptosporidium* infection (9.1%) than males (3.6%). The higher threat of *Cryptosporidium* to female cattle in this study may not be unconnected with the normal physiological state of pregnancy and lactation of the female cattle which often leads to stress and hormonal imbalance. The stress and hormonal imbalance

compromise their immune status thereby reducing their ability to withstand infections. Stress has generally been reported to facilitate animals, including cattle, to cryptosporidiosis (Díaz-Lee *et al*., 2011; Pam *et al*., 2013). The high mating ratio of female to male often adopted by farmers may also be responsible for the higher level of exposure of females to infections than male cattle (Adeiza *et al*., 2023). Bunaji breed dominate the sampled cattle in this study, 54.3% (217/400), while Cross breeds were the least sampled, 10.3% (41/400). The prevalence was however highest in the Sokoto gudali breed, 7.8% (11/142) which were 35.5% (142/400) followed by cross breeds (4.9%). The high infection rate in Sokoto gudali is like the previous reports of Nwoga, (2011), Maikai *et al* (2012) and Adeiza *et al*., (2023). The higher rate of *Cryptosporidium* infection in Sokoto gudali when compared to Bunaji or their cross may be because the Bunaji breeds are more resistant to diseases than Sokoto gudali (Tawah and Rege, 1996). Though the number of the cross breed in this study was small, it had relatively high prevalence which is consistent with reports of higher prevalence in cross breeds compared to indigenous zebu breeds in Ethiopia which was attributed to management practices employed in rearing cattle in the two different farms and area (Mohammed, 1999).

The factor of fecal texture in facilitating cryptosporidiosis in cattle in FCT was tested and the result showed diarrheic feces had the highest prevalence (21.4%) amongst other forms of feces. The difference in prevalence of *Cryptosporidium* in diarrheic feces and other fecal textures was not significant (0.335). Cattle raised through extensive management system predominated the sampled population (96.7%) against the lower population of those raised intensively (17.0%) but had higher prevalence of *Cryptosporidium* (19.1%). Satin (2020) had reported the recognizable role of *C. parvum* in the etiology of neonatal calf diarrhea. There is therefore, a correlation between this report and the high prevalence of the parasite in the diarrheic cattle. This current finding contradicts the previous reports (Ayinmode and Fagbemi, 2011; Dankwa *et al*., 2021; Adeiza and Nafarnda, 2021; Adeiza *et al*., 2023) where they variously reported diarrhea to be independent of *Cryptosporidium* infection. This result is however in tandem with the report of Ehsan *et al*. (2019) where he reported calves shedding oocysts as having 6.1 times the risk of being diarrheic. This current result also corroborates Mensah *et al* (2018) observing an association between *Cryptosporidium* infection and diarrheic cattle. The works of Das *et al* (2018) and Ayele *et al* (2018) further support the finding in this study. The type of water consumed by the cattle as a risk factor of Cryptosporidiosis in cattle reveals prevalence of *Cryptosporidium* to be highest in cattle that drank water from water bodies comprising of rivers, streams and surface water and run -offs compared to those that drank from other sources. This result is in accord with the previous report (Yang *et al*. 2008).This may be because water bodies is an open receptacle for *Cryptosporidium* contamination from oocysts-blowing winds, flying birds, grazing animal feces and run-offs.

In terms of distribution**,** *Cryptosporidium ryanae, C. bovis, C. andersoni* and *C. parvum* have been identified as the four frequently occurring species of *Cryptosporidium* in cattle worldwide, using molecular detection tools (Xiao, 2010; Santin, 2020). In this work, three of the species were identified in cattle in FCT. These were *Cryptosporidium ryanae* (3.3%), *Cryptosporidium andersoni* (1.5%) and *Cryptosporidium parvum* (0.8%) in order of frequency of occurrence. Predominant number of the species were found in the young cattle group (3.5%) compared to older cattle (1.5%). The dominance of *C. ryanae* in this study is in tandem with the report of Xiao (2010) that reported *C. ryanae* subtype as the most common in cattle the world over. The predominating prevalence of *C. ryanae* in this study contradicts the previous reports of the species occurring sporadically at lower prevalence worldwide (Meireles *et al*., 2011; Murakoshi *et al*., 2012; Smith *et al*., 2014).

We have no Subtyping tools of *C. ryanae* and *C. andersoni* to allow for their subtyping and understand their distribution and transmission pathways in cattle in the study area. *Cryptosporidium andersoni* comes second in frequency of occurrence in this study. *Cryptosporidium andersoni* is a gastric parasite often associated with reduced milk yield in dairy cattle and decreased weight gain in post weaned calves (Olson *et al*. 2004). It is however occasionally detected in humans (Jiang *et al*., 2014 and Liu *et al*., 2014). In some studies, *C. andersoni* was found to be the most prevalent *Cryptosporidium* species detected in humans (Jiang *et al*., 2014; Liu *et al*., 2014). *C. andersoni* has also been reported to be the most frequently dominant species in source and tap water in China and the UK (Nichols *et al*., 2010; Feng *et al*., 2011), suggesting that cattle could be the primary source of contamination.

Out of the fecal samples collected, 0.8% were C. parvum species. *Cryptosporidium* parvum predominated other species in the younger age cattle found in this study. Neonatal diarrhea in calves had been associated with Cryptosporidum in previous studies of O'Handley, (1999) and Naciri, (1999). The species has also been reported by Preiser, (2003) to be a zoonotic parasite with a potential for direct transmission between infected calves and humans. Although dairy and beef cattle were not discriminated in this study, both are reported to be usually infected at early age (Kvac, 2006).

Prevalence of C. parvum is highest (17.7%) in intensively managed cattle compared to those managed extensively and semi-intensively. This finding agrees with some previous studies that reported lower prevalence in cattle managed in the open range (Atwill, 1999; Gow and Wadner, 2006).

The sub genotyping of the three C. parvum isolates in this study through the analysis of their General Protein (GP60) gene, revealed sequences that conform to subtype IIa family. The IIa subtype family of C. parvum species is a zoonotic family (Wang *et al*., 2014) with worldwide distribution in mammals including humans, with cattle and sheep, according to Xiao, (2010) the most infected. Subtypes are many within family IIa each differing by the number of trinucleotide repeats (TCA or TCG) that code for amino acid serine. Subtypes may also have one or two ACATCA sequences coming after the nucleotide repeats.

The subtypes in the subtype family IIa detected in this study had only one ACATCA (R1) sequence after the trinucleotide repeats and three copies of TCG (G3) with eighteen copies of the TCA trinucleotide repeat (A18). This subtype is appropriately called IIaA18G3R1. This Allele IIaA18G3R1 has been previously reported in both humans and cattle worldwide especially Australia (Waldron *et al*., 2011b).

Conclusion

In conclusion, sex, type of water consumed, and the cattle management method were suggested as risk factors in *Cryptosporidium* infections based on the results. Genotypically, the study revealed the circulation of *Cryptosporidium parvum, C. ryanae* and *C. andersoni* in cattle in FCT. This confirms the report of worldwide distribution of the pathogen. The *Cryptosporidium parvum* detected in this study was subtype family IIa and its zoonotic allele IIaA18G3R1. The presence of these zoonotic variants justifies the need for public health surveillance for the pathogen in the human population of the study area.

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Conflict of Interest

The authors do not have any conflict of interest to declare.

Authors' Contribution

Conceptualization: AMA, NWD, TZO, ESI, BSY, GE and OC; Methodology: AMA and BSY; Formal analysis and investigation: AMA, TZO, GE and OC. Writing — original draft preparation: AMA, TZO, ESI and BSY, OC, GE Writing — review and editing: AMA., GE, ESI, BSY, NWD and TZO. All the authors contributed and accepted this publication.

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