

Detection, prevalence, and risk factors associated with *Cryptosporidium* infection among cattle in Kwara State, Nigeria

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Abstract

Cryptosporidium species cause high morbidity and sometimes high mortality rates among cattle, resulting in serious economic threats. This present study is aimed at investigating the presence, prevalence, distribution, and risk factors associated with *Cryptosporidium* species infection among cattle in Kwara State, Nigeria. To achieve this, 333 apparently healthy cattle were sampled from abattoirs and different cattle herds over a one-year period. Faecal samples were collected and subjected to the formalin-ethyl acetate (formol-ether) sedimentation technique. Sediments from the formol-ether sedimentation method were used for the modified Ziehl-Neelsen staining technique. The Microsoft Excel (2016) spreadsheet and the Statistical Package for the Social Sciences (SPSS, Chicago, Illinois, USA) for Windows version 22.0 were used for the data analysis. Of the cattle sampled, 99 were positive for the enteric protozoan (29.73%; 95% confidence interval (CI) = 25.07–34.85). There was no distinct pattern in the monthly prevalence of *Cryptosporidium* infection in cattle. Breeds of cattle, physiological status, and seasons were statistically associated with the prevalence of *Cryptosporidium* species in cattle. There is a need to educate pastoralists on the control and preventive measures of this enteric protozoan to improve the economy of the cattle industry and prevent possible transmission to humans in Kwara State and Nigeria in general.

Keywords: bovine; cryptosporidiosis; epidemiology; faecal samples; modified Ziehl-Neelsen stain

Introduction

Studies including risk factor investigation for gastrointestinal protozoa infection such as *Cryptosporidium* among cattle are vital as this protozoan poses a threat to the productivity, reproductivity, and survival of a wide range of animals and hence may cause negative impacts on the animal industry (Jacobson *et al.*, 2018; Abdullah *et al.*, 2019). *Cryptosporidium* is an intracellular but extracytoplasmic coccidian parasite that belongs to the Phylum Apicomplexa, Class Conoidasida, and Family Cryptosporidiidae (Taylor *et al.*, 2016; Ayele *et al.*, 2018). This enteric protozoan is ubiquitous and obligatory and is of veterinary and medical importance because it infects a wide range of animals (including birds, carnivores, fishes, marsupials, reptiles, rodents, ruminants, dugongs, horses, pigs, and rock hyrax) and humans globally (Taylor *et al.*, 2016; Amer *et al.*, 2013; Khan *et al.*, 2019). *Cryptosporidium* species are shed in the faeces of infected hosts, and transmission

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is through ingestion (the faecal-oral route), either directly through contact with faeces from infected hosts (human or animal) or indirectly via environmental contamination or ingestion of contaminated water or food (Thomson *et al.*, 2017; Adeiza and Nafarnda, 2020). Infection can also occur by ingesting sporulated oocysts in the air (Shallangwa *et al.*, 2022). Sporulation of *Cryptosporidium* oocysts takes place within the host so that oocysts are instantly infective when passed in faeces, making autoinfection possible (Taylor *et al.*, 2016). Infection is established when ingested oocysts excyst, releasing four sporozoites that then invade host epithelial cells in the small or large intestine, depending on the species (Taylor *et al.*, 2016; Chukwu *et al.*, 2019).

Cryptosporidium species infection in cattle is referred to as bovine cryptosporidiosis. The first report of *Cryptosporidium* infection in cattle was in the early 1970s (Panciera *et al.*, 1971), but the clinical signs observed were not solely attributed to *Cryptosporidium*, as there was indication of mixed infection with other bacterial and viral organisms (Thomson *et al.*, 2017). *Cryptosporidium andersoni*, *C. bovis*, *C. parvum*, and *C. ryanae* are the main species associated with cryptosporidiosis in cattle. However, other species, including *C. hominis*, *C. felis*, *C. muris*, *C. meleagridis*, *C. serpentis*, *C. suis*, *C. xiaoi*, and *C. ubiquitum* had also been identified in cattle (Gong *et al.*, 2017). Of the *Cryptosporidium* species that infect cattle, *C. hominis*, *C. meleagridis*, *C. muris*, and *C. parvum* have zoonotic potential (Taylor *et al.*, 2016; Gong *et al.*, 2017). *Cryptosporidium* species cause high morbidity and sometimes high death rates among cattle, resulting in serious economic threats. The economic impact and potential hazard that *Cryptosporidium* species pose to the cattle industry and public health in Nigeria make it necessary to investigate the presence and epidemiology of bovine cryptosporidiosis in different parts of the country.

This study is aimed at investigating the presence, prevalence, distribution, and risk factors associated with *Cryptosporidium* infection among cattle in Kwara State, Nigeria.

Materials and Methods

Study area

The study was conducted in four local government areas in Kwara State, namely Ilorin West, Ilorin South, Ilorin East, and Asa (Figure 1). Kwara State is situated in the north-central part of Nigeria within the forest-savanna region. The state covers a total area of 35,705 km² (13,947.27 sq. miles) and is positioned between latitudes 8°05'N and 10°15'N and longitudes 2°73'E and 6°13'E. Kwara State is bordered in the north by Niger State, in the east by Kogi State, in the west by the Republic of Benin, and in the south by Osun, Oyo, and Ekiti States. The state has two major seasons: the wet (raining) (April to July and September to November) and the dry (December to March and August). The state records a mean yearly temperature ranging between 22.1°C and 33.3°C and an average annual rainfall of 112.8 cm to 146.9 cm, with an average relative humidity of 49.6% (NBS, 2016; Ola-Fadunsin *et al.*, 2019).

Sample population and faecal collection

A total of 333 apparently healthy cattle were examined from abattoirs and different cattle herds over a one-year period. Cattle were sampled using the random sampling technique. At each sampling time, a clean polyethene bag was used to collect approximately 5 g of faecal sample from the rectum of each cattle, and the bag was labelled accordingly. All collected faecal samples were immediately placed in a cool box and transported to the Parasitology Laboratory of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Ilorin, Nigeria, for further parasitological processing. At the time of sample collection, the sampling date, age, breed, sex, physiological status, faecal consistency, and body condition score were recorded for each cattle on a recording sheet.

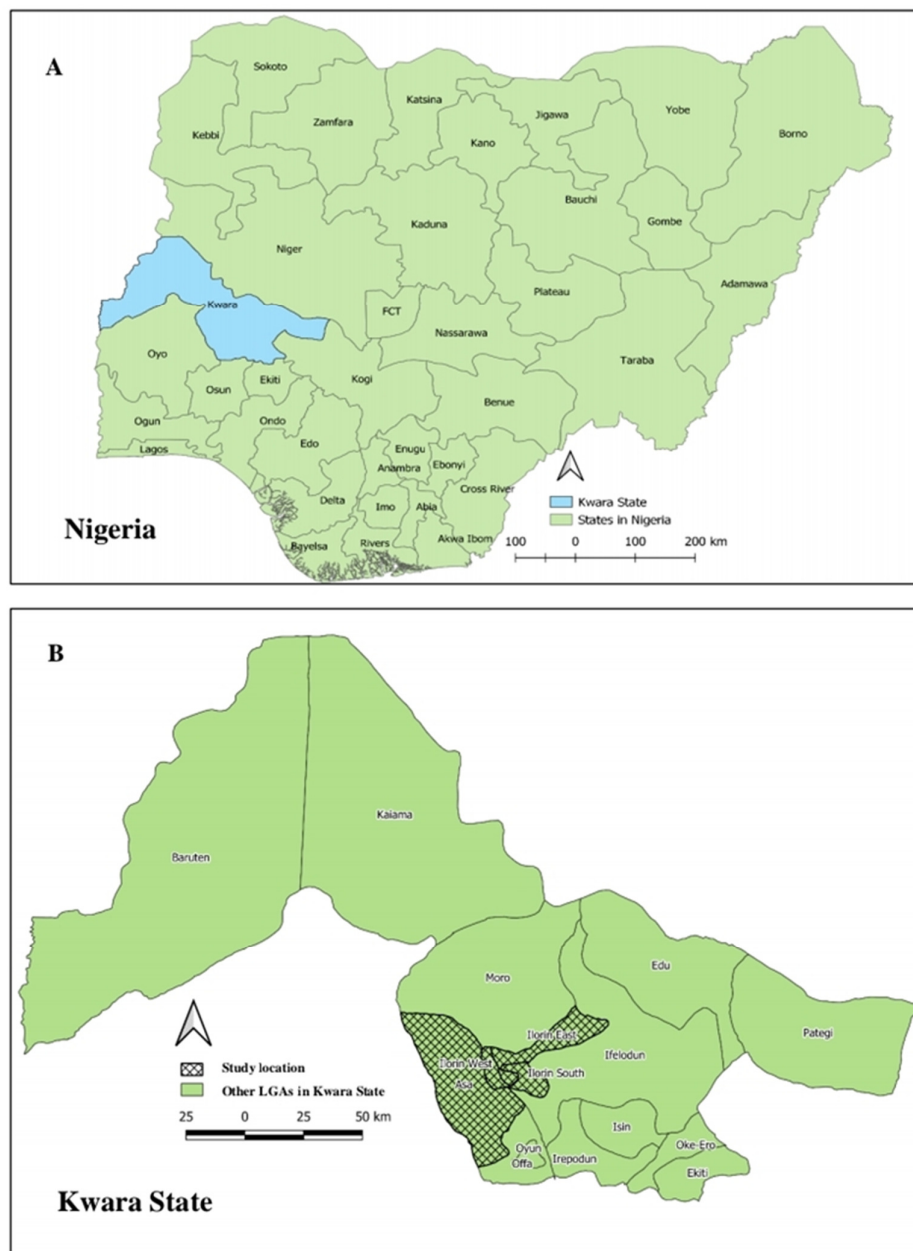


Figure 1. Map of Nigeria showing Kwara State (state of study) (A); Map of Kwara State showing the study location (B).

The age of each sampled cattle was determined using the guide as documented by Lasisi *et al.* (2002). Faecal consistency was evaluated immediately after each sample collection and classified as normal or diarrheal without any additional differentiation. Body condition scores (BCS) were determined using the protocol as recorded by Shittu *et al.* (2014). Information on each cattle was recorded accordingly.

Detection of Cryptosporidium oocyst

Cryptosporidium oocysts were detected by initially concentrating the faeces using the formalin-ethyl acetate (formol-ether) sedimentation method. Sediments from the formol-ether sedimentation method were

used for the modified Ziehl-Neelsen staining technique. The formol-ether sedimentation method and the modified Ziehl-Neelsen staining technique were carried out as documented by Cheesbrough (2009). In brief, 2 g of each faeces was dissolved in 10% formalin, sieved into a plastic test tube to the 7 ml mark, and allowed to stand for a few minutes before adding 3 ml of ethyl acetate. The tube was closed, vigorously shaken for 1 min, and centrifuged at 3000 rpm for 5 min. The debris plug was loosened, and the top three layers were discarded. About 2 drops of the sediment was used to make a smear on a clean glass slide and allowed to air dry. The air-dried smear was fixed in methanol for 2–3 minutes. It was then stained with carbol fuchsin for 15 minutes, rinsed with water, and decolourized with 1% acid alcohol for 10–15 seconds. Afterwards, it was rinsed with water and counterstained with methylene blue for 30 seconds. It was finally rinsed with water and allowed to air dry. The prepared smear was examined under the microscope (Olympus) using the oil immersion objective lens (total magnification = X 1000).

Statistical analysis

The Microsoft Excel (2016) spreadsheet and the Statistical Package for the Social Sciences (SPSS, Chicago, Illinois, USA) for Windows version 22.0 were used to analyse the data. Descriptive statistical analysis was conducted to calculate the prevalence using percentages and presented in tables and a figure. The Chi-square (univariate analysis) test and odds ratios (ORs) with a 95% confidence interval (CI) were used to determine the association between each risk factor and the absence or presence of *Cryptosporidium* oocysts. The ORs were calculated with respect to a reference category as shown in the respective tables. For the analysis, $p < 0.05$ was considered statistically significant.

Results

Cryptosporidium oocyst (Figure 2) was detected in 99 of the 333 cattle faecal samples examined, with a total prevalence of 29.73% (95% CI = 25.07–34.85). The occurrence of *Cryptosporidium* infection in cattle in other states in Nigeria ranged between 15.67% (Kaduna State) and 43.16% (Ogun State). There was a significant difference ($p < 0.05$) in the prevalence of *Cryptosporidium* infection in this study (Kwara State) compared to the prevalence in Adamawa, Borno, Kaduna, Kogi, and Ogun States. Infection in cattle was more likely to occur in the study state (Kwara State) than in Adamawa, Borno, Kaduna, and Kogi States, while the reverse was the case in Ogun State (Table 1).

Table 1. The prevalence of *Cryptosporidium* species among cattle in Kwara State and other states in Nigeria

State of study	N	n	Prevalence (%)	OR (95% CI)	<i>p</i> -value	Source
Adamawa	416	73	17.55	0.50 (0.36, 0.71)	<0.01 ^a	Shallangwa <i>et al.</i> , 2022
Borno	400	89	22.25	0.68 (0.48, 0.94)	0.02 ^a	Adamu <i>et al.</i> , 2015
Kaduna	194	31	15.98	0.45 (0.28, 0.70)	<0.01 ^a	Maikai <i>et al.</i> , 2011
Kaduna	300	47	15.67	0.44 (0.30, 0.65)	<0.01 ^a	Okojoku <i>et al.</i> , 2016
Kebbi	350	98	28.00	0.92 (0.66, 1.28)	0.62	Danladi and Ugbomoiko, 2015
Kogi	198	35	17.68	0.51 (0.33, 0.78)	<0.01 ^a	Adeiza and Nafarnda, 2020
Ogun	95	41	43.16	1.79 (1.12, 2.87)	0.02 ^a	Akinkuotu <i>et al.</i> , 2015
Ogun	200	75	37.50	1.42 (0.98, 2.05)	0.07	Akinkuotu <i>et al.</i> , 2014
Oyo	406	95	23.40	0.72 (0.52, 1.00)	0.05	Ayinmode and Fagbemi, 2010
Plateau	250	70	28.00	0.92 (0.64, 1.32)	0.65	Pam <i>et al.</i> , 2013
Sokoto	100	33	33.00	1.16 (0.72, 1.87)	0.53	Faleke <i>et al.</i> , 2014
Kwara [#]	333	99	29.73	1.00		

Chi-square (χ^2) value = 86.94; N = Number of cattle sampled; n = Number positive; OR = Odds ratio; CI = Confidence interval; ^a = Significant at $p < 0.05$; [#] = Reference category; [†] = Present study

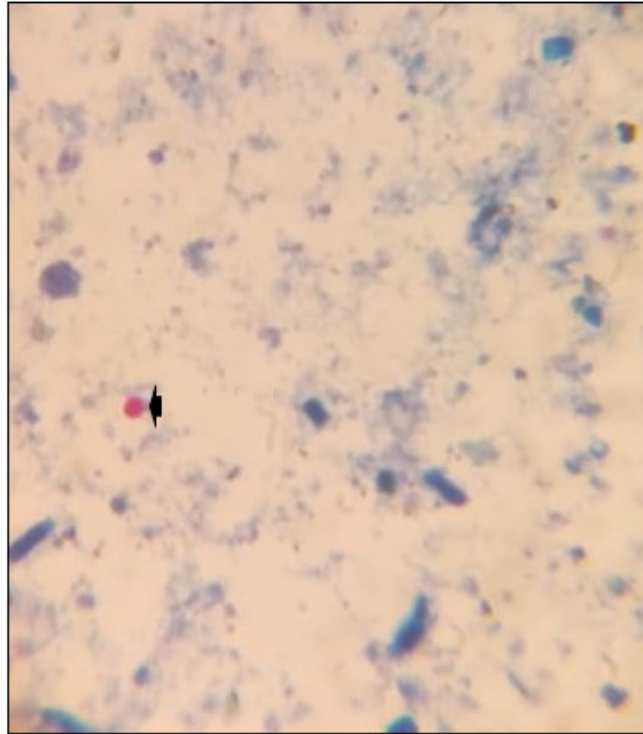


Figure 2. *Cryptosporidium* species oocyst (arrow head) of cattle, stained using the modified Ziehl-Neelsen staining technique (X1000)

The monthly prevalence of *Cryptosporidium* infection among cattle showed no distinct pattern. The highest prevalence of the enteric protozoan was recorded in October (64.29%), while the lowest prevalence was recorded in February (10.81%). The prevalence in the other months ranged from 14.86% in April to 57.89% in November (Figure 3).

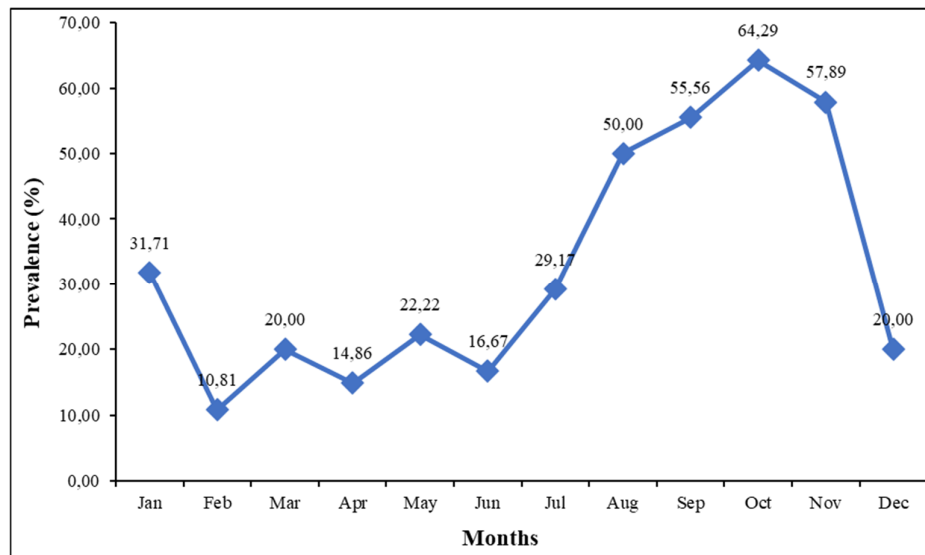


Figure 3. The monthly prevalence of *Cryptosporidium* species infection among cattle in Kwara State, Nigeria

Cattle less than a year old were the age group with the highest prevalence of the enteric protozoan, followed by those over 10 years old. The association with age was not statistically significant ($p > 0.05$). The Red Bororo breed of cattle was 2.56 times less probable to be infected with *Cryptosporidium* species compared to the White Fulani cattle breed, and this was statistically significant ($p < 0.05$). The Friesian cross breed was 3.66 times more likely to be infected with the enteric protozoan compared to the White Fulani breed; however, the association was not statistically significant ($p > 0.05$). Sex was not statistically associated ($p > 0.05$) with the incidence of *Cryptosporidium* species infection in cattle, as males were 1.26 times more probable to be infected compared to females. Cattle with poor and medium body condition scores were 1.46 and 1.14 times more likely to be infected with *Cryptosporidium* species compared to cattle with good body condition scores. The association between physiological status and the incidence of *Cryptosporidium* species in cattle was statistically significant ($p < 0.05$) with lactating cattle having a 1.96 chance of being infected compared to dry cattle. Young and matting cattle were 1.13 and 0.44 times more probable to be infected compared to dry cattle. Cattle with soft faeces were 0.99 times more probable to be infected with *Cryptosporidium* species compared to cattle with normal faeces, however, the association was not statistically significant ($p = 0.97$; $\chi^2 = 0.002$). *Cryptosporidium* species was 1.78 times more likely to be detected in cattle during the rainy (wet) season than the dry season, with a p -value of 0.02 and a χ^2 value of 5.293 (Table 2).

Table 2. Prevalence and epidemiological variables that were investigated as potential risk factors for bovine *Cryptosporidium* detection in Kwara State, Nigeria

Factors	N	n	Prevalence (%)	OR (95% CI)	p -value	χ^2 value
Age						
< 1 year	22	9	40.91	1.24 (0.45, 3.35)	0.66	
1 ≤ 4 years	106	26	24.53	0.59 (0.30, 1.14)	0.12	3.935
4 ≤ 10 years	135	39	28.89	0.73 (0.40, 1.36)	0.32	
>10 years	70	25	35.71	1.00		
Breeds						
Red Bororo	74	13	17.57	0.39 (0.19, 0.77)	0.01 ^a	
Sokoto Gudali	50	11	22.00	0.52 (0.24, 1.08)	0.08	
Friesian cross	6	4	66.67	3.66 (0.63, 29.30)	0.15	13.275
Keteku	38	13	34.21	0.96 (0.45, 2.01)	0.92	
White Fulani	165	58	35.15	1.00		
Sex						
Male	38	13	34.21	1.26 (0.60, 2.57)	0.52	0.412
Female	295	86	29.15	1.00		
Body condition score						
Poor	60	21	35.00	1.46 (0.72, 2.96)	0.29	
Medium	180	53	29.44	1.14 (0.65, 2.01)	0.66	1.166
Good	93	25	26.88	1.00		
Physiological status						
Young	30	9	30.00	1.13 (0.47, 2.59)	0.76	
Lactating	75	32	42.67	1.96 (1.13, 3.41)	0.01 ^a	
Matting	14	2	14.29	0.44 (0.07, 1.82)	0.30	12.346
Pregnant	10	0	0.00	X	X	
Dry	204	56	27.45	1.00		
Faecal consistency						
Soft	152	45	29.61	0.99 (0.62, 1.59)	0.97	0.002
Normal	181	54	29.83	1.00		
Seasons						
Wet	197	68	34.52	1.78 (1.09, 2.96)	0.02 ^a	5.293
Dry	136	31	22.79	1.00		

N = Number of cattle; n = Number positive; OR = Odds ratio; CI = Confidence interval; ^a = Significant at $p < 0.05$; χ^2 = Chi-square; X = Not applicable; * = Reference category

Discussion

In this study, the overall prevalence of *Cryptosporidium* infection in cattle was found to be 29.73%. This is in line with the reports of Danladi and Ugbomoiko (2015) and Pam *et al.* (2013), who documented a 28.00% prevalence among cattle in Kebbi and Plateau States of Nigeria. Other prevalences of the enteric protozoan infection among cattle in Nigeria ranged from 15.67% (Okojoku *et al.*, 2016) to 43.16% (Akinkuotu *et al.*, 2015), confirming that the prevalence in this study is within the range of prevalence in previous studies in Nigeria. Outside of Nigeria, an overall prevalence of 5.70%, 12.50%, 16.00%, 18.6%, 23.69%, 24.11%, 30.40%, 35.00%, and 45.00% was reported among cattle in the United States of America (Fayer *et al.*, 2007), Peninsular Malaysia (Abdullah *et al.*, 2019), Iran (Ranjbar and Fattahi, 2017), Ethiopia (Ayele *et al.*, 2018), Ghana (Dankwa *et al.*, 2021), Algeria (Hocine *et al.*, 2018), Egypt (Ghoneim *et al.*, 2017), Tanzania (Swai and Schoonman, 2010) and Brazil (Almeida *et al.*, 2010) respectively. The variation in the total prevalence of *Cryptosporidium* species among cattle in the different studies could be attributed to geographic and ecological differences; diagnostic techniques; study designs; management and production systems; herd size; age; and the seasons of the year the studies were conducted.

The detection of *Cryptosporidium* species in all the months of the year establishes that the enteric protozoan can be present in cattle herds all year. This is possible due to the peculiarity of its life cycle, in which sporulation of the oocyst occurs within the host, making cattle susceptible to infections all year.

Age is an important risk factor that is significantly associated with the incidence of *Cryptosporidium* species infection as young cattle (calves) are more at risk of the enteric protozoan infection compared to older cattle (Ayele *et al.*, 2018; Abdullah *et al.*, 2019). This information partially corroborates the finding of this study that reported a higher prevalence of *Cryptosporidium* species infection among cattle less than one year old compared to older cattle, but contradicts the fact that the difference was not significant. The reason for the lack of a significant difference in the occurrence of the enteric protozoan among the age categories may be attributed to the unique management system employed by most cattle farmers in Nigeria. Most pastoralists in Nigeria raise calves together with adult cattle under the same field conditions, making the older cattle serve as reservoirs for the infection.

The difference recorded among cattle breeds in relation to the occurrence of *Cryptosporidium* species infection substantiates the reports of Abdullah *et al.* (2019) and Birhanu *et al.* (2017), who documented a higher prevalence of the enteric protozoan in exotic and cross-breeds compared to indigenous breeds in their respective studies carried out in Ethiopia and Peninsular Malaysia, respectively.

The higher occurrence of *Cryptosporidium* species amongst males compared to females could be attributed to host intrinsic factors (physiology, genetics, and immunology) and extrinsic factors (management practices and environmental influences) (Ayinmode and Fagbemi, 2010). A higher prevalence of *Cryptosporidium* species has been reported among males compared to females (Ayele *et al.*, 2018; Shallangwa *et al.*, 2022), while Adeiza and Nafarnda (2020) and Dankwa *et al.* (2021) documented a contrary report.

The higher prevalence of *Cryptosporidium* species in poor and medium body condition scored cattle recorded in this study is in tandem with the reports of Ayele *et al.* (2018) and Birhanu *et al.* (2017) that documented a higher occurrence of the enteric protozoan in cattle with poor and medium body condition scores compared with those with good scores. This could be because cattle with poor body condition have lower immunity, and the protozoan is more susceptible in immunocompromised animals (Ayele *et al.*, 2018).

Stress has been documented to predispose animals, including cattle, to cryptosporidiosis (Díaz-Lee *et al.*, 2011; Pam *et al.*, 2013). In line with this, we report that lactating cattle had a higher prevalence of the enteric protozoan. This may be explained by the physiological stress associated with hormonal imbalances during lactation.

Faecal consistency was not statistically associated with the prevalence of *Cryptosporidium* species infection in cattle. Similarly, Akinkuotu *et al.* (2014), Ayinmode and Fagbemi (2010), and Hisamuddin *et al.* (2016) reported no statistical association between faecal consistency and the occurrence of *Cryptosporidium* species infection in cattle. This observation supports the suggestions of Ayinmode and Fagbemi (2010) and Bjorkman *et al.* (2003) that *Cryptosporidium parvum* may often be identified in non-diarrheic cattle and that diarrhea in *Cryptosporidium* species infections may be the result of other enteric pathogens that are usually associated with the infection.

Seasons play a vital role in the prevalence of *Cryptosporidium* species infection among animals, including cattle, with the protozoan being more prevalent during the wet (raining) season than the dry season in many countries around the world (Bern *et al.*, 2000; Naumova *et al.*, 2005; Ikiroma and Pollock, 2020). In line with this observation, we reported a higher occurrence of *Cryptosporidium* species in cattle during the rainy season than in the dry season. This observation could be attributed to the fact that wetness favours the survival of oocysts and the spread of the enteric protozoan between animals, including cattle (Manyazewal *et al.*, 2018).

Conclusions

This body of evidence revealed the presence of *Cryptosporidium* species among cattle in Kwara State. The study revealed that the prevalence of the enteric protozoan is within the reported prevalence in other states of Nigeria. Breeds, physiological status, and seasons were the risk factors associated with the enteric protozoan infection among cattle in the study area. There is a need to educate pastoralists on the control and preventive measures of this enteric protozoan to improve the economy of the cattle industry and prevent possible transmission to humans in Kwara State and Nigeria in general.

Authors' Contributions

Conceptualization: SDO; Data curation: SDO and IAG; Formal analysis: SDO, IAG, and KH; Investigation: SDO, IAG, and KH; Methodology: SDO; Writing - original draft: SDO; Writing - review and editing: SDO, IAG, and KH. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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