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Research Article

The Prevalence of Cryptosporidium Species in cattle in Lokoja, Kogi State, North Central Nigeria

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Abstract: The prevalence of *Cryptosporidium* spp. in cattle faeces in Lokoja, Kogi State, Nigeria was determined by a commercially produced enzyme-linked immunosorbent assay kit. Out of a total of 198 cattle faecal samples, 18.0% were positive for coproantigens. There were significant differences in infection rates in all the factors tested except the size of a cattle herd and sex. On herd size, the infection rate was highest in the 200-300 cattle herd size, 35% (8/23; χ^2 =5.397; Pv=0.06) ahead of 101-180, 0.00% (0/1) and 1-100 cattle herd group, 16% (27/174). For sex impact on infection, there were no significant differences (p 0.05) between the infection rates of 16.5% in males and 18.5% in females, (χ^2= 0.1346; Pv=0.714). Other factors tested that showed significant association with occurrence of Cryptosporidium were faecal consistency where diarrheic faeces had the highest prevalence of 73% (8/11) with a significant statistical difference (χ^2 =24.92; PV-0.00) amongst other forms of faeces such as loose faeces, 19% (8/43) and well-formed, 13.2% (19/144). Prevalence in the intensive management system of cattle was significantly higher than other methods of cattle rearing, 34% (21/61) with a significant statistical difference (χ^2=17.31: Pv=0.00). The highest rate of infection of 49.1% (26/53) was observed in calves up to 90 days of age while heifers between 271-360 days of age had the lowest rate of infection of 1.6% (1/62). The difference between the rates was significant (χ^2= 51.98; Pv=0.00). The result of the study showed that the prevalence of Cryptosporidium is high in cattle in Lokoja, Kogi State, Nigeria with calves being at the highest risk.
Keywords: Cryptosporidium, Intensive management, diarrheic feces.

Introduction

Cryptosporidiosis is caused by Cryptosporidium species, which is an important enteric apicomplexan parasite of zoonosis in the world (Parsons *et al*., 2015; Tanriverdi *et al*., 2007; Zhang *et al*., 2016). It is a critical emerging infectious disease in humans and animals that can lead to diarrhoea or other serious symptoms (Zhao *et al*., 2015). In general, cryptosporidiosis is transmitted through the faecal-oral route, when ingesting food or water contaminated with infective oocysts. Currently, there is no effective drug or vaccine to cure or prevent cryptosporidiosis. The disease has caused significant economic losses in animal husbandry. Infected animals may be a source of secondary infection, because they can serve as potential carriers for human and other animal infections via excreting faeces, including oocysts, that contaminate food and water (Deng and Cliver, 1999). Bovine cryptosporidiosis is widespread and studies have shown a wide range of oocyst shedding dynamics depending on the age, clinical situation and breeding system of the animals (Maldonado-Camargo *et al*., 1998). Currently, more than 30 species and genotypes have been identified (Baroudi, 2018; Ryan, 2014). Eleven of them, *C. muris, C. parvum,* muskrat II genotype, *C. hominis*-like genotype, caribou genotype, *C. hominis, C. bovis, C. ryanae,* deer genotype, *C.*

ubiquitum and *Cryptosporidium suis*-like genotype, have been identified in cervids, and cattle in China, Czech Republic, Japan, the United Kingdom, Spain, the United States, Norway and Poland (Huang *et al*., 2018; Kato *et al*., 2016; Kotkova *et al*., 2016; Wells *et al*., 2015; Garcia-Presedo *et al*., 2013). Cattle are infected with at least 4 Cryptosporidium parasites: *C. parvum*, *C. bovis*, *C. andersoni* and the *C. ryanae*. The occurrence of these species in cattle was shown to be age-related (Fayer *et al*., 2006).There were few earlier reports in Nigeria on bovine cryptosporidiosis based on microscopic detection of the oocysts on acid-fast stained faecal smears with reports of prevalence rates of 23.4% in Oyo State (Ayinmode and Fagbemi, 2010), 28.0% in Plateau State (Pam *et al*., 2013) and 33.0% in Sokoto State (Faleke *et al*., 2014). Fewer reports on the use of immunological methods of detection (especially ELISA) of Cryptosporidium in cattle have been documented (Ayinmode and Fagbemi, 2011) with none reported on Lokoja, North Central, Nigeria from my literature search. Although cattle are common domestic animals and major meat-producing animals in Nigeria with economic value, data on cryptosporidiosis in cattle especially Lokoja is scanty. Lokoja is a major transit point for most travellers between the North and southern parts of Nigeria. The city besides being a confluence of the two major rivers, Niger and Benue in Nigeria, is also a major livestock market. This study, therefore, is aimed at investigating the prevalence of infection in cattle in Lokoja, Kogi state of, Nigeria using the ELISA detection method. The findings will hopefully serve as a basis for screening the species and genotypes in cattle in Lokoja, thereby facilitating further studies on the possibility of zoonotic transmission of the disease.

Materials and Methods

Study Area and Study Design

The study was carried out in Lokoja. Lokoja has a population of over 90,000 inhabitants (Brittanica, 2018), and is the capital of Kogi State, Northcentral Nigeria. It lies about 7.8023° north of the equator and 6.7333° E east of the Meridian. It is about 165 km Southwest of Abuja. The town is situated in the tropical Wet and Dry savannah climate zone of Nigeria, and the temperature remains hot all year round. The hot season lasts for 2.7 months, with an average daily high temperature above 93°F. The cool season lasts for 4 months, with an average daily high temperature below 87°F. The occupation of the people includes farming, fishery and hunting. Lokoja is a trade center for agricultural products because it is situated at the confluence of the Niger and Benue rivers, and is close to the new federal capital of Nigeria in Abuja (Brittanica, 2018).

Sample Collection

A total of one hundred and ninety-eight (198) faecal samples from randomly selected cattle comprising of 79 male and 119 females of both young and adults were collected directly from the rectum of each cattle using a disposable plastic bag and emptied into a wide-mouthed disposable plastic container. The faecal samples collected were stored in 10% formalin and transported to the laboratory. They were preserved in 75% alcohol at room temperature until processed (Jongwutiwes *et al*., 2002).

Administration of Questionnaires

A well-structured questionnaire was used to obtain information for each cattle from which a faecal sample was collected. Information that may help identify risk factors for the faecal shedding of Cryptosporidium in cattle were also obtained. The questionnaire consisted of two sections: Section A covers the biodata of respondents and questions relating to transmission of the disease, while Section B contained questions on age, sex, breed, management system practised, source of drinking water, and presence of diarrhoea or loose faeces.

Sample Processing and Laboratory Procedures

The faecal samples were treated using the formol-ether concentration method. Each faecal sample collected was correspondingly examined for the presence of *Cryptosporidium* spp. antigens by Enzyme-Linked Immunosorbent Assay using a commercial kit (CoproELISA for detection of Cryptosporidium antigen in faeces). Samples with optical density (OD) higher than 0.5 were reported as positive.

Cryptosporidium oocyst detection

Data was obtained were summarised using descriptive statistics and presented as frequencies and proportions. The associations between Cryptosporidium infection and other explanatory variables such as potential risk factors were assessed using Fisher's exact test where appropriate. Chi-square and P-values were calculated to determine the association between the explanatory variables and Cryptosporidium infection. Values of $P<0.05$ were considered statistically significant.

Results

The overall *Cryptosporidium* spp. detection rate from this study was 18%. Females in this study were 57% (119/198), this dominated the cattle population sampled. Cattle between the ages of 271 and 360 days constituted 31% of the population while 73% had loose faeces. The majority (50%) of the cattle were reared on extensive breeding methods while 88% of the cattle were reared in small herds of 1-100 cattle. Data obtained revealed 34% drank from water bodies. Coproantigen detection was higher in female cattle (Table 1) in the current study, 18.5% (22/119) than the males, 16.5% (13/79). The difference is not however statistically significant (χ^2 =01346; PV=0.7136).

Table 1. Prevalence of Cryptosporidium according to sex in Lokoja, Nigeria

Sex	Number examined (%)	Coproantigen positive (%)	Chi square (χ2)	Df	P-value
Male	79 (43.3)	13 (16.5)	0.1346	1	0.7136
Female	119 (56.7)	22 (18.5)			
Total	198 (100.0)	35 (18.0)			

Coproantigen detection according to the age of cattle (Table 2) in the current study showed calves under 90 days had the highest prevalence of 49.1% (26/53) among the age groups. Following is the 91-180 age group with a detection rate of 16% (5/31). Others were 181-270 and 271-360 with 5.8% (3/52) and 1.6% (1/62) respectively. There is an association between age and Cryptosporidium infection in the study (χ2=51.9754; Pv=0.00).

Table 2. Prevalence of Cryptosporidium according to age distribution in Lokoja, Nigeria

Age (Days)	Number of fecal samples examined (%)	Coproantigen samples (%)	Chi square (χ2)	P-value
0-90	53 (26.5)	26(49.1)	51.9754	0.00
91-180	31(15.5)	5(16)		
181-270	52 (26)	3(5.8)		
271-360	62 (31)	1(1.6)		
Total	198 (100.0)	35 (18.0)		

Table 3 shows the coproantigen detection rate according to the different management practices tested in the Lokoja. The intensive method of rearing cattle was significantly higher

in the rate of antigen detection, 34% (21/61) than the other methods of breeding tested. Semi-intensive had a prevalence of 13.2% (5/38) while the Extensive method was 9.1% (9/99). An association was established between management practices and the occurrence of Cryptosporidium in the Cattle. ($\chi 2$ =17.3082: Pv=0.00).

Table 3. Prevalence of Cryptosporidium according to Cattle management practices in Lokoja state, Nigeria

Cattle management Practices	Number of samples examined	Positive samples (ELISA)	Chi square $\chi 2$	Df	P-value
Intensive	61 (31.0%)	21 (34%)	17.3082	2	0.00
Extensive	99 (50%)	9 (9.1%)			
Semi-intensive	38 (19.0%)	5 (13.2%)			
Total	198 (100)	35 (18.0%)			

Table 4 shows the detection rate of Cryptosporidium coproantigen in cattle based on different faecal consistencies. Diarrhoea faeces were highest, 73% (8/11) followed by loose stool, 19% (8/43) and least by the well-formed faeces, 13.2% (19/144). There is a significant difference in the prevalence rates among the different faecal consistencies (χ^2 =24.9217: Pv=0.00).

Table 4. Prevalence of Cryptosporidium according to faecal consistency of Cattle in Lokoja, Kogi state, Nigeria

Fecal Consistency	Number examined (%)	Coproantigen positive (%)	Chi square ($\chi 2$)	Df	P-value
Diarrhea	11 (5.6)	8 (73.0)	24.9217	2	0.00
Loose	43 (22)	8 (19.0)			
Well-formed	144 (53.8)	19 (13.2)			
Total	198 (100%)	35 (18.0)			

Fecal samples from cattle that drink from Water bodies constituting 35%, (69/198) of the total samples collected had the highest detection rate, 35% (24/69) of Cryptosporidium coproantigen compared to other sources from which cattle in the study drink water (Table 5). Detection rates from other sources were water and borehole, 15% (5/34); water bodies and well, 12% (2/17); None, 9% (4/45) and water bodies and tap, 0.0 (0/33). There was an association between water sources and the detection rate of Cryptosporidium in cattle of the study area, (χ^2 =23.9628; Pv=0.00).

Table 5. Effects of water on prevalence of Cryptosporidium in Lokoja, Nigeria

Type of water	Number of fecal samples examined (%)	Positive samples (%)	Chi square ($\chi 2$)	P-value
*Water bodies	69 (35)	24 (35)	23.9628	0.00
Water bodies and well	17 (9)	2 (12)		
Water bodies and borehole	34 (17)	5 (15)		
Water bodies and tap	33 (16.6)	0 (0.0)		
None	45 (23)	4 (9)		
Total	198 (100)	35 (18.0)		
Water bodies: Dam, pond, river, stream; Df = degree of freedom				

Cattle raised in large herds of 200-300 showed higher vulnerability to Cryptosporidium infection, 35% (8/23) than those raised in smaller herds of 101-200, 0.00 (0/1) and 0-100, 16% (27/174). There is no significant statistical difference in the detection rate of Cryptosporidium based cattle herd size, (χ^2 = 5.397; Pv=0.06). This implies that the size of the herd is not a risk factor in the detection of Cryptosporidium coproantigen in cattle (Table 6).

Table 6. Effects of size of cattle herd on prevalence of Cryptosporidium in Lokoja, Nigeria

Size of herd	Number of fecal samples examined (%)	Coproantigen Positive samples (%)	Chi square (χ2)	P-value
0-100	174 (88.0)	27 (16.0)	5.397	0.06
101-200	1 (1.0)	0 (0.00)		
200-300	23 (12.0)	8(35.0)		
Total	198 (100)	35 (18.0)		

Discussion

The overall prevalence of Cryptosporidium coproantigen in cattle obtained in this study was 18.6%. This figure is in tandem with the 17.6% reported in central Ethiopia (Abebe *et al*., 2008). The presence of coproantigen of Cryptosporidium in the cattle screened is an indication that these animals shed the oocysts of the parasite in the environment of the state. The figure is however lower than the prevalence reports of 37.5% by Akinkuotu *et al*. (2014), 32.2% by Ayinmode and Fagbemi, (2011) 27.8% by Alemayehu *et al*. (2013) and higher than the 7.8%, 13.6% and 15.8% prevalence reports by Wegayehu *et al*. (2013), Dinka and Berhanu (2015) and Wegayehu *et al*. (2016), respectively. Studies from other parts of the world reported varied prevalence rates. Those with comparable values include 18.8% reported by Budu-Amoako *et al.*, (2012) and 17% by Keshavarz *et al*. (2009). A higher prevalence of between 27% to 86.7% was however reported by Santin *et al*., (2004), Nguyen *et al*., (2007) and Venu *et al*., (2012). Lower values of between 12% and 11.7% were reported by Hamnes *et al*. (2006) and Khan *et al*. (2010), respectively.

The difference in the overall prevalence of Cryptosporidium among different studies could be due to differences in potential risk factors in the area of study. These factors may include, study design, season, sample size, management system, ecology, age, herd size and detection techniques employed. Age appears to be a critical factor in the epidemiology of Cryptosporidium in Lokoja. Calves under the age of 180 days had the predominant antigen circulation in the study area accounting for 37% of the population under study. Cattle under 90 days were 49.1% infected, this tallies with the observation that suckling calves had a higher rate of infection supported by (Ayinmode and Fagbemi, 2011); (Díaz-Lee *et al.,* 2011) and (Del Coco *et al.,* 2008) who reported the highest *C. parvum* infection rates in calves to be 7-14 days and 8-21 days of age respectively and generally observed that the prevalence of *Cryptosporidium* spp. in pre-weaned calves is usually high. This may be attributed to the higher susceptibility of calves to infection due to lack of previous exposure to the parasite. It suggests further that the management practices by pastoralists in Nigeria, where calves are grazed with the adults, may increase their risk of infection by consumption of contaminated feed and water by the calves (Ayinmode and Fagbemi, 2010). An acquired immunity towards Cryptosporidium in adults due to infections in early life (Harp *et al*., 1990) may account for the low incidence of clinical condition and thus the low shedding of Cryptosporidium oocysts in this age group. Calves of high infection rate may also be of zoonotic concern as calves

have been reported to be important reservoirs of the infection. Fayer *et al.*, (2007) and Xiao, (2010), thereby serving as an important source of contamination of water through the shedding of the oocysts (Xiao, 2010).

Data from the current study has shown Intensive management practice in rearing cattle as disposing cattle to the threat of Cryptosporidium infection in the Lokoja. Intensive and semi-intensive methods employ some varying degrees of confinement to a restricted area of the cattle reared. Animals reared under an intensive management system were more affected by Cryptosporidium (34%) than those under the extensive system (9.1%). This may be due to differences in breeds of animals as well as the degree of confinement, higher stocking rate and crowding in the intensive dairy farms favouring more contamination of barns, high contact of animals and rapid dissemination of oocysts compared to extensive farms. Restriction may lead to continuous contamination of the surroundings (Maikai *et al*., 2011). This result is in agreement with the findings of Geurden *et al.*, (2006) that reported a prevalence of 42.8% for animals reared under the intensive system and 6.3% for animals under the extensive system. Comparable lower prevalence had been reported in extensive farms compared to intensive farms (Ralston *et al*., 2003; Santin *et al*., 2004). Cattle with diarrheic faeces had a higher prevalence of 73% (8/11) than other forms of faecal consistencies. Many reports from previous studies have shown a similar association Maddox-Hyttel *et al.*, 2006; Geurden *et al.*, 2006; Singh *et al.*, 2006; Karanis *et al*., 2010). Other works identified the parasite as one of the pathogens most commonly found in scouring calves which may be detected either alone or with other entero-pathogens (Reynolds *et al.*, 1986 and de la Fuente *et al.*, 1999). Contrarily, reports of studies from Abebe *et al*., 2008 and Rieux *et al*., 2013 did not attest to an association between the occurrence of Cryptosporidium and the diarrheic status of cattle.

The differences in the findings may be due to the differences in pathogenicity of the strains or the effect of co-infections by other diarrhoea causing entero-pathogens which may have masked the effect of Cryptosporidium in the other studies. There is a significant association in the frequency of detection of *Cryptosporidium* antigen (35%) in the faeces of cattle that drank from water bodies in the current study. Increased risk of *Cryptosporidium* was seen in farms using river/stream water sources. This could be due to exposure of these water sources to faeces of humans, domestic and wild animals contaminated with oocysts of *Cryptosporidium*. This, therefore, alludes to the findings of Yang *et al.*, (2008) that river water is heavily contaminated with oocyst of Cryptosporidium.

Data obtained in this study indicated that cattle herd size is not associated with the occurrence of Cryptosporidium. Large herd management however showed higher vulnerability to Cryptosporidium infection as the highest antigen detection was recorded in the group. The higher rate of detection may be due to the difficulties of management of large size herds than smaller herds. The sanitary state of the farms due to improper or untimely evacuation of the waste in the farm leads to heavy contamination with oocysts. The rearing method where adult cattle run with calves may also result in heavy Cryptosporidium infection burdens in such large farms. Furthermore, inadequate and lack of commensurate water and feeding points may also contribute to the rate of exposure to the infection.

Conclusion

There is a high presence of Cryptosporidium in Lokoja, the capital of Kogi State, North Central, Nigeria with varying degrees of prevalence across the study area. The overall prevalence is 18%. Cattle under 19 days showed high vulnerability to Cryptosporidium

infection with the highest detection rate of 49.1% (26/53). Age, management practices, fecal consistency and the sources of water consumed by cattle are all risk factors in the spread of Cryptosporidiosis in the study area.

The potential for the spread of the parasite in the territory is high, as a good number of cattle across the area may be asymptomatic carriers of the parasite. Further studies to evaluate the genotypes and subtypes of the parasite will assist in the understanding of the transmission dynamics and the zoonotic potential of the parasite.

Conflicts of interest

The authors declare no conflicts of interest.

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