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**Research Article**

# Associated Risk Factors of Cryptosporidium Infection in Cattle in the Federal Capital Territory, Nigeria

**[1]Adeiza Musa Abdulrahman and [2]Nafarnda Daniel Wesley**

[1&2]Department of Veterinary Public Health and Preventive Medicine
[1]Email: musa.adeiza@uniabuja.edu.ng; [2]Email: wesley.nafarnda@uniabuja.edu.ng

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**Abstract:** Cryptosporidium is a zoonotic protozoan that can cause gastrointestinal illness with diarrhoea in a wide range of hosts. This study investigated the prevalence and associated risk factors of Cryptosporidium in the cattle of the Federal Capital Territory (FCT) of Nigeria, using the Copro-Elisa Cryptosporidium antigen detection technique. Four hundred cattle faecal samples were analyzed to elucidate the prevalence of Cryptosporidium, while a well-structured questionnaire was administered and evaluated to determine the potential risk factors for Cryptosporidium infection and spread in the Federal Capital Territory of Nigeria. The investigation revealed a prevalence of 13% in the FCT of Nigeria. Among the potential risk factors identified are, Age, cattle of between 1-100 days had the highest prevalence of 24% (95% CI, 0.019-5.163). A significant statistical relationship (PV = 0.00) was established between age and Cryptosporidium infection in cattle in the FCT. Faecal consistencies showed that well-formed faeces had the highest prevalence of 42% with a significant statistical difference (PV-0.00). Three breeds of cattle were tested in this study. Sokoto Gudali had a significantly higher prevalence, 31% than the other breeds (PV=0.00). Based on the management system, the prevalence among cattle under extensive management system was significantly higher than other methods of rearing cattle in the FCT. This implies that cattle under extensive management system are more vulnerable to Cryptosporidium infections than those reared under other systems. Herd size was found to be a risk factor as a smaller herd size of 1-100 cattle had the highest infection rate (PV = 0.00) amongst others. Area council location showed no significant impact on Cryptosporidium infection in the FCT (PV=0.55). Prevalence of Cryptosporidium in Kwali Area Council of the FCT was highest, 17.3% (9/52) amongst the six local area councils. This study revealed the widespread of Cryptosporidium in the FCT as the antigen of the parasite was detected across the study area.

**Keywords:** Cryptosporidium, Risk factors, Prevalence, Parasite.

## Introduction

Cryptosporidium is an apicomplexan intestinal protozoon, which infects animals and the human gastrointestinal tract and causes cryptosporidiosis (Huang and White, 2006). The genus Cryptosporidium is composed of at least 13 species and cattle are infected with at least four Cryptosporidium species: *C. parvum*, *C. bovis*, *C. andersoni* and the *C. ryanae*. The occurrence of these Cryptosporidium species in cattle was shown to be age-related. Cryptosporidium is of public health importance causing diarrhea in humans and domestic animals, including livestock, dogs, cats and wildlife (Caccio, 2005). Some of the zoonotic Cryptosporidium species (*C. parvum*, *C. meleagridis*, and *C. canis*) usually cause self-limiting diarrhoea in humans and animals (Joachim, 2004). Contact with infected calves has been implicated as the cause of many cryptosporidiosis outbreaks in veterinary students, research technicians and children attending agricultural shows (Del Coco *et al.,* 2009).

Infected animals can act as asymptomatic carriers and shed large numbers of oocysts into the environment and remain a source of infection to other domestic and wild animals (Xiao and Feng, 2008). In newborn animals' cryptosporidiosis cause severe diarrhoea that is sometimes accompanied by anorexia, reduced milk intake, dehydration, growth retardation, stiffness, hyperpnea, slow gait and depression (Casemore *et al*., 1997; Fayer, 2004).

Cattle have been implicated as a major source of *C. parvum* in pasture runoff, which is responsible for environmental contamination and human infection either by direct or indirect contact through faecal contamination of food or water for human consumption (Current and Garcia, 1991).

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). Although cattle are the most common domestic animals and the major meat-producing animals in Nigeria with economic value, data on cryptosporidiosis in cattle especially in Federal Capital Territory (FCT) is scanty. FCT is the strategically located administrative capital of Nigeria where most of the indigenous communities indulge in a variety of agricultural activities.

## Materials and Methods

### Study Area and Study Design

The study was carried out in cattle in the Federal Capital Territory (FCT) of Nigeria. FCT consists of six Area Councils namely: Abaji, Abuja Municipal Area Council (AMAC), Bwari, Gwagwalada, Kwali and Kuje, with a total population of 2,238,800 according to the 2006 Nigerian official census figure. It covers a landmass of 7,315km$^2$ and 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, 2021). It lies in the Guinean forest-savannah mosaic zone of the West African sub-region (Britannica, 2018). The area experiences three weather conditions annually; warm, humid rainy season and extremely hot dry season (Britannica, 2018).


**Figure 1. Map of Federal Capital Territory, Nigeria showing the sampling sites.**
**Source: www.researchgate.net (2020)**

### Sampling, Transportation and Storage of Samples

A total of four hundred faecal samples of 2g each were randomly sampled from selected cattle comprising of 127 males and 273 females of both young and adults in the FCT. To ensure the humane handling of the animals, the herdsmen restrained the animals while samples were being collected. Faecal samples were collected directly from the rectum of each cattle using disposable plastic bags and emptied into a wide-mouthed disposable plastic container. Potassium dichromate (2.5%) was added to each container for preservation (according to the manufacturer's instructions)

and transported on ice packs to the Laboratory for analysis. Analysis was carried out immediately and, where analysis was not carried out immediately, the samples were preserved at $4^0$C for a maximum of 24 hours before it is subjected to ELISA protocol.

**Administration of Questionnaires**

Structured questionnaires were used to obtain information on each cattle from which faecal sample was collected. Information that may help identify risk factors for the faecal shedding of Cryptosporidium in cattle were obtained through the questionnaire from the owners or those who take care of the cattle.

The questionnaire consisted of section A, which contained questions related to 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.

**DNA extraction and Cryptosporidium detection**

The presence or absence of Cryptosporidium antigens in the faecal samples was detected using a commercial enzyme-linked immunosorbent assay kit, "CoproELISA" Cryptosporidium by Savyon Diagnostics Limited, Israel. The analysis was carried out according to the manufacturer's instructions. About 0.15 g or 150 µl of 10% formalin preserved faecal samples were homogenized in 400 µl stool diluent in 2.0 ml Eppendorf tubes and vortexed.

One hundred microlitres of the positive control were added to a well of the microtiter plate coated with anti-Cryptosporidium antibodies, while two wells of the negative control diluent each received 100 µl. One hundred microlitres of faecal samples already homogenized in the diluent were added to the wells and incubated at $37^0$C for 1 hour. The plate was washed five times with 300 µl of washing buffer followed by the addition of 100 µl of horseradish peroxidase (HRP) conjugate and then incubated at $37^0$C for another 1 hour.

After washing with buffer for 5 times, 100 µl of tetramethylbenzidine (TMB) substrate was added and incubated for 15 minutes, followed by the addition of 100 µl of stop solution to the wells. The plate was then read at 450 nm using the ELISA reader (Thermo Scientific Multiskan EX).

Sample absorbance was compared to a cut-off value obtained from the average absorbance in the negative control wells to determine the presence or absence of Cryptosporidium antigens. Cryptosporidium antigens were detected (positive) if the sample absorbance was equal or higher than the cut-off value and non-detectable (negative) when sample absorbance was lower than the cut-off value.

**Data Analysis**

Data obtained was analyzed using IBM SPSS Statistics for Windows, version 25.0 (Armonk, NY: IBM Corp.). The data were summarized using descriptive statistics and presented in frequencies and proportions with their confidence intervals. The associations between Cryptosporidium infection and other explanatory variables such as potential risk factors were assessed using Fisher's exact test where appropriate. Odds ratios at 95% confidence intervals were calculated to determine the strength of the association between the explanatory variables and Cryptosporidium infection. Values of $P<0.05$ were considered statistically significant.

**Results**

Table 1 showed the faecal Cryptosporidium antigen distribution across the six Area Councils of FCT, Nigeria. Abaji local council area represents 13% (52/400) faecal samples collected for the study and had 17.3% (9/52) positive samples as the highest positive faecal Cryptosporidium antigen detected among all council sampled.

**Table 1. The prevalence of Cryptosporidium in cattle based on the location of the Federal Capital Territory using ELISA**

| Subject Studied | Location (Area council) | Number Examined (N) | ELISA Positive Samples (%) | Chi square ($X^2$) | P-value |
|---|---|---|---|---|---|
| CATTLE | Abaji | 76 (19%) | 12 (15.8) | 3.977 | 0.55 |
| | AMAC | 76 (19%) | 7 (9.2) | | |
| | Bwari | 60 (15%) | 6 (10) | | |
| | Gwagwalada | 68 (17%) | 7 (10.3) | | |
| | Kuje | 68 (17%) | 11 (16.2) | | |
| | Kwali | 52 (13%) | 9 (17.3) | | |
| **Total/Prevalence** | | **400 (100%)** | **52(13)** | | |

The difference was not statistically significant ($X^2$ = 3.977; PV=0.55), this therefore, indicates that the Local Council Area location of cattle is not a risk factor for bovine cryptosporidiosis in the FCT.

Table 2 showed cattle of 1-100 days old as the most sampled age group in this study representing 44.8% (179/400). This group of calves had the highest prevalence of Cryptosporidium, 24% (43/179) above other cattle age groups. The difference between the prevalence of Cryptosporidium among 1-100 days old and the other age groups was statistically significant (95% CI =0.019-5.163, PV = 0.00). This implies an association between the age of cattle and Cryptosporidium infection in the FCT.

Cattle reared on intensive management practice formed 95% (379/400) were the most sampled amongst other management practices in this study. Only 10.3% (39/379) of the samples were positive. Those raised on an extensive management system which constituted 3% (12/400) of the animal samples were 92% (11/12) positive for faecal Cryptosporidium antigen. There was an association between management practices and the Cryptosporidium antigen in cattle of the FCT as there was a significant statistical difference between the detection rate in the extensive management system and the other mode of cattle rearing in the FCT (95% CI=0.351-345.077: PV=0.00).

Female cattle formed 68.1% (273/400) of total sampled cattle and accounted for 16.1% (44/273) of the positive samples detected in this study. An association (95% CI=0.012-3.130, PV=0.00) was established between sex and the occurrence of Cryptosporidium among cattle in the FCT.

Data generated from the study on breeds indicated the highest Cryptosporidium antigen detection was in the local breed of cattle, Sokoto Gudali, 22% (31/142) followed by crossbreed, 15% (6/41) and the least was 7% (15/217). An association was established in this study between Cryptosporidium infection and breeds (95% CI, 0.017-4.594; PV=0.00). Well-formed faeces were significantly highest in Cryptosporidium antigen detection amongst other forms of faecal consistencies. It had 42% (37/89) prevalence while the second was 8.6% (6/70) from diarrhoeic faeces and the least was the loose faeces 4% (9/241). The difference between the well-formed faeces and the rest forms of faeces was significant (95% CI=0.004-1.247; PV=0.00).

The herd size of cattle is a determinant of Cryptosporidium infection in FCT, Nigeria. The herd with cattle population of 1-100 recorded the highest prevalence of Cryptosporidium antigen 30% (29/98), followed by the cattle in the 200-300 population group, 11.2% (14/123).

The least detection rate was in the cattle of 101-200 population group, 5% (9/179). There is a significant statistical difference in the distribution of the Cryptosporidium across the various age groups of cattle (95% CI=0.025-6.950; PV=0.00).

**Table 2. The associated risk factors of Cryptosporidiosis in cattle in the Federal Capital Territory, Nigeria using ELISA detection technique**

| Variables | No of samples (%) | No of positive Samples (%) | Confidence Interval @ 95% | P-value |
|---|---|---|---|---|
| **Age (Days)** | | | | |
| < 90 | 179 (44.8%) | 43 (24%) | 0.019-5.163 | 0.00 |
| 91-180 | 82 (21%) | 6 (7.3) | 0.004-1.426 | |
| 181-270 | 121 (30.1%) | 1(0.8) | 0.000-0.250 | |
| 271-360 | 18 (41%) | 2 (11.1) | 0.005-2.881 | |
| **Sex** | | | | |
| Males | 127 (32%) | 8 (6.3) | 0.004-1.177 | 0.00 |
| Females | 273(68.1%) | 44 (16.1) | 0.012-3.130 | |
| **Faecal consistency** | | | | |
| Loose | 241 (60.1%) | 9 (3.7) | 0.016-0.3441 | 0.00 |
| Diarrhoea | 70 (18%) | 6 (8.6) | 0.005-1.696 | |
| Well-formed | 89(22.3%) | 37 (42.0) | 0.043-11.745 | |
| **Breed** | | | | |
| Bunaji | 217 (54.1%) | 15 (7.0) | 0.004-1.247 | 0.00 |
| Gudali | 142 (36%) | 31(22.0) | 0.017-4.594 | |
| Cross-breed | 41 (10.1%) | 6 (14.6) | 0.009-3.128 | |
| **Management practices** | | | | |
| Intensive | 379 (95%) | 39 (10.3) | 0.007-1.871 | 0.00 |
| Extensive | 12 (3%) | 11 (92.0) | 0.351-345.077 | |
| Semi-intensive | 9 (2.3%) | 2 (22.2) | 0.010-5.985 | |
| **Size of herd** | | | | |
| 1-100 | 98 (25%) | 29 (30%) | 0.025-6.950 | 0.00 |
| 101-200 | 179 (45%) | 9 (5.0%) | 0.003-0.917 | |
| 200-300 | 123 (31%) | 14 (11.4%) | 0.008-2.170 | |

**Discussion**

Cryptosporidium is an important zoonotic protozoan parasite with a cosmopolitan distribution (Ryan and Hijjani, 2015). The most vulnerable species of animals to Cryptosporidium infection in cattle (Angus, 1990). The transmission routes of Cryptosporidium species are thought to result from faecal-oral transmission of oocysts via direct contact with infected humans or animals, or through the ingestion of contaminated water or food (Holubowa *et al.*, 2019). However, the contribution of animal reservoirs to human infections remains unclear and requires clarification (Ryan *et al.*, 2016). Very little has been documented about the infections involving this parasite in the cattle of the Federal Capital Territory. This study is therefore to establish the occurrence of this parasite in the cattle of the FCT using Enzyme Linked-Immuno Sorbent Assay (ELISA) detection protocol.

In this study, the prevalence of Cryptosporidium infection in cattle in the Federal Capital Territory of Nigeria was found to be 13%. This finding falls within the range of prevalence reported for Cryptosporidium infection in cattle in Africa (Samra *et al.*, 2016; Sultane *et al.*, 2007). This is also in tandem with the 12% and 15% reported by Julius *et al.*, (2016) in Kaduna and Kafanchan in Nigeria respectively using Zeehl Nelson microscopy. The figure is however substantially higher than the 9.3% reported in Nigeria by Pam *et al.*, (2013). However, prevalence higher than the one established in this study have been established in some studies in Nigeria (Ayinmode *et al*., 2010; Maikai *et al*., 2011; Akinkuotu *et al*., 2014; Adamu *et al.*, 2015).

The differences in the prevalence in these studies and the current findings may be due to the differences in the season of sampling, the geographic and ecological location of the study areas, laboratory techniques employed in the detection of the parasite, type of animal management system

practised by the farmers, the breed of the animals, and hygiene status of the farms as well as the climate of the area under study (Ayele *et al*, 2018; Mensah *et al.,* 2018).

Locational disposition to Cryptosporidium showed cattle in Kwali Area council having the highest prevalence of 17.3% amongst the six council areas of the FCT. There was however a widespread presence of the parasite across all the council areas of FCT implying a high level of asymptomatic carrier cattle status of Cryptosporidium in the territory. Prior information about the prevalence of Cryptosporidium in cattle in any of the area councils of the FCT has not been reported before the current study. Kwali is a suburban Area Council with heavy reliability on agribusinesses. This involves a huge amount of water on irrigation farming leading to surface overflow of water and spread of Cryptosporidium oocysts. The indiscriminate extensive breeding of ruminants particularly cattle may sum up to account for the high prevalence in the Kwali Area council.

Data from this study showed both sexes of cattle are vulnerable to the infection of the parasite. Female cattle however had a higher prevalence of 16.1% than the 6.3% obtained in the Males. The reason for this disparity is not known, the outcome could be attributed to the usual practice of having a higher female: male ratio in a herd and also the retention of female animals for breeding and milk production. It could also be related to host intrinsic factors (genetics, physiology and immunology) and extrinsic factors (environment and management practices). The underlying mechanisms of the effect of these factors are poorly understood and require clarification (Ayinmode *et al*., 2010).

Cattle under the age of six months appeared to account for the substantial percentage of, 19% of the Cryptosporidium infection in cattle by age distribution. Calves under 90 days of age, however, had the highest prevalence of 24%, correlating the assertion that Cryptosporidium in cattle is age-related. Findings from this study widely agree with the established Cryptosporidium-age distribution pattern of 4.9% to 9.0% in adult cattle, 12.69% and 19.5% in post weaned, Juvenile and pre-weaned cattle in Nigeria respectively (Chukwu *et al*., 2019). This association is demonstrated by the four main species of the parasite in cattle. *Cryptosporidium andersoni* was reported to prefer older animals, while *Cryptosporidium parvum*, *Cryptosporidium ryanae*, and *Cryptosporidium bovis* were predominant in younger calves (Santin *et al.,* 2004; Brook *et al.,* 2009). Other reports have also shown the predisposition of younger ages to Cryptosporidium infection (Fayer *et al*., 2007). This may not be unconnected to certain immunological incompetence usually found in the age group under discussion. Cattle especially calves have been implicated as a risk factor for human cryptosporidiosis in the United States, United Kingdom, Ireland, and Australia (Goh *et al*., 2004; Hunter *et al*., 2004; Roy *et al*., 2004).

The group of cattle with well-formed feces in this study had the highest prevalence of Cryptosporidium, 42% among those with non-diarrhoeic samples reported by Dankwa *et al.,* (2021) in Ghana. The finding in this study however runs contrary to the reported finding of significant association between Cryptosporidium species infection and diarrhea, with calves that are shedding oocysts having 6.1 times the risk of being diarrheic as uninfected calves (Ehsan *et al.,* 2011).

The prevalence of Cryptosporidium was highest in the Gudali breed of cattle among other breeds tested in this study, Gudali is an indigenous breed. Although many studies have been done on the prevalence of Cryptosporidium in both dairy and beef cattle (Geurden *et al*., 2006), literature on molecular studies on Cryptosporidium in Bunaji and Gudali breeds are scanty. The prevalence obtained in this study is consistent with that of Maikai *et al.,* (2012) where Sokoto Gudali recorded the highest rate of prevalence (20%). The higher rate of Cryptosporidium infection in Gudali above Bunaji or their cross maybe because the Bunaji breeds are more resistant to diseases than Gudali (Tawah and Rege, 1996). Reports from Ethiopia, however, showed a higher prevalence of Cryptosporidium infection in cross-bred than the indigenous zebu. Such contradiction was likely due to the management differences between the large size management of the Crossbreed and the local Zebu (Mohammed *et al.,* 1999).

An extensive management system employed in rearing cattle at the FCT, Nigeria has shown greater vulnerability to Cryptosporidium infection than the other systems of cattle management. The prevalence of Cryptosporidium in the extensive management system was 92% indicating a high level of morbidity of cryptosporidiosis in cattle under an extensive management system in the Federal Capital Territory. This result is at variance with the 42.8% in the intensive management system and 6.3% in the extensive system reported by Geurden *et al.,* (2006). The result however agrees with the study conducted in Kaduna State by Maikai *et al.,* (2012) wherein a higher prevalence of Cryptosporidium in extensive management was recorded. The high prevalence of Cryptosporidium in an extensive system of management could be attributed to the fact that animals on extensive management may randomly pick the oocysts on their path and from the contaminated water they drink along their grazing routes.

Cryptosporidium distribution across the various herd size of cattle in the Federal Capital Territory showed a cattle herd size of 1-100 had 30%. This shows that cattle of smaller herd sizes are more likely to be infected with Cryptosporidium than those of moderate and large sizes. This may be due to better sanitary practices in farms with larger herds due to their being managed by professionals compared to medium and small-sized farms run by owners or non-professionals. ELISA, the only diagnostic technique employed to detect Cryptosporidium in the study, was unable to distinguish between species of the parasite. The inability of the test to detect species of the parasite in this study did not allow us to understand the transmission routes and possible zoonotic potential. Further studies, using polymerase chain reaction and restriction fragment length polymorphism or sequencing would help identify the species and genotypes of the parasite in the Federal Capital Territory, Nigeria. This will be significant for a deeper understanding of the epidemiology of the parasite in the study area. Low levels of antigens in samples may go undetected and may have been reported as negative. This together with the intermittent shedding of the parasite in the host may lead to an underestimation of the prevalence of the parasite (Dankwa *et al.,* 2021). In addressing this shortcoming, obtaining repeated samples from participants would have been the best option, but this was not done in the current study. Besides, the current study also failed to capture the seasonal variation in the prevalence of the parasite as sampling was for only part of the year.

## Conclusion

There is a widespread of Cryptosporidium in the FCT of Nigeria with varying degrees of prevalence across the six Area Councils and an overall prevalence of 13%. Kwali Area council had the highest detection rate of 17.3% (9/52). Age, management system, sex, breed of cattle was found to be the major 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 territory are asymptomatic carriers of the parasite. Further studies to evaluate the genotypes and subtypes of the parasite is hereby recommended for a better understanding of the transmission dynamics, more risk factors and the zoonotic potential of the parasite.

**Conflicts of interest:** There is no conflict of interest of any kind.

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