Veterinary Medicine and Public Health Journal (VMPH)

Volume.4, Issue.1, pp: 1-9 Article 1
Jan (2023)

Assessment of *Cryptosporidium* burden in cattle in Federal Capital Territory, Nigeria

Abdulrahman Musa Adeiza[a*], Nuhu Abdulazeez Sani[b], Alhaji Nma Bida[a], Wesley Daniel Nafarnda[a], Enid Godwin[a], Elizabeth Chinwe Okoli[a]

[a] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Abuja, Nigeria
[b] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja, Nigeria
[a] ovavu64@gmail.com

Received: 3/10/2022 Revised: 18/10/2022 Accepted: 17/12/2022

DOI: https://doi.org/10.31559/VMPH2023.4.1.1

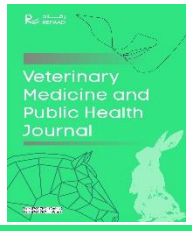
Available online at www.refaad.com
VMPH 4(1) 2023: 1-9
Research Article

www.refaad.com

Veterinary Medicine and Public Health Journal (VMPH)

Journal Homepage: https://www.refaad.com/Journal/Index/7

E-ISSN 2707-7195 | P-ISSN 2707-7187



Assessment of Cryptosporidium Burden in Cattle in Federal Capital Territory, Nigeria

Abdulrahman Musa Adeiza[a*], Nuhu Abdulazeez Sani[b], Alhaji Nma Bida[a], Wesley Daniel Nafarnda[a], Enid Godwin[a], Elizabeth Chinwe Okoli[a]

[a] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Abuja, Nigeria.
[b] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja, Nigeria.

*** Corresponding Author:** Abdulrahman Musa Adeiza Email: ovavu64@gmail.com

How to cite this article: Adeiza, A. M. et al. (2023). Assessment of Cryptosporidium Burden in Cattle in Federal Capital Territory, Nigeria. Veterinary Medicine and Public Health Journal, 4(1), 1-9.

Abstract

This study was undertaken to undertaken to assess the *Cryptosporidium* burden in cattle in the Federal Capital Territory (FCT), Nigeria. A total of 400 cattle fecal samples were screened for *Cryptosporidium* oocysts using Safranin-Methylene Blue staining technique. A questionnaire was used to analyze the possible factors driving the prevalence of *Cryptosporidium* infection in cattle in the study area. The overall prevalence of *Cryptosporidium* infection was 17.8% (71/400). The prevalence of *Cryptosporidium* in cattle was highest (32.4%, 22/68) in the Gwagwalada Area Council and lowest (12.1%, 8/66) in AMAC. There was a significant difference in prevalence of *Cryptosporidium* in cattle in the Area Councils ($P = 0.010 < 0.005$). There was a statistical difference in prevalence of *Cryptosporidium* and age, consistency of feces, breed, management practices and size of herd of cattle ($P < 0.05$) in the study area. There was no significant difference in sex and prevalence of *Cryptosporidium* infection in cattle in the study area ($P > 0.05$). This study demonstrated a widespread prevalence of *Cryptosporidium* infection in cattle in Federal Capital Territory. More worrisome is their prevalence in asymptomatic cattle as reservoirs that may sustain zoonotic cycle of transmission in the humans.

Keywords: *Cryptosporidium; Cattle; Feces; Federal Capital Territory; Nigeria; Prevalence; Questionnaire; Safranin Methylene Blue.*

1. Introduction

Cryptosporidiosis is a parasitic gastrointestinal infection caused by *Cryptosporidium* affecting a wide range of vertebrates, including mammals, fish, reptiles, and birds (Velasquez et al. 2018; Mi et al, 2013). The most vulnerable species of animals to *Cryptosporidium* infection is cattle (Abeywardena et al., 2015; Diaz et al., 2018). The important *Cryptosporidium* species which infect cattle are *C. parvum, C. bovis,* and *C. andersoni* (Thompson et al., 2017). Cattle are the biological reservoir for *Cryptosporidium* and commonly implicated in outbreaks of human cryptosporidiosis (Caffarena., 2020). Cryptosporidiosis ranks 5th amongst the 24 most important food borne parasites globally (Nj et al., 2011; Ryan et al., 2014). *Cryptosporidium* is responsible for 8–19% of cases of diarrheal disease in developing countries, causing a wide range of infections in vertebrate hosts including humans (Certed et al., 2015). *Cryptosporidium* infections in cattle have been reported to be more prevalent in calves as compared to the adult animals (Witto et al., 2021). Cryptosporidiosis in cattle occurs 3–5 days post-infection and the disease is characterized by profuse watery diarrhea, gastrointestinal discomfort, nausea and fever (Diptyanusa & Sari 2021). These episodes normally result in weight loss and occasionally death (Rajendran et al., 2011). In addition to farmers incurring production losses, farmers may acquire infections from their animals (Witto *et al*., 2021).

Cryptosporidium is transmitted via the feco–oral route through the ingestion of water or food contaminated with oocysts (Ponka et al., 2009). *Cryptosporidium* has a low infective dose with as few as 9 oocysts capable of causing disease (Bouzid et al., 2013). Oocysts are discharged into water by various animal hosts (Lamb, 2018). The sporocysts are resistant to most chemical disinfectants but are susceptible to drying and ultraviolet portion of the sunlight (USFDA, 2005).

Nigeria has 13.9 million cattle and much of this population is in the northern part of the country (Lawal-Adebowale, 2021) where Federal Capital Territory (FCT) is located. These cattle generally grazed in the open fields where they litter the environment with their wastes. This poor environmental sanitation and general lack of safe drinking water have led to enhanced burden of cryptosporidiosis in Africa (Aldeyarbi, 2016). Studies on *Cryptosporidium* in cattle, a natural reservoir, is inevitable in view of its public health potentials of spreading the parasite further to cattle and man in the FCT.

This study was to determine the prevalence of *Cryptosporidium* infection in cattle in FCT. We hypothesized that intrinsic and extrinsic factors cannot drive occurrence of the infection in cattle in Nigeria. Outcomes of this investigation are expected to provide preliminary information to knowledge towards mitigation of the impact of cryptosporidiosis in Federal Capital Territory, Nigeria.

2. Materials and Methods

2.1. Study Area

This study was conducted in the Federal Capital Territory (FCT) of Nigeria that covers a landmass of 7,315km^2 and 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). FCT consists of six area councils: Abaji, Abuja Municipal Area Council (AMAC), Bwari, Gwagwalada, Kuje, and Kwali (Fig. 1). With a total population of 2,238,800 (NPC, 2006), it lies in the Guinean forest-savannah mosaic zone of the West African sub-region. It experiences three weather conditions annually; warm, humid rainy season and extremely hot dry season (Britannica, 2018).

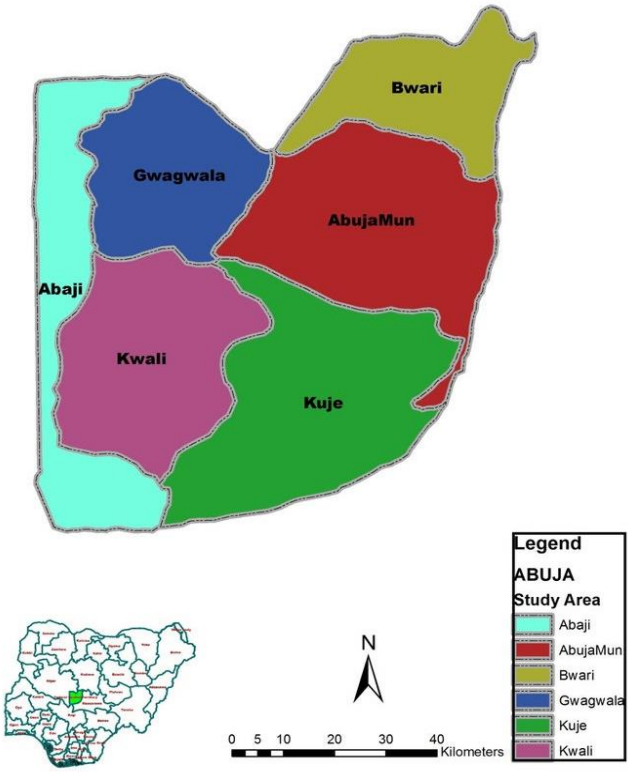


Fig (1): Map of Nigeria showing the location of the Federal Capital territory, Abuja.
Source: Modified from the Administrative map of Nigeria.

2.2. Study Design and Population

A cross-sectional study was carried out on randomly selected cattle populations domiciled in the Federal Capital Territory between September 2017 and April 2018. Inclusion criteria included all cattle within the FCT, whose owners had consented to be used for the study. Exclusion criteria are the cattle outside the FCT or cattle within FCT belonging to owners who did not give their consent.

2.3. Sample Size Determination and Sampling Procedure

The number of samples to be collected was determined using this formula: $n = Z^2pq / d^2$ (Mugo 2008), Where: n = sample size, p = proportion of the population that is positive to *Cryptosporidium* infection (A prevalence rate from an earlier study), Z=desired confidence 1.96, q=1- p, d (desired absolute precision) = 5%. For cattle fecal samples, a prevalence rate of 37.5% for ruminant animals *Cryptosporidium* infection in Northcentral Nigeria (Akinkuotu et al., 2014) was used. Thus, n = 361. However, 10.8% (n=38.9) contingency was added to make up for non-response, and the final sample size, n, was 400.

Cattle farms and abattoirs were identified from the records kept in the Area Councils Agriculture Departments. The identified farms and abattoirs in the kept records were purposively selected in field under each Area Council. However, cattle designated for biological samples were randomly selected by balloting and sampling with replacement.

2.4. Checklist Design and Administering

An open-ended checklist was designed to collect information on the individual farm management practices. The questions focused on themes that include animals' biodata information like age, sex, breed, husbandry system (intensive/extensive), number of animals in each farm, type of animals living in contact with the cattle, herd size and presence of diarrhea on all participating animals and other information that will be helpful in identifying risk factors of the disease. Animals were classified on the basis of their age range: less than 90 days of age (179), between 91-180 days (82), 181-270 days (121) and greater than 271 days (18) while fecal consistency was categorized based on visual observation into diarrheic, loose and well-formed. The checklist was administered through a semi-structured interview exercise in a Focus-group interview. Furthermore, informed consent of the farmers was verbally obtained from each participant before commencement and they were assured of confidentiality of all responses.

2.4. Sample Collection and Processing

Visitations to farms were made on a weekly basis until the desired number of samples were

collected. Fecal samples were collected from ten to twenty randomly selected cattle from each of the cattle farms depending on cattle population in the farm. A total of 400 samples, at least ten per farm, of 2g for each cattle were randomly taken from selected cattle comprising of 127 males and 273 females of both young and adults in the FCT. To ensure humane handling of the animals, the herdsmen restrained the animals while samples were being collected. A disposable plastic bag was used to take a single fecal sample from the rectum of each animal and emptied into a wide-mouthed disposable plastic container. Alcohol (75%) was added to each container for preservation at room temperature and transported on ice packs to the parasitology laboratory of Department of Public Health and Preventive Medicine, University of Abuja, Nigeria for screening (Jongwutiwes et al., 2002).

2.5. Detection of *Cryptosporidium* oocysts

Concentration of fecal specimen was done using formalin-ethyl acetate sedimentation About 5 mL of the formalin-treated stool specimen was washed in 10% Formalin-saline. The sediment was centrifuged at 650 × g for 5 min, suspended in 8 mL of formalin-saline in 3 mL of ethyl acetate. The mixture was mixed thoroughly for 3 min and centrifuged at 500 × g for 5 min, resulting in four layers of ethyl acetate, a plug of debris, a layer of formalin-saline, and the sediment. The top three layers were decanted. One portion (1g) of the sediment was placed on a microscope slide and dried for Safranin methylene blue staining in line with the protocol of Baxby et al. (1984) on an air-dried slide. The slide was examined under the microscope using the x40 and oil immersion objective. Each sample was screened three times (3x) to eliminate the possibility of false positives. The oocysts of *Cryptosporidium* appeared as small spherical to round bright orange to reddish mass within a halo. This was considered positive (Baxby et al., 1984). *Cryptosporidium* control slides used for the study were obtained from Dr. Bruce Anderson of University of Idaho U.S.A.

2.6. Statistical Analysis

The obtained data was summarized and entered into Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheets, and statistical analysis was conducted in EpiInfo 3.5.3 (CDC, Atlanta, USA). Descriptive and analytic statistics were used. Percentages were used for descriptive analysis while Chi-square test (or Fisher Exact test where necessary) was used to determine associations. All analyses were carried out with p-value set at 0.05. Differences were expressed as significant at 95% confidence level (Hennekens & Buring 1987).

3. Results

3.1. Burden of *Cryptosporidium* infection at the Area Councils

Four hundred (400) fecal samples of cattle in Federal Capital Territory, Nigeria were collected and screened for *Cryptosporidium* oocysts **(Fig 2)**.

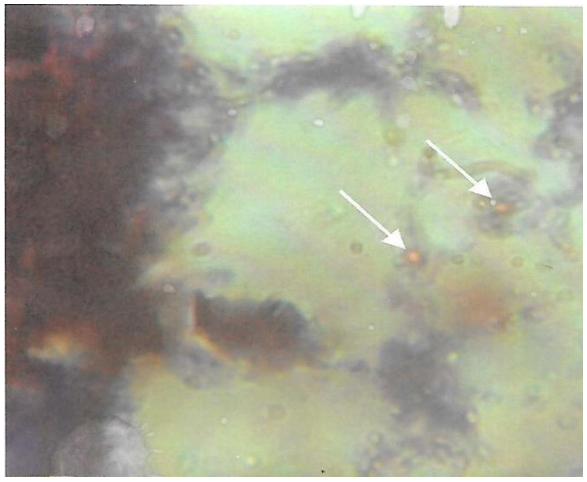


Fig. (2): Scanned photomicrograph of *Cryptosporidium* oocysts isolated from cattle feces using safranin-methylene-blue staining technique x40.

The overall prevalence of *Cryptosporidium* was 17.8% (71/400). Eleven samples (2.8%; 11/400) that initially tested positive was negative after repeated screenings. The Area Council specific prevalence of *Cryptosporidium* infection in cattle was 13.2% in Abaji, 10.5% in Abuja Municipal Area Council (AMAC), 14.3% in Bwari Area Council (BAC), 32.4% in Gwagwalada Area Council (GAC), 13.2% in Kuje Area Council (KAC) and 23.1% in Kwali Area Council (Table 1).

Table (1): *Cryptosporidium* infection rates in cattle by Area Councils of the Federal Capital Territory, Nigeria

Area Council	Total samples	Positive samples	Negative samples	Prevalence (%)	Chi-square (X^2)	P-value
Gwagwalada	68	22	46	32.4	15.00	0.010
Kwali	52	12	40	23.1		
Bwari	70	10	60	16.7		
Kuje	68	9	59	13.2		
Abaji	76	10	66	13.2		
AMAC	66	8	58	12.1		

3.2. Factors associated with occurrence of *Cryptosporidium* infection in cattle population

The summary of the infection rates of *Cryptosporidium* in cattle in FCT according to the various factors tested and using multivariate regression technique is as presented in the **Table 2** below. Analysis showed overall infection to be 17.8% in the Cattle feces examination using Safranin staining technique. Infection was highest in cattle in Gwagwalada area council 32.4% (71/400). There was no significant difference in the infection rates of both sexes of cattle though, cows had higher rates than bulls (19.8%; P = 0.109). Analysis of all the results showed age, breed, fecal consistency, management practices and size of herds as having positivity to *Cryptosporidium*. In the age categories, infectivity declined as cattle advanced in age. All the age categories showed an association with *Cryptosporidium* infection, however, cattle in the 181 -270 days' category were more likely to be infected (OR: 7.04; 95% CI: 3.08 -16.09) than those in age group < 90 days. The result of this survey showed *Cryptosporidium* infection in cattle based on fecal consistency in the order 60% (42/70), 12.4% (11/89) and 7.4% (18/241) for diarrheic, well-formed and loose feces respectively. There was no significant difference in the *Cryptosporidium* infection rate in the well-formed compared to the loose fecal samples. The loose feces were less likely (OR: 0.05; 95% CI: 0.03- 0.11; P: <0.001) to be infected with *Cryptosporidium* than the diarrheic category.

Regression analysis showed significant association in *Cryptosporidium* infection and breed of cattle in this survey. The infection rate was less likely in the bunaji breeds of cattle than the cross breeds (OR: 0.16; 95% CI: 0.16 – 0.91). In terms of management practices, infection was significantly associated with cattle rearing in all the methods used.

Infection was, however, less likely in the intensive than the extensive rearing method (OR: 0.32; 95%CI: 0.17 - 0.60). On the effects of herd size on *Cryptosporidium* infection, there was no significant difference in *Cryptosporidium* infection in 001 - 100 cattle herds and the other groups. The cattle in 201 – 300 herd size showed 5 times vulnerability to infection than those in 101 -200 herd size (OR: 5.05; 95%CI: 2.59 - 11.66).

Table (2): Multivariable logistic regressions analysis - Intrinsic and extrinsic factors that influence occurrence of *Cryptosporidium* infection in cattle in the Federal Capital Territory, Nigeria

Variables	Number positive (%)	Number negative (%)	Odd ratios	95% Confidence interval	P-value
Age (Days)					
< 90	54	125	1.00		
91-180	9	73	3.51	1.64, 7.51	0.001
181-270	7	114	7.04	3.08, 16.09	<0.001
271-360	1	17	5.83	1.34, 25.40	0.005
Fecal Consistency					
Loose	18	223	1.00		
Diarrhea	42	28	0.05	0.03, 0.11	<0.001
Well-formed	11	78	0.57	0.26, 1.27	0.170
Breed					
Bunaji	21	196	1.00		
Gudali	41	101	0.26	0.15, 0.47	0.001
Cross-breed	9	32	0.38	0.16, 0.91	0.030
Management practices					
Intensive	49	270	1.00		
Extensive	19	33	0.32	0.17, 0.60	0.001
Semi-intensive	3	26	1.57	0.46, 5.40	0.500
Size of herd					
200-300	27	75	1.00		
101-200	11	168	5.50	2.59, 11.66	0.001
001-100	33	86	0.94	0.52, 1.70	0.837

4. Discussion

Substantial works have been done on *Cryptosporidium* in Nigeria using varieties of detection procedures but there is little or no information on the occurrence of the parasite in cattle in the Federal Capital Territory, Nigeria. This study confirms the detection of *Cryptosporidium* infection in cattle in the Federal Capital Territory of Nigeria. Many reports have earlier implicated cattle as veritable agent of transmission of *Cryptosporidium* (Hunter et al., 2004; Roy et al., 2004).

After screening of the fecal samples in this study, 71 out of the 400 (17.8%) fecal samples were positive to *Cryptosporidium* oocysts. About 11 samples that were positive on first screen returned negative (False positives) at subsequent screening. False positivity of samples may be due to the presence of fungal debris in the feces or inadequate number of oocysts in the fecal samples. This is in line with the finding of Guerrant et al. (2005) who reported that stain can only be picked by feces only if it has more than 500,000 oocysts/g. The detection rate of 17.8% of *Cryptosporidium* obtained in this study was in the neighborhood of the 16% prevalence obtained in Kaduna (Maikai et al., 2011). The figure is also within the reported world range of detections in cattle 6.3% and 39.7% (Joule et al., 2016). This detection is higher than the 13.0% obtained in FCT, Nigeria in *Cryptosporidium* antigen surveillance (Adeiza and Nafarnda, 2021), 7.8% in Central Ethiopia (Wegayehu et al., 2016) and 3.6% in Iran (Reza and Rooholah, 2015). The figure is, however, lower than the 32.3% reported in Oyo State, Nigeria (Ayinmode & Fagbemi, 2011), 37.5% in Ogun State, Nigeria (Akinkuotu et al., 2014), 61.0% reported in New York State (Starkey et al., 2006) and the 27.3% reported in Ontario, Canada (Coklin et al., 2007). The differences in detection of *Cryptosporidium* from these studies could be due to the methods used for detection, ecology of the study area, and sampling techniques employed.

Prevalence of *Cryptosporidium* in this study appears to decline with the age of cattle. Cattle under 90 days of age in the study area had the dominant presence of *Cryptosporidium*. Calves under 180 days generally had higher prevalence of *Cryptosporidium*, 24.14% (63/261) compared to the adult cattle of between 181 and 360 days, 5.6% (8/139). Specifically, prevalence was highest in the calves under 90 days, 30.2% (54/179) compared to the rate of detection from older age groups. The distribution of *Cryptosporidium* across the ages of cattle in this study therefore suggests some age relatedness to infection. Calves have generally been reported to have higher vulnerability to *Cryptosporidium* infection than cattle of older ages On This high detection of *Cryptosporidium* in calves may not be unconnected to certain immunological incompetence usually found in younger age-group. Of the numerous outbreaks of human cryptosporidiosis reported worldwide annually (Efstratiou et al., 2017), many have been linked to cattle especially calves as sources of infection (Caffarena et al., 2022). *Cryptosporidium* was detected in both sexes in this study. Though there was no statistically significant difference in the level of occurrence between both sexes, the detection was however higher in the female cattle (cows) than the males (Bulls). This report compares with a study done among native cattle herds in Nigeria (Ayinmode & Fagbemi, 2011) report from coastal and forest savannah transition zone of the Greater Accra region of Ghana (Dankwa et al., 2021) and report from the Aizawl district in India (Das et al., 2018) where female cattle had higher prevalence compared to the males. This result from this study however contrasts report of other studies in Nigeria which recorded a significantly higher detection of *Cryptosporidium* among male animals (Ayinmode & Fagbemi 2011; Maikai et al. 2011) than females. The higher prevalence amongst females in this study is probably due to mating ratio of male to female cattle that permit more females in the herd than the males. This increases the probability of higher exposure of female cattle to *Cryptosporidium* infections.

In this study, there was no significant difference in the *Cryptosporidium* infection rate in the well-formed compared to the loose fecal samples. The loose fecal consistency category was less likely (OR: 0.05; 95% CI: 0.03- 0.11; P: <0.001) to be infected with *Cryptosporidium* than the diarrheic category. Detection of *Cryptosporidium* was however highest in diarrheic feces compared to the results from samples of well-formed and loose fecal consistencies screened in this study. This result is consistent with the findings of Ehsan et al. (2019) that reported calves shedding oocysts as having 6.1 times the risk of being diarrheic. This result however contrasts the reports of Adeiza and Nafarnda (2021) from FCT, Nigeria, Ayinmode and Fagbemi (2011) from Oyo state, Nigeria, and Dankwa et al. (2021) from Central Ghana where they variously reported diarrhea to be independent of *Cryptosporidium* infection. Differences in prevalence may be due to the detection method, age of cattle sampled, season and ecology of the study area. All the breeds screened for *Cryptosporidium* in this study showed significant association with *Cryptosporidium* infection. The infection rate was less likely in the bunaji breeds of

cattle than the cross breeds (OR: 0.16; 95% CI: 0.16 – 0.91) compared to the Sokoto gudali breed that appeared to be the most vulnerable to *cryptosporidium* infection. This report is similar to the findings of Nwoga (2021) where he observed Sokoto Gudali breeds of cattle to be weaker in resistance to diseases than Bunaji and their crossbreeds. Reports from Ethiopia however showed higher of infection rate in crossbred than the indigenous zebu. Such contradictions may be due to the management differences between the large size management of the Cross breed and the local Zebu (Mohammed, 1999). *Cryptosporidium* infection was significantly associated with management practices employed in rearing cattle in the study area. Infection was, however, less likely in the intensive than the extensive rearing method (OR: 0.32; 95%CI: 0.17 - 0.60). The high prevalence of *Cryptosporidium* in cattle raised through extensive husbandry practices may be due to the wider exposure of the cattle to contaminated environment and water during grazing thereby, subjecting the cattle to random picking of *Cryptosporidium* oocysts from the water they drink along their grazing routes. This finding agrees with the reports of Adeiza and Nafarnda (2021) but disagrees with the reports of Innes et al., (2020) where cattle raised intensively had higher prevalence of *Cryptosporidium* oocysts.

Cattle herd of 201-300 cattle heads was five times more likely to be infected with *Cryptosporidium* than those in the 101-200 cattle herds (OR: 5.05; 95%CI: 2.59 - 11.66). Detection of *Cryptosporidium* in large herd cattle is highest compared to the prevalence in moderate and smaller cattle herds. This finding is in contrast with the earlier reports of Adeiza and Nafarnda (2021) where small herds had higher occurrence. The high rate of detection of *Cryptosporidium* in the large size herds may be due to poor management and poor sanitation sometimes associated with managing herds of large number of cattle.

5. Conclusion

This study showed high detectable presence of *Cryptosporidium* across all the risk parameters tested in cattle in Federal Capital Territory, Nigeria using Safranin-Methylene blue staining and microscopy. Location of cattle as defined by the local council area in the FCT, the age, fecal consistency, breed management practices and size of cattle herd are all drivers/risk factors in the spread of *Cryptosporidium* and cryptosporidiosis in cattle of the Federal Capital Territory, Nigeria. This pervading presence of *Cryptosporidium* and its many risk factors underscore its public health importance in the federal capital of Nigeria.

5.1. Recommendation

We recommend further detection and confirmation of the parasites in cattle in the same study area using molecular tools to deepen our knowledge of the parasite's epidemiology and its possible route of transmission to humans.

5.2. Financial Disclosure

This work was not funded by any research grant. Financial contributions were from individuals, especially the lead researcher.

5.3. Author Contribution

Abdulrahman Musa Adeiza: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. Nuhu Sani: Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. Enid Godwin and Elizabeth Chinwe: Methodology, Supervision, Writing – review & editing. Nma Bida Alhaji, Writing – review & editing. Wesley Daniel Nafarnda: Supervision, Writing – review & editing. Gabriel Kehinde Omeiza- Methodology, review and editing. Andrew Musa Adamu: Supervision, Writing – review & editing.

5.4. Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

5.5. Code Availability

Not applicable.

5.6. Ethical Approval

Ethics approval Protocols of sampling of the animals were approved by the Faculty of Veterinary Medicine, University of Abuja Research Committee No 0202. Verbal informed consent from animal owners was given prior to start of the sample collection.

5.7. Consent to Participate

Verbal informed consent was obtained from all participants in the study especially the owners of the cattle prior to sample collection from their cat. Consent for publication is Not applicable.

5.8. Declaration of Competing Interest

The authors declare that they have no competing interests.

5.9. Acknowledgements

We deeply express our sincere appreciation to the participants in this study for their invaluable contributions. We are grateful to the Veterinary attendants that the facilitated the collection of the fecal samples.

References

Abeywardena, H., Jex, A.R. & Gasser, R.B., (2015). A perspective on *Cryptosporidium* and Giardia, with an emphasis on bovines and recent epidemiological findings. *Advance Parasitology*. 88, 243–301. https://doi.org/10.1016/bs.apar.2015.02.001

Adeiza, M.A. & Nafarnda, D.W., (2020). The Prevalence of *Cryptosporidium* species. in cattle in Lokoja, Kogi State, North Central Nigeria. *International Journal of Recent Innovation and Academic Research*. 4(1), 49–58.

Adeiza, M.A. & Nafarnda, D.W., (2021). Associated Risk Factors of *Cryptosporidium* Infection in Cattle in the Federal Capital Territory, Nigeria. *International Journal of Recent Innovation and Academic Research.* 5(5), 31–39.

Ayele, A., Seyoum, Z. & Leta, S., (2018). *Cryptosporidium* infection in bovine calves: prevalence and potential risk factors in northwest Ethiopia. *BioMed Central Research.* 11, 1–6. https://doi.org/10.1186/s13104-018-3219-7

Ayinmode, A. & Fagbemi, B., (2011). Cross-reactivity of some *Cryptosporidium* species with Cryptosporidium parvum coproantigen in a commercial ELISA kit. *Nigerian Veterinarian Journal*. 32, 1–4. https://doi.org/10.4314/nvj.v32i1.69004

Ayinmode, A.B., Fagbemi, B.O. & Xiao, L., (2011). Molecular Characterization of *Cryptosporidium* in Children in Oyo state, Nigeria: Implication for infection sources. *Parasitology Research*. 110(1), 479–481. https://doi.org/10.1007/s00436-011-2531-0

Baxby, D., Blundell, N. & Hart, C.A., (1984). The development and performance of a simple, sensitive method for the detection of *Cryptosporidium* oocysts in feces. *Journal of Hygiene. (Cambridge)*, 93 (2), 317–323. https://doi.org/10.1017/s0022172400064858

Bouzid, M., Paul, R., Hunter, R.M. & Chalmers, K.T.M., (2013). *Clinical microbiology Review.* 26(1), 115–134.

Britannica, (2018). *The Editors of Encyclopaedia. "FederalCapital Territory"., Encyclopedia Brittanica*. Com/ace/Abuja-federal-capital-territory-Nigeria. *https://www.brittanica.*

Caffarena, D., Meireles, M., Carrasco-Letelier, L., Picasso-Risso, C., Santana, B., Riet-Correa, F., & Giannitti, F., (2020). Dairy Calves in Uruguay Are Reservoirs of Zoonotic Subtypes of *Cryptosporidium parvum* and Pose a Potential Risk of Surface Water Contamination. *Frontier for Veterinary Science*. https://doi.org/10.3389/fvets.2020.00562

Certad, G., Dupouy-Camet, J., Gantois, N., Hammouma-Ghelboun, O., Pottier, M. & Guyot, K., et al., (2015). Identification of *Cryptosporidium* Species in Fish from Lake Geneva (Lac Léman) in France. *PLOS ONE* 10(7). https://doi.org/10.1371/journal.pone.0133047

Coklin, T., Uehlinger, F.D., Farber, J.M., Barkema, H.W., O'Handley, R.M. & Dixon, B.R., (2009). Prevalence and molecular characterization of *Cryptosporidium* species. in dairy calves from 11 farms in Prince Edward Island, Canada. *Veterinary Parasitology.* 160(3-4), 323–326. https://doi.org/10.1016/j.vetpar.2008.10.096

Dankwa, K., Nuvor, S. V., Obiri-Yeboah, D., Feglo, P. K. & Mutocheluh, M., (2021). Occurrence of *Cryptosporidium* Infection and Associated Risk Factors among HIV-Infected Patients Attending ART Clinics in the Central Region of Ghana. *Tropical Medical and Infectious Disease*. 6(4), 210. https://doi.org/10.3390/tropicalmed6040210

Das, P., Deka, D., Borthakur, S.K., Roychoudhury, P. & Das, M., (2018). Studies on occurrence, molecular detection and genotyping of *Cryptosporidium* parvum along with associated risk factors in cattle and human from Aizawl district, Mizoram, India. *Biology Rhythm Research.* 51(2), 238–253. https://doi.org/10.1080/09291016.2018.1526501

Diaz, P., Varcasia, A. & Pipia, A.P. (2018). Molecular characterization and risk factor analysis of *Cryptosporidium* species in calves from Italy. *Parasitology Research.* 117, 3081–3090. https://doi.org/10.1007/s00436-018-6000-x

Díaz, P., Navarro, E., Remesar, S., García-Dios, D., Martínez-Calabuig, N., Prieto, A., López-Lorenzo, G., López, C.M., Panadero, R. & Fernández, G. (2021). The Age-Related *Cryptosporidium* Species Distribution in Asymptomatic Cattle from North-Western Spain. *Animals,* 11(2), 256. https://doi.org/10.3390/ani11020256

Diptyanusa, A. & Sari, I.P., (2021). Treatment of human intestinal cryptosporidiosis: A review of published clinical trials. *International Journal for Parasitology: Drug Resistance.* 17, 128–138. https://doi.org/10.1016/j.ijpddr.2021.09.001

Efstratiou, A., Ongerth, J.E. & Karanis, P., (2017). Waterborne transmission of protozoan parasites: review of worldwide outbreaks - An update 2011–2016. *Water Research,*114, 14–22. https://doi.org/10.1016/j.watres.2017.01.036

Ehsan A., Geurden, T., Casaert, S., Paulussen, J., De Coster, L., ShoeMaker, T., Chalmers, R., Grit, G., Vercruysse, J. & Claerebout, E., (2015). Occurrence and potential health risk of *Cryptosporidium* and *Giardia* in different water catchments in Belgium. *Environmental Monitoring Assessment.,* 187(2). https://doi.org/10.1007/s10661-014-4157-z

Garcia, L. S. & Shimizer, R. (1981). Comparison of clinical results for the use of ethyl acetate and diethyl ether in the formalin-ether sedimentation technique performed on polyvinyl alcohol-preserved specimens.

Journal of Clinical Microbiology. 13(4), 709-713. https://doi.org/10.1128/jcm.13.4.709-713.1981

Guerrant, L.R.O., Bushen, O.Y., Patrick, P.D., Houpt, E. Lima, A.A.M., (2005). Global Impact of Diarrheal Diseases That Are Sampled by Travelers: The Rest of the Hippopotamus. *Clinical and Infectious Disease*. 41(8), S524–S530. https://doi.org/10.1086/432946

Hunter, P.R., Hughes, S., Woodhouse, S., Raj, N., Syed, Q., Chalmers, R.M., Verlander, N.Q. & Goodacre, J. (2004). Health sequelae of human cryptosporidiosis in immunocompetent patients. *Clinical and infectious Disease*. 39(4), 504–510. https://doi.org/10.1086/422649

Innes, E.A., Bartley, P.M., Rocchi, M., Benevides-Silvan, J., Burrells, A., Hotchkiss, E., Chianini, F., Canton G. & Katzer, F., (2011). Developing vaccines to control protozoan parasites in ruminants: dead or alive? *Veterinary Parasitology*. 180(1-2), 155–163. https://doi.org/10.1016/j.vetpar.2011.05.036

Ireland-Perry, R.L. & Stallings, C.C., (1993). Fecal Consistency as Related to Dietary Composition in Lactating Holstein Cows. *Journal of Dairy Science*. 76(4), 1074–1082. https://doi.org/10.3168/jds.s0022-0302(93)77436-6

Jongwutiwes, S., R. Tiangtip, S. Yentakarm, N. & Chantachum (2002). Simple method for long-term copro-preservation of *Cryptosporidium* oocysts for morphometric and molecular analysis. *Tropical Medicine and International Health* 7(3), 257-264. https://doi.org/10.1046/j.1365-3156.2002.00854.x

Lawal-Adebowale, O.A., (2012). *Dynamics of Ruminant Livestock Management in the Contextof the Nigerian Agricultural System. In (Ed.)*, Livestock Production. IntechOpen.

Maikai, B.V., Umoh, J.U. & Kwaga, J.K.P. (2011). Molecular characterization of *Cryptosporidium* species. in native breeds of cattle in Kaduna state, Nigeria. *Veterinary Parasitology*. 178(3-4), 241–245. https://doi.org/10.1016/j.vetpar.2010.12.048

Mi, R., Wang, X. & Li, C. (2013). Prevalence and genetic characterization of *Cryptosporidium* in yaks in Qinghai Province of China. *Public Library Science One*, 8(9), e74985. https://doi.org/10.1371/journal.pone.0074985

Mohammed, H.O., Wade, S.E. & Schaaf, S., (1999). Risk factors associated with *Cryptosporidium parvum* infection in dairy cattle in southeastern New York State. *Veterinary Parasitology*. 83(1), 1–13. https://doi.org/10.1016/s0304-4017(99)00032-1

Natalie, L., (2018). *Cryptosporidium species*. In book: The Global Water Pathogen 10.14321/*waterpathogens*.33.

Ng, J., Yang, R., McCarthy, S., Gordon, C., Hijjawi, N. & Ryan, U., (2011). Molecular characterization of *Cryptosporidium* and *Giardia* in pre-weaned calves in Western Australia and New South Wales. *Veterinary Parasitology*. 176(2-3),145-50. https://doi.org/10.1016/j.vetpar.2010.10.056

Nwoga, C., Ikeh, N., Onodugo, M., Baiyeri, P. & Machebe, N., (2021). *Assisted Reproductive Technologies as Veritable Tools for Improving Production Efficiencies of N'dama and Muturu Cattle Breeds in Nigeria-A Review*. In (Edition), Bovine Science – Challenges and Advances.

Pignata, C., Bonetta, S., Bonetta, S., Caccio, S.M., Sannella, A.R., Gilli, G. & Carraro, E., (2019). *Cryptosporidium* contamination in drinking water: A case study in Italy. *International Journal of Environment Research in Public Health* 16(11), 2055. https://doi.org/10.3390/ijerph16112055

Ponka A., Kotilainen H., Rimhanen-Finne R., Hokkanen P., Hanninen M.I., Karna A., Meri T. & Kuusi, M., (2008). A foodborne outbreak due to *Cryptosporidium parvum*. *Eurosurveillance* 14, pii:19269.

Reza, R. & Roohollah, F., (2015). Prevalence of *Cryptosporidium* species. in calves under one-year-old in Ilam County (Iran). *Electrical Physician Review*. 47 84–96.

Roy, S.L., DeLong, S.M. & Stenzel, S.A., (2004). Risk factors for sporadic cryptosporidiosis among immunocompetent persons in the United States from 1999 to 2001. *Journal Clinical and Microbiology*, 42(7), 2944–2951. https://doi.org/10.1128/jcm.42.7.2944-2951.2004

Ryan, U.N.A., Fayer, R. & Xiao, L., (2014). *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology*, 141(13), 1667–85. https://doi.org/10.1017/s0031182014001085

Thompson, S., Hamilton, C.A., Hope, A., Morrison, L.J. & Innes, E.A., (2017). Bovine cryptosporidiosis: impact, host parasite interaction and control strategies. *Veterinary Research*. 48(1), 42. https://doi.org/10.1186/s13567-017-0447-0

Velásquez, J.N., Pantano, M.L. & Vittar, N., (2018). First Detection in Cryptosporidium DNA in blood and cerebrospinal fluid of HIV-infected patients. *Parasitology Research*. 117(3), 875–881. https://doi.org/10.1007/s00436-018-5766-1

Weber, R., Bryan, R.T. & Bishop, H.S. (1991). Threshold of detection of *Cryptosporidium* oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. *Journal of Clinical Microbiology*, 29, 1323–1327. https://doi.org/10.1128/jcm.29.7.1323-1327.1991

Wegayehu, T., Karim, R., Anberber, M., Adamu, H., Erko, B. & Zhang, L., (2016). Prevalence and genetic characterization of *Cryptosporidium* species in dairy calves in Central Ethiopia. *Public Library Science One*, 11(5). https://doi.org/10.1371/journal.pone.0154647

Witto, G.S., Kankya, C., Akurut, G., Mugasa, M.C., Kazibwe, A. & Ochwo, S., (2021). The prevalence and genetic characterization of *Cryptosporidium* isolates from cattle in Kiruhura District, Southwestern Uganda. *Journal of Parasitology Diseases*, 45, 778-789. https://doi.org/10.1007/s12639-021-01361-6