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https://dx.doi.org/10.4314/nvj.v45i1.3 **ORIGINAL ARTICLE**

Cryptosporidium Infection Among Slaughtered Cattle in Igboora, Oyo State, Nigeria

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SUMMARY

Cryptosporidium is an enteric protozoan of gastrointestinal tracts that cause life threatening diarrhea in the young, as well as, malnourished or immune-compromised adult animals. It is sustained in the environment by continual shedding through asymptomatic carrier via contamination. This study evaluated the presence of 250 cattle prior to slaughtering at Igboora slaughter slabs, Oyo State, Nigeria. Faecal samples were collected per rectum processed and analyzed for *Cryptosporidium* oocyst which were confirmed by their Pinkish red spherical ovoid shape with halo against a green background. The age, sex, breed and body condition score of each of cattle were also noted. Out of the 250 sampled cattle, 22% (55/250) were positive for *Cryptosporidium*. Higher prevalence of *Cryptosporidium* oocysts was detected in females (22.2%) compared to males (21.2%), and in the younger (23.3%) compared to older cattle (15%). Among the breeds of cattle screened, the highest prevalence was recorded in Sokoto Gudali (41.7%) while Cross breeds had the least prevalence (19.4%). Also, the highest prevalence of *Cryptosporidium* oocysts was detected in cattle with lean Body Condition Score (29.4%). No statistically significant difference was observed among all the variables ($p>0.05$). This study has shown the occurrence of *Cryptosporidium* infection in cattle slaughtered in Igboora. It is therefore advised that sanitation measures including proper sewage and dung disposal be put in place to prevent further transmission among cattle and to human beings

Key words: Igboora; Cattle; *Cryptosporidium* oocysts; Ziehl Neelsen stainin

INTRODUCTION

Cryptosporidium is an intestinal protozoon which infects the gastrointestinal tract of both animals and humans causing cryptosporidiosis (Huang and White, 20061). This parasite produces oocycts which are hardy and are ubiquitous in the environment which when ingested usually from contaminated food or water infect the gastrointestinal tract of the host (Amer *et al*., 2013). Cryptosporidiosis is considered to be of socioeconomic and of public health importance. The infection usually causes diarrhea which is commonly short term but can be severe in an immunocompromised host (Collinet-Adler and Ward, 2010). It is known that *Cryptosporidium* species can differ in their host range. Some species are restricted to particular types of hosts while others have a broad host range including man such as *C. parvum, C. meleagridis*, and *C. canis* constituting zoonotic significance (Thompson, 2008, Adamu *et al*., 2015).

Cryptosporidium parvum, Cryptosporidium bovis, C. ryanae and *C. andersoni are common species* with *Cryptosporidium parvum associated with* life-threatening neonatal diarrhoea, abdominal pain, anorexia, loss of weight and retarded growth in calves (Akinkuotu *et al*., 2014, Dankwa *et al*., 2021). Infected adult animals serve as asymptomatic carriers that shed large numbers of the oocysts into the environment (Adamu *et al*., 2015).

Cattle is the major source of transmission of Cryptosporidium *parvum.* This occurs through pasture runoff that contaminates the environment, human food and water by direct or indirect contact with fecal contaminant (Current and Garcia, 1991). Previous studies have shown that cattle cryptosporidiosis is widespread with oocyst shedding dynamics that depend on age, clinical situation and breeding system of the animals (Maldonado-Camargo *et al*., 1998). In Nigeria, cattle are reared mainly by the extensive and semi-intensive management systems which allow animals to come in close contact with farmland and water bodies used by humans, which makes it a public health concern (Adamu *et al*., 2015).

The aim of this study was to investigate the prevalence of *Cryptosporidium* infection among cattle in Igboora, Southwestern, Nigeria. The knowledge from this study can in turn be used to facilitate further studies on circulating strains of cryptosporidium and possible zoonotic transmission of this disease.

MATERIALS AND METHODS

Study design

A cross-sectional study was conducted to detect infections with *Cryptosporidium* spp and the associated factors that impact their presence among slaughtered cattle. The study was carried out between February and April, 2022 in Igboora, Oyo State, Nigeria.

Study area

This study was conducted in two slaughter slabs in Igboora: Towobowo (latitudes 7°25.8´N° & longitudes 3°17.41´E) and Igboora Central slaughter slabs (latitudes 7°26.5´N° & longitudes 3°17.34´E). Each of the slab slaughter on the average between seven to eight cattle on daily basis. Igboora is located approximately 80km north of the coast of Lagos and about 95km west of Ibadan. Igboora is the headquarters of Ibarapa Central Local Government Area. The major economic activity in Igboora is farming. Many pastoralist settlements and their cattle herds are

situated there due to the abundance of green pastures all year round.

Sample collection and handling

A total of 250 cattle faecal samples (200g) were collected per rectum before slaughtering. The samples were then transported on ice packs to the Parasitology Laboratory, Federal University of Agriculture, Abeokuta, where they were immediately processed. The age, sex, breed, and body condition score of sampled cows were recorded using distinct codes. The cattle ages were grouped into ≤3 and >3years according to Bakre *et al.* (2020). Male and female cattle were randomly selected. The breeds were White Fulani, Sokoto Gudali and Crossbreed. The body condition was scored as lean, moderate and good as previously described by Adedipe et al. (2014). Sampling was done twice in a week and five samples per location.

Laboratory analysis
Formol-ether sedimentation method

An application stick was used to homogenize one gram of faeces in 10ml of 10% formalin in a universal bottle. The homogenized sample was decanted into a centrifuge tube through a gauze and funnel. Three ml of dimethyl ether was then added to remove fat from the filtrate. The mixture was shaken properly to ensure thorough mixing and thereafter centrifuged for 2 minutes at 2000g. The supernatant was then decanted (WAOH, 2022).

Modified Ziehl- Neelsen Acid-Fast Staining method

The mixed sediment was used to obtain thin smears on glass microscope slides. They were further air-dried at room temperature then fixed with absolute methanol for 5 minutes. The microscope slides were stained with concentration of Ziehl-Neelsen: Carbol Fuschin (1:10) for 3 to 5 minutes and thereafter washed under running water. De-colorization was done with 3% acid alcohol (3% HCl in ethanol) for 10 to 15 minutes. This was followed by counter-staining with Malachite green (0.5%) solution for one minute. The slides were washed under running water and air-dried (WAOH, 2022).

Detection of *Cryptosporidium* oocysts

Each slide was examined under x100 microscopic objective lens (oil immersion object magnification). *Cryptosporidium* oocysts appeared pinkish-red with small spherical or ovoid organism having a halo, against a green to purplish background (Casemore, 1991).

Data analysis

Data was entered into Microsoft Excel version 2013 and analyzed with Stata 14.2 for Windows (StataCorp, College Station, TX, USA). Data was summarized using frequencies and proportions with their respective confidence intervals. The associations between *Cryptosporidium* infection and other explanatory variables such as age, sex, breed and Body Condition Scores were assessed using Pearson's Chi-square. Since *Cryptosporidium* infection outcome has prevalence greater than 10% in this study, a log-binormal model using the logit function in STATA was fitted to elucidate the predictors of *Cryptosporidium* infection among cattle in Igboora, Oyo State, Nigeria. The level of statistical significance was set at a 2-tailed p-value of 0.05 or less.

RESULTS

The overall prevalence of Cryptosporidium infection among the cattle population in this study was 22.0% (95% CI, 17.0 – 27.7). Female animals formed 79.2% of the sampled population. Majority of the sampled cattle were three years or younger (84.0%) and of White Fulani Breed (80.8%). In addition, 24.4% were fat, 68.8% were moderate and 6.8% were lean (Table 1). The prevalence of Cryptosporidium infection in faecal samples was higher among female cattle, 22.2% (95% CI, 16.6-28.7) compared to that of males, 21.2% (95% CI, 11.1- 34.7) even though the difference was not significant (p = 0.869). The prevalence of Cryptosporidium was 23.3% (95% CI, 17.8-29.7) among animals that are three years or younger compared to the 15.0% (95% CI, 5.7-29.8) found in animals older than 3 years. The difference in this group was also not statistically significant (p = 0:244). As shown in Table 1, Cryptosporidium was detected among all cattle breeds sampled for this study with the highest prevalence among Sokoto Gudali, 41.7% (95% CI, 15.2- 72.3), followed by White Fulani, 21.3% (95% CI, 15.9- 27.6) and Cross breeds, 19.4% (95% CI, 8.2- 36.0). Based on Body Condition Score, the prevalence of Cryptosporidium infection was highest among cattle that were lean, 29.4% (95% CI, 10.3- 56.0), followed by those that were fat, 24.6% (95% CI, 14.5- 37.3) and those that were moderate, 20.4% (95% CI, 14.6-27.2). None of the explanatory variables was a predictor of Cryptosporidium infection (Table 2).

DISCUSSION

Cryptosporidium has been reported to be a major enteric parasite associated with neonatal diarrhea and mortality in farm animals (De Graaf, 1999), environmental contamination (in water supplies), and risk of zoonotic transmission. Healthy and adult animals have been documented to shed oocysts, often in large numbers, providing additional potential reservoirs of infection (WAOH, 20222), Prevalence rate among cattle observed in this study is consistent with some previous reports in Nigeria; 23.4% in Oyo State (Ayinmode and Fagbemi, 2010), 22.3% in Maiduguri, Bornu State (Adamu *et al*., 2015), 28% in Jos, Plateau (Pam and Ogbu Dadki, 2013) and 28% in Zuru, Kebbi State (Danladi and Ugbomoiko, 2015). The result of this current study is however lower than 37.5% in Ogun State (Akinkuotu *et al*., 2014) and 58% in Yenagoa, Bayelsa State (Mondebo *et al*., 2022).

TABLE I. Occurrence of Cryptosporidium infection by age, sex, breed and Body Condition Score

Type	Variables	Number of samples (%) `	Number of positives (%)	95% CI	P- value
Age (years)	> 3	40 (16.0)	6 (15.0)	5.7 - 29.8	0.244
	≤ 3	210 (84.0)	49 (23.3)	17.8 - 29.7	
Sex	Female	198 (79.2)	44(22.2)	16.6 - 28.7	0.869
	Male	52 (20.8)	11(21.2)	11.1 - 34.7	
Breed	Cross	36 (14.4)	7 (19.4)	8.2 - 36.0	0.234
	Sokoto Gudali	12(4.8)	5 (41.7)	15.2 - 72.3	
	White Fulani	202(80.8)	43 (21.3)	15.9 - 27.6	
Body Condition Score	Fat	61 (24.4)	15 (24.6)	14.5 - 37.3	0.59
	Moderate	172 (68.8)	35 (20.4)	14.6 - 27.2	
	Lean	17 (6.8)	5 (29.4)	10.3 - 56.0	
Total		250	55 (22.0)	17.0 - 27.7	

TABLE II. Predictors of Cryptosporidium infection among cattle in Igboora, southwestern Nigeria

Type	Variable	Prevalence (%)	Odds ratio (95% CI)	P-value
Age (years)	> 3	6 (15.0)	1	
	≤ 3	49 (23.3)	0.66 (0.30 - 1.45)	0.303
Breed	Cross	7 (19.4)	1	
	Sokoto Gudali	5 (41.7)	2.12 (0.83 - 5.45)	0.119
	White Fulani	43 (21.3)	1.16 (0.56 - 2.38)	0.689

Other studies that have been reported in different countries of the world include: 30.40% in Brazil (Almeida *et al*., 2010), 16.00%, in Iran (Ranjbar and Fattahi 2017), 24.11% in Algeria (Hocine *et al*., 2018), 12.50% in Malaysia (Abdullah *et al*., 2019), 23.7% and in Ethiopia (Manyazewal *et al*.,

2018). The variations in the infection may be due to several possible reasons like sample size, climate, age of animals, geographical and ecological location, diagnostic methods used, hygiene conditions, management practices, the type of animals, and hygiene status of the farms or kraals (Squire *et al*., 2013, Ayele *et al*., 2018).

The occurrence of this infection in cattle as revealed by this study may be attributed to factors such as poor sanitation and hygienic practices, which exposes the animals to the risk of infection. The result of this study revealed a higher prevalence among female cattle in comparison to their counterpart males. This is similar to the findings of (De Graaf, 1999, Ayinmode and Fagbemi, 2010, Adamu *et al*., 2015, Ayele *et al*., 2018, Adeiza and Nafarnda, 2020). The reason for this difference is not certain. However, the outcome could be attributed to the usual practice of slaughtering more female cattle or stress related with hormonal imbalances. Findings from this study is in agreement with Akinkuotu *et al*. (2014) that reported decreasing prevalence in *Cryptosporidium* infection with increasing age of cattle. Younger animals, majorly calves, are known to have reduced immunity and are therefore susceptible to infection (Brook *et al*., 2009). Also, adaptive immunity acquired for *Cryptosporidium* in adult life due to prior infections in early life (Harp *et al*., 1990) might be responsible for the low incidence of clinical status, which results in low shedding of *Cryptosporidium* oocysts in adult cattle.

Sokoto Gudali breed of cattle had the highest prevalence of *Cryptosporidium* infection which is in consonance with the report of (Nwoga *et al.*, 2021). The lower rate of *Cryptosporidium* infection in White Fulani and Cross breed screened in this study might be because they are hardy and resistant to diseases than Sokoto Gudali (Tawah and Rege, 1996). Cattle with lean body condition scores had higher prevalence of *Cryptosporidium* infection compared to cattle with good and fat body condition scores. This correlates with the reports of (Birhanu *et al.,* 2017; Ayele *et al.,* 2018) that documented a higher occurrence of the enteric protozoan in cattle with poor body condition scores compared with those with good scores. This could be because cattle with poor body condition have lower immunity, and immunocompromised (Ayele *et al*., 2018).

Limitation

The diagnostic technique employed in this study (formalin-ethyl acetate sedimentation) reflect its limitations and it is suggestive of the need for effective techniques that can be used in further studies to determine the prevailing species in the study area and their zoonotic importance.

CONCLUSION

This study confirms the presence of *Cryptosporidium* infection among cattle in Igboora, southwestern, Nigeria. Hence, there is a need to educate pastoralists on preventive measures, in order to improve the health of their cattle and themselves. Further studies are required to determine the circulating species of *Cryptosporidium* in the study area to aid in understanding of the transmission and the zoonotic potential of the parasite.

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