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Epidemiology of *Cryptosporidium* sp. infection among free-range and intensive farm birds in Akure South LGA, Ondo State, Nigeria

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Abstract

Background: *Cryptosporidium* spp. is an intracellular zoonotic protozoan parasite that causes cryptosporidiosis, a diarrhoeal disease of humans and domestic animals. Transmission of Cryptosporidiosis to humans and other animals is by ingestion of oocysts of the parasite and as low as ten oocysts can cause clinical infections in otherwise healthy persons. The aim of this study is to investigate the prevalence of Cryptosporidiosis and compare the rate of infection between free range bird and poultry bird reared in Akure South LGA, Ondo State, Nigeria.

Result: The overall prevalence of *Cryptosporidium* reported in this study was 11.9%. Free-range birds show a higher prevalence rate 13.2% of *Cryptosporidium* oocysts than 10.9% in poultry birds. Aule recorded the highest prevalence of *Cryptosporidium* oocysts infection (16.1%) followed by Ipinsa (12.2%), Onigari (10%), and FUTA (8.1%). The highest prevalence 15.9% was recorded in broilers, while turkey showed no infection (0%) by *Cryptosporidium*. Semi-intensive system of farming was showed to be more susceptible to *Cryptosporidium* oocysts infection at 13.3% followed by the 12.6%, 10.3% in deep litter and battery cage. The female birds recorded higher *Cryptosporidium* oocysts infection (12.2%) than the male (11.6%).

Conclusion: The study established the presence of *Cryptosporidium* oocysts infection among studied birds in Akure South LG of Ondo State, Nigeria.

Keywords: Epidemiology, *Cryptosporidium* oocysts, Infection, Free-range, Poultry, Birds

Background

Cryptosporidiosis is an emerging zoonotic disease, resulting in intestinal and extraintestinal disorders in both humans and animals (Fayer et al. 2000). Transmission of *Cryptosporidium* oocysts infections is through multiple routes. Infections may be transmitted through person to person, which is particularly important in daycare settings with children; by direct contact with infected animal or via faecal-oral route or by ingestion of oocysts contaminated water and food (Khan et al. 2004). Transmission of Cryptosporidiosis to humans and other animals is by ingestion of oocysts of the parasite (Fayer

2010) and as low as ten oocysts can cause clinical infections in otherwise healthy persons (DuPont et al. 1995). These oocysts are resistant to most common disinfectants and are not readily killed by routine chlorination of water (LeChevallier et al. 1991). *Cryptosporidium* can infect more than 30 avian species. However, three different *Cryptosporidium* species (*C. parvum*, *C. meleagridis*, and *C. galli*) were considered the major pathogens of birds. The occurrence of animal *Cryptosporidium* spp. in humans indicates that humans are constantly at risk of contracting cryptosporidiosis from these reservoir hosts. *Cryptosporidium* infections in humans account for up to 6% of all diarrhoea cases in immune competent persons and 24% of persons with both HIV and diarrhoea worldwide (Bialek et al. 2002; UNICEF 2007). *Cryptosporidium* was included in the World Health Organization's

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Neglected Diseases Initiative in order to enhance knowledge on the epidemiology and host-parasite interactions especially through molecular techniques (Savioli et al. 2006). In poultry houses, shared water source is an effective means of sharing the oocysts among birds and hence infecting a large number. *Cryptosporidium* infections continue to be a significant health problem in both developed and developing countries (Harp 2003), where it is recognized as an important cause of diarrhoea in both immunocompromised and immunocompetent people (Kjos et al. 2005). They are different free range and poultry farms that serves as major producers of birds and eggs for public consumption in the study area Akure. Although the prevalence of *Cryptosporidium* has been studied in various parts of Nigeria, there is paucity of information on the epidemiology and comparison of *Cryptosporidium* infection especially in this part of the country. Thus, the aim of this study is to investigate and compare *Cryptosporidium* infections among free range and intensive chicken and turkey farms reared in the study area.

Method

Study areas

The study was conducted in four locations within Akure South Local Government Area in Ondo State, Nigeria (Fig. 1) and with a total population of 486,300. The city covers an estimated area of 331 km² and is located at 7.2050°N latitude and 5.1877E longitude.

Study design

This was a cross-sectional study. A total of 437 birds including 247 from farms and 190 local breeds from free range (Tables 1 and 3) reared in Aule, Ipinsa, FUTA, and Onigari (Table 2) were sampled for this study. Furthermore, samples were collected from three (3) species of birds present in the poultries selected. Sampling sites were conveniently selected at random.

Laboratory analysis of samples

Laboratory method, namely Modified Ziehl Neelsen, is used to diagnose *Cryptosporidium* infection.

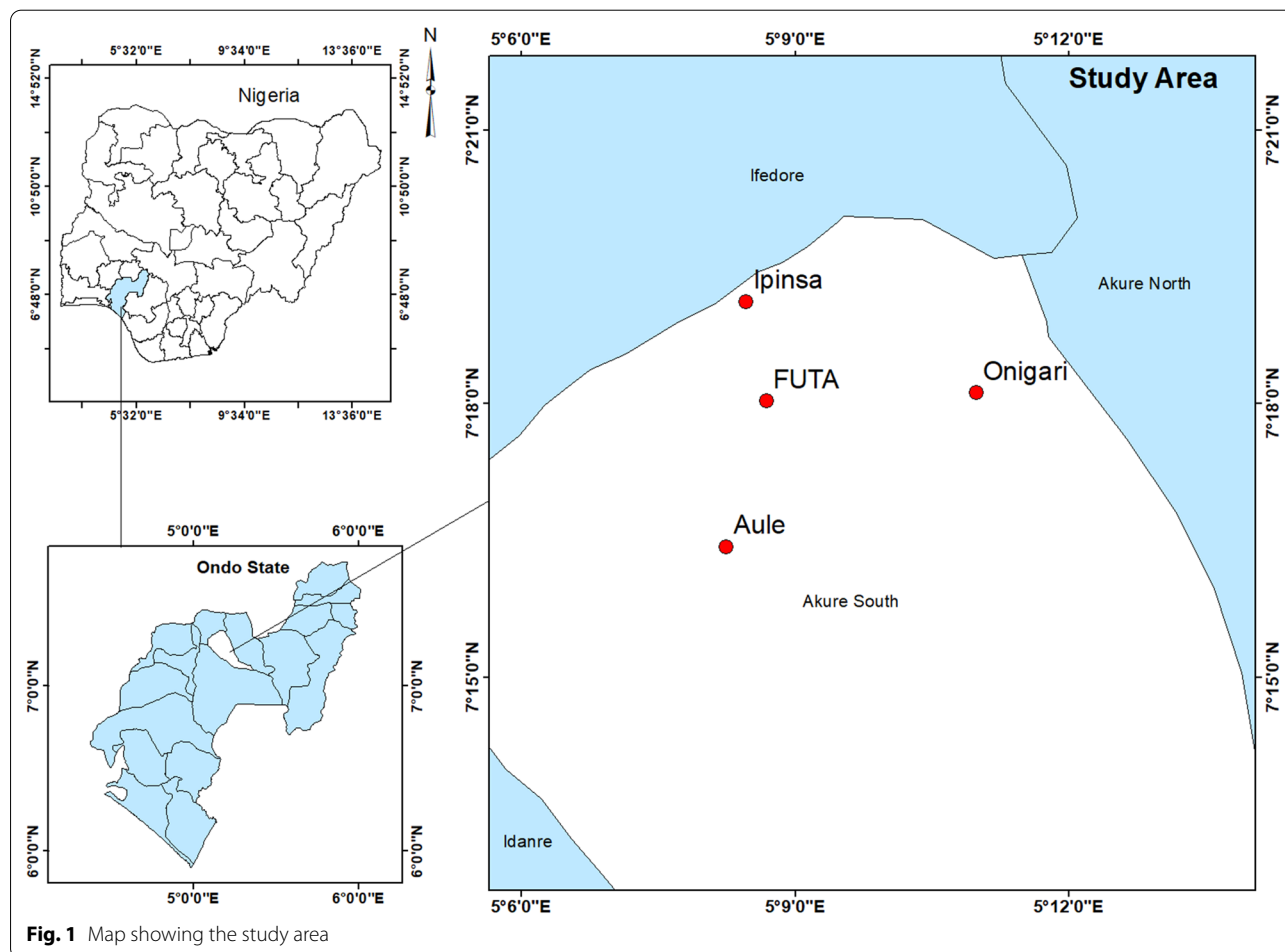


Fig. 1 Map showing the study area

Table 1 Prevalence of *Cryptosporidium* oocysts in relation to method of rearing

Rearing method	Number examined	Number (%) infected	χ^2	df	P value
Poultry	247	27 (10.9)	0.508	1	0.476
Free range	190	25 (13.2)			
Total	437	52 (11.9)			

Table 2 The prevalence of *Cryptosporidium* oocysts according to sampling location

Sampling location	Number examined	Number (%) infected	χ^2	df	P value
Aule	31	5 (16.1)	2.044	4	0.128
Ipinsa	82	10 (12.2)			
FUTA	74	6 (8.1)			
Onigari	60	6 (10)			
Total	247	27 (10.9)			

Faecal collection

The sampling exercise was conducted from October 2020 to April 2021. Chickens in steel cages contained 2–3 birds whose faeces were collected on the tray under the cages were considered as one sample, collected by the use of plastic sample bottle, marked with the poultry farm name, bird species, and collection date while that of free-range birds are collected early morning from birds housing in various homes. The sampling process was conducted to collect the fresh droppings to the best of my ability. Samples were kept in a sampling bottle until they were transported to the laboratory, then stored in the refrigerator at 4 °C and processed as soon as possible. The samples were transported to the undergraduate research Laboratory where they were processed and examined. After smears were prepared, the remaining samples were preserved in 10% formalin solution and in 2 ml cryo vials. Samples were preserved by freezing at – 20 °C.

Slide preparation

Using the applicator stick, about 0.2 g of faecal sample was emulsified onto the slide to make a thin smear which was dried on a slide warmer at 60 °C for 5 min. The fixed smear was put on the staining rack (accommodating 12 slides per rack at a time). The smears were flooded with Kinyoun’s carbol fuchsin for 1 min after which they were rinsed in distilled water and drained. The smears were then decolourized in 1% acid-alcohol for 2 min after which they were washed again with distilled water and counterstained with

methylene blue for 2 min. Lastly, the smears were washed with distilled water and drained before drying them on slide warmer at 60 °C for about 5 min. The stained slides were examined under the microscope using oil immersion objectives (×100 objectives) (Sunnotel et al. 2006).

Microscopy detection

Samples of *Cryptosporidium* oocysts were examined by optical microscopy observation under ×40 objective then with ×100 oil immersion magnification based on the shape of oocysts and the shape index measured. Oocysts seen were identified with the aid of an atlas encyclopedia of parasitology photographs and manual of parasitology (Bowman 2009).

Data analysis

Data were entered in Microsoft Excel® spreadsheet and analysed using Statistical Package for the Social Sciences (SPSS) version 16. Proportions of positives, with 95% confidence intervals, were estimated. The relationships between the presence of *Cryptosporidium* and hypothesized risk factors were investigated using Chi-square or Fisher’s exact test in univariate analyses where appropriate. Results were presented in percentages/proportions, and the multiple effects of predictor variables were investigated using the logistic regression. A significance level of 5% was used for all tests.

Results

A total of 437 samples were examined for *Cryptosporidium* oocysts. Two hundred and forty-seven (247) samples were examined in poultry birds, and one hundred and ninety (190) sample were examined in free range birds. Table 1 showed that out 247 poultry birds’ sample, twenty-seven (27) were positive for *Cryptosporidium* oocysts with prevalence of 10.9%, while out of the 190 samples examined, twenty-five (25) were positive for *Cryptosporidium* oocysts with prevalence of 13.2%. Generally, a total number of fifty-two (52) sample were observed to be positive *Cryptosporidium* oocysts given a total prevalence rate of 11.9%. The prevalence of *Cryptosporidium* oocysts was higher in free range bird’s sample (13.2%) than in poultry bird’s sample (10.9%). Chi-square analysis of data showed that there was no significant difference in the prevalence of *Cryptosporidium* oocysts observed among samples collected among Poultry and Free-range birds ($\chi^2 = 0.508; p = 0.476, p > 0.05$).

Table 2 showed that out of the thirty-one (31) sample examined from Aule, five (5) were positive for *Cryptosporidium* oocysts with prevalence of 16.1% while out the eighty-two (82) sample collected in ipinsa, 10 were positive *Cryptosporidium* oocysts with prevalence of 12.2%. Seventy-four (74) samples were examined from Futa, and

six (6) of them were positive for *Cryptosporidium* oocysts given a prevalence of 8.1%. From the sixty (60) samples collected from Onigari, six (6) were positive for *Cryptosporidium* oocysts with prevalence of 10%. Chi-square analysis of data showed that there was no significant difference in the prevalence of *Cryptosporidium* oocysts observed among samples collected in the different sites. ($\chi^2 = 2.044$; $p = 0.128$, $p > 0.05$).

Result presented in Table 3 showed that 18 out of the 113 Broilers examined were infected with *Cryptosporidium* oocysts with prevalence of 15.9%, 9 out of the 100 Layers birds examined were seen to be infected with *Cryptosporidium* oocysts given a prevalence of 9%, while none of the 34 sample examined from turkey birds were positive to the presence of *Cryptosporidium* oocysts given it a zero (0%) prevalence. Of the ninety-five (95) cockerel examined, 15 were positive to *Cryptosporidium* oocysts with a prevalence of 15.8%. Ninety-five (95) Hen species were examined of which 10 were positive to *Cryptosporidium* oocysts with a prevalence of 10.5%. Chi-square analysis of data showed that there was no significant difference in the prevalence of *Cryptosporidium* oocysts observed among bird's species ($\chi^2 = 6.377$; $p = 0.173$, $p > 0.05$).

Prevalence of *Cryptosporidium* oocysts in relation to System of Bird Farming is presented in Table 4. From the 190 samples collected from semi-intensive system of bird farming, 25 were positive to *Cryptosporidium* oocysts given a prevalence of 13.2%. 11 out the 107 sample examined from battery cage system were positive to *Cryptosporidium* oocysts infection leaving the prevalence at 10.3%. In deep litter system of bird farming, 16 were positive from the 127 samples examined given a 12.6% prevalence. Chi-square analysis of data showed that there was no significant difference in the prevalence of *Cryptosporidium* oocysts observed among the system of bird farming ($\chi^2 = 2.017$; $p = 0.365$, $p > 0.05$).

The prevalence of *Cryptosporidium* between males and female birds is presented in Fig. 2. The Modified

Table 3 The prevalence of *Cryptosporidium* oocysts according to species of bird

Birds species	Number examined	Number (%) infected	χ^2	df	P value
Broiler	113	18 (15.9)	6.377	4	0.173
Layer	100	9 (9)			
Turkey	34	0 (0)			
Cock	95	15 (15.8)			
Hen	95	10 (10.5)			
Total	437	52 (11.9)			

Table 4 The prevalence of *Cryptosporidium* oocysts according to system of bird farming

System	Number examined	Number infected (%)	χ^2	df	P value
Battery cage	107	11 (10.3)	2.017	2	0.365
Deep litter	127	16 (12.6)			
Semi-Intensive	190	25 (13.3)			
Total	437	52 (11.9)			

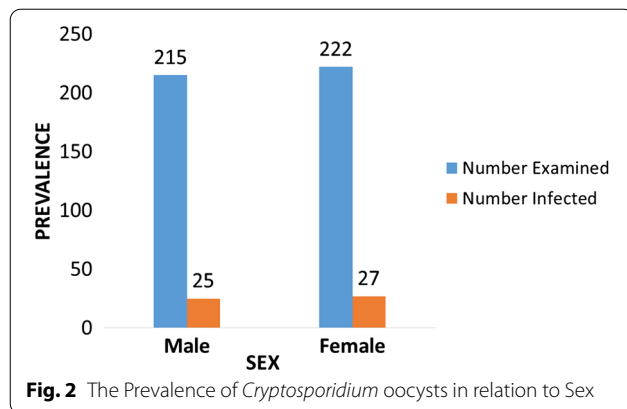
Ziehl Neelsen method detected 25 (11.6%) positive samples in males, slightly lower than the 12.2% (27/222) detected in female birds. The prevalence of *Cryptosporidium* between males and females' birds was not significant different ($p = 0.863$, $p > 0.05$).

Discussion

The prevalence of *Cryptosporidium* species in both poultry and free-range birds in Akure South Local Government of Ondo State, Nigeria was determined in this study. Other studies have previously confirmed the presence of *Cryptosporidium* species in birds with variations in prevalence from place to place, which could be due to climatic, breed, sex, and the bird farm management differences used. The overall prevalence of *Cryptosporidium* infection reported in this current study was 11.9% which was higher than that recorded in Tunisia (4.5%) by Soltane et al. (2007), 5.1% reported in Germany according to Helmy et al. (2017), 7.4% prevalence result of Bamaiyi et al. (2013) in Zaria, Nigeria, and 8.1% publication by Meng et al. (2011) in China, previous studies use both diagnostic approach of microscopic examination and molecular genotyping. However, slightly lower to the 14.8% in Brazil by Da Cunha et al. (2018) and 19.8% in Bangladesh by Kabir et al. (2020). The difference in the prevalence rates may be attributed to the use of different detection methods (histology, serology, and microscopy), management system, farm control practice, and seasonal difference in the study areas.

The prevalence of *Cryptosporidium* oocysts according to rearing method

Free-range birds show a higher prevalence rate 13.2% of *Cryptosporidium* oocysts than poultry birds, and this was in agreement with the findings of Bamaiyi et al. (2013) in Zaria, Nigeria. This higher infection rate may be attributed to the fact that free-range birds are allowed to roam about freely thereby exposing them to multiple sources of infection through sporulated oocysts from water, livestock, and human during feeding (Ryan 2010) compared to the exotics poultry bird which are kept in cage and confide



compartment that restrict wide range of roaming about. Furthermore, it could be as a result of accumulation of the parasite from smaller intermediate host like cockroaches which have been confirmed to be positive to *Cryptosporidium* during feeding compared to the poultry birds which are fed with processed feeds (Kumar et al. 2014).

The prevalence of *Cryptosporidium* oocysts according to sampling location

The present study reveals that irrespective of the site, birds are all susceptible to *Cryptosporidium* infection. *Cryptosporidium* oocysts prevalence was higher at Aule and Ipinsa with 16.1% and 12.2% prevalence, respectively, than every other location, in my personal observations this may be as a result of various favourable factors that support the infection of *Cryptosporidium* to thrive in Aule and Ipinsa farm which may include poor hygiene, farm management where deep litter system of farm management which can encourage oocysts survival and increase the viability (Gascon et al. 2000).

The Prevalence of *Cryptosporidium* oocysts according to species of bird

The prevalence rate of *Cryptosporidium* oocysts varies between different bird species examined. The higher prevalence was recorded in broilers birds in this study than their cock, layers, and hen counterpart, and this finding was in contrast with the work of Wang et al. (2010) and Helmy et al. (2017). However, was in agreement with the report of Kabir et al. (2020) where they recorded a higher prevalence in broiler than in layer chicken. This is also in agreement with reports by Nnadi and George (2010) in Southeastern, Nigeria; Nematollahi et al. (2009) in Iran; Jatau et al. (2012) in Zaria, North-western Nigeria and Naphade, 2013 in India. This might be connected with higher stocking densities and intensive husbandry management systems practiced in broiler production in the study area.

The prevalence of *Cryptosporidium* oocysts according to system of bird farming

Semi-intensive system of farming was showed to be more susceptible to *Cryptosporidium* oocysts infection, and this may be attributed to the fact that the birds will have access to more sources of *Cryptosporidium* oocysts as they roam about and feed on soil content, livestock faeces, and other vector that harbour *C. oocysts* (Ryan 2010). The result of this study also revealed that from the intensively managed poultry farm with deep litter system and constructed battery cage, the prevalence was relatively higher in the deep litter system than the battery cage management system. This was in agreement with the finding of Lawal et al. (2016) which can be attributed to sporulation of buildup oocysts resulting from spillage of water and humid environment which lead to relatively high oocysts accumulation according to the report of Dakpogan and Salifou (2013) and Taylor et al. (2007) as most of the farms with deep litter left the floor dust for more than a week before replacement. The Battery Cage system of farming was showed to have the least prevalence, and this may be as a result of the iron cage structure use to house the birds as birds do not have access to pick infected faeces because they drop direct under the cage as the cage are raised above the ground level in the study area.

The prevalence of *Cryptosporidium* oocysts according to sex

The finding from this present study is in agreement with the work of Bamaïyi et al. (2013) that acclaimed that both sex of birds is susceptible to *Cryptosporidium* oocysts infection. The prevalence of *Cryptosporidium* oocysts was higher in female birds than in male birds, and this may be attributed to the high susceptibility of female to infection due to reduced immunity at certain period of reproductive cycle as reported by Gbemisola et al. (2016). This also indicated that females were more likely to contract intestinal protozoa than male (Davoust et al. 2008). However, this was in contrast to the findings of Zhang et al. (2015) in a work done in North china. The prevalence indicates that both sexes have equal chance of becoming infected with *Cryptosporidium* oocysts during feeding and outbreak of infection (Oljira et al. 2012).

Conclusions

Although the identification of *Cryptosporidium* species was not decided in this study but most of the sample observed morphological resemble *C. baileyi* which is probably the most common avian *Cryptosporidium* species. The results of the present study established the presence of *Cryptosporidium* infection among birds surveyed in Akure South LG of Ondo State Nigeria. The overall

prevalence of *Cryptosporidium* reported in the study was 11.9%, and it also revealed that all species of birds, sex of birds, and system of bird's management are susceptible to *Cryptosporidium* sp. infection which can act as a source of human Cryptosporidiosis. Poor management practice is the main risk factor favouring the onset of *Cryptosporidium* such as oocysts build-up, oocysts sporulation, and the humid environment.

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Authors' contributions

OAA and OTA contributed to the research design and involved in field and laboratory work. OAA carried out statistical analysis and interpret the result of the study. OAA write the first draft of the manuscript. OTA review the manuscript. All author read and approved the final manuscript.

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Available of data and materials

All analysed data involved in this study are included in this manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing for interests

The authors declare that they have no competing interests.

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