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Prevalence of Cryptosporidium infection in chickens in Maiduguri Metropolis, Borno State, Nigeria

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Abstract

Cryptosporidiosis is a neglected parasitic zoonotic disease known to cause diarrhoea in humans, domestic animals, and wild vertebrates and has serious public health threats. Globally, poultry production is one of the most lucrative businesses and serves as a major source of protein due to its high demand. This study aimed to determine the prevalence of Cryptosporidium oocysts in chickens in Maiduguri metropolis. A total of one hundred and seventy faecal samples were collected from live bird markets, chicken slaughter slabs, and backyard poultry farms and surrounding environmental samples. Sex, age, and diarrhoea condition of the chickens were recorded. Samples were processed and analysed according to a standard operating procedure. The study recorded an overall prevalence of 2.4% Cryptosporidium oocysts in chickens. This prevalence was higher in females (3.4%) and local chickens (4.5%), compared to males (1.2%) and exotic birds (0%). Only slaughter slabs had a prevalence of 5%. There was no statistically significant ($p>0.05$) association between location, sex, chicken type, and Cryptosporidium oocysts prevalence. The study recommends that poultry faeces and contaminated poultry meat should be hygienically handled to prevent infection. Poultry farmers, consumers and handlers should be educated on the hygienic measures to be taken.

Keywords: Chicken, Cryptosporidium, Maiduguri, Prevalence, Zoonosis

Introduction

The Poultry industry occupies an important position in the provision of animal protein (meat and egg) to man and generally plays a vital role in the national economy as a source of revenue. Poultry is one of the

most intensively reared of the domesticated species and one of the most developed and profitable animal production enterprises (Heise *et al.,* 2015; Mottet & Tempio, 2017; Erdaw & Beyene, 2022).

Chickens and eggs are in constant demand and have become a lucrative agricultural practice, providing a significant source of animal proteins worldwide. This could be attributed to the fact that it has low production costs, with little or no religious or cultural restrictions on the use of the products in most nations (Mottet & Tempio, 2017; Danladi & Garba, 2021; Attia *et al.,* 2022).

Cryptosporidium, a coccidian protozoan parasite, has been described in sixteen different species of domestic and wild mammals, birds and reptiles (Zaheer *et al.,* 2021; Ali *et al.,* 2024; Golomazou *et al.,* 2024), as an important causative agent of human and animal gastrointestinal infection globally (Zaheer *et al.,* 2021). Most human infection is mainly caused by *Cryptosporidium parvum*; however, the two main avian species, *Cryptosporidium baileyi* and *Cryptosporidium meleagridis,* demonstrate unique zoonotic potentials*. C. meleagridis* is reported to have potential effects on avian species, particularly chickens and pigeons, as well as humans, with significant implications for public health (Santana *et al.*, 2018; Liao *et al.,* 2018; Altamimi, 2020; Lin *et al.,* 2021; Ryan *et al.,* 2021; Hao *et al.,* 2024). Conversely, *C. baileyi* is rarely developed in immunocompromised individuals and cancer patients, primarily functioning as a parasite of birds. Moreover, field surveys consistently characterise its zoonotic potential as low or uncertain, lacking definitive evidence of regular cross-contamination between avians and mammals (Liao *et al*., 2018; Iijima *et al.,* 2018; Robertson *et al.,* 2020; Altamimi, 2020; Feng *et al.,* 2023). The parasite is responsible for acute gastrointestinal and less frequent respiratory infections in humans. It is self-limiting in immunocompetent people but prolonged and potentially life-threatening for the immunocompromised populations (Fayer, 2004). The incubation period is usually about one week, with clinical signs of watery diarrhoea which may be accompanied by abdominal pain, vomiting and fever (Fayer, 2004). According to Experimental and observational studies by King & Monis (2007), Guechtouli *et al.* (2022), Berhanu *et al*. (2022), and Wang *et al.* (2024), indicated that Cryptosporidium oocysts flourish in cool, moist environments and retain infectivity for months on damp litter or wet soils, whereas desiccation significantly reduces their lifespan.

Poultry Cryptosporidiosis caused by the protozoan zoonotic parasite of the genus Cryptosporidium, is an important parasitic disease in the poultry industry. The distribution and economic significance of the disease have been reported in different countries such as Algeria, China, Germany, and Bangladesh (Baroudi *et al.,* 2013; Wang *et al.,* 2014; Helmy *et al.,* 2017; Kabir *et al.,* 2020; Helmy & Hafez*,* 2022). The infection occurs through ingestion of food or water contaminated with viable, environmentally resistant oocysts. So far, infection in birds is predominantly caused by four important species: *Cryptosporidium baileyi, Cryptosporidium galli, Cryptosporidium meleagridis* and *Cryptosporidium avium* (Nakamura & Meireles, 2015). The parasite has been reported in wild, domestic and captive birds (Bamaiyi & Redhuan, 2016).

The commonest clinical signs associated with *C. meleagridis* infection in chickens are greenish diarrhoea and decreased growth (Baroudi *et al.,* 2013). The parasite infects several animal and human hosts. From previous studies, infection has been reported in over 170 host species (Danladi & Ugbomoiko*,* 2015).

In developed countries, waterborne outbreaks of cryptosporidiosis have a significant economic impact (Checkley *et al.,* 1998). In Nigeria, human cryptosporidiosis has been reported earlier from Northern (Kwaga *et al.,* 1988), Central (Banwat *et al.,* 2003), Eastern (Okafor & Okunji, 1994 and 1996) and Western (Reinthaler *et al.,* 1987) parts of the country. The disease is a major public health problem in both developing and developed countries (Saidu *et al*., 2023). In developing countries, the disease probably exerts most of its impact on young children resulting in stunted growth (Bushen *et al.,* 2007).

The increasing consumption of poultry and poultry products (meat and egg) as a source of protein as well as a source of livelihood have increased the frequency of contact between poultry and humans. The zoonotic implication of poses a lot of uncertainties among the human population. The consumption of undercooked and other food preparations against the background of poor hygiene in production, processing and marketing of these products in Maiduguri exposes the consumer to enormous risks of Cryptosporidium infection, particularly among immunocompromised individuals. There is a paucity of information on the prevalence of Cryptosporidium infection in chickens and limited awareness of the disease in the study area. Hence, this necessitated the need to determine the incidence/prevalence of Cryptosporidium oocyst in poultry at various live

bird's markets and slaughter slabs in Maiduguri. This will therefore provide baseline data on the prevalence of Cryptosporidiosis in chicken in the study area and inform appropriate control and public health interventions.

Materials and Methods

Study area

The study was carried out in Maiduguri Metropolis, Borno State capital, located in the Northeastern part of Nigeria on latitude 11.8311" and longitude 13.1510" of the equator. It has a landmass area of 70,898Km^2 and borders Republic of Niger to the North, Lake Chad and Tchad Republic to the Northeast, Cameroon Republic to the East and borders the Nigerian states, Adamawa, Gombe and Yobe on the South and West, respectively. It is a semi-arid zone characterized by a harsh climate condition with dry season starting from November to early June, during which the temperature varies from 30℃ to 41℃ daily especially from March to June (Aliyu *et al.,* 2025). The rainy season usually starts from late June to October with low relative humidity and short wet seasons. The population of Maiduguri is estimated to be 845,000 in 2023, and that of the State is 5,860,200 as of 2016, with a population density of 83.0 inhabitants per square kilometre. Borno State consist mostly of farmers, civil servants, fishermen and livestock keepers as there are abundant water bodies (rivers, streams and dams), and marketplaces for fish, livestock and goods across the State. The study involve collection of samples at the LBMs in the study area, which were known for purchasing and selling birds such as chickens, pigeons, guinea fowl, turkeys, ducks, rabbits, quails, and other animals.

A cross-sectional study was designed to determine the prevalence of Cryptosporidium oocysts in chickens. The study was conducted between the months of August and September 2023.

Sample size

An Epi-Info™ 7 was used to determine the sample size, a baseline prevalence of 11.9% reported by Olonisakin & Olusi (2021). Therefore, at 95% confidence level, the sample size was 161. Most of the poultry meat processors and their leaders at various locations of live birds markets (LBMs) and poultry farmers were informed of the objectives of the study, and their cooperation was requested before sampling. Oral consent was sought before sample collection; those who declined were excluded from the study.

Sample collection and transportation

The samples were randomly collected from three live bird market locations (Monday market, Baga Road, and Tashan Bama), chicken slaughter slabs within these markets, and poultry farms around the study area. The samples were collected on a weekly basis.

The samples were randomly obtained from three locations of live bird marketplaces (Monday market, Baga road, and Tashan Bama), chicken slaughter slabs within these markets, and poultry farms across various locations in the study area. The samples were collected weekly. A total of one hundred and seventy faecal samples were collected from live and slaughtered birds either as bird`s fresh droppings or by cutting open an eviscerated intestine of slaughtered chicken while noting their sex, age and diarrhoeal condition. All faecal samples were placed into sample bottles immediately and appropriately labelled. In addition, 15 environmental samples (soil) around the poultry farm, slabs and live bird market were collected for the determination of Cryptosporidium oocysts. All the samples collected were then transported to the Veterinary Parasitology Laboratory in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri for further processing.

Laboratory analysis

Acid-fast staining technique for the detection of Cryptosporidium oocysts

Samples were processed and analysed according to the procedure developed by Paul Ehrlich in 1882 (Ehrlich, 1892). One to two drops of concentrated stool were smeared on the slide and air-dried. This was fixed with absolute methanol for one minute. The slide was flooded with carbol fuchsin solution and intermittently heated from underneath using a spirit lamp for five minutes until fumes arose. The slide was then rinsed thoroughly with water and decolourized with acid alcohol for ten minutes, after which it was rinsed with water. The smear was then counterstained with methylene blue solution or malachite green solution for one to two minutes, which was then rinsed again, air dried and viewed under the microscope using a x100 objective with oil immersion.

Identification of Cryptosporidium ocysts

Each of the slides was examined using a light microscope at x 40 and x100 magnification. Cryptosporidium oocysts appeared as bright pink

spherules. Oocysts were confirmed under a higher power magnification (x100).

Data analysis

The prevalence rate of Cryptosporidium infection was calculated using the formula: Prevalence = (Number of samples positive)/ (Total sample analysed) ×100 (Spronk *et al.*, 2019).

Results were presented in percentages/proportions, tables and charts. The proportion of positives with 95% confidence interval was estimated. The relationships between the presence of Cryptosporidium and hypothesized risk factors were analyzed using Chi-square. The data were presented in tables and charts. An association between age, sex, management system, location of samples, and Cryptosporidium infection were determined using the Chi-square (χ2) test of association and odds ratio (OR); confidence intervals (95%) were calculated for odds ratios. P-values less than 0.05 were considered significant. Statistical Package for the Social Sciences (SPSS) version 20 was used to analyze the data.

Ethical approval and consent

Verbal consent was obtained from poultry farmers, and approval was granted by the management of LBMs and chicken slaughter slabs prior to the initiation of the study. Farmers who declined were omitted from the research.

Verbal consent was sought from poultry farmers, and approval was given to us by the Management of LBMs and poultry slaughter slabs before the commencement of the study. No human sample was involved in this study.

Results

Sex distribution of Cryptosporidium oocysts in chickens

This study recorded an overall prevalence of 4 (2.4%) Cryptosporidium oocysts in chickens in the study. Out of the 89 hen samples examined, 3 (3.4%) were positive, while only 1 (1.2%) was positive out of 81 cocks. There was no statistically significant (p=0.62 association between sex and Cryptosporidium oocyst detection (Table 1).

Distribution of Cryptosporidium oocyst in chickens at slaughter slabs, live birds market and commercial poultry farms, Maiduguri Metropolis

Out of the 170 faecal samples collected at 3 different sampling units, only poultry slaughter slabs recorded 5% prevalence of Cryptosporidium oocyst (Table 2).

Distribution of Cryptosporidium oocysts in Maiduguri metropolis based on chicken type

Out of 93 local chickens examined, 4 (4.3%) were positive for Cryptosporidium oocysts compared to exotic chickens with 0% prevalence. There was no statistically significant association (p=1.3%) between chicken types and Cryptosporidium oocysts. None of the 15 soil environmental samples tested for Cryptosporidium oocysts was positive (Table 3).

Table 1: Sex distribution of Cryptosporidium oocysts in chickens at Maiduguri Metropolis

Sex	Number tested	Number Positive	Prevalence (%)	χ^2	p-value
Female	89	3	3.4	0.8	0.62
Male	81	1	1.2		
Total	170	4	2.4		

Table 2: Distribution of Cryptosporidium oocysts in chickens at slaughter slabs, Live Birds Market and commercial poultry farms, Maiduguri Metropolis

Sampling Unit	Total number of sampled examined	Number of samples positive	Prevalence (%)
Commercial poultry farm	50	0	0.0
Live Bird markets	40	0	0.0
Slaugher slabs	80	4	5.0
Total	170	4	2.4

Table 3: Distribution of *Cryptosporidium* oocysts in Maiduguri metropolis based on chicken type and environmental samples

Types	Number tested	Number Positive	Prevalence (%)	χ^2	p-value
Broilers	77	0	0.0	3.25	1.3
Locals	93	4	4.3		
Total	170	4	2.4		
Soil	15	0			

Discussion

The prevalence of Cryptosporidium oocysts in chickens in Maiduguri metropolis, Borno state, Northeastern Nigeria may seem low, but it might be significant as it may pose a public health threat, particularly among immunocompromised individuals, and also the poultry industry in the study area. This is because Cryptosporidium oocysts can remain infective under cool, humid conditions for many months, especially where water temperatures in ponds, lakes, and rivers remain low but above the freezing point (Fayer, 2004). This reported prevalence was lower than 7.4% reported by Bamaiyi *et al.* (2013) in Zaria and 11.9% reported by Olonisakin *et al.* (2021) in chickens in Akure, Ondo state. In addition, this also disagrees with the findings of Mustapha *et al.* (2016), who reported 6.4% prevalence of Cryptosporidium oocysts in chickens in Kano state. This variation may be attributed to differences in the periods of sample collection, sample size, environmental conditions, hygienic measures, and other management practices of the study area. This is because Ondo state is known to have high humidity, which may improve oocyst survival in the environment and subsequent transmission to a susceptible host. Adding to that, Zaria has relatively higher humidity and lower ambient temperature compared to Maiduguri; as such, this may serve as the reason for the higher prevalence in the former.

The higher prevalence reported in females may be attributed to the fact that females stay longer in the flock, making them have more chances of being exposed to Cryptosporidium oocysts, particularly during the period of high soil moisture and also, during laying, females stay longer in the cage than males, and as such have a higher risk of being exposed. This finding agrees with that of Olonisakin *et al.* (2021), who reported a higher prevalence of 12.2% in females as compared to males. Though sex has not been statistically associated with Cryptosporidium oocyst. This agrees with the findings of Bamaiyi & Redhuan (2016), Shahbazi *et al.* (2020), Gong *et al.* (2021) and Feng *et al.* (2023).

The prevalence of Cryptosporidium oocysts in local chickens in this study may be linked to poor management practices attached to rearing local chickens. This is comparable with the findings of Bamaiyi *et al.* (2013), who reported a higher prevalence of 9.5% in local Chickens in Zaria. The absence of Cryptosporidium oocysts in environmental samples in this study may be attributed to the higher temperature in the study area, which halted the growth and life cycle of the parasite, contrary to other areas, which may have an enabling environment of high humidity and lower temperature. This is in contrast to findings of Mohaghegh (2016), who reported 30.9% prevalence of Cryptosporidium oocysts in soil in northern Iran. The disparity may be due to differences in the environmental conditions of the study area. This is because northern Iran is a cold semi-arid climate zone and most of the precipitation is in the form of snow, which may favour the soil survival of Cryptosporidium oocysts.

The presence of Cryptosporidium oocysts in chicken slaughter slab may not be unconnected with constant presence of moisture around the vicinity of the slaughter slabs, together with poor hygienic measures, which gives a moist, protective, conducive microhabitat in which oocysts may persist for 4-12 months or longer (Olson *et al.,* 1999). This is contrary to the findings of Mustapha *et al.* (2016), who reported a higher prevalence of 10% in LBMs in Zaria. The disparity may be due to differences in sample size, locations, period of sample collection and study area.

This study concluded that the low prevalence recorded in this study, which is also higher in males in local chickens than in exotic ones, and in hens than in cocks, could still pose a public health threat in the study area.

Lack of constant electricity supply to store samples and effectively identify the oocyst microscopically, and lack of funds to further characterize the oocyst have formed part of the limitations of this study.

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Conflict of Interest

The authors declare no conflict of interest.

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