NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., December 2016 Vol 37 (4): 247-253.
ORIGINAL ARTICLE

Occurrence and Risk Factors Associated with Faecal Shedding of Cryptosporidium Oocysts in Avian Species in Kano Metropolis, Nigeria

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SUMMARY
The aim of this study was to determine the occurrence and risk factors associated with faecal shedding of Cryptosporidium oocysts in avian species in Kano Metropolis. Faecal samples from 330 different avian species were examined using flotation technique and Modified Ziehl Neelson staining technique. Structure questionnaires were administered to bird owners to determine risk factors associated with faecal shedding of Cryptosporidium oocysts in avian species. An overall prevalence of 6.4% was determined. Live bird market (LBM) had the highest prevalence rate of 10%, while slaughter slabs and commercial poultry farms had 4.55% and 4.55%, respectively. Based on bird species, turkeys had the highest prevalence rate of 18.75%, followed by ducks (9.09%) and chickens (6.61%). Occurrence of Cryptosporidium oocysts in samples based on Local Government Areass revealed that Dala had 8 (12.12%) positive samples, followed by Tarauni 6 (9.09%), Nasarawa 4 (6.06%), Municipal 2 (3.03%) and Kumbotso 1(1.52%). Cryptosporidium oocysts were 1.3 times more likely to occur in flocks of those that source their birds from outside the State, 1.86 times more likely to occur in flocks of sellers that do not routinely clean their cages, 2.6 times more likely to occur in flocks of those that get their feed from sources other than commercial feed, 1.7 times more likely to occur in flocks that drink well water, and 1.18 times more likely to occur in flocks kept in wooden cages. There is a need for close monitoring of the infection in farm animals because of the public health and economic importance of the disease.

Key words: Cryptosporidium, risk factors, avian species, Kano Metropolis.

INTRODUCTION
Cryptosporidium is a coccidian parasite found infecting a wide range of mammals including man, birds and lower vertebrates. Cryptosporidiosis is an emerging zoonotic disease, resulting in intestinal and extra-intestinal disorders in both humans and animals (Fayer *et al*., 2000). The disease is well known in veterinary medicine and has been recognized as a leading protozoal cause of human diarrhea (Goldsmith *et al*., 1989). In poultry, the disease is known to cause heavy economic losses. It is highly probable that the disease is often misdiagnosed in poultry in Nigeria since it is not routinely

diagnosed and the condition can persist in a farm and even be confused with Coccidiosis. Transmission of Cryptosporidiosis to humans and other animals is by ingestion of oocysts of the parasite (Fayer *et al.*, 2010). 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 species of *Cryptosporidium* (*C. baileyi, C. meleagridis*, and *C. galli*) were considered the major pathogens of birds (Ryan *et al.*, 2003; Xiao *et al.*, 2004). The Kano Metropolis live bird markets (LBMs) are of traditional structure in which poultry and poultry products are displayed in an open system for buyers and sellers (AICP, 2008). Considering the possibility of exposure of human beings to *Cryptosporidium* still exists and the fact that the oocysts are resistant to most common disinfectants, the prevention of Cryptosporidiosis is important for public health (LeChevallier *et al.,* 1991; Karanis *et al.,* 2007). Although studies on Avian Cryptosporidiosis have been conducted in some places in Nigeria, for instance in Zaria (Bamaiyi *et al.*, 2013), it is important to note that there is paucity of information on Avian Cryptosporidiosis in Kano Metropolis. Therefore, the aim of this study was to determine the occurrence and associated risk factors for faecal shedding of *Cryptosporidium* oocysts in live bird markets, slaughter slabs and commercial poultry farms in Kano Metropolis, Nigeria.

MATERIALS AND METHODS

Study Area

Kano Metropolis is located in the central western part of Kano State between latitude 11059"59.57 and 12002"570N of the equator and between longitudes 8033"19.69 and 8031"59.690E. Its population is the second largest in Nigeria after LAGOS. The metropolitan area comprises eight local government areas (LGAS); Municipal, Gwale, Dala, Tarauni, Nasarawa, Fagge, Ungogo and Kumbotso (KSMI, 2005).

Sample Size Determination and Sampling Units

The sample size was determined using an established prevalence of 7.4% (Bamaiyi *et al.,* 2013) using the formula described by Thrusfield (1997): $N = Z^2pq/d^2$, where N is sample size, Z is constant (1.96), p is prevalence (7.4%), q is 1- p, and d is level of significance (0.05). Therefore, a total of 330 faecal samples (110 each from LBMs, slaughter slabs and commercial poultry farms) were collected disposable plastic bags. The samples were taken to Protozoology Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University (A.B.U), Zaria, for analysis.

Detection and Identification of *Cryptosporidium* oocysts

Detection of *Cryptosporidium* oocysts was done using Flotation technique according the method described by Urquhart *et al* (1987). Oocysts seen were identified with the aid of an Atlas Encyclopedia of Parasitology photographs and Manual of Parasitology (Levine *et al.*, 1985; Bowman, 2009). Positive oocysts were further subjected to modified Ziehl Neelsen stain for confirmation of *Cryptosporidium* oocysts (Lynne and Garcia, 1999).

Identification of Associated Risk Factors for Faecal Shedding of *Cryptosporidium* oocysts

A structured questionnaire was designed for this study. The questionnaire contained some variables covering information about LBMs location, feed and water sources, source of birds, frequency of cleaning cages and type of cages. The questionnaire was administered to poultry sellers through interview during on-site visits. Personal observation was used to validate the responses.

TABLE I: Occurrence of *Cryptosporidium* oocysts in live bird markets, slaughter slabs and commercial poultry farms in Kano Metropolis, Nigeria

Sampling Unit	Total number of faecal samples examined	Number of positive samples	Occurrence (%)
Live bird market	110	11	10
Slaughter slab	110	5	4.55
Commercial poultry farm	110	5	4.55
Total	330	21	6.4

$X^2 = 3.662$, $p = 0.210$

TABLE II: Occurrence of *Cryptosporidium* oocysts in some avian species in Kano Metropolis, Nigeria

Bird specie	Total number of faecal samples examined	Number of positive samples	Occurrence (%)
Turkeys	16	3	18.75
Chickens	257	17	6.61
Ducks	11	1	9.09
Guinea fowls	38	0	0
Pigeons	8	0	0
Total	330	21	6.4

Fisher exact test = 6.650, $p = 0.094$

TABLE III: Occurrence of *Cryptosporidium* oocysts in avian species based on Local Government Areas in Kano Metropolis, Nigeria

Bird specie	Total number of faecal samples examined	Number of positive samples	Occurrence (%)
Kumbotso	66	1	1.52
Municipal	66	2	3.03
Nasarawa	66	4	66.06
Dala	66	8	12.12
Tarauni	66	6	9.09
Total	330	21	6.4

$X^2 = 8.340$, $P = 0.084$

Data Analysis

The prevalence of *Cryptosporidium* oocysts was determined: P = number of positive samples/ Total number of samples x 100. Results were tabulated. Statistical Package

Cryptosporidium oocytes and associated risk factors. Values of $p<0.05$ were considered significant.

RESULTS

for Social Sciences (SPSS) version 20 was used to analyze the data. Chi-square test and Odds Ration (OR) at 95% confidence interval were used to determine the association between occurrence of

An overall prevalence of 6.4% was determined for *Cryptosporidium* oocysts in Kano Metropolis from the 330 faecal samples collected. Prevalence of 10% was

recorded for *Cryptosporidium* oocysts in LBMs, 4.55% in slaughter slabs and 4.55% in poultry commercial farms (Table I). The difference in prevalence of *Cryptosporidium* oocysts based on sampling units was not significant (p = 0.210). Occurrence of *Cryptosporidium* oocysts based on bird species were 18.7% in turkeys, 9.09% in ducks, 6.61% in chickens, 0% in both guinea fowls and pigeons (Table II). Occurrence of *Cryptosporidium* oocysts in samples based on LGAs revealed that Dala had 8 (12.12%) positive samples, followed by Tarauni 6 (9.09%), Nasarawa 4 (6.06%), Municipal 2 (3.03%) and Kumbotso 1 (1.52%) (Table III).

TABLE 4: Risk factors associated with faecal shedding of *Cryptosporidium* oocysts in avian species in Kano Metropolis, Nigeria

Factors	No. of samples	No. positive (%)	p value	Odds Ratio	95% CI
Local Government Areas					
Municipal	22	1 (4.5)	0.618	1.000	0.059-17.065
Kumbotso	22	1 (4.5)			
Nasarawa	22	2 (9.1)		0.476	0.040-5.671
Tarauni	22	3 (13.6)		0.302	0.029-3.152
Dala	22	4 (18.8)		0.214	0.022-2.095
Conventional LBM: Yes	100	11 (11.0)	0.594	0.890	0.831-0.953
No	10	0			
Source of bird: Intra-State					
	34	4 (11.8)	0.735	1.314	0.358-4.828
	76	7 (9.2)			
Outside State					
Frequency of cleaning cages:					
Daily	55	7 (12.7)	0.263	1.859	0.512-6.756
	55	4 (7.3)			
Others					
Source of feed:					
commercial	29	5 (17.2)	0.126	2.604	0.720-9.298
others	81	6 (7.4)			
Source of water:					
Borehole	46	6 (13.0)	0.521	1.770	0.0506-6.196
Well	74	5 (7.8)			
Type of cage:	44	4 (9.1)	0.533	1.186	0.326-4.320
Metallic					
	66	7 (10.6)			
Wooden					

Chi-square test, $p<0.05$ significant

The difference in occurrence of *Cryptosporidium* oocysts between the LGAs was not significant (p = 0.084). The questionnaire survey for associated risk factors revealed that *Cryptosporidium* oocysts were 1.3 times more likely to occur

in flocks of those that source their birds from outside the State, 1.86 times more likely to occur in flocks of sellers that do not routinely clean their cages, 2.6 times more likely to occur in flocks of those that get their feed from sources other than commercial feed, 1.7 times more likely to occur in flocks that drink well water, and 1.18 times more likely to occur in flocks kept in wooden cages (Table IV).

DISCUSSION

Even though, the prevalence of *Cryptosporidium* oocysts was low, the fact that they are present in avian species could pose risk for the spread of infection in the area, possibly through feed and water. The finding is similar to that of Bamaiyi *et al* (2013) who also determined a prevalence of 7.4% in bird species in Zaria. Even though, the species of *Cryptosporidium* oocysts isolated from birds of different species were not specifically identified in this study, it is important to note that most cases diagnosed in chickens were *C. baileyi* which can slow down growth rate in broilers and cause decrease in egg production in layers as reported by Wang *et al* (2014). However, *C. meleagridis* may also cause deadly disease in immunocompromized humans, and this could be of serious public health importance (Ryan, 2010). The finding that the highest occurrence of the oocysts was observed in LBMs may be attributed to the multiple species of birds sampled, unlike in slaughter slabs and commercial poultry farms where only chickens were sampled. The implication is that LBMs might serve as potential reservoirs for the spread of *Cryptosporidium* oocysts to different species including humans. It is also important to note that LBM is a place where people gather and might be in close or direct with *Cryptosporidium* oocysts, and this have highlighted the risk of exposure to both birds owners and the buyers (Ryan, 2010). It has been reported that exposure to *Cryptosporidium* oocysts directly through contact with infected birds may lead to acute diarrhoea in man, which presumably may begin from the small intestines where the emerging sporozoites infect enterocytes and after multiplication spread through the epithelial surface of both villi and crypts (Tzipori and Ward, 2002; Latif and Rossle, 2015). The eventual loss of microvilli border and villus height diminishes intestinal surface and uptake of fluids, electrolytes and nutrients from the gut lumen (Tzipori and Ward, 2002). Clinical disease has been reported in turkeys, quails, pigeons, gulls and sea birds in which the parasite when ingested invaded oesophageal gland, proventriculus, small intestine, cloaca and bursa of Fabricius (Nguyen *et al.*, 2013).

The finding that the highest prevalence of *Cryptosporidium* oocysts was reported in turkeys than in other species is a matter of concern, even though the species of *Cryptosporidium* oocysts recovered from the turkeys was not identified. However, the possibility of the oocysts being that of *C. meleagridis* should not be overlooked. This is in view of the fact that *C. meleagridis* has zoonotic potential (Ryan, 2010; Wang *et al.*, 2014). The finding that Dala LGA (in comparison to the other LGAs) appeared to have the highest prevalence of *Cryptosporidium* oocysts may not be unconnected with the fact that the poultry birds in this area are predominantly the local breeds that are usually maintained on free Range or semi-intensive system of management (Jellison *et al.*, 2004). Therefore there is high possibility of exposure of these birds to the oocysts.

It was also observed that *Cryptosporidium* oocysts are more likely to be spread to poultry through well water than other sources. This may be attributed to the fact that well water is the most common source of drinking water for most poultry in the area. The implication is that, contamination of well water by *Cryptosporidium* might result in major water-borne outbreaks of Cryptosporidiosis not only in poultry, but

even in humans (Putignani and Manichelia, 2010). The high risk estimate associated with wooden cages may also be attributed to the nature of the wood which can provide conducive environment for the *Cryptosporidium* oocysts survival in that, wood is often difficult to wash and disinfect when compared to metallic cages. In addition, wooden cage may retain high level of moisture that is known to be a predisposing factor for oocysts survival.

CONCLUSION AND RECOMMENDATIONS

Based on the fact that *Cryptosporidium* oocysts were detected in birds in this study, which is a one of the causes of diarrhoeal illnesses in man and animals worldwide, there is a need for close monitoring of the infection in farm animals because of the public health and economic importance of the Disease. Further studies should also be conducted to determine the species of *Cryptosporidium* oocysts detected in birds in the study area.

ACKNOWLEDGEMENTS

The Authors are grateful to the technical staff of Protozoology Laboratory, A.B.U., Zaria for their assistance and the bird owners for their cooperation.

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