

The Prevalence of *Cryptosporidium* Oocysts in Birds in Zaria, Nigeria

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ABSTRACT

A study was conducted to elucidate the prevalence of *Cryptosporidium* oocysts in birds in Zaria, Nigeria. A total of 890 faecal samples comprising 132, 305 and 453 from wild, local and exotic birds respectively from different parts of Zaria were examined using the formol-ether concentration technique with safranin–methylene blue stain and auramine phenol stain using light microscopy and fluorescent microscopy respectively. The total prevalence rate was 7.4%. However, Samaru had the highest prevalence rate of 20.6% and Tudun Wada the lowest rate of 2.8%. The difference in the prevalence rates between the different localities of Zaria was found to be statistically significant ($P < 0.001$). Among the different birds sampled, local birds had the highest prevalence rate of 9.5% followed by exotic birds 6.6% and the wild ones with 5.3%. The difference was not statistically significant ($P > 0.05$). In Tudun Wada, where the different sexes were noted, there was no significant statistical difference ($P > 0.05$) in the prevalence rate between male and female birds and none between the different species of wild birds sampled ($P > 0.05$). This study confirms the presence of avian *Cryptosporidium* in Zaria, Nigeria and indicates that whereas location may influence infection, breed, sex and species of birds may not be significant factors in the epidemiology of the infection.

Keywords: Prevalence, *Cryptosporidium*, oocysts, birds, Nigeria

INTRODUCTION

Cryptosporidium is a zoonotic coccidian protozoan parasite that has gained significant attention in the last 25 years as a clinically important human and animal pathogen (Sevá *et al.* 2011; Sréter & Varga 2000).

There are 3 main *Cryptosporidia* affecting birds namely *Cryptosporidium baileyi*, *Cryptosporidium galli* and *Cryptosporidium meleagridis* (Qi *et al.* 2011; Wang *et al.* 2011).

For many years *Cryptosporidium* was thought to be a rare opportunistic animal pathogen until the first case of human cryptosporidiosis was reported in 1976 involving a 3-year-old girl from rural Tennessee suffering from severe gastroenteritis for two weeks (Nime *et al.* 1976). Using electron microscopy of the intestinal mucosa *C. parvum* was implicated as the infectious species in man. In the 1980s, the significant association between cases of cryptosporidiosis and individuals with immuno-deficiency (e.g.

those with the acquired immuno-deficiency syndrome – AIDS) brought the parasite to lime light as a ubiquitous human pathogen (Guerrant 1997; Monis & Thompson 2003). From the 1990s to date the increasing population of immuno-compromised individuals and the various outbreaks of cryptosporidiosis through infection by water-borne and food borne *Cryptosporidium* oocysts, even in immuno-competent individuals, have placed even a greater emphasis on this pathogen (Guerrant 1997; Qi *et al.* 2011).

Unlike most intestinal pathogens *Cryptosporidium* can infect several different animal and human hosts, in fact infection has been reported in over 170 host species (Nagano *et al.* 2007; O'donoghue 1995; Sevá *et al.* 2011). Although human infection is mainly due to *C. parvum*, the two main species found in birds, *C. baileyi* and *C. meleagridis*, have also been reported to infect humans (Ditrich *et al.* 1991; Pedraza-Díaz *et al.* 2001; Plutzer & Tomor 2009; Qi *et al.* 2011).

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Cryptosporidium parvum has also been found to retain its viability and infectivity after passing through the intestine of birds (Graczyk *et al.* 1996). Therapeutic remedies for this infection has not been very effective but the drug paromomycin has promising effects for treatment and prophylaxis (Johnson *et al.* 2000).

Previous workers have elucidated significant association between *Cryptosporidium* infection and age, season, land use and habitat and no association with sex of the bird (Ziegler *et al.* 2007). In a poultry house and on shared water sources, drinking water is an effective means of sharing the oocysts among birds and hence infecting a large number (Sréter & Varga 2000). Qi *et al.* (2011) described the prevalence of 3 species (*C. baileyi*, *C. meleagridis* and *C. galli*) and 2 genotypes (*Cryptosporidium* avian genotype III and *Cryptosporidium* avian genotype V) of *Cryptosporidium* from pet birds with an overall prevalence of 8.1% out of 434 faecal samples taken from 14 families of birds from different bird pet shops. In another study using 242 faecal samples from wild birds 16 (6.6%) were positive for *Cryptosporidium* oocysts but factors associated with prevalence were not elucidated. The infection usually varies from one geographical location to another depending on many factors that may favour or limit the infection (Ryan 2010; Sréter & Varga 2000).

In Nigeria, Some workers have reported *Cryptosporidium* spp. in calves (Ayinmode *et al.* 2010; Maikai *et al.* 2011) but there is paucity of literature on the status of *Cryptosporidium* infection in birds. The potential threats of *Cryptosporidium* species in birds to man and animals should not be underestimated. This study was therefore, conducted to determine the prevalence of *Cryptosporidium* oocysts in birds in Zaria and elucidate some factors associated with the infection.

MATERIALS & METHODS

Sample Collection

A total of 890 faecal samples were collected from birds in Zaria (11°4'0"N/7°42'0"E), Nigeria (Travelmath 2011). The samples from

Samaru, Tudun Wada and Sabon Gari areas of Zaria were collected mainly from their various markets while the samples from Shika, Zaria were collected from the Poultry Farm of the National Animal Production Research Institute (NAPRI) Shika, Zaria. The rainy season was defined as April to September and the dry season as October to March. Wild birds were trapped into cages (with feed inside) within Samaru and Tudun Wada areas of Zaria, Nigeria. Faecal samples were either directly collected into universal bottles and 10% formol saline immediately added as a preservative before processing or they were collected using the disposable leather gloves and processed immediately on return to the laboratory.

Processing of Samples

About 1 g of the faecal sample (sometimes with intestinal contents) were homogenised in 10 ml of 10% formol saline using a pestle and mortar or using an applicator stick in a universal bottle. This was sieved through gauze on a funnel into a centrifuge tube. 1 ml of diethyl ether was then added to the filtrate to extract fat and minimize debris and the extract was centrifuged at 5,000 revolutions/minute for 10 minutes. The supernatant was decanted and a thin smear was made from thoroughly mixed sediment on a clean micro slide and air-dried (Baxby *et al.* 1984).

Staining Techniques

Safranin-methylene blue staining technique

The air-dried smear on a clean micro slide was fixed with 3% acid alcohol for 3 – 5 minutes. The slide was then flooded with 1% aqueous safranin and heated from bottom with a spirit flame or bunsen flame for 1 minute. It was then rinsed gently in clean tap water and counterstained with 1% methylene blue for 1 minute. The methylene blue was thoroughly rinsed off with clean tap water. The slide was air-dried and examined under the microscope using the X 40 objective and oil immersion objective. The oocysts of *Cryptosporidium* appear as small spherical to round bright orange to reddish mass within a halo (Baxby *et al.* 1984).

Auramine phenol staining technique

The air-dried positive faecal smears using the safranin-methylene blue staining technique on clean micro slides were fixed with methanol for 3 minutes and then exposed to formalin vapour at 37°C for 30 minutes. Slides were stained with auramine phenol (auramine O, 0.03 g, phenol 3 g, distilled water 100 ml) for 10 minutes, washed in tap water, decolourised with 3% acid alcohol for 5 minutes, washed, counterstained with 0.1% potassium permanganate for 30 seconds, washed, air dried, and examined with a Leitz incident light fluorescence microscope. Specimens were screened at X 50 magnification and *Cryptosporidium* oocysts were clearly visible as yellowish discs against a dark background (Casemore *et al.* 1984).

Identification

Identification Standard

Faecal samples smears that were positive using the safranin-methylene blue staining technique and also positive with the auramine phenol staining technique were considered truly positive and recorded as the positive samples.

Positive slides of *Cryptosporidium* oocysts provided by Dr. Bruce Anderson of University of Idaho U.S.A. and Dr. Liisa Jokipii of Institutum Serobacteriologicum Universitatis, Helsinki served as control throughout the study.

Statistical Analysis

Data generated were analysed on the computer statistical package SigmaStat and Epi Info™ using Chi-square and Odds ratio analysis and differences expressed as significant at 95% confidence level (Thrusfield 2005).

RESULTS

Table 1 indicates the prevalence of *Cryptosporidium* oocysts according to location. The highest frequency of detection was obtained in Samaru (20.6%). The distribution of the frequency of detection by location was statistically significant ($X^2=21.45$, $P<0.05$).

Local chickens had a higher rate of detection (9.5%) than either exotic birds (6.6%) or wild birds (5.3%) (Table 2). However, the difference in the distribution of positive cases among the types of birds was not statistically significant ($X^2=2.76$, $P>0.05$).

The infection was more common in broilers (19.1%) than in cocks (8.5%), layers (3.9%) and breeders (0.8%) (Table 3). The distribution of infection rates in the different types of exotic chickens was statistically significant ($X^2= 22.05$, $P<0.001$).

Table 4 shows the prevalence of *Cryptosporidium* among the different species of wild birds sampled. The village weaver (*Ploceus cucullatus*) had the highest prevalence rate (14.3%).

Table 5 indicates that the infection was more common in the local (9.5%) than in the exotic birds (6.6%) and was 1.5 times more likely to occur in the local than in the exotic birds although the difference in their prevalence rates was not statistically significant ($P>0.05$). The infection was also more common in the local birds as compared with the wild birds (5.3%) and was 1.9 times more likely to occur in the local than in the wild birds (Table 6) but the difference in their prevalence rates was not statistically significant ($P>0.05$).

Table 1. The prevalence of *Cryptosporidium* oocysts according to location of local and exotic birds.

Location	Sample size	Number positive	% Positive
Samaru	97	20	20.6
Sabon Gari	102	6	5.9
Tudun Wada	106	3	2.8
NAPRI – Shika	453	30	6.6
Total	758	59	7.8

$P<0.001$ $X^2 = 21.453$

Table 2. The prevalence of *Cryptosporidium* oocysts according to types of birds sampled.

Birds	Sample size	Number positive	% Positive
Wild birds	132	7	5.3
Local chickens	305	29	9.5
Exotic chickens	453	30	6.6
Total	890	66	7.4

P = 0.252 $X^2 = 2.759$

Table 3. The prevalence of *Cryptosporidium* oocysts according to the different types of exotic chicken sampled.

Exotic chickens	Sample size	Number positive	% Positive
Layers	127	5	3.9
Breeders	129	1	0.8
Cocks	129	11	8.5
Broilers	68	13	19.1
Total	453	30	6.6

P < 0.001 $X^2 = 22.049$

Table 4. The prevalence of *Cryptosporidium* oocysts among the different species of wild birds sampled.

Species of birds	Sample size	Number positive	% Positive
Speckled pigeons (<i>Columba guinea</i>)	41	1	2.4
Laughing doves (<i>Streptopelia senegalensis</i>)	37	2	5.4
Mourning doves (<i>Streptopelia decipiens</i>)	15	0	0
Village weavers (<i>Ploceus cucullatus</i>)	28	4	14.3
Brown babblers (<i>Turdoides plebejus</i>)	2	0	0
Black crakes (<i>Limnocorax flavirostra</i>)	3	0	0
Red bishops (<i>Euplectes orix</i>)	4	0	0
Bush fowls (<i>Francolinus bicalcaratus</i>)	2	0	0
Total	132	7	5.3

P > 0.05

Table 5. The prevalence of *Cryptosporidium* oocysts between the local and exotic chickens.

	Positive	Negative	Total	% Positive
Local	29	276	305	9.5
Exotic	30	423	453	6.6
Total	59	699	758	7.8

P = 0.188 $X^2 = 1.732$ OR = 1.48

Table 6. The prevalence of *Cryptosporidium* oocysts between the local and wild birds.

	Positive	Negative	Total	% Positive
Local	29	276	305	9.5
Wild	7	125	132	5.3
Total	36	401	437	8.2

P = 0.201 $X^2 = 1.635$ OR = 1.88

Comparing the exotic and wild birds (Table 7) the infection was more common in the exotic birds (6.6%) than in the wild birds (5.3%) and was 1.3 times more likely to occur in the exotic than in the wild birds although the

difference between the prevalence rates was not statistically significant (P > 0.05).

In Tudun Wada where the sex of the local birds was recorded there was no statistical

significant difference ($P>0.05$) in the prevalence rates between them although the infection was 1.1 times more likely to occur in the female (2.9%) than in the male (2.8%) (Table 8).

The infection was more common in the dry season (10.4%) than in the wet (rainy) season (6.2%). The infection was 1.8 times more likely to occur in the dry season than in the wet season (Table 9) and the difference in the prevalence rates between the seasons was statistically significant ($P<0.05$).

DISCUSSION

The prevalence rate of *Cryptosporidium* oocysts in Samaru (20.6%) as compared with other locations was higher as the prevalence of the infection varies with geographical locations and depends on favourable factors for the infection to thrive (Sréter & Varga 2000).

The local birds showed a higher prevalence rate of *Cryptosporidium* oocysts than other birds. This should be expected considering the main faecal-oral route of transmission of the infection since local birds are restless,

indiscriminate scavengers for food and water as they are allowed to mostly roam freely, they are more likely to come in contact with sporulated oocysts of the organism from different sources including man and livestock (Ryan 2010).

However, the difference in the prevalence rate of *Cryptosporidium* oocysts among the local, exotic and wild birds was not statistically significant since all of them can equally be infected and none have been shown to have a higher resistance or higher susceptibility than others (Sréter & Varga 2000). Wild birds may seem more likely to contaminate water bodies with *Cryptosporidium* oocysts through aerial deposition of infected faeces. Many outbreaks of cryptosporidiosis have been associated with drinking water such as the one in Milwaukee in 1994 in which about 403,000 people were infected (Mackenzie *et al.* 1994). Therefore, the detection of *Cryptosporidium* oocysts in wild birds is very significant as many of them move from one water source to another and hence may contaminate them with viable oocysts.

Table 7. The prevalence of *Cryptosporidium* oocysts between the exotic and wild birds.

	Positive	Negative	Total	% Positive
Exotic	30	423	453	6.6
Wild	7	125	132	5.3
Total	37	548	585	6.3

$P = 0.730$ $X^2 = 0.119$ $OR = 1.27$

Table 8. The prevalence of *Cryptosporidium* oocysts in local chickens in Tudun Wada according to sex.

	Positive	Negative	Total	% Positive
Female	1	33	34	2.9
Male	2	70	72	2.8
Total	3	103	106	2.8

$P = 1.000$ Fisher Exact test

Table 9. The prevalence of *Cryptosporidium* oocysts according to rainy and dry season.

	Positive	Negative	Total	% Positive
Dry season	27	232	259	10.4
Rainy season	39	592	631	6.2
Total	66	824	890	7.4

$P = 0.040$ $X^2 = 4.219$ $OR = 1.77$

KEY: P = P value
 X^2 = Chi-Square
 OD = Odds ratio

Broilers seem to have higher prevalence rates of *Cryptosporidium* spp. than the other birds (Sréter & Varga 2000). Similar observation was also made in this study. There was a statistically significant difference in the prevalence rate of *Cryptosporidium* oocysts between layers, breeders, cocks and broilers. This may be due to the high metabolic rate of broilers as they are raised for meat coupled with their inherent genetic poor ability to resist infections, hence intestinal and other infections are very common in broiler chicken (Rao *et al.* 2009; Ryan 2010; Sréter & Varga 2000). The deep litter system of raising the broilers also exposes them more to the infective oocysts in faeces and contaminated water. Breeders had the lowest prevalence rate of 0.8% as they may be more resistant to infection and are raised mostly in cages. The high prevalence rate of 6.6% in the exotic birds agrees with the findings of a worker who diagnosed *Cryptosporidium* in 6.8% of 1000 consecutive cases of chicken in Georgia diagnosed histologically (Goodwin & Brown 1987).

Many of the wild birds sampled share similar feeding and drinking habits and hence may be equally exposed to many parasitic infections (Ziegler *et al.* 2007). The difference in the prevalence rates of *Cryptosporidium* infection in the wild birds examined was not statistically significant in spite of the variation in the prevalence rate of the village weaver (*Ploceus cucullatus*) as compared to the rest of the birds. The low number of samples taken from the different wild birds may account for the seeming wide variation between the prevalence rate of *Cryptosporidium* infection in the village weaver and other wild birds and may not allow conclusive statement to be made on the findings.

From large scale surveys by various workers in various animal species it was concluded that both sexes are equally susceptible to *Cryptosporidium* infection (Ryan 2010; Sréter & Varga 2000; Wang *et al.* 2011). Findings from the present study seem to corroborate these observations. Sex is therefore not a very important factor for susceptibility to *Cryptosporidium* infection.

Cryptosporidium infection is prevalent all year round (Atwill *et al.* 1999), but our study indicates a significantly higher prevalence during the dry season. Oocysts of *Cryptosporidium* are also found in dust particles which are easily carried about from one place to another during the dry season especially harmattan, this prevalence rate may be expected. It is known that *Cryptosporidium* oocysts sporulate within the body and need no vectors or favourable conditions and can be carried about in the dust particles by the wind and other mechanism (Fayer *et al.* 2000; Plutzer & Tomor 2009). Since the harmattan period is part of the dry season it may be that more oocysts were transported by this means leading to higher infection. The dry period may also mean going longer distances, especially, by the scavenging local birds to get feed and water which helps to spread the infection as they scavenge along. However, more work needs to be done for a longer period (at least 3 years) to determine if season is a factor in the prevalence of *Cryptosporidium* infection in birds.

CONCLUSION

This study has established the presence of *Cryptosporidium* infection in birds in Zaria. In view of the zoonotic potentials of the infection, the threats to human life should not be underestimated especially with the increasing number of pet birds and poultry and availability of susceptible groups such as newborn infants, the elderly, patients on immunosuppressive drugs, people infected with HIV, young animals, who are at a higher risk of infection. All breeds of birds are equally susceptible to *Cryptosporidium* infection. However, local birds and broilers seem more susceptible. Season and location may be significantly associated with *Cryptosporidium* infection.

RECOMMENDATION

More work needs to be done to establish the association of season with cryptosporidiosis. *Cryptosporidium* oocysts should be identified to species level especially using molecular techniques and to establish if *C. parvum*

infection did occur in Nigerian birds under natural conditions apart from the *C. baileyi* and *C. meleagridis* infections. Experimental infection of our local birds especially, with *C. parvum* should be carried out to determine their role in the predominant human cryptosporidiosis which is mainly caused by *C. parvum*. In order to safeguard human life from cryptosporidiosis it is important to boil drinking water or use standard filtration techniques that are effective against the oocysts. Food should be properly cooked before consumption. Animals should be given water free of the oocysts. Animal farm houses should be managed with strict hygiene. Keeping birds in cages could minimize their chances of exposure to infected oocysts in the faeces.

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