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Seroprevalence of anti-*toxoplasma gondii* antibodies in free-range chickens in Kaduna metropolis, Nigeria

IN Nzelu[1]*, BD Shingyu[2] & JKP Kwaga[2]

1. *Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, Joseph Sarwuan Tarka University Makurdi, Nigeria*
2. *Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

*Correspondence: Tel.: +2348037727690; E-mail: nancynzelu@gmail.com

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Abstract

Free-range chickens play a vital role in the epidemiology of toxoplasmosis because they feed on the ground, exposing them to infective oocysts shed by cats that contaminate the environment. The role of chickens in the epidemiology of toxoplasmosis in Nigeria is understudied. Therefore, in the present study, we surveyed 222 free-range chickens slaughtered for human consumption in Kaduna metropolis, Nigeria, for the presence of anti- *T. gondii* antibodies using indirect enzyme-linked immunosorbent assay (iELISA). Of the total birds sampled, 27.9% (62/222) were seropositive. Results showed no statistically significant association between seroprevalence and sex ($p > 0.05$). The study has demonstrated the presence of anti-*T. gondii* antibodies in free-range chickens in Kaduna, Nigeria, indicative of exposure of the sampled chickens to *T. gondii* oocysts. Proper cooking of poultry meat obtained from the study location is advocated to avoid exposure to *T. gondii*.

Introduction

Toxoplasma gondii infection is distributed worldwide. People become infected by ingestion of infected undercooked or raw meat, accidental ingestion of infective oocysts from water or food, and contaminated soil (Dubey, 2010a). Human infection can also occur through blood transfusion, organ transplantation and transplacentally from an infected mother to the foetus (CDC, 2020). Felids are the definitive host of the causative agent, *Toxoplasma gondii*. They become infected by eating infected tissues from intermediate hosts and excrete environmentally resistant oocysts (Dubey, 2010b).

Free-range chickens play an essential role in the epidemiology of *T. gondii* infection. As they feed on the ground, they are considered one of the best indicators of soil contamination with *T. gondii. They*

are important sources of infection for cats that shed infective oocysts that contaminate the environment (Dubey, 2010b). *Toxoplasma gondii* rarely causes clinical disease in chickens (Dubey, 2010b), but chickens can harbour mice-virulent *T. gondii* strains (Dubey *et al*., 2002).

The role of free-range chickens in the epidemiology of *T. gondii* infection in Nigeria has not been adequately investigated; only a few studies have been conducted thus far, with varying seroprevalence reported using various techniques (Aganga & Belino, 1984; Ayinmode & Dubey, 2012; Ayinmode & Olaosebikan, 2014; Ayinmode & Akinboboola, 2015; Aliyu *et al*., 2020).

On the other hand, there are several reports of human *T. gondii* infection in Nigeria, including Kaduna state (Bello *et al*., 2017; Lawal *et al*., 2018). Chickens being an indicator for establishing soil contamination with *T. gondii* oocyst and the absence of any published report about the infection in chickens in Kaduna state, the current study set out to assess the seroprevalence of anti-*T. gondii* antibodies in free-range chickens slaughtered at Sokoto Road live bird market in Kaduna city, Nigeria.

Materials and Methods

Ethical approval

The present study was a pilot study within a larger research project with approval obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), with approval number: ABUCAUC/2016/0017.

Study area

The study was conducted in Kaduna city, the capital of Kaduna State, located in the north-western region of Nigeria. The study location was the Sokoto Road live bird market within the city centre, with geographic coordinates of 10.53011, 7.43481. Sokoto road live bird market is one of the largest live bird markets in Kaduna city.

Study design and sampling procedure

From September to December 2016, a cross-sectional study approach was used to determine the seroprevalence of anti-*Toxoplasma gondii* antibodies in free-range chickens in the study area. On each sampling day at the market, birds to be sampled were selected by obtaining an equal number of birds from each recruited seller, and birds were selected by convenience from each batch of birds from each seller.

Sample collection

Blood samples were collected from 222 free-range chickens bought by customers at Sokoto Road live bird market in Kaduna and sent for slaughter at the market's slaughter slab. Samples were collected into blood collection tubes without EDTA, placed in a cold box and transported to the Veterinary Public Health and Preventive Medicine Laboratory, Ahmadu Bello University, Zaria, for processing. The sex of the birds was noted. Serum was extracted from blood samples by centrifugation at 5,000 rpm for 10 minutes and stored at -20 ^{o}C until required for serology.

Serology

Sera samples were subjected to indirect ELISA using a commercial kit (ID Screen® Avian Toxoplasmosis Indirect, ID.vet, France) to detect anti-*T. gondii* antibodies. The assay was validated and carried out according to the manufacturer's instructions. Samples presenting an S/P ratio greater than 50% were considered positive.

Statistical analysis

Data were analysed using a statistical package for social science (SPSS) version 23.0 (SPSS Inc. Chicago, IL, USA). Statistical methods employed included descriptive statistics utilising frequencies and percentages. A Chi-square test was used to establish an association between the infection status of the sampled birds and sex. Statistical significance at a probability of 5 % ($P<0.05$) with a confidence interval of 95 % was adopted.

Results and Discussion

Of the total birds sampled, 27.9% (62/222) were *T. gondii* seropositive. About equal seroprevalence was observed in male and female chickens at 27.9% and 28.0%, respectively. Results showed no statistically significant association between seroprevalence and sex ($p > 0.05$) (Table 1).

Table 1: Sex-specific seroprevalence of anti-*Toxoplasma gondii* antibodies in free-range chickens slaughtered for human consumption in Kaduna, Nigeria

Sex	Total sampled	No. positive	Specific prevalence (%)	*p value
Male	129	36	27.9	0.993
Female	93	26	28.0	
Total	222	62	27.9	

*Statistical method used: Chi-square test of association, df = 1, $\alpha = 0.05$

The findings of the present study have demonstrated the presence of anti- *T. gondii* antibodies circulating in free-range chickens in the Kaduna metropolis, suggesting that the sampled birds were exposed to environments contaminated with *T. gondii* oocysts. This finding implies that meat from birds in the study area may contain cysts of *T. gondii*. This situation puts the health of those who work with slaughtered chickens in the study area at risk because they can become infected with *T. gondii* if proper hand hygiene is not practised.

This study's seroprevalence is low in comparison to previous studies (Ayinmode & Dubey, 2012; Ayinmode & Olaosebikan, 2014), but higher than reports by Ayinmode and Akinboboola (2015). These studies suggested that MAT is a more sensitive method for detecting anti-*T. gondii* antibodies than IFAT (Ayinmode and Akinboboola, 2015). In comparison to a previous study (Aliyu *et al*., 2020) that used iELISA, the current study had a higher seroprevalence. The differences in prevalence reported in the various studies may be due to the type of technique used. Anti-*T. gondii* antibodies in chickens can be detected using a variety of serological assays, each with its own cut-off titre for determining clinically significant levels of anti-*Toxoplasma* antibodies. Variation among different serological tests was reported in a study in Brazil (Casartelli-Alves *et al*., 2014) where different seroprevalence was obtained for different methods using the same samples. Studies have shown that ELISAs are generally suitable for the detection of anti-*T. gondii* antibodies in domestic and wild animals (Gamble *et al*., 2005; Gamble *et al*., 2019) and having comparable detection levels with MAT (Gamble *et al*., 2019). The ELISA kit used in the present study is a validated test kit manufactured for use in birds. Variations in seroprevalence could also be due to differences in study location, as the previous studies were conducted primarily in the southwestern region of the country, whereas the current study was done in northwest Nigeria.

When compared to surveys conducted outside of Nigeria that used ELISA as the diagnostic method, the current study had a lower prevalence than Barakat *et al*. (2012) but a higher prevalence than Ding *et al*. (2012). This suggests that *T. gondii* seroprevalence varies by continent. The current study found that sex is not a determinant in *T. gondii* infection in free-range chickens. This indicates that both male and female chickens are equally susceptible to *T. gondii* infection. This finding is consistent with Ayinmode and Akinboboola (2015) and Grebremedhin *et al*. (2015).

In conclusion, the study has demonstrated that anti-*T. gondii* antibodies are circulating in free-range chickens in Kaduna, indicative of exposure of the birds to an environment contaminated with *T. gondii* oocysts. Because free-range chickens are important sentinels in the parasite's epidemiology and potential sources of human infection, it is critical that high-risk individuals in the state take adequate precautionary measures to avoid infection, as toxoplasmosis can be fatal in immunocompromised patients. To avoid infection, the authors recommend that meat from free-range chickens in the study area be properly cooked or frozen overnight before consumption and that meat handlers properly wash their hands after handling meat to avoid the risk of infection with *T. gondii*.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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