

SERO-PREVALENCE OF *TOXOPLASMA GONDII* IN NIGERIA CHICKENS IN SOME LOCAL GOVERNMENT AREAS OF PLATEAU STATE, NIGERIA

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Abstract

Toxoplasma gondii is an intracellular protozoan parasite that infects mostly warm-blooded animals including man. Birds act as reservoir hosts. *Toxoplasma gondii* is the causative agent of the disease toxoplasmosis. This study determined the sero-prevalence of *Toxoplasma gondii* in Nigeria chickens (*Gallus gallus domesticus*) brought to markets in some Local Areas of Plateau State, Nigeria. A total of 300 blood samples were collected at slaughter points in EDTA sample bottles, placed in plastic container containing ice packs to maintain temperature at -4°C and transported to the Parasitology laboratory of the Federal College of Animal Health and Production Technology, National Veterinary Research Institute Vom. Sera were separated and assayed for the presence of *Toxoplasma gondii* with Toxo-lax test kits (TL) strictly adhering to the manufacturer's instruction. There was an overall prevalence of 65.33% for *Toxoplasma gondii* antibodies. Sex specific seroprevalance was 24.33% for male and 43.33% for female respectively. There was significant differences ($p < 0.05$) in the seroprevalence of *Toxoplasma gondii* among the markets and between the male and female birds from Kugiya and Miango and no significant difference between the male and female birds from Tunkus. The high prevalence of *Toxoplasma gondii* in this study is a pointer to public health problem to the handlers of these birds. Recommendation is made for proper cooking of these indigenous birds before consumption as well as maintenance of good hygiene and sanitation practices to reduce the risk of infection.

Keywords: Seroprevalence, *Toxoplasma gondii*, Nigeria chickens

Introduction

Toxoplasma gondii is a protozoan parasite which belongs to the phylum Apicomplexa, subclass Coccidiasina and family Sarcocystidae (Welss & Kim, 2011; Halonen & Weiss, 2013). This coccidian parasite infects mostly species of warm-blooded animals including man and birds (Mohammed & Abdullahi 2013; Khalife *et al.* 2022) causing the disease toxoplasmosis, a zoonotic parasitic infection caused by *Toxoplasma gondii* and is estimated to have infected about

a third of the population (Saadatinia & Goalkar, 2012; Adams *et al.* 2013). Birds act as reservoir hosts (Tilahun *et al.* 2013; Ibukunoluwa & Daniel, 2021). There are three (3) strains of *Toxoplasma gondii* (Type I, Type II and Type III). Type I is extremely virulent for mice and humans, seen in AIDS patients; Type II non-virulent in mice, virulent in humans and has been associated with the majority of toxoplasmosis cases in AIDS patients; Type III is non-virulent in mice, virulent mainly in animals but seen to a lesser degree in humans and has been detected in AIDS patients (Dimie *et al.* 2013; Ibukunoluwa and Daniel, 2021).

Toxoplasma gondii is generally transmitted to humans either congenitally, through renal transplant or via ingestion of undercooked or raw meat from infected animals, or ingestion of food or water contaminated with oocysts excreted by infected felids (Martina *et al.* 2011; Scallan *et al.* 2011; Singh, 2016). Ingestion of infected chickens' meat can be a source of infection for *Toxoplasma gondii* in humans and other animals (Sarkikawa *et al.* 2012). There is a high prevalence of toxoplasmosis throughout the world (20%—90%), as well as a high resistance and persistence of the parasite in a broad spectrum of biological matrixes which constitutes public health significance (Vaz *et al.* 2010). Chickens are considered resistant to clinical toxoplasmosis. There are only a few reports of clinical toxoplasmosis in chickens worldwide (Dubey *et al.* 2012; Mohammed & Abdullahi 2013). There are several reports indicating that both chickens raised in backyard and commercial free-range systems harbour viable *Toxoplasma gondii* (Dubey *et al.* 2015; Feng *et al.* 2016; Sabri *et al.* 2019).

According to Ayinmode & Dubey (2012); Liu *et al.* (2017) chickens play important role in the epidemiology of *Toxoplasma gondii* in the rural environment, perhaps more than rodents, because they are clinically resistant to *Toxoplasma gondii* and live longer than rodents. Same author stated that chickens can harbour mouse-virulent *Toxoplasma gondii* without showing any clinical signs. Most chickens raised under free range and backyard operations in developing countries are usually slaughtered at home without supervision. This can allow the transmission of *Toxoplasma gondii* infection to humans if care is not taken to wash hands thoroughly after cutting meat and during cooking of meat. Ayinmode and Dubey (2012) reported that viscera of such chicken are usually not properly disposed but left for scavengers thereby encouraging the transmission of *Toxoplasma gondii* to

reservoirs like rodents or even cats which are the final host. Dubey, (2010b), reported that the probability of humans getting infected from ingesting eggs from infected chicken is not yet substantiated, although *Toxoplasma gondii* has been shown to survive in egg yolk and albumin of boiled egg and yolk of egg fried for 3 minutes, but there are evidences that showed that raw eggs are not likely to be a source of infection for humans.

Infections with *Toxoplasma gondii* has long been reported to be widespread in West Africa (Ayeh-Kumi *et al.* 2010; Dubey 2010b). In sub-Saharan Africa, toxoplasmosis often remains undetected and untreated due to insufficient diagnostic procedures (Dubey 2010b). In Nigeria, toxoplasmosis has been reported both in man and some important animals (Lovetta *et al.* 2013; MC Conkey *et al.* 2013). Studies revealed the high preponderance and spread of veterinary toxoplasmosis in Northern Nigeria with reported high seroprevalence of toxoplasmosis among some animals of economic importance, such as sheep and dogs, (Dubey 2010; Kamani *et al.* 2010). According to Emmanuel *et al.* (2013), animals reported as having high seroprevalence may represent possible animal sources of infection to humans. The keeping of poultry in high human populated areas increases the risks of transmission of zoonosis. Research involving farmers in Plateau State, Nigeria reported that free-range chickens that obtain food directly from the ground are more exposed to risks of infection through contamination and may serve as indicators of the presence of the parasites in the environment and as source of infection to other animals including man (Ogendi *et al.* 2013).

Infection with *Toxoplasma gondii* is difficult to diagnose due to high percentage of subclinical infections and the persistence of the parasite in tissues which complicates tests

with PCR. Hence, serology is often used for diagnosis (Ibukunoluwa & Daniel, 2021). The detection of chronic infection with *Toxoplasma gondii* in animals relies primarily on serological assays. There is no gold standard test for the screening of the large diversity of *Toxoplasma* host species. The sensitivity and specificity of the techniques depend on the animal species (Dubey, 2011). The epidemiology of *Toxoplasma gondii* revealed a worldwide distribution but with varying prevalence. Serological studies have indicated infection rates of 10-17% in Western Europe, 34-93% in Central Europe, 16-40% in North America, 35-76% in Central America 2.3-58% in South east Asia and 7-80% in Africa (Robert-Gangeux and Darde 2012; Emmanuel *et al.* 2013).

A worldwide survey of *Toxoplasma gondii* infection in chickens carried out showed seroprevalence rate varying from 36.3-65.5% in Argentina, 40-66% in Brazil, 18.7-40.4% in Egypt, 64% in Ghana, 16.9-100% in USA, 27.1% in Portugal, 13.3% in Kenya and 13.7% in Italy (Malaysian Agricultural Research Institute, 2012; Robert-Gangeux and Darde 2012; Emmanuel *et al.* 2013). The distribution of this parasite depends on regions and weather condition where the oocysts survive in environment (Knoll *et al.* 2019,;Khalife *et al.* 2022). Another factor that determines distribution of the disease is the cooking culture of the people. In France, for example, about 88% of the populations are carriers, probably due to a high consumption of raw and lightly cooked meat. High prevalence rates of between 67% and 80% have been reported in Germany, the Netherlands and Brazil. While in Britain and South Korea, about 22% and 4.3% respectively are carriers (Adam *et al.* 2013).

Chickens are considered resistant to clinical toxoplasmosis. There are only a few reports of clinical toxoplasmosis in chickens worldwide.

Clinical signs observed were anorexia, emaciation, diarrhea, blindness and death. Microscopically, lesions observed include sciatic nerve involvement, chronoretininitis, encephalitis, myocarditis and pericarditis. Others include inability to stand, torticollis and lateral decumbency. At Necropsy, lesions were limited to the brain which had multiple areas of necrosis; perivascular had multiple areas of necrosis, perivascular lymphocytic cuffs and gliosis. A strong finding was the presence of numerous tissue cysts and tachyzoites in the lesions, the *Toxoplasma gondii* antibodies. Also, in Illinois, three birds died suddenly out of a group of 14 backyard chickens. (Markovitz *et al.* 2015; Sabri *et al.* 2019). Ocular prevalence of *Toxoplasma gondii* has also been reported (Dela-Torez *et al.* 2011).

Several serological tests reported to be used in the diagnosis of chicken toxoplasmosis, include Sabin-Feldman dye test, Indirect fluorescent antibody assay (IFA), Complement fixation test (CFT) and the Enzyme-linked immunosorbent assay (ELISA) and specific enzyme-linked immunosorbent assays (ELISA) have been developed for some domestic animal species such as goats and chickens. The ELISA test has the advantage that it can be automated and is convenient for large scale surveys. Criteria such as the given color of tachyzoites under a microscope (Dye Test and IFA test). The principal of agglutination of *Toxoplasma* tachyzoites (Direct agglutination test, DAT), Red blood cells (indirect haemagglutination test, IHA) or latex particles (Latex agglutination, LA) and also the degree of color change which defines the quantity of specific antibody in a given solution (ELISA) are important in the detection of *Toxoplasma gondii* (Mohammed & Abdullahi 2013; Ferguson & Dubremetz 2014; Dubey *et al.* 2015).

According to Dubey *et al.* (2013); Parks *et al.* (2018), specific genetic sequence of a given MHC molecules differs dramatically between individuals, which is why these molecules are involved in transplant rejection. Individuals carrying certain genetic sequences of MHC molecules are much more likely to be infected with *Toxoplasma*. In their study, over 1600 individuals found with *Toxoplasma gondii* infection was common among people who expressed certain MHC or else HLA-B 08:01, HLA —CO4:01 HLA-DRB 03: 01, HLA-DQA 05: 01 and HLA- DQB 02: 01.

MATERIALS AND METHODS

Study Area

The study was carried out at Miango market in Bassa L.G.A with a fluctuating average temperature of 14.2°C (57.6 °F) and 30.5 °C (86.9 °F) bordering Bauchi and Kaduna States to the North, Kugiyia market in Jos South L.G.A with an average temperature varying from 14.2°C (57.6°F) to 34.5°C (94.1°F) bordering Bauchi to the South and Tunkus market in Mikang L.G.A with varying temperatures from 18.3°C (65°F) to 37.8°C (100°F) but rarely 16.1°C (61°F) bordering Nasarawa and Taraba States to the South (<https://www.weather-atlas.com> > miango. Miango, Nigeria - Climate & Monthly Weather Forecast (Accessed December 27th, 2022 7:48 pm); [weatherspark.com](https://www.weatherspark.com). Climate and Average Weather Year Round in Tunkus, Nigeria (Accessed December 27th, 2022 7:50 pm); [weather-atlas.com](https://www.weather-atlas.com). Climate and Monthly Weather Forecast Jos, Nigeria (Accessed December 27th, 2022 7:56 pm))

Study design

A cross — sectional study approach was used for determining the seroprevalence of *Toxoplasma gondii* in slaughtered chickens in the study areas. Markets used for the sample

collection represented of markets where villagers across Plateau State go to sell Nigerian indigenous chickens.

Sample Collection

100 blood samples each were collected at slaughter from birds brought to the markets under study into anticoagulant sample bottles and labeled. Samples were transported on icepack to the microbiology laboratory, Federal College of Animal Health and Production Technology, Vom. Each sample was centrifuged at 300 revolution per minute (rpm) for 10 minutes to separate the serum from the whole blood. The serum was kept at the temperature of — 20°C until analyzed.

Sero Diagnosis

Toxoplasma gondii antibodies were detected using *Toxoplasma* Latex kit following procedures described by the Manufacturers (Jacobs *et al.*, 1973). 50µL of each sample and one drop each positive and negative controls were dropped into separate circles on the test slides. The toxo — latex reagent was mixed vigorously with vortex mixer. 25µl of toxo-latex was added on the wells containing serum. Serum and the reagent drops stirred, spreading them over the entire surface of the circle using different stirrers for each sample. The slide was placed on a mechanical rotator at 80 — 160 rpm, for 4 minutes and reactions observed were recorded. False positive result could appear if the test is read later than 4 minutes.

Data Analysis

Data obtained was subjected to Chi — square test to determine the seroprevalence association of *Toxoplasma gondii* antibodies in Nigeria indigenous chickens based on markets and sex.

RESULTS

Result showed an overall seroprevalence of 65.33 % of *Toxoplasma gondii* in Nigerian indigenous chickens slaughtered at some markets in the study area. Of the 65.33 %, 38.78% was from Kugiyia in Jos South L.G.A; 32.14% was from Miango market in Bassa L.G.A and 29.08% was from Tunkus market the female birds from Miango and 43.86% in the male and 56.14% in female birds from Tunkus (Table 2).

in Mikang L.G.A of Plateau State (Table 1). There was 76%, 63% and 57% seroprevalence of the parasite from Kugiyia, Miango and Tunkus markets respectively. Sex-based seroprevalence of *Toxoplasma gondii* in the study area revealed 42.11% in male and 57.89% in the female birds from Kugiyia, 14. 29% in the male and 85.71% in

Table 1: Seroprevalence of *Toxoplasma gondii* in Nigeria Chickens Slaughtered at some Markets in Plateau State, Nigeria

Market (L.G.A)	No. (%) Examined	No. (%) Positive
Kugiyia (Jos South)	100 (33.33)	76 (38.78)
Miango (Bassa)	100 (33.33)	63 (32.14)
Tunkus (Mikang)	100 (33.33)	57 (29.08)
Total	300 (100)	196 (65.33)

$p = 0.034$

Table 2: Sex-Based Seroprevalence of *Toxoplasma gondii* in Nigeria Chickens Slaughtered at some Markets in Plateau State, Nigeria

Variables		Markets									
		Kugiya		Miango				Tunkus			
Sex	No. Examined	(%) Psitive	No. Examined	(%) Psitive	No. Examined	(%) Psitive	No. Examined	(%) Psitive	No. Examined	(%) Psitive	
Male	34 (34.00)	32 (42.11)	37 (37.00)	9 (14. 29)	46 (46.00)	25 (43.86)					
Female	66 (66.00)	44 (57.89)	63 (63.00)	54 (85.71)	54 (54.00)	32 (56.14)					
Total	100 (100)	76 (76.00)	100 (100)	63 (63.00)	100 (100)	57 (57.00)					

$p = 0.043$ for Kugiyia and Miango markets

$p = 0.058$ for Tunkus



Plate 1: A = Positive control and B = Negative control Agglutination Reactions of *Toxoplasma gondii* Antibodies on the Wells



Plate 2: A = Negative and B = Positive *Toxoplasma gondii* Antibodies Samples

DISCUSSION

The overall seroprevalence of 65.33% *Toxoplasma gondii* in Nigerian chickens slaughtered at some markets in the study area indicated chickens' meat may serve as possible source of *Toxoplasma gondii* for consumers. The chickens were slaughtered for human consumption at the markets some of which could take home for others to use. This is similar with the reports of Dubey, (2010b); Sarkikawa *et al.* (2012) who stated that ingestion of infected chickens' meat can be sources of infection for *Toxoplasma gondii* in humans and other animals. The findings in the present study also conforms to the global widespread of *Toxoplasma gondii* reported by Robert-Gangeux & Darde (2012); Emmanuel *et al.* (2013); Knoll *et al.* (2019) with varying infection rates ranging from 10% - 90%.

The high seroprevalence of *Toxoplasma gondii* in this study could be attributed to either sensitive or specific nature of the toxo-latex reagent or inability to record the coagulation reaction in 4 minutes of mixing the sera with the toxo-latex reagent which could lead to false positive results. This is in line with the reports of Jacob *et al.* (1973) who reported that false positive could appear if the test is read later than 4 minutes, Dubey, (2011); El-Massey SR, Metawea F.Y (20130); Ibukunoluwa & Daniel (2021) who stated that infection with *Toxoplasma gondii* is difficult to diagnose due to high percentage of subclinical infections and the persistence of the parasite in tissues which complicates tests with PCR. Hence, serology is often used for diagnosis and that detection of chronic infection with *Toxoplasma gondii* in animals relies primarily on serological assays. However the authors stated that there is no gold standard test for the screening of the large diversity of *Toxoplasma* host species and that the sensitivity and specificity of the techniques depend on the animal species.

The prevalence of infection in Nigeria chickens, free-range scavengers used for this study, also concurred with the report of Ogendi *et al.* (2013) who reported that free-range chickens that obtain food directly from the ground are more exposed to risks of infection through contamination and may serve as indicators of the presence of the parasites in the environment and as source of infection to other animals including man.

The seroprevalence significant differences ($p < 0.05$) that existed among the markets could be attributed to different weather conditions of the areas under study. This affirms to the works of Environmental Science and Research (2010); Knoll *et al.*, (2019); Khalife *et al.* (2022) who reported that the distribution of *Toxoplasma gondii* depends on regions and weather condition where the oocysts survive in environment.

The seroprevalence significant differences ($p < 0.05$) that existed between the male and female birds from Kugiya and Miango markets could be due to numerical variations in the male and female examined. On the other hand, the insignificant difference observed could be attributed to the almost equal numbers of male and female birds examine. Although this study did not consider age, the result related with the report of Khalife *et al.* (2022) who reported significant differences in the sex and age of sheep and goat ($p < 0.05$ and $p < 0.01$) respectively.

The study concluded by reporting the spread of *Toxoplasma gondii* by Latex-kit serological technique in Nigeria chickens destined for human consumption brought to Kugiya, Miango and Tunkus markets of Jos South, Bassa and Mikang Local Government Areas, Plateau State, Nigeria.

Recommendations are made for further studies to cover more markets and inclusion of humans and other animals in the study in

Plateau State; taking larger sample size; public enlightenment campaigns on the public health risks of consuming improperly cooked chicken meat and taking prompt control measures against the spread of *Toxoplasma gondii* infections in the study areas.

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