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Toxoplasma gondii infection and risk factors associated with its spread at live bird markets in Katsina Metropolis, Nigeria

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Copyright: © 2020	Abstract
Aliyu <i>et al</i> . This is an	Toxoplasmosis occurs in most species of warm -blooded animals. This study aimed
open-access article	to determine the sero-prevalence of Toxoplasma gondii antibodies in local chickens
published under the	(Gallus gallus domesticus) in Katsina metropolis. A total of 460 sera were collected
terms of the Creative	from two live bird markets (LBMs) at slaughter points and samples were analyzed by
Commons Attribution	Indirect Enzyme Linked Immuno-Sorbent Assay (ELISA) test kits specific for avian
License which permits	toxoplasmosis. Structured questionnaires were administered to the workers at LBMs
unrestricted use,	to evaluate their attitudes and practices toward Toxoplasma infection. The overall
distribution, and	prevalence for <i>T. gondii</i> antibodies was 7.83% (36/460). Gobarau yan kaji had a
reproduction in any	higher prevalence 9.06% (25) than Central market 5.98% (11). Mean score was
medium, provided the original author and	26.42±15.9 and 21.74±9.21 for attitude and practice respectively. There was a
source are credited.	significant correlation r=0.717 (p <0.05) between attitude and practice score of the
source are created.	respondents. The level of education and specific duty at the slaughter houses
	correlated significantly (p <0.05) with attitude and practice mean ranks. There was
	no association (0.137) between experience on the job and practice score. Attitude
	and practice ranked as poor, fair and good in this study were 60% (30), 12% (6), 28%
	(14) and 80% (40), 6% (3), 14% (7) respectively. This result has shown the presence
	of T. gondii antibodies in local chickens slaughtered for human consumption in
Publication History:	Katsina metropolis and this warns on the public health safety problems. There is
Received: 04-12-2019	urgent need for the implementation of public awareness campaign on toxoplasmosis
Accepted: 02-03-2020	for workers at LBMs in Katsina metropolis.

Keywords: Attitude, ELISA, Live Bird Market, Practice, Seroprevalence, Toxoplasmosis

Introduction

Toxoplasmosis, which is caused by *Toxoplasma* gondii, is one of the most common zoonoses around the world, affecting warm-blooded animals, including birds and humans (Dubey, 2010). *T. gondii* is a protozoan parasite which belongs to the phylum *Apicomplexa*, subclass *Coccidiasina* and family *Sarcocystidae* (Pereira *et al.*, 2010). There are three major genotypes (type I, type II, and type III) of *T*

gondii. These genotypes vary in prevalence and pathogenicity among individuals. Type II genotype is responsible for most cases of congenital toxoplasmosis in Europe and United States (Lindsay & Dubey, 2011). Toxoplasmosis is usually subclinical or asymptomatic in immunocompetent individuals (Wang *et al.*, 2017).

The two major routes of transmission of T. gondii are vertical and horizontal. Vertical transmission involves tachyzoite infection through the placenta or semen from infected male during artificial insemination (Lopes et al., 2013). Horizontal transmission involves ingestion of food and water contaminated with oocysts, tachyzoite infection by blood transfusion, ingestion of raw milk and cheese or consumption of undercooked meat containing bradyzoites (Dubey et al., 2014). Ingestion of infected chicken meat can be a source for T. gondii infection in humans and other animals (Dubey, 2010). Chickens are considered resistant to clinical toxoplasmosis. There are only a few reports of clinical toxoplasmosis in chickens worldwide (Dubey & Jones, 2008). Chickens play an 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 (Dubey & Jones, 2008). In developing countries, local chickens are usually slaughtered at home and live bird markets without supervision by health inspectors. Hygienic measures are not usually observed when processing such chickens, thus the likelihood of transmission of Toxoplasma gondii infection to humans. The offals of chicken are not properly disposed, there is a possibility of it being scavenged upon by rodents (reservoir) and cats (final host), thus playing a major role in the transmission of Toxoplasma gondii (Ayinmode & Dubey, 2012).

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. Several serological tests have been reported to be used in the diagnosis of chicken toxoplasmosis; they include Sabin-Feldman dye test, Indirect Fluorescent Antibody Assay (IFA), Complement Fixation Test (CFT) and the Enzyme-linked Immunosorbent Assay (ELISA) (Hill & Dubey, 2002). Specific enzyme-linked immunosorbent assays (ELISA) have been developed for some domestic animal species such as goats and chickens (Dubey, 2011).

This study aimed to determine the seroprevalence of *Toxoplasma gondii* in local chickens at live bird markets in Katsina metropolis, Katsina State, Nigeria. Also, to assess the risk factors associated with its spread at the live bird markets.

Materials and Methods

Study area

Katsina State is located on latitude 12°59'N and longitude7°36'E with altitude of 182.82 -457 metres above sea level. The population of Katsina State is 5,792,578. The average rainfall is 600mm, there is a difference of 217mm of precipitation between the driest and wettest months. It has a minimum and maximum temperature range between 21°C and 42°C and it also has temperature variations, where it is cool in the morning, hot in the afternoon and cool again in the night in the months of December to March.

Study design

A cross-sectional study approach was used for determining the seroprevalence of Toxoplasma gondii in slaughtered chickens in the study area within January to March 2015. Using proportional random sampling, 2mls each of whole blood was collected from 276 chickens (different cages owned by different marketers) at Gobarau LBM and 184 chickens from Central LBM. The 460 whole blood samples were transported in ice packs at 4°C to laboratory and centrifuged at 3000rpm for 5 mins to separate out the serum. Structured questionnaires were applied to 30 repondents at Gobarau LBM and 20 respondents at Central LBM over the period of sample collection. The respondents were selected based on willingness to participate. There were 28 close ended questionaires which were used to obtain information on respondent's demographic data, attitudes, practices towards Toxoplasma gondii infection and biosecurity practices. The questionnaires were scored by the method adopted from lyor (2005).

Indirect Enzyme Linked Immuno-sorbent Assay (ELISA) was carried out for the detection of anti-*Toxoplasma* antibodies in chicken sera using TOXOS-AV ver0714GB (ID Screen®, ID.vet, Paris, France) using the manufacturer's description. The ELISA kit has a specificity of 100%. Data generated were entered into Microsoft Excel® (Microsoft Corporation, USA) and subjected to descriptive analysis using Statistical Package for Social Science (SPSS version 16 standard version, SPSS Chicago, Illinois).

Descriptive statistics were used to analyze the demographic data. Mean and standard deviations of attitude and practice scores were obtained. Kruskal Wallis H test was applied to compare mean ranks of the domains with various demographic factors. Pearson's correlation test was used to test relationship between practice and attitude of the respondents. The value of p < 0.05 was considered significant.

Results

Serological analyses

Out of a total of 460 sera of chickens collected f-rom two major slaughter points in Katsina metropolis, 36 (7.83%) had antibodies against *Toxoplasma gondii*. The seroprevalence of *Toxoplasma gondii* in local chickens in the two live bird markets showed that Gobarau LBM had higher prevalence of 25 (9.06%) out of the 276 samples tested than Central market LBM where 11 (5.98%) samples out of the 184 were positive (Table 1).

Specificity of positive samples from Central LBM shows that percentage specificity ranged from 53 - 115%, among which two samples had percentage specificity above 100% (105% and 115%) (Figure 1). Positive samples from Gobarau LBM indicate percentage specificity varying from 50 - 222%, with four samples having percentage specificity of over 100% (Figure 2).

Demographic profile of respondents

The demographic characteristics of the respondents including level of education, specific duty and experience on job are shown in Table 2. Majority (66%) of the respondents had education up to secondary school level, while 6% had no formal education and another 6% were educated to tertiary level. Those that had primary education were 11(22%). Most of the respondents (78%) had been working at the live bird market for over five years, while those with less than one-year experience were 4(8%). Those that had worked for between 1 and 5 years were 7 (14%). Most of the respondents interviewed were within 21-30 age group (42%) while the least number of respondents was in the age group that are less than 20 years (12%). Other age groups included 31-40 (22%) and those greater than 41 years (24%). The mean age was 21.52± 24.76 years. All the 50(100%) respondents interviewed were males.

Attitudes and practices of live bird markets workers in relation to demographic factors

Respondents with tertiary level of education had mean scores of 29.5 and 33.0 for attitude and practice which was higher than mean scores for respondents with secondary education (17.50, 17.18) and was statistically significant (p<0.05). Higher mean scores were observed for poultry processors (31.63, 28.56)

regarding attitude and practices, whilst live bird marketers had mean scores of 15.50 and 20.50. Respondents that have been working at the market for 1 - 5 years had mean score of 13.50 for attitude, 18.50 for practice, which was statistically insignificant. Mean score for respondents working for over 5 years was higher with attitude (25.29) and less with practice (24.40) (Table 3).

 Table 1. Seroprevalence of Toxoplasma gondii in

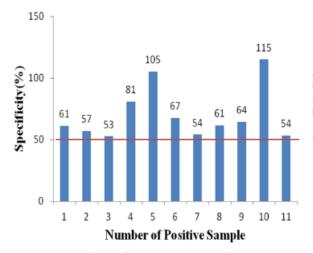
 local chickens slaughtered at live bird

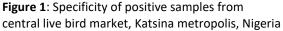
 markets in Katsina metropolis. Nigeria

Market	Number of	Number positive for	Prevalence (%)		
	samples	antibodies			
Central	184	11	5.98		
Gobarau	276	25	9.06		
yan kaji					
Total	460	36	7.83		

Table2.DemographiccharacteristicsofrespondentsatlivebirdmarketsinKatsinametropolis, Nigeria (n=50)

Variables	Frequency market	at	Percentage (%)	
Age group (year				
<20	6		12	
21 – 30	21		42	
31-40	11		22	
>41	12		24	
Sex				
Male	50		100	
Female	0		0	
Level of education	n			
None	3		6	
Primary	11		22	
Secondary	33		66	
Tertiary	3		6	
Specific duty				
Marketer	19		38	
Poultry	31		62	
processor				
Experience on job				
<1 year	4		8	
1 – 5 years	7		14	
>5 years	39		78	





Attitudes of respondents at live bird markets that could enhance the transmission of Toxoplasma gondii infection

Frequency of responses for specific questions on attitude is shown on Table 4. The mean score for attitude was 26.42±15.9. Majority 76% (38) did not use gloves when handling raw chicken meat. Few 13/50 (26%) of the respondents ate or drank while processing chicken. 64% (32/50) felt that raw chicken meat does not pose any threat to their health. Keeping cat at live bird market was considered safe by majority 92% (46/50). Feeding dogs and cats with raw poultry meat and offals was considered safe by 66% (33/50) while 76% (38/50) felt it was proper to use poultry feathers and offals as manure.

Practices of respondents at live bird markets that could enhance the transmission of Toxoplasma gondii infection

Mean score for practice was 21.74±9.21. Majority 60% (30/50) used well/borehole water. Raw chicken meat was handled with bare hands by 80% (40/50), while only 2% (1/50) used face mask and boots while processing chicken. Majority 96% (48/50) did not wash knife with soap and water after use on a particular chicken, while 84% (42/50) had been injured with knife while processing chickens. Minority of respondents 16% (8/50) felt it was proper to dispose poultry waste and offals at refuse dump (Table 5).

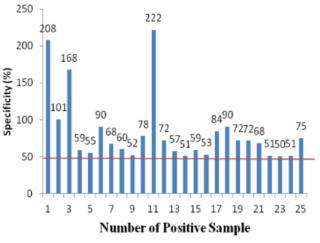


Figure 2: Specificity of positive samples from gobarau live bird market, Katsina metropolis. Nigeria

Categorization and correlation of attitudes and practices of respondents

Frequencies from categorization of scores on attitude and practice by the respondents revealed that majority were categorized as poor 60% (30/50), 80% (40/50) for attitude and practice respectively. The respondents categorized to have good attitude were 28% (14/50), while 14% (7/50) had good practice at the slaughter house. Those categorized as fair were 12% (6/50) for attitude and 6% (3/50) for practice. The relationship between attitude and practice of the respondents revealed a positive pearsons correlation 0.717 which was statistically significant (p <0.05) (Table 6).

Discussion

The 7.83% (36/460) prevalence of Toxoplasma gondii infection in indigenous chickens at the two main slaughter houses in Katsina metropolis, indicates chicken meat may serve as a possible source of Toxoplasma gondii infection for consumers in this region. The finding in the present study is similar to that of Velmurugan et al. (2008) who reported a 6.3% (5/79) prevalence in Nigeria. The prevalence was however lower than that reported by Aganga et al. (1985) 44% in Zaria, 33% in Ibadan (Ayinmode & Dubey, 2012), and 57% in Iran (Zia Ali et al., 2007). This could be due to the fact that duration of infectivity decreases with increasing temperature, with infectivity maintained for at least 200 days at temperature range of 10-25°C, 1 month at 35°C and 1 day at 45°C (Dubey et al., 1998). Thus, considering that Katsina State normally records temperature of up to 38-43°C during hot season, this may shorten the exposure period of chickens to sporulated oocyts. All the above studies reported that chickens served as an intermediate host and source of infection to human especially when meat is consumed undercooked. The higher seroprevalence rate at Gobarau 9.06% (25/276) as compared to Central market 5.97% (11/184), may be due to the fact that Gobarau is a bigger live bird market with a larger collection of pooled indigenous chickens from weekly markets around the locality. Variation of seroprevalence of Toxoplasma gondii in domestic chickens between and within countries can be due to the testing method, number of samples examined, type and hygiene of animal breeding (Dubey, 2010). The differences between the results of this study and other findings may likely be connected to the test kit used, as the ELISA kit used in this study is still in the process of being developed for commercial use. It has been tested for specificity on negative specific pathogen free (SPF) sera in Paris. It showed specificity ranging from 0-30%, in contrast to IHAT which had specificity of 25% (Frenkel, 1981). On sensitivity, 10 positive samples of control group in Thailand showed a result of Specificity ranging from 50-133%, thus more sensitive in contrast to IHAT that showed 46% sensitivity (Frenkel, 1981). The cost of ELISA is lower as compared to MAT, but it is usually automated and very convenient for large scale surveys for avian toxoplasmosis. In similarity to ELISA kits for human toxoplasmosis, it is subjected to standardization (Shaapan *et al.*, 2008).

The epidemiological significance of *Toxplasma gondii* in chickens indicates that cats may have a role to play, because they disseminate the oocysts on the soil (Asgari *et al.,* 2006). Most of these cats become infected by eating infected rodents, chickens and wild birds. Factors such as management practice, hygiene standard and density of cat may play a very important role in the infection of chickens with *T. gondii* oocyst (Jones *et al.,* 2007).

Environmental conditions such as humidity, altitude and temperature also affect the survival of oocysts. The study area which is Katsina has a dry weather, Saharan-like environment which may also have contributed to the relatively low prevalence. Management practice of chickens in the region is mainly semi-intensive.

Variable	Attitude	P value	Practice	P value	
	mean rank		mean rank		
Level of Education					
Secondary	17.50	0.04*	17.18	0.002*	
Tertiary	29.50		33.00		
Specific duty					
Seller	15.50	0.00*	20.50	0.006*	
Dresser	31.63		28.56		
Experience on the job					
1 – 5 years	13.50	0.016*	18.50	0.137	
>5 years	25.29		24.40		

Table 3: Mean score rank of attitudes and practices with respect to demographic data of respondents at live birds Market. Katsina Metropolis. Nigeria.

*Statistically significant at p < 0.05

Table 4: Attitude of live bird market workers that may predispose them to *Toxoplasma gondii* infection in Katsina metropolis, Nigeria (n=50).

Questions	Yes	No	No response
Do you feel its safe using gloves when handling raw chicken meat?	12(24%)	38(76%)	
Do you think it's proper to wash your hands with soap and water after contact with raw chicken meat?	17(34%)	33(66%)	
Do you feel it is safe to eat or drink while processing chicken?	13(26%)	37(74%)	
Do you think raw meat of chicken can be a threat to your health?	9(18%)	32(64%)	9(18%)
Do you feel it is safe keeping cat at live bird market?	46(92%)	4(8%)	
Do you think it is safe to feed the market dog and cat with poultry meat and offals?	33(66%)	5(10%)	12(24%)
Is it proper to use poultry feathers and offals as manure?	38(76%)	7(14%)	5(10%)
If you were ill, will you admit working at chicken slaughter house to the health officer?	40(80%)	9(36%)	1(2%)

Questions	Yes	No	No
			response
Is well or borehole water your source of water?	30(60%)	20(40%)	
Do you think it is proper to wash your hands with soap and water after contact with raw chicken meat?	43(86%)	7(14%)	
Do you handle raw chicken meat with bare hands?	1(2%)	49(98%)	
Do you wash knife with soap and water after use on a particular chicken?	2(4%)	48(96%)	
Have you been injured with knife while processing chicken?	42(84%)	8(16%)	
Is it proper to dispose poultry offal at refuse dump?	8(16%)	35(70%)	7(14%)

Table 5. Practices of live bird market workers that may predispose them to *Toxoplasma gondii* infection in Katsina metropolis, Nigeria (n=50).

The demographic data of the slaughterhouses showed most of the respondents are within the age group of 21-40 years, thus indicating they are mostly youths within the age group responsive to awareness and training. All the respondents were males mainly due to the culture and tradition of the region, in which such jobs are strictly for males. The live bird marketers were predominantly males, contrary to reports from southern part of the country where women were dominant (AICP, 2008). Significant proportion of the respondents had certain levels of education, hence enabling ease of communication and ability to create the required awareness, considering the fact that level of literacy is strongly related to risk cognition and comprehension of public health campaigns (Abdullahi et al., 2009). Most of the respondents have been working at the live bird markets for over 5 years, which may be enough to establish some level of equilibrium to exposure to parasite in the markets.

The attitudes and practices of respondents were poor overall. The use of protective gears and hand washing with soap was not adopted by majority of the respondents. A common negative attitude was the perception of the respondents towards keeping cats in the market and disposal of poultry offals as manure. Poor disposal of carcass is not peculiar to Nigeria as it has been reported in other developing countries such as Thailand (Kilpatrick et al., 2006). Cats serve as definitive host for animal and human toxoplasmosis, thus their presence is a very important risk factor (Dubey et al., 1995). Re-infection of the cat may occur due to the attitude of feeding dogs and cats with poultry meat and offal. Cats can shed tens of millions of oocysts into the environment in several days, which can potentially infect birds before being sold and also pose huge risk to the human population in the market (Dubey, 2010). The only source of water at Central market was mainly well water (40%), which may pose a high chance of contamination of the meat. Well water has been reported to be contaminated with oocysts (Villena et al., 2004). The degree of contamination of well water may depend upon the degree of environmental hygiene and the depth of the well. The slaughtering at the slaughterhouses were not supervised, thus no examination of carcass and no strict observation of hygiene standards and practices. Slaughter and poor disposal of carcasses of birds in markets have particularly been associated with contamination of market environment (Indriani et al., 2010). Waste and viscera at the slaughter house are mostly bagged and sold to farmers as manure. Organic manure is usually used due to its relative availability, low cost and improvement of urban food production, thus leading to increased access to food by urban population (Baumgartner & Belevi, 2001).

From the aforementioned findings, it can be concluded that *Toxoplasma gondii* infection was present in Local chickens (7.82%) slaughtered for human consumption in Katsina metropolis.

It is thus necessary to create public health awareness among workers and other individuals at live bird markets on *Toxoplasma gondii* infection in chickens and the environment.

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Conflicts of Interest

The authors declare no conflict of interest.

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