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Seroprevalence of *Toxoplasma gondii* in dogs slaughtered for food in Southwestern Nigeria and assessment of consumer's knowledge and behavior

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ABSTRACT:

Nigeria has communities where dogs are consumed as food and there is no data available on the prevalence of Toxoplasma gondii in dogs slaughtered for food. In this study, serum antibodies prevalence to T. gondii were investigated in dogs slaughtered for food in two Southwestern Nigeria states using the modified agglutination test (MAT, cut off 1:20) and the knowledge, habits and practice of consumers that related to T. gondii infection were accessed using structured questionnaire. Antibodies to T. gondii were found in 55 (19.8%) of 278 dogs with titres of 1:20 in 37, 1:40 in 6, 1:80 in 8, 1:160 in 1, 1:320 in 2, 1:640 in 0 and 1:1280 in 1. T. gondii was significantly associated with gender and sampling location ($P< 0.05$), but no association was found with age and body condition of dogs. In addition, it was observed that few individuals were found to have negative behavior like eating of raw dog meat and feeding of dog meat to cat, and most people in the sampling location have no knowledge of possibility of getting infected with T. gondii through eating of dog meat. The obtained results in the current study showed that dogs slaughtered for food in Ondo and Ekiti states, Nigeria are exposed to T. gondii infection and also revealed the need for proper health education of dog consumers on toxoplasmosis.

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1. Introduction

Toxoplasma gondii, an intracellular apicomplexan protozoan with a worldwide distribution in a wide range of animals' hosts and humans is one of the most important zoonotic parasite. *T. gondii* may be transmitted from cat and other felids (primary hosts) to humans and other animals (intermediate hosts) and vice versa, as well as between different intermediate hosts (Tenter, 2009). Ingestion of food or water contaminated with sporulated oocysts from cat faeces, and also the consumption of undercooked or raw meat containing tissue cyst may be responsible for most natural infection (Tenter et al., 2000). Although, *T. gondii* infection is usually asymptomatic, it can cause severe illness in immunosuppressed patients and when it is transmitted congenitally to fetus.

Dogs, an important domestic animal are now known to be an important intermediate host of *T. gondii* and could transmit the oocyst to humans mechanically (Lindsay et al., 1997; Frenkel et al., 2003).

Seroprevalence of *T. gondii* antibodies in dogs have been widely studied and varying levels have been reported around the world (Dubey, 2010). It has been established that continuous exposure of outdoor dogs to feeding from the ground accounts for their usually high seropositivity to *T. gondii* (Tenter, 2009). Outdoor dogs are therefore used as indirect bio-indicator of environmental contamination by *T. gondii* oocysts (Meireles et al., 2004; Salb et al., 2008). Although, there is paucity of information on dog to human transmission of *T. gondii*, there are evidences to show that dogs harbouring tissue cysts in their organs including skeletal muscles play important role in foodborne transmission to humans who consume their meat (Tassi, 2007; Tenter, 2009). Studies conducted where dog meat are consumed as food will be invaluable towards understanding the role of dogs in epidemiology of *T. gondii* infection in humans as well as the implications for prevention and control in such community.

The two main objectives of the present study conducted in two south-western states of Nigeria, were to investigate the seroprevalence of *T. gondii* infection in dogs slaughtered for food by humans, and to evaluate dog eaters' knowledge of *T. gondii* infection and habits that could subject them to contracting the infection.

2. Materials and Methods

Sampling location

Study Area

The study was carried out in Ekiti and Ondo states, Southwest Nigeria located between latitude 7°40′N and 7°10′ N; and longitude 5°05′E and 5°5′E (ZODML, 2013). Ekiti state has a land area of about 5887.890 sqKm and estimated population of 2.39 million people as at 2006, while Ondo state has a landmass of 15,195.2 sqKm with estimated population of 3.46 million people as at 2006 (ZODML, 2013). Both states are located within the rainforest area of Nigeria. Sampling was randomly carried out in Ado and Ikere-Ekiti in Ekiti state, while Idanre and Akure were the sampling locations selected in Ondo state.

Sampling

A total of 278 blood samples were randomly collected (after obtaining necessary permits) from dogs during slaughtering between July and October 2014 in selected towns within the two states. The sera were separated and stored at –20°C until assayed for antibodies to *T. gondii*. Data on age, gender, location and body condition were obtained.

Questionnaire design and data collection

A structured questionnaire was designed and administered to dog consumers in the two states where dog samples were collected to assess the knowledge, behavior and practice that could affect exposure to *T. gondii* infection in humans. Questions for measuring knowledge (on if they could contract disease from eating dogs), attitude (regarding how often they eat dog and preference for dog meat over beef) and practice (related to eating dog meat with family members; rearing dog for food and contact of such dogs with cat; eating of hunting and sick dogs; eating of raw dog meat; duration of cooking dog meat; cleaning of utensils used for processing dog meat) were asked from the respondents. The questionnaire contained closed-ended questions (and was scored by the number/ percentage of response to true or false statements) to facilitate processing and to improve precision of responses.

Detection of Toxoplasma gondii antibodies

Dog sera were screened using two-fold dilutions from 1:20 to 1:2560 for the presence of *T. gondii* antibodies using the Modified Agglutination Test (MAT) as described by Dubey and Desmont (1987). Briefly, formalin-killed whole *T. gondii* tachyzoites (RH strain) were used as antigen and 2-mercapthoethanol was added to eliminate the IgM-like substances that interfere with the specificity of the test (Dubey and Desmont, 1987). Dilutions of dog sera were performed using the serum-diluting buffer, and agglutination was performed in U-bottom 96-well microtitre plates using a mixture of 50µl antigen and 50µl diluted sera. The plates were incubated at 37°C overnight with positive and negative controls included in each plate. The test was considered positive when a layer of agglutinated parasites was formed in wells at dilutions of 1:20 or higher, while a clear, button-shaped deposit of parasite suspension at the bottom of the well was interpreted as a negative reaction.

Statistical analysis

Statistical analysis of data was performed using Vassar Stats software developed by Richard Lowry, U.S.A. (Lowry, 1998). Differences between groups of animals were analyzed by Fisher exact test. Bivariate analysis was fitted to determine factors associated with *T. gondii* infection and statistically significant difference was observed if the p value was < 0.05. Descriptive statistics were performed for the data obtained from the questionnaire and results expressed as frequencies and percentages.

3. Results

T. gondii antibodies were found in 55(19.8%) of 278 dogs examined by MAT. Antibody titres were 1:20 in 37 dogs, 1:40 in 6 dogs, 1:80 in 8 dogs, 1:160 in 1 dogs, 1:320 in 2 dogs and 1:1280 in 1 dog. No dog was found positive at 1:640 and 1:2560. The distribution of antibody titre among various categories is summarized in Table 1.

The seroprevalence of dogs from Ondo state was significantly higher ($P< 0.05$) than those from Ekiti state (Table 2). The seroprevalence of *T. gondii* was higher in adults than in young dogs and also higher in dogs with poor body condition than those with good body condition, but the differences were not statistically significant. Seroprevalence of *T. gondii* was significantly higher ($P<0.05$) in males than in females (Table 2).

The analysis of the questionnaire (Table 3), showed that most dog consumers always eat dog meat along with their family members and majority also eat hunting dogs when slaughtered for food. Most people cook dog meat very well and also clean used utensils properly after processing dogs for food. However, most people have no knowledge of the possibility of contracting disease from eating dog meat. Fewer individuals prefer dog meat to beef, eat sick dogs, eat raw dog meat, feed dog meat to cat and allow the dogs reared for food to have contact with cat (Table 3).

4. Discussion

T. gondii infection has been reported worldwide with varying prevalence in domestic animals. The overall prevalence (19.8%) found in this study is lower but comparable with 25% obtained in dogs from Bornu state, Northern Nigeria (Kamani et al., 2010). The present study also showed variation in seroprevalence of *T. gondii* antibodies in dogs from the two states where sampling was carried out in Southwestern Nigeria (Ondo state, 23.3% and Ekiti state, 12.4%). However, all the findings so far in Nigeria are lower than 89% reported in a country like Brazil (Lindsay et al, 1997). Variation in seroprevalence across the world has been attributed mainly to difference in serological test or cutoff (Luciano et al., 2011). The MAT used in this study is considered reliable, sensitive and specific test for the detection of antibodies to *T. gondii* in various hosts (Dubey and Desmonts, 1987). Other factors like the population of cats in sampled location, age of animals, management practices, ecological and weather conditions, (Smith, 1999) have also been associated with prevalence rate.

In our study, although age was not significantly associated with *T. gondii* infection, antibodies to *T. gondii* infection in dogs were found to increase with age, suggesting that the animals acquired the infection progressively after birth, some previous studies have found exposure to *T. gondii* infection to be directly related to the age of dogs, suggesting cumulative acquisition rather than congenital transmission (Hill and Dubey 2002; Dubey, 2010; Yan et al. 2012). However, a report from Durango City, Mexico observed no increase in seroprevalence of *T. gondii* infection with age (Dubey et al. 2007).

The present study showed that male gender was significantly associated with seroprevalence of *T. gondii*. This is in contrast to report by Kamani et al. (2010) who reported that gender was not associated with *T. gondii* infection in dogs. However, our finding could be as a result of increase exposure of local male dogs to environments contaminated with oocysts when they roam in search for females during mating seasons.

Our result indicated that sampling location is significantly associated with *T. gondii* infection, with higher seroprevalence in Ondo state than Ekiti state. The difference in environmental conditions, population of infected cats and rodents and other factors that modulate infection pressure in the environment might account for difference in *T. gondii* seroprevalence in the sampled location (Hill and Dubey, 2002; Tenter, 2009). Moreover, the result of quantification of antibody titre by MAT also showed that dogs from Ondo state have higher antibody titre, which is indicative of higher exposure of dogs in Ondo than those from Ekiti state. This might indirectly also suggest that the environment in Ondo has higher infection load and/ or sources.

Our findings did not obtain any significant association between body condition of dogs and *T. gondii* infection, although dogs with poor body condition had higher seroprevalence. Body condition may not be a crucial factor in Toxoplasmosis, since it is known that production of tissue cyst (the terminal life-cycle stage in intermediate host) is less frequent in dogs (Tenter et al., 2000), and infected dogs may not show signs of illness.

The questionnaire assessment of humans where dogs were slaughtered showed that most people eat dog meat along with their family members, but not as a preference to beef. A large proportion of respondent also enjoy eating hunting dogs. This suggests that the human population in studied area could be exposed to *T. gondii* infection if infected dogs are consumed (Tenter, 2009); and there is therefore the need for public education on meat hygiene in the studied area that should also focus on dog meat along side with other common sources of protein like beef, pork, mutton and others. This study showed that majority of dog consumers eat well-cooked meat, do not eat sick dog and would always clean the utensil used to processing the dog meat properly. These practices will obviously reduce the risk of being infected with *T. gondii* through the tachyzoites in body fluid or tissues cyst (Hill and Dubey, 2002). Although, the study also showed that some people deliberately eat dog meat. Further studies are needed to ascertain if eating of raw dog meat will significantly correlate with seropositivity to *T. gondii* infection. Our study also observed that majority of dog consumers do not feed dog meat and offal to cat. This is good practice that should be encouraged wherever dog meat is consumed, since it will help to limit the access of cat (the final host) to infected dog meat. This study showed that most people in studied area have no knowledge of the possibility of contacting disease from eating dog meat. Our finding reveals gaps in public knowledge on *T. gondii* infection and suggests the need to improve public health education on Toxoplasmosis and other foodborne zoonosis.

In conclusion, the present study which to the best of our knowledge is the first to study the seroprevalence of *T. gondii* in dogs slaughtered for

food and to also access the consumer's perception and preventive behavior in Southwestern Nigeria, provides evidence that dogs slaughtered for human consumption in both Ondo and Ekiti states of Nigeria are exposed to *T. gondii* and there exist the risk for human infection. It also reveals the need for proper public health education to close the gaps in knowledge of dog consumers on toxoplasmosis and other foodborne zoonosis in the studied region.

Table 1. Seropositivity of Food dogs to *Toxoplasma gondii* infection in Two Southwestern states of Nigeria

Factor	Category	Positive	Number positive at difference titres							
			1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Age	Adult	27	18	5	2	1	1	0	0	0
	Young	28	19	1	6	0	1	0	1	0
Sex	Male	48	30	6	8	1	2	0	1	0
	Female	7	7	0	0	0	0	0	0	0
Health	Good	38	25	4	7	1	0	0	1	0
	Poor	17	12	2	1	0	2	0	0	0
Location	Ekiti	11	10	1	0	0	0	0	0	0
	Ondo	44	27	5	8	1	2	0	1	0
Total		55	**37**	6	8	1	2	0	1	0

Table 2. Seroprevalence of *Toxoplasma gondii* infection in food dogs in Two Southwestern states of Nigeria

Factor	Category	No tested	Positive (%)	Odd ratio (95% Confidence interval)	P - value
Age	Adult	119	27 (22.7)	1.373 (0.760 – 2.482)	0.3614
	Young	159	28 (17.6)	0.728 (0.403 – 1.132)	
Sex	Male	190	48 (25.3)	3.912 (1.691 – 9.048)	0.0011*
	Female	88	7 (8.0)	0.256 (0.111 – 0.591)	
Health	Good	205	38 (18.5)	0.750 (0.393–1.432)	0.3952
	Poor	73	17 (23.3)	1.334 (0.699 – 2.548)	
Location	Ondo	189	44 (23.3)	2.152 (1.052 –4.402)	0.0362*
	Ekiti	89	11 (12.4)	0.465 (0.227 – 0.951)	
Total		278	55 (19.8)		

* Significant, P< 0.05

Table 3. Knowledge, attitude and practice of dog consumers in Two Southwestern states of Nigeria

Characteristic	No of respondent (%)		Total
	Yes	No	
Always eat dog meat	117 (75.5)	38 (24.5)	155
Prefer dog meat that beef	72 (47.1)	81 (52.9)	153
Feed dog meat to cat	12 (10.3)	105 (89.7)	117
All family members eat dog meat	90 (76.9)	27 (23.1)	117
Dog reared for food has contact with cat	44 (38.6)	70 (61.4)	114
Eat hunting dogs	87 (74.4)	30 (25.6)	117
Eat sick dogs	76 (66.1)	39 (33.9)	115
Clean utensil properly after processing dog for food	110 (94.0)	7 (5.9)	117
No Knowledge of getting infected with disease from eating dogs	91 (59.5)	62 (40.5)	153
Eat raw dog meat	39 (33.3)	78 (66.7)	117
Cook dog meat very well	96 (85.7)	16 (14.3)	112

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