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Original article

SEROLOGIC SURVEY OF *TOXOPLASMA GONDII* ANTIBODIES IN CATS (*FELIS CATUS*) SOLD AT LIVE ANIMAL MARKETS IN SOUTHWESTERN NIGERIA

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Summary

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Toxoplasma gondii is a zoonotic parasite causing infection in humans and a wide range of mammals, with cats being the final and only host that excrete *T. gondii*-resistant oocysts to the environment. This study was designed to investigate the seroprevalence of antibodies to *T. gondii* in cats sold at Live Animal Markets (LAMs) in Ibadan, Osogbo and Offa, Southwestern Nigeria. Blood samples were randomly collected from 226 cats at these markets and separated sera were tested for the presence of antibodies (IgG) to *T. gondii,* using two-fold dilutions from 1:20 to 1:320 by the Modified Agglutination Test (MAT). Attributes were analysed using Chi-square and Fisher's exact tests at $P<0.05$. Median age of cats was 4.0 months (range: 1–36 months). Of the 226 samples tested, only 10 (4.4%) were positive for anti-*T. gondii* IgG antibodies at the cut-off titre of 1:20. Nine (4.0%) gave a titre of 1:20; one (0.4%) gave 1:80 while none was positive at 1:40, 1:160 and 1:320 dilutions. Age and gender of cats was not significantly associated with *T. gondii* infection. Our results showed low prevalence of *T. gondii* infection in cats sold at LAMs in studied area and suggests confinement as an efficient way of limiting exposure of cats to infection sources.

Key words: cats, modified agglutination test (MAT), Nigeria, *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii is a zoonotic parasite that causes infection in humans and a wide range of mammals and birds (Dubey, 2010). *T. gondii* infection occurs mainly through the consumption of food or water contaminated with sporulated oocysts shed by cats or by consumption of undercooked meat of infected animal containing bradyzoites (Dubey, 1998; 2010). The shedding of resistant oocysts by cats (the only known definitive host) to the environment has been associated with out-

breaks of disease in humans (Teutsch *et al*., 1979; Benenson *et al*., 1982; Bowie *et al*., 1997; De Moura *et al*., 2006) and infection through the ingestion of resistant oocysts from environmental sources (water, soil, fruit and vegetables) is thought to have more epidemic consequences than having direct contact with cats (Santos, 2010).

T. gondii infection in cats is usually asymptomatic (Elmore *et al*., 2010; Bastos *et al*., 2014), but may occasionally cause non-suppurative encephalomyelitis, pancreatitis and pneumonia-like respiratory disorders. The life cycle of *T. gondii* can be expressed in two phases mainly: a sexual phase occurring in the intestinal epithelium of the cat where oocysts are shed in faeces, and an asexual phase which occurs in the extra-intestinal tissues of mammals and birds (Dubey, 2010). The disease in cats is usually disseminated and more likely to occur when cats consume infected meat and water (Elmore *et al*., 2010). Clinical infection can also occur when cats are immuno-compromised or suffer from immune-suppressing infections such as feline immunodeficiency virus and feline leukemia virus infections. Symptoms mostly observed in cats infected with *T. gondii* include fever, diarrhoea, icterus, cough, dyspnea, loss of appetite and neurological signs (Elmore *et al*., 2010; Bastos *et al*., 2014).

Serological methods of detecting specific antibodies (IgG or IgM) to *T. gondii* have been used to investigate the parasite in cats. Methods such as the enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence (IIF), direct agglutination test (DAT) and modified agglutination test (MAT) have been widely used as indicators of exposure to *T. gondii* (Zarnke *et al*., 2001; Kikuchi *et al*., 2004; Mucker *et al*., 2006; Ayinmode & Dubey, 2012). However, the MAT has recently been shown to be a more reliable, sensitive and specific test over the DAT for serological diagnosis of *T. gondii* infections (Dubey, 2010).

T. gondii is distributed worldwide and factors such as geographical, environmental and cultural habits have been known to influence the prevalence of the parasite especially in tropical and subtropical regions of the world (Dubey, 2010). Serological studies carried out worldwide indicate varying prevalence rates of *T. gondii* infection with 7% in Nigeria (Alayande *et al*., 2012), 57.8% in China (Qian *et al*., 2012), 63% in Albania (Silaghi *et al*., 2014) and 10% – 87% in Brazil (Dubey *et al*., 2012; Cerro *et al*., 2014).

In Nigeria, there are many reports on the seroprevalence of *Toxoplasma gondii* infection in humans and other animals (Uneke *et al*., 2007; Ishaku *et al*., 2009; Kamani *et al*., 2009; Ayinmode & Dubey, 2012; Ayinmode *et al*., 2015) but only few studies (Kamani *et al*., 2010; Alayande *et al*., 2012) are available on the infection in cats. This study was therefore aimed at investigating the occurrence of antibodies to *T. gondii* in cats sold at markets in southwestern Nigeria.

MATERIALS AND METHODS

Sampling location

Samples were randomly collected between January to November 2014 after obtaining necessary permits at cat markets in Ibadan, Oyo state (7°22N, 3°58E), Osogbo, Osun state (7 °48N, 4 °37E) and Offa, Kwara state (8°13N, 4°42E), Nigeria (Fig. 1) (Blexxonmak, 2010). These markets were situated within the larger community markets; the cats were obtained from both urban and rural areas and were kept and

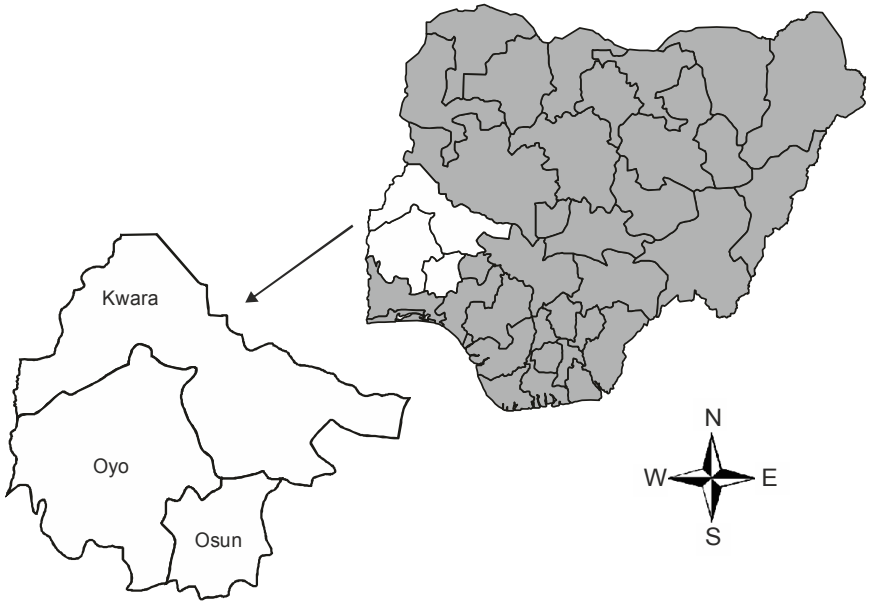

Fig. 1. Map of Nigeria, showing the location of states where cats were sampled.

fed in cages made either of wood or palm fronds till they were sold.

Sampling method

About 3.0 mL blood was collected from the saphenous vein of each cat and allowed to clot at room temperature. Sera separated from the samples were then stored at −20°C before being used for serology.

Serological assay

Sera obtained from the cats were tested for the presence of antibodies (IgG) to *T. gondii* using formalin-killed whole *T. gondii* tachyzoites (RH strain) and two-fold dilutions of each serum from 1:20 to 1:320 in a modified agglutination test (MAT) as previously described (Dubey, 2010).

Statistical analysis

Attributes obtained were organised and grouped into quantitative and qualitative variables that were fed into SPSS (version 20) statistical software package (Chicago, IL) for meaningful statistical analysis. Data were then summarised in tables using descriptive and inferential statistics. Categorical variables were reported as frequencies and percentages while continuous variables were examined for skewness, kurtosis and presence of univariate outliers. The continuous variables were reported as median and range for variables not conforming to a normal distribution curve. The concept of relationship that existed between variables was explored using Chi-square analysis and Fisher's exact test at $P<0.05$. This was done to ascertain the degree of association between categorical variables and the significance of such association.

RESULTS

A total number of 226 cats were obtained from animal markets in Ibadan (137), Offa

(46) and Oshogbo (43). The males and females were 112 and 114 respectively. All the cats were local breed and have their sexes intact.The median age of the cats was 4.0 months (range: 1–36 months) while their mean temperature and median weight was 37.4±0.976 ^{o}C and 490 g (range: 200–2100 g) respectively. The majority (69.9%) of cats were less than six months of age and only few (8.4%) were within the 13–36 months age range. Although most (96.9%) of the cats were apparently healthy, only few were presented with lice (2.2%) and ocular discharges (0.9%). Interestingly, none of the cats with visible signs of ill-health (such as lice and ocular discharges) was positive for anti-*T. gondii* IgG antibodies.

Out of the 226 samples collected, only 10 (4.4%) were positive for anti-*T. gondii* IgG antibodies by MAT at the cut-off titre of 1:20, with 9 samples (4.0%) having a titre of 1:20, one sample (0.4%) having 1:80 titre and no positive sample at 1:40, 1:160 and 1:320 dilutions.

Comparative analysis between outcome of the infection and explanatory variable revealed that all samples positive for anti-*T. gondii* IgG antibodies were from cats less than six months of age. Age was however not a statistically significant consideration for *T. gondii* infection.

Table 1. Comparison of anti-*Toxoplasma gondii* IgG antibody status with physical characteristics of cats sold in Southwestern Nigerian markets (n=226)

Variable	Anti-*Toxoplasma* IgG antibody		χ^2	*P* value
	Positive (%)	Negative (%)		
Age:				
<6 months (Kittens)	10 (6.3)	148 (93.7)	4.503	0.105
6 – 12 months (Juvenile)	0 (0)	49 (100)		
13 – 36 months (Young adults)	0 (0)	19 (100)		
Gender:				
Male	5 (4.5)	107 (95.5)	0.001^{FET}	1.000
Female	5 (4.4)	109 (95.6)		
Temperature:				
Below normal range	2 (2.2)	88 (97.8)	1.967	0.374
Within normal range	8 (6.0)	125 (94.0)		
Above normal range	0 (0)	3 (100)		
Physical condition:				
Apparently healthy	10 (4.6)	209 (95.4)	0.334	0.846
Ocular discharge	0 (0)	2 (100)		
Lice	0 (0)	5 (100)		
Location:				
Ibadan	9 (6.6)	128 (93.4)	4.068	0.131
Offa	0 (0)	46 (100)		
Osogbo	1 (2.3)	42 (97.7)		
Total	10 (4.4)	216 (95.6)		

FET = Fishers Exact Test.

Comparison of seropositivity based on gender showed that 5 male (4.5%) and 5 female (4.5%) cats were positive for anti-*T. gondii* IgG antibodies, indicating that sex was also of no statistical significance for *T. gondii* infection in the present study (Table 1). Similarly, location from which samples were obtained showed no statistically significant association with outcome of *T. gondii* infection as 9 positive samples were from Ibadan, 1 positive sample was from Osogbo and none was found positive from Offa (Table 1).

DISCUSSION

The seroprevalence rate of 4.4% for *T. gondii* antibodies obtained in this study is lower than the 36.2% (Kamani *et al*., 2010) and 7.0% (Alayande *et al*., 2012) rates reported from previous studies in Nigeria. The differences in seroprevalence could partly be attributed to the fact that most seropositive cats documented in these studies were stray or free-roaming while those sampled in the present study were in confinement (cages) with no information on their living conditions before they were bought. Cats in confinement are likely to have low infection rate because of restricted access to infection sources in the environment.

This study also showed that there was no statistically significant association between gender and *T. gondii* infection in cats. This finding is similar to the report of some previous studies (Gauss *et al*., 2003; Alayande *et al*., 2012; Cerro *et al*., 2014) but contrary to the suggestion that there is the likelihood of higher exposure for the male cat due to its territorial dominance (Smith *et al*., 1992). Our study revealed that kittens less than six months of age had higher antibodies to *T. gondii* infection than other age groups. This finding differs from the report of Alayande *et al*. (2012) where cats aged between 6–12 months were the most exposed group. Although no statistically significant association was found between *T. gondii* infection and age, it is likely that the seropositive kittens in the studied population may have acquired the infection from their mothers (vertically). However, more studies are needed to verify the possible role of vertical transmission of *T. gondii* infection in kittens.

Our study showed that cats with physical evidence of ill-health were negative for *T. gondii* antibodies while seropositive ones had body temperatures within normal range. This can be attributed to the fact that *T. gondii* infection in cats has been known to be asymptomatic (Elmore *et al*., 2010; Bastos *et al*., 2014) with the cats periodically shedding oocysts during primary infection and in the absence of any form of immune incompetence. The absence of clinical symptoms in seropositive cats in this study might also be attributed to the fact that the IgG-based test used could only detect previous exposure to *T. gondii* and not current infection which an IgM-based assay would detect.

In conclusion, age, gender and locations where cats were sampled from were not significantly associated with *T. gondii* infection in the present study, and infected cats did not show clinical symptoms of the infection. Furthermore, the detection of low prevalence of *T. gondii* infection in the studied population of confined cats may substantiate the fact that confinement is an efficient way of limiting the exposure of cats to infection sources. Cat owners are therefore advised to institute appropriate preventive measures that would, as much as possible, restrict cats from having access to and consuming tissues of infected animals, especially rodents.

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