Seroprevalence of anti-*Toxoplasma gondii* antibodies in cattle and pigs in Ibadan, Nigeria

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Abstract Toxoplasmosis is an important food borne parasitic zoonoses. However, data on *Toxoplasma gondii* infection in domestic animals mostly used for human consumption in Nigeria are scarce. We thus conducted a survey on the seroprevalence of *T. gondii* infection in a representative sample of cattle and pigs in farms and the abattoirs between June and December 2012. Sera from 210 cattle (both sexes) and 302 pigs (both sexes), were examined for *T. gondii* specific IgG antibodies by indirect Enzyme linked immunosorbent assay (ELISA). The seroprevalences determined were 13.91 % in cattle and 29.14 % in pigs. The antibody levels ranged from 0.499 to 2.103 in cattle, and 0.544 to 3.020 in pigs. The prevalence of IgG anti-*T. gondii* antibodies was positively correlated with the age. The seroprevalences (26.23 %) and (36.54 %) for adult cattle and pigs respectively, were higher compared to younger groups. However the difference was not significant ($P > 0.05$) in cattle but significant ($P < 0.05$) in pigs. In Nigeria, exposure to raw or undercooked foods is a risk factor for *T. gondii* infection. Knowledge of the prevalence of toxoplasmosis will help to target prevention efforts.

Keywords Epidemiology · Cattle · Nigeria · Pig · Protozoa · *Toxoplasma gondii*

Introduction

Toxoplasma gondii is a zoonotic, heteroxenous obligate intracellular protozoan parasite that can infect almost all

mammals including man. It has a worldwide distribution. Cats, including all felines, are its definitive hosts and excrete environmentally resistant oocysts in their faeces (Dubey et al. 2005a). The importance of this parasite in food safety, human health and animal husbandry has been well recognized. Although the parasites remain dormant in people with normal immune competence, they do pose threats to individuals who are immunocompromized such as patients with AIDS or organ transplantation and cause mental retardation and loss of vision in congenitally-infected children and abortion in pregnant women and livestock (Dubey 2008).

There are two major modes of transmission of *T. gondii*. Infection may occur by ingestion of food or water contaminated with oocysts excreted by infected cats or by ingesting uncooked or undercooked meat containing tissue cysts of *T. gondii*. The proportion of the human population that acquires infection by ingestion of oocysts in the environment or by eating contaminated meat is not known, and there are no tests available that can determine the infection source (Dubey et al. 2005b). However, sero-epidemiologic data suggest that ingesting improperly cooked meat containing *T. gondii* is a major source of infection for humans in the United States (Dubey and Beattie 1988).

Cattle have high natural resistance to the parasite. *Toxoplasma gondii* causes subclinical infection in cattle (Dubey and Thulliez 1994). Several epidemiological studies have mentioned that the consumption of raw or undercooked beef could be considered a risk for *T. gondii* infection in humans (Cook et al. 2000). Information on different part of the world shows a prevalence of 28.9 % in fattening pigs in Serbia (Klun et al. 2006), 20 % in Argentina (Venturini et al. 2004), 16.3 % in Italy, (Villari et al. 2009), 15.6 % in Portugal (De Sousa et al. 2006) and 44.5 % in Ibadan (Okewole 2007) using modified agglutination test (MAT).

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A relative estimate of the risk to consumers of acquiring *T. gondii* infection from ingestion of contaminated fresh meat might be gathered from information on prevalence of these pathogens in live animals (Dubey et al. 2005c). Human activities that are suspected to increase the risk of exposure to *T. gondii* include handling of cats and cat litter, gardening, handling raw meat or contaminated animal feed, whereas washing hands after conducting these activities is believed to decrease the risk of transmission (Weigel et al. 1995). Transmission of *T. gondii* to humans is more likely in environments where the risk of exposure is high (Weigel et al. 1995). Studies of risk factors for *Toxoplasma gondii* infection in humans have shown that the relatively high prevalence is associated mainly with consumption of undercooked meat and/or meat products. However, data on *T. gondii* infection in domestic animals mostly used for human consumption in Nigeria are scarce. We thus conducted a survey on the seroprevalence of *T. gondii* infection in a representative sample of cattle and pigs in Ibadan.

Materials and methods

Study area

The study was conducted in Ibadan, the largest city in West Africa and the second largest in Africa with an estimated population of about 2,550,593 million, growing rapidly with industries and residential houses. Ibadan city lies on the longitude 3°5′ East of Greenwich meridian and latitude 7°23′ North of the Equator (Filani et al. 1994). However, the city is characterized by low level of environmental sanitation, poor housing, and lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations. Samples were collected from small scale farms, Bodija municipal abattoir and Moniya slaughter house in Ibadan (Fig. 1). The animals were sampled using simple random sampling method. The age, sex and breeds of the animals were considered in the process of sampling.

Collection of blood sample

Labeled sterile vacutainer tubes which contained no anticoagulant or preservatives was used to collect blood from animals at the time of slaughter from severed jugular vein. Sterile needle and syringe was used to collect blood from the anterior vena cava from live pigs. The blood sample was kept in slanted positions in the containers to avoid haemolysis of blood cell and to assist in serum separation. It was transported to the laboratory in ice pack and

centrifuge at 3,000 rpm for 10 min using a bench centrifuge (Centromix, UK). The separated serum was decanted into a labeled ependoff tube for storage in the freezer at −20 °C until required for use.

Serology

The cattle and pig sera samples were tested using commercial indirect ELISA Kits (ID Screen® Toxoplasmosis Indirect Multi-Species from ID Vet Innovative Diagnostic, Montpellier, France) to determine the presence of Toxoplasma specific IgG antibodies, resulting from infection with *T. gondii*. To avoid differences in incubation times between specimens, a 96-well plate was prepared containing the test and control specimens, before transferring them into the ELISA microplate using a multichannel pipette. Optical densities (OD) were read at 450 nm. The test was validated if the mean value of the positive control O.D. (ODPC) is greater than 0.350 (ODPC > 0.350) and the ratio of the mean O.D. values of the positive and negative controls (ODPC and ODNC) is greater than 3.5 (ODPC/ODNC > 3.5). According to the manufacturers recommendation, sera or meat juice with S/P % less than or equal to 40 % are considered negative, between 40 and 50 % are considered doubtful and greater than or equal to 50 % are considered positive. Doubtful samples were repeated for certainty.

Statistical analysis

The results are expressed in percentages. The prevalence for *T. gondii* antibodies was statistically analyzed using student *t* test and one way analysis of variance (GraphPad Prism version 5) considering the variables; sex, age and breeds. The differences were considered statistically significant at $P \leq 0.05$.

Results

Cattle

The prevalence of anti-*T. gondii* antibodies of the cattle are presented in Table 1. The overall seroprevalence of *T. gondii* antibodies was 13.81 %, with optimal density for the positive sample ranging from 0.499 to 2.103. The highest prevalence (26.23 %) was observed in >5 years old category while the lowest prevalence (2.13 %) was observed in the 0–1.5 years old group. There was no significant difference ($P > 0.05$) between the ages of cattle examined. The prevalence was significantly ($P < 0.05$) higher in the female (14.53 %) compared to the male (12.90 %) (Table 2). Out of 95 White Fulani breed of cattle

Fig. 1 Map inset, Nigeria/Oyo state and Ibadan metropolis the study area

examined, 17(17.89 %) were positive while 1 (4.55 %) out of 22 were positive for the cross breeds of cattle. All the 10 N'dama cattle examined were negative for *T. gondii* antibody (IgG). There was no significant difference ($P > 0.05$) between breeds (Table 1).

Pigs

The estimated overall prevalence of *T. gondii* antibodies (IgG) in pigs was 29.14 %. However, antibody levels were usually high. Detection of antibodies to *T. gondii* by

Table 1 Prevalence of *Toxoplasma gondii* antibodies in Cattle in Ibadan, Nigeria

Age (years)	Number examined	Positive (%)	Doubtful (%)	Negative (%)
0–1.5	47	1 (2.13)	0 (0)	46 (97.87)
2–2.5	27	3 (11.11)	1 (3.70)	23 (85.19)
3–3.5	40	4 (10)	0 (0)	36 (90)
4–4.5	35	5 (14.29)	2 (5.71)	28 (80)
>5	61	16 (26.23)	4 (6.56)	41 (67.21)
Total	210	29 (13.81)	7 (3.33)	174 (82.86)
Sex				
Male	93	12 (12.90)	2 (2.15)	79 (84.95)
Female	117	17 (14.53)	5 (4.27)	95 (81.19)
Total	210	29 (13.81)	7 (3.33)	174 (82.86)
Breed				
White Fulani	95	17 (17.89)	2 (2.11)	76 (80)
Sokoto Gudali	40	5 (12.5)	1 (2.5)	34 (85)
Red Bororo	43	6 (13.95)	3 (6.98)	34 (79.07)
N'dama	10	0 (0)	0 (0)	10 (100)
Cross	22	1 (4.55)	1 (4.55)	20 (90.91)
Total	210	29 (13.81)	7 (3.33)	174 (82.86)

Significant level was set at ($P < 0.05$)

Table 2 Prevalence of *Toxoplasma gondii* antibodies in Pigs in Ibadan Nigeria

Age	Number examined	Positive (%)	Doubtful (%)	Negative (%)
Weaners	62	13 (20.97)	3 (4.84)	46 (74.19)
Growers	188	56 (29.79)	6 (3.91)	126 (67.02)
Finisher	52	19 (36.54)	4 (7.69)	29 (55.77)
Total	302	88 (29.14)	13 (4.30)	201 (66.56)
Sex				
Male	126	22 (17.46)	5 (3.97)	99 (78.57)
Female	176	66 (37.5)	8 (4.54)	102 (57.95)
Total	302	88 (29.14)	13 (4.30)	201 (66.56)
Breed				
Large white	223	61 (27.34)	8 (3.59)	154 (69.06)
Hampshire	31	8 (25.81)	1 (3.23)	22 (70.97)
Local	16	8 (50)	3 (18.75)	5 (31.25)
Cross	32	11 (34.38)	1 (3.13)	20 (62.5)
Total	302	88 (29.14)	13 (4.30)	201 (66.56)

Significant level was set at ($P < 0.05$)

ELISA was positively correlated with the age of pigs measured as parity numbers ($r_s = 0.50$; $P < 0.05$). With the prevalence significantly higher in the adult ($P < 0.05$, 36.54 %) followed by the growers (29.79 %) and weaners (20.97 %). Seroprevalence was significantly higher in the female ($P < 0.05$, 37.5 %) than in the male (17.46 %). The highest prevalence of seropositive pigs was observe in the indigenous breed (50 %), followed by the cross breeds (34.38 %), large white (27.34 %) and hampshire (25.81 %) (Table 2). However there was no significant difference

($P > 0.05$) between the breeds of pigs examined. The optimal density for all the positive pig samples ranged from 0.544 to 3.020.

Discussion

Of the various research methods, serological techniques are most often used for the assessment of *T. gondii* infection in animals (Gamble et al. 1999). This is because diagnosis of

toxoplasmosis by demonstration of *T. gondii* in tissue is difficult. Therefore, the detection of antibody response by screening of cattle and pig sera appears to be the conclusive tool for proper diagnosis of toxoplasmosis. The seroprevalence of 13.81 % in cattle reported in this study using ELISA is consistent with the observation of previous investigators. Joshua and Akinwumi (2003) reported a prevalence of 16.9 % using latex agglutination test (LAT) while Okewole (2007) reported a prevalence of 10.1 % in Ibadan using MAT. The low prevalence of toxoplasmosis in cattle could be attributed to genetic resistance of cattle to *T. gondii* and the management system that reduced contact of cattle with cat or cat wastes. These factors were also speculated to be responsible for the higher seropositivity of the nomadic white Fulani cattle breed than the sedentary N'dama, (Joshua and Akinwumi 2003).

The antibody levels in most pigs were very high possibly due to continued infection with *T. gondii* in pigs over time. There is only scanty information on the time course of antibody levels to *T. gondii* in naturally infected pigs (Lind et al. 1995), but it has been shown using ELISA that IgG antibodies appear from 10 to 21 days after experimental infection and may persist on a similar level for more than 2 years (Dubey et al. 1997). Another explanation for high levels of antibodies to *T. gondii* antigens could be environmental contamination and the presence of infected rats in the premise of the farms and abattoirs. The prevalence of 29.13 % observed in pigs infected with *T. gondii* in this study is similar to the reported prevalence (28.9 %) in Serbian pigs by Klun et al. (2006) and 28.8 % by Fan et al. (2004) in Taiwan. However, higher prevalence rate of 41.5 % was reported by Okewole 2007 in Ibadan using modified agglutination test. These discrepancies might not be unrelated to the variation in the sensitivity and specificities of diagnostic assays used by the investigator. The ELISA kit used is highly sensitive, specific and does not cross react with other coccidian parasites. The prevalence of *T. gondii* antibodies was highest in the adult (36.54 %) than in the grower (29.79 %) and weaners (20.97 %). This is because the longer animals stay in vicinity with the predisposing factors, the greater their chances of picking the infection. In this study the prevalence of toxoplasmosis was higher in the female compared to the male cattle and pigs. Alexander and Stinson (1988) reported that the female animals are more susceptible to protozoan parasites as compared to male. The hormonal differences between males and females play an important role in determining susceptibility to parasitic infection. It is now widely accepted that many hormones including the sex-associated hormones directly influence the immune system (Roberts et al. 2001).

Different breeds showed varying levels of susceptibility and resistance to parasitic diseases. The indigenous breeds

were more seropositive compared to the others. This could be due to the fact that the indigenous breeds mostly reared extensively have free access to rodents and soil contaminated with oocysts. They are usually given more access to scavenge on garbage to meet their nutritional needs and as a result they pick up oocysts from contaminated environment. Manuel and Tubongbanua (1977) found in the Philippines higher percentage of serologically positive pigs in indigenous breeds compared to exotic or crossbreds. Generally, prevalence based on breeds of animals could be due to genetic variation (Van der Puije et al. 2000).

In Nigeria, toxoplasmosis has been described in human patients (Alayande et al. 2012) but there are few references to the source of infection to humans. Isolation of viable *T. gondii* appears to be less successful from slaughter pigs with low levels of antibodies (Dubey et al. 1995) and failed in some studies (Gajadhar et al. 1998). However, it should be noted that the currently available viability tests for *T. gondii* vary greatly in their sensitivities (Dubey et al. 1995) and also that viable *T. gondii* stages were occasionally isolated from meat of seronegative pigs (Dubey et al. 2002). Therefore, seropositivity of pigs does not accurately reflect the infection risk for humans, but it is likely that a seropositive pig harbours infective tissue cysts of *T. gondii* and hence the consumption of under-cooked meat or meat products derived from these animals is a potential risk factor for *T. gondii* transmission to humans.

Conclusion

Toxoplasma gondii is a widely prevalent parasite that is potentially responsible for significant morbidity and mortality in the congenitally infected child and the immunosuppressed. In the present study, we have determined the prevalence of toxoplasmosis in cattle and pigs. Reduction of *T. gondii* contamination of meat and knowledge of the prevalence of the disease in food animal and the environment could help to further reduce the burden of toxoplasmosis in Nigeria.

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