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Seroprevalence of *Toxoplasma gondii* infection in slaughtered pigs in Makurdi, Nigeria

IN Obijiaku*, N Ornguga & AA Adikwu

Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria

*Correspondence: Tel.: +2348179502022; E-mail: nancynzelu@gmail.com

Abstract

Toxoplasmosis is a parasitic disease/infection of medical and veterinary importance. The causative agent; *Toxoplasma gondii*, can infect warm blooded animals, birds as well as humans. This study was designed to determine the seroprevalence of *Toxoplasma gondii* infection in slaughtered pigs in Makurdi, Nigeria. A cross-sectional study was designed in which 181 blood samples were collected from two pig slaughterhouses in Makurdi (Wurukum and Railway) from September to December 2014. Sera were harvested and stored at -20 ^{o}C until required for processing. Indirect ELISA test kit (ID-Vet, France) was used to determine the presence of anti-*Toxoplasma gondii* antibodies. Sex, age and breed of each sampled pig was recorded. A total seroprevalence of 4.4% was obtained. Sex specific seroprevalence was 5.4 and 4.0 % for male and female respectively. Breed specific seroprevalence was 4.5 and 4.2 % for indigenous and exotic breeds respectively. Age specific seroprevalence was 4.7 and 0 % for pigs aged greater or equal to 8 months (≥ 8 months) and less than 8 months (< 8 months) respectively. This study found no significant association between sex, breed, age and presence of anti-*Toxoplasma gondii* antibodies ($p > 0.05$). The study provided preliminary information on *Toxoplasma gondii* infection in some pigs slaughtered for human consumption in Makurdi metropolis.

Keywords: Makurdi, Pigs, Seroprevalence, Slaughterhouse, *Toxoplasma gondii*
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Introduction

Toxoplasmosis is a parasitic zoonotic disease/infection caused by an obligate intracellular protozoan, *Toxoplasma gondii*, which belongs to the phylum Apicomplexa (Dubey, 2010). The disease has a worldwide distribution and is of public health importance (Dubey, 2010). Members of *felidae*, especially cats, serve as the definitive host of the parasite, while warm-blooded animals, birds and humans play a role as intermediate hosts in the transmission cycle (Dubey, 2010). Cats become infected with *T. gondii* by eating infected tissues containing cysts from intermediate hosts, while food animals such as pigs, sheep, goats and chickens most commonly become infected by ingestion of oocyst from cat faeces in contaminated environments (Dubey, 2010; CDC, 2017). These animals in turn serve as sources of infection to man when raw or undercooked meat is consumed. Symptoms in animals are usually subclinical, although abortion is common in pigs, sheep and goats (Tenter *et al*., 2000; WHO, 2014). Outbreaks of lethal toxoplasmosis have been reported on pig farms and most of these cases occurred during hot and humid weather due to the consumption of food contaminated with oocysts from cat faeces (Li *et al*., 2010; Jones & Dubey, 2012).

In Nigeria, few studies on toxoplasmosis in pigs have been reported and mainly in the south western region of the country. In Ibadan, Onyiche & Ademola (2015) reported seroprevalence of 29.14 % in pig farms and abattoirs, while Ayinmode & Olaosebikan (2013) reported seroprevalence of 25 % in backyard

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and wandering pigs. Akande *et al*. (2016) reported a prevalence of 24.2 % in Ogun state. However, Giegbefumwen *et al.* (2013) recorded seroprevalence of 16.6 % in slaughtered pigs and farm pigs in some parts of Kaduna state, northern Nigeria. In a large-scale study conducted in Ibadan amongst various livestock species, pigs had the highest seroprevalence of 45.2 % (Ayinmode & Abiola, 2016).

Primary acquired infection with *T. gondii* in immunocompetent individuals is generally asymptomatic but some patients may develop fever, cervical lymphadenopathy, myalgia and other non-specific clinical signs (Robert-Gangneux & Darde, 2012). Toxoplasmosis has a higher impact in immunocompromised individuals and in congenital infections due to the involvement of the central nervous system (Furtado *et al*., 2011).

The inhabitants of Benue state, of which Makurdi is the capital, commonly consume pork. Pork is prepared in several ways in homes and displayed at restaurants and roadside by food sellers for patronage by consumers. There is little or no published data on the prevalence of *T. gondii* infection in Makurdi to the best of our knowledge. Moreover, routine testing of slaughtered pigs at slaughterhouses to identify seropositive pigs or inspection of meat for viable cysts is lacking. Consequently, the prevalence, source of infection and risk factors remain unknown. Therefore, we conducted this survey to determine the seroprevalence of *T. gondii* infection in slaughtered pigs in Makurdi, Benue state, Nigeria.

Materials and Methods

Study area

The study was conducted in two pig slaughterhouses in Makurdi (Railway and Wurukum). Makurdi is the capital of Benue state, Nigeria. The city is located within the north central agricultural zone of Nigeria along River Benue. Makurdi lies between 7°44'1.50"N and 8°31'17.00"E. Wurukum and Railway are the main slaughter locations for pigs in Makurdi metropolis. Although, pockets of slaughter occurs in few other locations, but most pork sold in the various markets in Makurdi metropolis are processed from these two locations.

Sampling

Blood samples were collected from 181 randomly selected pigs, slaughtered for human consumption in the two main slaughterhouses. After proper restraint, 4 ml of blood were collected from each animal during thoracic stick exsanguination, and immediately transferred into a properly labeled plain vacutainer. The samples were kept in a cold box containing ice and transported to the Veterinary Public Health and Preventive Medicine Laboratory, University of Agriculture Makurdi, for processing. Data on sex, age and breed of each sampled pig were recorded. Age was classified into less than 8 months and greater or equal to 8 months. The ages of the animals were determined using dentition, while breed was classified into local and exotic. During processing, serum was extracted from blood samples with no coagulant by centrifugation at 5,000 rpm for 10 minutes and stored at -20 ^{o}C until required for serology.

Serology

Sera samples were subjected to indirect enzyme linked immunosorbent assay (ELISA) using a readily available commercial kit (ID-Vet, France) to detect specific anti-*Toxoplasma* IgG antibodies. The assay was validated and carried out according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using statistical package for social science (SPSS) version 16.0 (SPSS Inc. Chicago, IL, USA). Statistical methods employed included descriptive statistics employing frequencies and Percentages. Fisher's exact test was used to establish association between the infection status of the sampled pigs and variables such as age, sex and breed. Statistical significance at a probability of 5 % ($P<0.05$) with a confidence interval of 95 % was adopted.

Results and Discussion

Out of 181 sampled pigs, the overall seroprevalence was 4.4 % (8/181), while 95.6 % (173/181) were negative. Sex specific seroprevalence for male and female were found to be 5.4 and 4.0 %, respectively. The result showed no statistically significant ($p > 0.05$) association between toxoplasmosis in pigs and sex (Table 1). The seroprevalence of *T. gondii* based on breed indicated a slightly higher seropositivity in indigenous breed of 4.5 % (7/157) than in exotic breed with 4.2 % (1/24). However, there was no statistically significant ($p > 0.05$) association between seropositivity and breed (Table 1). An increased seroprevalence with age of 0 and 4.7 % for pigs aged less than 8 months and greater or equal to 8 months respectively was observed, although there was no significant association between seropositivity and age ($p > 0.05$) (Table 1).

Table 1: Relationship between toxoplasmosis and identified factors in pigs slaughtered for human consumption in Makurdi, Nigeria, 2014

Factors	Total sampled	No. positive	Specific prevalence (%)	*p value
Sex				0.704
Male	56	3	5.4	
Female	125	5	4	
Total	181	8	4.4	
Breed				1.000
Indigenous	157	7	4.5	
Exotic	24	1	4.2	
Total	181	8	4.4	
Age (months)				
< 8	11	0	0	1.000
≥ 8	170	8	4.7	
Total	181	8	4.4	

*Statistical method used: Fisher's exact test, $\alpha = 0.05$

This study has confirmed the presence of circulating *T. gondii* antibodies in slaughtered pigs from Makurdi metropolis, Nigeria. This is an indication that these pigs have been exposed to *T. gondii* oocysts, probably from contaminated soil or other sources. Results from this study revealed a low prevalence compared to previous studies. Onyiche & Ademola (2015) reported a seroprevalence of 29.14 % in pig farms and abattoirs in Ibadan, Nigeria, during the late rainy and early dry season. Epidemiologically, the infection is said to be more prevalent in warm climates and in humid areas than in cold climates and dry areas (Dubey, 2010; Kistiah *et al*., 2011). These conditions are reported to favour sporulation and survival of oocysts in the environment (Dubey, 2010; Kistiah *et al*., 2011).The present study was conducted during the dry season (September to December) which may have accounted for to the low prevalence observed. In addition, the high prevalence in the former study could be attributed to the geographical location where the study was conducted. Ayinmode & Olaosebikan (2013) reported a similar prevalence of 25 % in a study conducted within the same geographical location as Onyiche & Ademola (2015). Animals reared outdoors are more prone to parasitic infections because they pick up parasites from contaminated environment.

Toxoplasma oocysts were reported to be able to survive in the environment for about 1.5 years (Frenkel *et al*., 1975) and for 4.5 years at 4 ^{o}C (Dubey, 1998). In the our study, slaughtered pigs were bought by dealers, from small-scale farms from various locations such as Jos (Plateau state), Kafanchan (Kaduna state) and Nasarawa state and transported to Makurdi. These farms usually practice semi-intensive management system of pig husbandry, and this could have been responsible for the low seroprevalence obtained in this study.

Our study indicates an increased seroprevalence of toxoplasmosis with age. This is in agreement with the reports of Zemene *et al.* (2012) and Onyiche & Ademola (2015), who reported statistically significant association between age and toxoplasmosis. Nonetheless, the present study showed no significant association between age and toxoplasmosis. Interestingly, sex and breed were also not associated with presence of anti-*Toxoplasma gondii* antibodies and so, they are not important determinants of toxoplasmosis in the present study.

In conclusion, the study provides preliminary information on *Toxoplasma gondii* infection in pigs slaughtered for human consumption in Makurdi metropolis. The work highlights the need for public enlightenment on the importance of proper cooking of pork sourced from the study area, to prevent the risk of being exposed to *T. gondii* cysts. There is the need for further studies to assess the prevalence of *T. gondii* infection in pigs in Benue state as a whole, through antigen detection techniques and if possible, identify and compare the prevalent strains responsible for infections in both pig and human populations.

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