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Detection of *Toxoplasma gondii* antibodies in trade donkeys (*Equus asinus*) at Ganawuri district market, Riyom Local Government Area, Plateau State, North Central Nigeria

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Abstract

Background and Aim: *Toxoplasma gondii* is a parasite of public health significance due to its possible transmission to humans through ingestion of tissue cysts in raw or undercooked meat or food or water contaminated with oocysts shed by felids and transplacental transmission. This study was carried out to provide information on the possible risk of transmission through eating undercooked donkey meat by conducting a market based cross-sectional study design to determine the presence of *T. gondii* antibodies in trade donkeys in Ganawuri district, Riyom Local Government Area, Plateau State, North Central, Nigeria.

Materials and Methods: One hundred and thirteen serum samples were collected from trade donkeys at the market and analyzed for *T. gondii* antibodies using latex agglutination test (LAT). Serum samples with LAT titer >10 µl/ml were considered positive.

Results: The distribution of the donkeys based on their sources showed that greater proportions (61.1%) were from North Eastern part of the country. The study showed that 31 of the serum samples collected and analyzed were positive for *T. gondii* antibodies given an overall prevalence of 27.4%. The prevalence ranges between 22.2 and 33.3% across the states where the donkeys were sourced. The study showed that seropositivity increases with age (p=0.003; OR=11.8) and ranges between 12.5 and 47.2%. The prevalence did not vary significantly based on sex and source/location (p=0.494, OR=0.05; 0.920, 1.45, respectively).

Conclusion: This study showed that trade donkeys at Ganawuri district market have antibodies to *T. gondii* and suggest a public health risk from the consumption of undercooked donkey meat.

Keywords: antibodies, cross-sectional, donkeys, prevalence, public health, *Toxoplasma gondii*, trade.

Introduction

Toxoplasma gondii is an obligate intracellular apicomplexan protozoan that can infect virtually all warm-blooded animals [1,2]. The parasite has been reported to have a worldwide distribution [3]. Felids, both domestic and wild are the only known definitive hosts. The parasite is of public health significance due to its possible transmission to humans through ingestion of tissue cysts in raw or undercooked meat or food or water contaminated with oocysts shed by felids and transplacental transmission [4,5]. It is also known to cause great economic losses in the livestock industry due to abortion, premature, and stillbirth. Infection with *T. gondii* in humans is usually asymptomatic; however, the infection may cause abortion in pregnant women or occasionally toxoplasmic encephalitis or even death in patients with an immune-suppression disease like AIDS [1,6].

Aside from being useful to humans in sports competition, police work, carriage, donkeys, and horses serve as sources of protein and traditional delicacies in many parts of the world [7]. Infection of *T. gondii* in horses is usually subclinical, but there may be fever, ataxia, retinal degeneration, and abortion or stillbirth in pregnant equine. Even though infection with *T. gondii* is usually asymptomatic, viable parasites have been isolated from the meat of animals such as sheep, goat, cow, pig, chicken, horse, and donkey [8]. Meat from birds and warm-blooded animals traditionally has been considered major sources of *T. gondii* infection [9]. More so, cases of human toxoplasmosis associated with consumption of horse and donkey meat have been reported in some countries [2,10].

At the moment donkeys are reared majorly on extensive management system with few managed intensively in this part of the country. These practices expose the animal to various infective stages of parasites (some of which are zoonotic) with attendant health impact. Donkeys are routinely brought to

Ganawuri district market to be sold while some are slaughtered for human consumption. Data on *T. gondii* in trade donkeys in Ganawuri district market, Riyom Local Government Area, Plateau State, North Central Nigeria are scarce.

This study was carried out to provide information on the possible risk of transmission through eating undercooked donkey meat by conducting a market based cross-sectional study design to determine the presence of *T. gondii* antibodies in trade donkeys in Ganawuri district, Riyom Local Government Area, Plateau State, North Central, Nigeria.

Materials and Methods

Ethical approval

This study was approved by the Animal Health Department, Federal College of Animal Health and Production Technology, National Veterinary Research Institute Vom, Nigeria, before sample collection. Animals were handled in strict compliance with good animal practice.

Study area

The study was conducted in Ganawuri district, Riyom Local Government Area, Plateau State (Figure-1). It is located at an elevation of 1265 m above sea level. Its geographical coordinates are 9°41°0" North, 8°42'0" East. Riyom Local Government Area has an area of 807 km^2 and a population of 131,557 at the 2006 census which is predominantly Berom. The Local Government Area has coordinates of 12° 08' 00'' N, 8° 46' 00''E. It shares boundaries with Kaduna and Nasarawa State. Ganawuri is the place where agricultural farming is practiced. Donkeys serve as a source of protein and income to the majority of the people in the area (NIPOST, 2009). Donkeys were brought from the northeastern and northwestern part of the country on every market day.

Study design

A cross-sectional market-based study design was carried out while a systematic random sampling technique was adopted for the inclusion of animals into the study. Donkeys brought to the market were first tied to a stake. About 20-30 donkeys were brought from different parts of the country (mostly northeastern and northwestern part of the country) during each market day. In each market day, donkeys presented were assigned numbers, and those with even numbers were included in the study and for sample collections. Approximately 13-15 samples were collected per visit. Information on sex and source of donkey was recorded. The age of each donkey was estimated using dentition. The sample collection was between the months of December 2017 and April 2018

Sample collection and handling

Blood samples were collected from 113 donkeys. Samples were labeled and transported on ice to the Parasitology Laboratory, Federal College of Animal Health and Production Technology, National Veterinary Research Institute, Vom. Samples were centrifuged at 1000 rpm for 3 min to separate serum. Serum was stored at −20°C until used.

Serology

Antibodies to *T. gondii* were determined using the latex agglutination test (LAT) kits as described by the manufacturer (Biokit, Barcelona-Spain). A semi-quantitative analysis was carried out. Briefly, 50 µl of normal saline was placed on slide sections

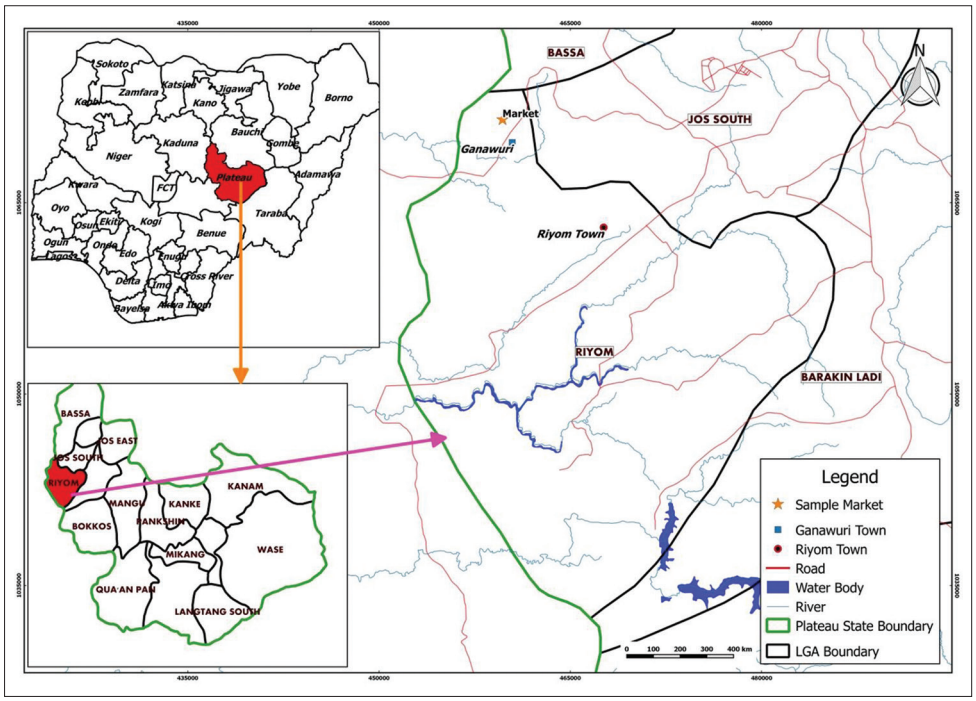

Figure-1: Map of Nigeria showing the study area.

2 through 6. A 50 µl of the sample was placed on slide sections 1 and 2 using an automatic pipette. The sample and the saline solution on section 2 were mixed several times, and 50 µl of the mixture made on section 2 was transferred to section 3 and repeated through to section 6, thereafter discarding 50 µl. One drop of reagent was then added and mixed with a stirrer covering the whole surface of the slide section. The slide was rotated for 5 min on a rotary shaker set at 80-100 rpm. The presence of agglutination at titer >10 µl/ml was considered positive. Positive and negative controls were included in each test.

Statistical analysis

Data obtained were analyzed using statistical package for the social sciences (SPSS) version 21(IBM Corp. USA). For categorical data, Chi-square test was used to determine the association between the presence of *T. gondii* antibodies and factors such as age, sex, and source of trade donkeys in Ganawuri district market. $p<0.05$ was considered statistically significant.

Results

The study showed that greater proportions (61.1%) of the donkeys at Ganawuri market were from Northeastern part of the country(Figure-2). Of the 113 serum samples collected from donkeys, 31 were positive for *T. gondii* antibodies using LAT kit giving an overall prevalence of 27.4% (Table-1). The result showed that the seroprevalence was higher (29.0%) in male than in female (26.8%); though not statistical significant (p=0.494, df=1, OR=0.05) (Table-2). More so, the result showed that *T. gondii* seropositivity increases with age (p=0.003, OR=11.8) and ranges between 12.5 and 47.2% (Table-3). There was no statistically significant difference between *T. gondii* seropositivity and the sources of the donkeys. The seroprevalence however based on the sources of donkeys ranges between 21.4 and 31.8% with Yobe, Bauchi, Kaduna, Maiduguri, Kano, and Gombe state having a prevalence of 21.4, 22.2, 22.7, 31.2%, 31.8, and 33.3%, respectively (p=0.920; OR 1.45) (Figure-3).

Discussion

Ganawuri district market is a major market in North Central Nigeria where donkeys are sold and or slaughtered for human consumption. This study was, therefore, carried out to determine the prevalence of *T. gondii* antibodies in trade donkeys to ascertain their role in the transmission of the parasite. Studies carried out by Bártová *et al.* [11] reported a prevalence of 17.0% in donkeys sourced from Bauchi State, Nigeria. Studies conducted in Egypt (Monofia Province) reported a prevalence of 25.6% [12] while prevalence of 25.6, 23.6, and 20.3% were reported in Spain, Northeastern, and Southwestern China, respectively [5,13,14]. Similarly, Machacova *et al.* [15] reported a seroprevalence of 5 and 8% using LAT and IFAT, respectively, in donkeys in Italy while Alvarado-Esquivel *et al.* [16] reported a prevalence of 10.9% in donkeys slaughtered for human consumption in Mexico. The seroprevalence of *T. gondii* in donkeys in the study area is comparatively higher than those in other regions. Consumption of undercooked donkey meat may pose a possible health risk to humans in the area. The differences in the hygienic conditions, management practices, climatic conditions, and cat abundance may explain the differences in the seroprevalence reported in the different regions/areas with the present study. More so, differences in the sensitivity of the serological test and sample size may account for the variation in the seroprevalence in different studies. The limitation of our study is that the validity of LAT kits for use in animals is unknown and needs to be put into account while interpreting our findings.

Studies in some species of animals showed that *T. gondii* seropositivity increases with age [17,18]. This study has revealed that *T. gondii* seropositivity

Table-1: Overall prevalence of *T. gondii* antibodies in trade donkeys in Ganawuri district market.

No. of samples collected	No. of positive	% prevalence
113	31	27.4

T. gondii=Toxoplasma gondii

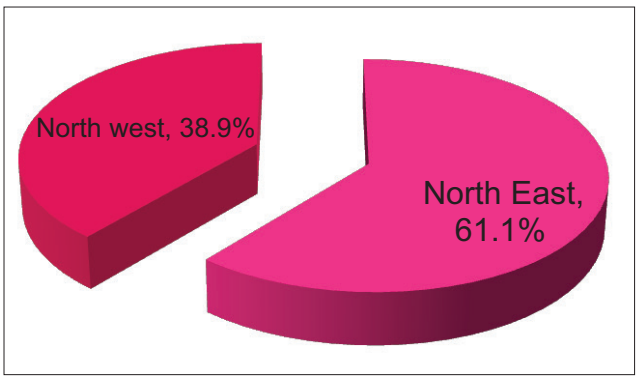

Figure-2: Sources of donkeys at Ganawuri market.

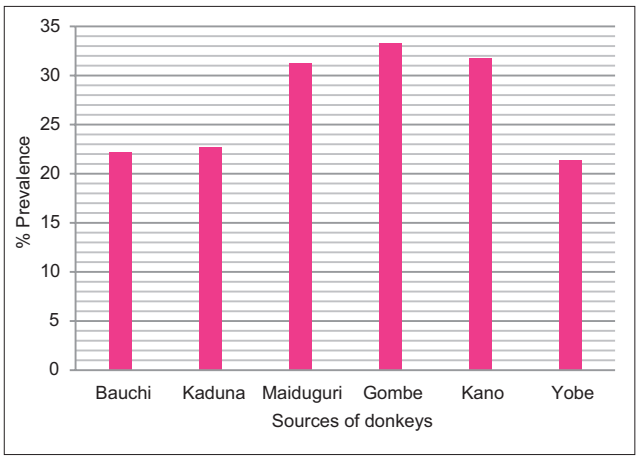

Figure-3: Seroprevalence of *T. gondii* in trade donkeys at Ganawuri district based on the source.

Table-2: Seroprevalence of *T. gondii* in trade donkeys in Ganawuri district market based on sex.

Sex	No. examined	No. of positive	% prevalence	p-value	OR
Male	31	9	29.0		
Female	82	22	26.8	0.494	0.05
Total	113	31			

T. gondii=Toxoplasma gondii

Table-3: Seroprevalence of *T. gondii* in trade donkeys at Ganawuri district market based on age.

Age (years)	No. examined	No. of positive	% prevalence	p-value	OR
4-6	40	5	12.5		
<6≤10	37	9	24.3	0.003	11.8
>10	36	17	47.2		

T. gondii=Toxoplasma gondii

differs significantly among the age group examined. Increased in age usually, portents increase in contact period of an animal with the contaminated environment [18]. This may explain the reason for the higher prevalence in those older than 10 years. Miao *et al.* [5], in contrast, reported no significant difference between the age group examined in their study. The seropositivity of *T. gondii* in donkeys did not differ significantly in relation to sex and source. This is in agreement with the report of Miao *et al.* [5] who reported a lack of significant difference in relation to sex and location or sources of donkeys in a study conducted in Southwestern China. This may suggest that both male and female donkeys have equal chances of being exposed to the parasite. In contrast, El-Ghaysh *et al.* [12] and Machacova *et al.* [15] in their studies reported a higher prevalence in females than in males.

Greater proportions of the donkeys sold at Ganawuri district market for slaughter were from the Northeastern part of the country. *T. gondii* seropositivity in donkeys in this study did not vary according to the source of the donkeys, although the prevalence rate differs with reports from other parts of the world. Similarities in the vegetation, climatic condition, cat abundance, and management practices in these areas (Northeastern and Northwestern part of Nigeria) where the donkeys were sourced might be the reason for lack of significant differences in the seropositivity.

Conclusion and Recommendations

This study revealed a relatively high prevalence of *T. gondii* in trade donkeys in Ganawuri district market, Riyom Local Government Area compared to those reported in other regions. The seropositivity increased with age of donkey and did not differ according to sex and source. Undercooked donkey meat in the area may represent a potential health risk for humans. Donkey meat should, therefore, be properly cooked before consumption. Proper washing of hands by meat handlers is also recommended. The impact of *T. gondii* on the productivity of donkeys in the area should be investigated while studies should be carried out to determine the seroepidemiology of the parasite in a larger sample population of donkeys and other species of animals. An attempt should also be made to isolate *T. gondii* cysts in the tissues of donkeys using bioassay to ascertain the presence of viable tissue cysts. More so, the strains circulating in the area should be identified.

Authors' Contributions

IBS conceived and designed the study. IBS and SBK developed the project. SBK collected the samples. SBK and IBS carried out the serology and SBK produced the tables. SBK and IBS wrote the draft of the article. IBS carried out the data analysis. Both authors read and approved the final draft of the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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