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Seroprevalence and Risk of *Toxoplasma gondii* Infection among Pregnant Women at Federal Teaching Hospital Gombe, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FMB, BVM and AAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ABS, AUEN and YTS managed the analyses of the study. Authors AAA, HUF and FS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Numerous studies have been published on *Toxoplasma gondii* infection, but no studies have been conducted on toxoplasmosis in the study area. Blood samples were obtained from pregnant women and sera were assayed using *Toxoplasma* kit (ToxG-046) and 112 out of the 400 women were positive with an overall prevalence of 28%. The highest prevalence was recorded among those in second trimester 57(33.5%), those within the age group of 0-19 years (<20 years) 74(52.86%) and also those that owned cats 50(55.6%). There was a significant association between the prevalence of *T. gondii* antibodies (IgG) with trimester, age and cat ownership.

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1. INTRODUCTION

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii,* an obligate intracellular protozoan parasite in the phylum Apicomplexa. The parasites infect most genera of warm blooded animals, including humans, but the primary host is felid (cat) family [1,2,3]. Toxoplasmosis is found worldwide, infections are particularly common in warm, humid climate at lower altitude [4]. It is an opportunistic infection associated with acquired immune deficiency syndrome (AIDS) and has been described as a neglected disease [5,6]. The disease can manifest as, acute, latent or cutaneous toxoplasmosis. During acute toxoplasmosis, symptoms are often an influenza-like: swollen lymph nodes or muscle aches and pains that last for months or more, people who received an organ transplant may develop severe toxoplasmosis [7].

Infections acquired during pregnancy can lead to congenital toxoplasmosis [8,3]. During the third trimester a fetus has increased risk of becoming infected, but the risk of damage to the fetus is decreased since most of the important developments have already occurred [9]. *T. gondii* is one of the most widespread parasites in nature and the importance of the parasite to obstetricians lies in the fact that although the great majority of infants born to mothers acquiring toxoplasmosis during pregnancy are asymptomatic, effects such as abortions, early infant mortality and blindness are known to be associated with this zoonosis, when mothers are infected during the first and second trimesters [10].

In sub-Saharan Africa, toxoplasmosis often remains undetected and untreated due to insufficient diagnostic procedures, man power and financial status of the patients. A good number of congenital malformations occur without knowing the specific cause and toxoplasmosis is one disease that causes congenital malformation. There is no documented information on the disease distribution among women of childbearing age in the study area. Women in the study area keep cats either as pets, or to drive away rats in the home. Thus the close association between them and cats which may provide the medium for transmission of the disease. This study was aimed at ascertaining sero-prevalence of *T. gondii* antibodies among pregnant women attending antenatal clinic at Federal Teaching Hospital Gombe.

2. MATERIALS AND METHODS

2.1 Ethical Consideration

Ethical approval was obtained from Federal Teaching Hospital, Gombe, Gombe State (NHREC:/25/10/13).

2.2 Study Area and Population

The prospectice cross-sectional study was carried out from January to April, 2016 at the Federal Teaching Hospital (FTHG), Gombe. It is localized within the city of Gombe, the capital of Gombe State, located between latitude 10º & 11º N within the Sahel Savannah belt. It has a population of about 2.1 million people and an area of 18, 000 square km. The temperature averages 30ºC with an annual rainfall of 1200 mm. A total of 400 pregnant women was recruited in this study. These preganant women were with and without HIV and within the age ranges 15-49, attending Antenatal Clinic (ANC) at Federal Teaching Hospital Gombe State. The consents were obtained from these subjects prior to study commentmence. Data form was used to record demographic profiles (e.g., age), risk factor (e.g., cat ownership) and obstretic history (e.g., Abortion, retained placenta and Stillbirth…..).

2.3 Sample Collection

About 5 mls of blood was collected from venepuncture of each pregnant woman and was immediately taken to the hematology laboratory for processing. The blood was centrifuged at 3,000 rpm for 5 mins, the sera were harvested, numbered and stored at -20ºC deep frozen till further used.

2.4 Anti-toxoplasma Antibodies Detection

All sera samples were screened using a commercial ELISA kit for anti-*Toxoplasma* IgG antibodies (IBL International GmbH, Hamburg, Germany) according to the manufacturer's instruction. In brief, it has a sensitivity of 96.6% and specificity 98.2%. The frozen samples were brought out and allowed to thaw and a clean disposable tip was used to dispense 90 µl of diluents and 10 µl sample into another container vortex tube mixture was used to thoroughly mix

the samples and diluents. One hundred microliters of each standard A-E provided with the kit and diluted samples were dispensed into respective wells of the microplate, leaving well A as substrate blank. The wells were covered with foil supplied with the kit and samples were incubated for 1 hour at 37ºC in water bath. After the incubation, foil removed and contents aspirated off and washed three times with washing interval of 5 seconds. *Toxoplasma* anti-IgG conjugate was dispensed and incubated for 30 mins at room temperature (20-25ºC). The plate micro-wells were washed and 100µl of Tetramethylbenzidine substrate solution was dispensed into all wells except the blank well and incubated for 15 mins at room temperature (20-25ºC) in the dark. Finally, when stop solution was added, the positive samples turned yellow from blue and the negative samples remain blue. The absorbance was measured at 620 nm. The Cut-off Index was calculated by determining the ratio of the absorbance of each sample to the absorbance of the standard B. Samples presenting Cut-off index values less than 0.8 were considered negative, 0.8 to 1.2 were considered equivocal and greater than 1.2 were considered positive.

2.5 Statistical Analysis

All data were entered into the statistical package for social sciences SPSS version 22 Association between various risk factors and the prevalence of *Toxoplasma* infection was determined using chi- square test where appropriate. Prevalence rates of Toxoplasmosis in various risk factors were also calculated by dividing the number of positive samples over the number of samples examined for each factor.

3. RESULTS

Table 1 shows the prevalence of *T. gondii* infection among pregnant women in different trimesters. Most of the women (44.5%) sampled were in their first trimester followed by second (42.5%) and the least sampled were in the third trimester (13.0%). The highest prevalence of *T. gondii* infection among the pregnant women was recorded among those in their second trimester 57 (33.5%), followed by first 38 (21.3%) and the least was in the third trimester 17 (32.7%). There was statistical significance (P=0.029) between the prevalence of *T. gondii* infection and the trimester of the pregnant women. Seventy four (52.86%) of pregnant women within the (<20) were positive for the presence of *T. gondii antibodies*, 29 (19.33%) of age group 20-39 were positive, while the least prevalence 9 (8.18%) was seen in (≥40) group. There was statistical significance (P=0.0001) between the prevalence of *T. gondii* infection and the age groups of the pregnant women in the study area.

The association of cat ownership and the prevalence of *T. gondii* antibodies is shown in Table 3. Three hundred and ten (77.5%) pregnant women sampled did not own cats while 90 (22.5%) owned cats. The prevalence of *T. gondii* antibodies was higher among women that own cats 50 (55.6%) than women that did not own cats 62 (20.0%). There was a significant association between prevalence of *T. gondii* antibodies and cat ownership among the pregnant women (OR= 5.00; 95% CI on OR: 3.032< OR < 8.246).

Table 4 shows the effect of medical history of the pregnant women on the prevalence of *T. gondii* antibodies. Thirty three (8.25%) of the pregnant women had previous history of abortion while the remaining 367 (91.75%) had never experienced abortion. Twenty (60.6%) of the women that had experienced abortion were positive for *T. gondii* antibodies while a greater percentage of the women with higher *T. gondii* antibodies 92 (25.1%) were among those women that have never had aborted pregnancies. There was a significant association between the prevalence of *T. gondii* antibodies and previous history of abortion (OR= 4.60; 95% CI on OR: 2.005< OR < 9.611).

Table 1. Prevalence of *T. gondii* antibodies in pregnant women attending antenata clinic of Federal Teaching Hospital Gombe, Gombe State

Trimester	Number examined (%)	Number positive (%)	Chi square		
			(χ^2)	Df	P-value
First	178 (44.5)	38 (21.3)	7.053	2	0.029
Second	170 (42.5)	57 (33.5)			
Third	52 (13.0)	17 (32.7)			
Total	400 (100.0)	112 (28.0)			

Df: Degree of freedom

Table 2. Association between prevalence of *T. gondii* antibodies and age group of pregnant women recruited in the study

Age (years)	Number examined (%)	Number positive (%)	Chi square		
			(χ^2)	Df	P-value
(<20)	140 (35.0)	74(52.86)	269.071	2	0.0001
20-39	150 (37.5)	29(19.33)			
(≥40)	110 (27.5)	9(8.18)			
Total	400 (100.0)	112 (28.0)			

Df: Degree of freedom

Table 3. Association of cat ownership and the prevalence of *T. gondii* antibodies among pregnant women

Cat ownership	Number examined (%)	Specific rate (%)	Odds ratio (OR)	95% confidence interval (CI) on OR
No[ref]	310 (77.5)	62 (20.0%)	1.00	3.032-8.246
Yes	90 (22.5)	50 (55.6%)	5.00	
Total	400 (100.0)	112 (28.0%)		

Table 4. Association between obstretic medical history and the prevalence of *T. gondii* antibodies among pregnant women

Medical history	Number examined %	Number positive %	Odds ratio	95% confidence interval on OR
Abortion				
Yes[ref]	33(8.25)	20(60.6)	1.00	
No	367(91.25)	92(25.1)	4.60	2.005-9.611
Retained placenta				
Yes[ref]	48(12)	5(10.4)	1.00	
No	352(88)	107(30.4)	0.27	0.103-0.691
Still birth				
Yes[ref]	44(11)	3(6.8)	1.00	
No	356(89)	109(30.6)	0.17	0.050-0.547

Ref: Reference category

4. DISCUSSION

The 28% prevalence of *T. gondii* antibodies recorded in this study suggests that the pregnant women in Gombe may be at risk of *T. gondii* infection. This is not particularly consistent with high endemicity observed from some previous studies carried out in certain parts of the country. In 1996, prevalence rates of 75% and 88% of *T.gondii* antibodies were observed in pregnant and postpartum women respectively [11]. A similar prevalence rate of 27.9% was obtained among pregnant women in Sokoto [12]. This is perhaps due to similar techniques used and in addition it could be associated with similar cultural and religious behave in the two study areas. A higher infection rate of 52.86% (74\140) was observed among women under the age group of <20 years as compared to other age groups. This may be a reflection of possible exposure by this age group of women to the environment which might have been contaminated with sporulated oocysts and hence serves as a medium that is critical for the transmission of parasites. This age group is fond of different social activities which may include keeping cats as pets, indiscriminate eating and drinking without caution, although no study was done to compare between age groups and toxoplasmosis. Based on trimester, the women mostly affected are in the second trimester, the reason may be that most women do not go for antenatal at first trimester because cultural believe. The prevalence of *T. gondii* antibodies was higher among women that own cats and this indicated that those having cats in their households are more likely to have *T. gondii* antibodies in their blood than those who do not keep cats. There was a significant association between prevalence of *T. gondii* antibodies and cat ownership among the pregnant women that attended antenatal clinic at the Federal Teaching Hospital, Gombe. This study showed that abortion, retained placenta as well as

stillbirth were highly significant. The reproductive problems exhibited by the women examined in this study are of public health significance which require more epidemiologic investigation.

5. CONCLUSION

In conclusion, the findings in this research revealed overall 28% prevalence of *T. gondii* antibodies and cat ownership are found to be associated with the infection. Age, gestational age, Abortion, retained placenta, as well as stillbirth were significantly associated with toxoplasmosis by the pregnant women attending the antenatal clinic at the Federal Teaching Hospital, Gombe, Gombe state, Nigeria. Thus, there is a need for wider surveillance of the disease in the state and Nigeria as a whole. Enlightment campign should be orgnaised to educate the communities on the disease and the associated risk factors. Screening of toxoplasmosis should be included as part of antenatal clinic for pregnant women in order to avoid congenital transmission associated with Toxoplasma infection during pregnancy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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