

Seroprevalence of *Toxoplasma gondii* infection in pregnant women attending selected General Hospitals in Yobe State, Nigeria

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Abstract

Toxoplasma gondii is an obligate intracellular parasite which affects warm blooded animals including humans and causes toxoplasmosis. It is an endemic zoonotic disease which can be transmitted by consuming undercooked meat or contaminated foods, its primary host is the domesticated cats. The parasite causes wide range of diseases which is unapparent and asymptomatic in immune-competent individuals but leads to serious health problems in immunosuppressed individuals and pregnant women. It is the main cause of congenital toxoplasmosis in pregnant women. This study was carried out to detect the presence of *T. gondii* in pregnant women attending selected general hospitals in Yobe State. A total of 360 samples were obtained from 3 senatorial district which are Gashua, Potiskum and Gaidam general hospitals with 120 samples each from each district. Gashua recorded the highest prevalence of 18.3% while Potiskum and Gaidem recorded 15.0% and 5.0% respectively with an overall prevalence of 12.77%. It showed a statistical significance at $p \leq 0.05$ using *Toxoplasma elisa* IgM capture kit. The mean differential and white blood cell count recorded from pregnant women in the study was relatively low as participants from Gaidam hospital recorded the lowest mean value at 2.50×10^9 cells/L while participants from Gashua hospital recorded 4.00×10^9 cells/L and pregnant women from Potiskum hospital recorded 3.30×10^9 cell/L. In conclusion, this zoonotic infection obtained from domesticated cats can be identified to cause congenital toxoplasmosis which can be fatal to the foetus.

Keywords: Congenital infection, Elisa kit, Pregnant women, *Toxoplasma gondii*, Toxoplasmosis,

INTRODUCTION

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Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii* (*T. gondii*), an obligate intracellular protozoan parasite in the Phylum Apicomplexa. The parasite infects warm blooded animals, including humans, but its primary host is felid (cat) family (Urquhart *et al.*, 2003; CDC, 2015; Tymoshenko *et al.*, 2015; Ohiolei & Clement, 2016, Obijiaku *et al.*, 2019, Silva-Díaz *et al.*, 2020). Toxoplasmosis has a widespread distribution in the world, infections are particularly common in warm, humid climate at lower altitude (Ahmad *et al.*, 2014, Obijiaku *et al.*, 2019). It is an endemic zoonotic parasitic disease which could be transmitted to man by consumption of infected undercooked meat and contaminated food items including drinking water (Ohiolei & Clement, 2016; Rouatbi *et al.*, 2019). The routes of disease transmission to humans include accidental oral ingestion of food or water contaminated with infectious oocysts, the consumption of raw or undercooked meat containing bradyzoite tissue cysts and transplacental transmission to the foetus during a primary infection in a pregnant woman (Wong & Remington, 1994; Singh, 2016); Of these modes of transmission, the oral route is considered more common (CDC, 2011; Zhou *et al.*, 2011; Silva-Díaz *et al.*, 2020).

Toxoplasma gondii infection is responsible for a range of human and animal diseases and have considerable medical and economic impact worldwide (Black & Boothroyd, 2000, Silva-Díaz *et al.*, 2020). Primary infection with this parasite is initially unapparent or asymptomatic and lead to serious complications in pregnant women (Ocak *et al.*, 2007; Linguissi *et al.*, 2012; Koki *et al.*, 2014), a significant cause of neonatal mortality and infant morbidity in the world (Binnicker *et al.*, 2010; Mehmet *et al.*, 2015). In addition, the signs and symptoms of congenital toxoplasmosis are macrocephaly, hydrocephaly, miscarriage, ocular malformation (such as chorioretinitis), hepatosplenomegaly, lymphadenopathy and central nervous system abnormalities. It becomes an opportunistic infection in association with other infection (Rodier *et al.*, 1995; Ferreira & Borges, 2002; Hill & Dubey, 2015).

Congenital infections are the most important causes of perinatal morbidity and mortality, particularly in developing countries (Aynioglu *et al.*, 2015; Sharma *et al.*, 2015). The transient immunosuppression increases the vulnerability of pregnant women to various infectious agents. The ability of the foetus to resist infection is limited and the foetal immune system is unable to prevent the dissemination of infectious microorganisms to various tissues (Sebastian *et al.*, 2008; Parlak *et al.*, 2015). A prevalence of 22% was reported by Ohiolei & Isaac (2016) in north eastern Nigeria and data on toxoplasmosis is sparse in the selected study area therefore, this study is aimed at detecting the seroprevalence of *T. gondii* in pregnant women attending selected general hospitals in Yobe State.

MATERIALS AND METHODS

Study area/Ethical consideration

A total of 360 blood samples were obtained from three general hospitals in Yobe state, Nigeria. 120 blood samples from each senatorial zone which are North (Gashua), South (Potiskum) and East (Gaidam). Ethical approval was obtained from the Research Ethical Committee of Ministry of Health Damaturu, Yobe state and Individual Informed Consent was sought from each pregnant woman prior to collection of samples. Confidentiality was ensured for consented participants

Collection of Blood sample

Three milliliters of blood was collected from participating pregnant women by trained laboratory scientists for a period of 4 months from December 2019- June 2020. About 2ml of blood sample was dispensed into a serum separator container for serology and 1ml was dispensed into an EDTA container for differential cell count.

Detection of *T. gondii* Antibody among Pregnant Women

The method as described by Ballah *et al.*, 2017 for anti-toxoplasma antibodies detection was adopted. TOXOPLASMA ELISA IgM CAPTURE was used to capture antibodies against *Toxoplasma gondii* in human serum or plasma (Vircell, Parque Tecnológico Granada, Spain) by following manufacturer's instructions. Incubator was set at 37±1°C and all reagents were brought to room temperature approximately an hour without removing the plate from bag before use, refrigerated samples were brought to room temperature prior to use. All components were shaken, plates were removed from package and the numbers of wells in plates were counted in four wells for the control (two for cut-off serum and one each for positive and negative sera). One hundred microlitre of sample dilution was added into all wells except the four assigned controls, 5µl of each sample was added and 100µl of positive control was added to all components. One hundred microlitre of cut-off in duplicate and 100µl of negative control was added into the corresponding wells. The plates were covered with a sealing sheet and incubated at 37±1°C for 60 minutes. Conjugate preparation was done according to manufacturers' instruction. Seal was removed, liquid aspirated from all wells and washed five times with 0.3ml washing solution and plates were drained of any remaining liquid. Immediately, 100µl of reconstituted conjugate was added into each well and covered with a sealing sheet, incubated at 37±1°C for 60 minutes. Seal was removed, liquid aspirated from all wells, washed five times with 0.3ml of washing solution per well and the remaining liquid drained off. Immediately, 100µl was added of substrate solution into each well, incubated at room temperature for 20 minutes and protected from light. 50µl of stopping solution was immediately added into all wells. Plates were read with a spectrophotometer at 450nm within an hour of stopping. Interpretation was done according to manufacturers' guidance as samples with index below nine (<9) were considered negative with no IgM specific antibody against *T. gondii* and those with index above eleven (>11) were considered positive for IgM specific antibody against *T. gondii* and those with 9-11 are equivocal which means samples to re-run for confirmation.

Differential leucocyte count

The method described by Cheesebrough (2006) was adopted for differential cell count. A drop of blood was thinly smeared on a clean glass slide and the slide was allowed to air dry. Three drops of Giemsa stain was added to the slide, allowed for seven minutes before adding two drops of water to dilute the stain, the slide was allowed to stand for three minutes before washing with water and allowed to air dry. The film was examined microscopically and the different leucocytes were counted using mechanical differential cell counter.

Statistical analysis of data

The results were presented as percentages and presented in Tables and Figure. The relationship between the data were analysed using chi square (χ^2) and odds ratio (OR). Values of $p < 0.05$ were considered significant at 95% confidence interval.

RESULTS AND DISCUSSION

Seroprevalence of *Toxoplasma gondii* infection in pregnant women attending selected General Hospitals in Yobe State, Nigeria.

The prevalence of *T. gondii* infection at 12.77% was recorded in 46 pregnant women attending three selected General Hospitals in Yobe State, Nigeria as shown in fig 1. The percentages obtained from 46 sero positive pregnant women showed that 6 women at 5.0% were attending General hospital Gaidam while 22 women at 18.3% were attending General Hospital Gashua and 18 women at 15.0% were attending General Hospital Potiskum as shown in Table 1. The highest seroprevalence was recorded in Gashua general hospital which showed statistical significance at $p \leq 0.05$ as seen in Table 1.

Table 2 shows the mean differential leucocyte counts for pregnant women with seropositive *Toxoplasma gondii* infection from selected hospitals. Pregnant women attending Gaidam general hospital recorded a higher mean value for lymphocytes 30 as compared to the record of 28 and 25 for Gashua and Potiskum general hospitals. Pregnant women from Gashua hospital recorded a lower mean for neutrophils 61 as compared to 70 and 65 for Gaidam and Potiskum hospitals. The mean monocyte count for consented pregnant women attending Potiskum general hospital recorded 8 while Gashua hospital recorded 6 with no value recorded for Gaidam hospital. The mean eosinophil value recorded from consented participants showed that Gashua hospital had 4 and 2 for Potiskum while Gaidam hospital recorded 0 value. The pregnant women from Gashua hospital recorded 1 mean value for basophil while Potiskum and Gaidam hospitals recorded 0 value. The mean WBC counts obtained for seropositive participants showed that Gaidam recorded the lowest mean at 2.50×10^9 cell/L while participants from Gashua recorded the highest mean at 4.00×10^9 cells/L.

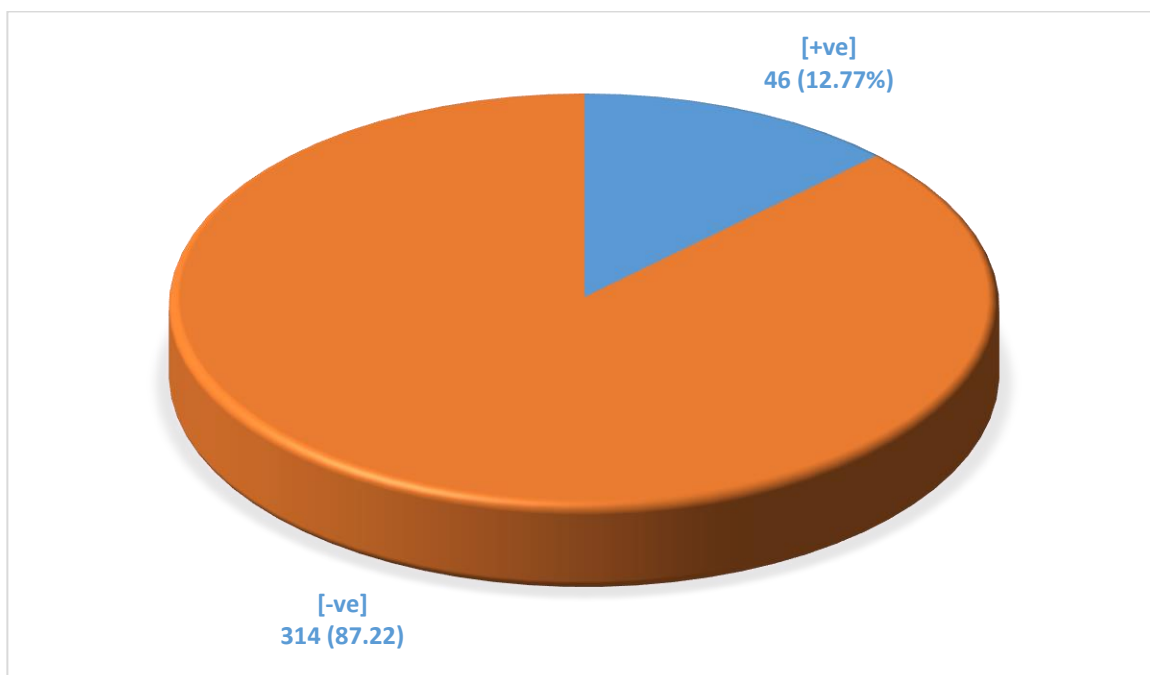


Figure 1: showing seroprevalence of *T. gondii* infection in pregnant women attending selected General hospitals in Yobe State.

Table 1: The seroprevalence of Toxoplasmosis among pregnant women from each General Hospital in the selected study area.

Seroprevalence of *Toxoplasma gondii* infection in pregnant women attending selected General Hospitals in Yobe State, Nigeria.

Hospitals Examined	No Examined pregnant women	No of positive Women	Prevalence (%)	X ²	p-value
GDM	120	6	5.0		
GSH	120	22	18.3		
PKM	120	18	15.0		
Total	360	46		10.3683	0.0056*

Key: GDM- Gaidam, GSH- Gashua, PKM- Pokiskum.

*Statistical significant at $p \leq 0.05$.

Table 2: The mean differential leucocyte count for pregnant women from selected hospitals in study areas.

Hospitals	No examined	No positive	Mean differential count					Mean WBC(Cells/L)
			L	N	M	E	B	
Gaidam	120	6	30	70	0	0	0	2.50x10 ⁹
Gashua	120	22	28	61	6	4	1	4.00x10 ⁹
Potiskum	120	18	25	65	8	2	0	3.30x10 ⁹

Key: L-Lymphocyte, N-Neutrophil, M-Monocyte, E-Eosinophil, B- Basophil.

WBC- White blood cell count.

This study showed a significant relationship between *T. gondii* infection and pregnant women from the selected study area with Gashua having highest prevalence and Gaidam lowest value. This is an indication of active infection and also the prevalence clearly varies among the study area. The overall seroprevalence of 12.77% reported in this study is relatively lower than the report of Ballah *et al.* (2017) with a prevalence of 28% in Gombe state, 27.9% was obtained among pregnant women in Sokoto by Alayande *et al.* (2013). Oyinloye *et al.* (2014) also recorded a prevalence of 22.2% in Maiduguri municipal council, Borno State and 31.3% by Bello *et al.*, 2017 in Kaduna state, comparable to this report are the findings in Lagos and Maiduguri of 7.6% (Deji-Agboola *et al.*, 2011; Obijiaku *et al.*, 2019) and 8.9% (Nasir *et al.*, 2015; Obijiaku *et al.*, 2019). In comparison with other studies conducted outside Nigeria, a lower prevalence has also been reported in different countries: United Kingdom, 7.7%-9.1%, Norway, 10.9%; Spain, 18.8% and Sweden, 14-25.7% (Gulden *et al.*, 2009). The presence of IgM detected among the pregnant women is indicative of either a recent *T. gondii* infection or reactivated dormant toxoplasmosis (Ishaku *et al.*, 2009). Cats are the definitive hosts that shed oocysts which infects humans and animals, therefore, pregnant women who have close contact with domestic cats have the highest risk of contracting the disease which can lead to congenital toxoplasmosis, perinatal morbidity and mortality also, poor eating food habits, personal hygiene and management practice in animal husbandry (rearing of animals) plays important roles in the spread of *T. gondii* infection. Therefore, educating pregnant women on food hygiene, avoidance of eating/tasting undercooked meat or raw vegetables and exposure to cat faeces during antenatal care is of utmost importance.

The white blood cell plays different role in protecting the body against foreign pathogens, defend the body and checks the overall white blood cell in the body and its proportion. The threshold for white blood cell count varies between individuals from 4,000 to 11,000 cells per blood but below 4,000 cells per blood in an adult is an indication of pathogenic infection. The mean differential and white blood cell count recorded from pregnant women in the study was relatively low as participants from Gaidam hospital recorded the lowest mean value at 2.50x 10⁹ cells/L while participants from Gashua hospital recorded 4.00x 10⁹ cells/L and pregnant women from Pokiskum hospital recorded 3.30x 10⁹cell/L. This results correlates with the report of Hopkins (2017) and Silva-Díaz *et al.* (2020). The low mean value observed in this

study could be due to pre-existing underlying diseases predisposing the seropositive pregnant women to opportunistic pathogens, thus weaken the immune system.

CONCLUSION

In conclusion, this study shows the presence of *T. gondii* antibodies in pregnant women attending selected hospitals in Yobe State. This zoonotic infection obtained from domesticated cats can be identified to cause congenital toxoplasmosis which can be fatal to the foetus. Therefore, it is important to educate pregnant women on the modes of transmission and prevention of *T. gondii* during routine antenatal care.

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