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MOLECULAR DETECTION OF TOXOPLASMA GONDII B1 GENE AMONG PREGNANT WOMEN ATTENDING ANTE NATAL CLINICS IN ZARIA, KADUNA STATE, NIGERIA.

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ABSTRACT

Toxoplasmosis is caused by *Toxoplasma gondii* an obligate intracellular parasite which cause severe complications to an infected mother if the primary infection was acquired during pregnancy. This includes spontaneous miscarriage, low birth weight, congenital deformities and intrauterine death. The aim of the study was to determine the seroprevalence of *Toxoplasma gondii* among pregnant women attending antenatal clinics in Zaria, Kaduna State. This Hospital-based and cross-sectional study was carried out in three Hospitals. The samples were collected from Gambo Sawaba General Hospital Kofan Gayan, Ahmadu Bello University Medical Centre and Major Ibrahim Bello Abdullahi Memorial Hospital. A total of 182 blood samples were collected. Enzyme Linked Immunosorbent Assay (ELISA) was used to determine the seroprevalence of *Toxoplasma* IgM antibodies. A structured questionnaire was also used to collect information on socio-demographic characteristics and factors associated with *Toxoplasma* infection. The study established an overall seroprevalence of 19.2%. There were no significant associations between *T. gondii* infection with all demographic and risk factors measured in this research ($p>0.05$), except ownership of cat (OR=0.23, 95%CI=0.1 – 0.5, p 0.00) and blood transfusion (OR=0.04, 95%CI=0.04 – 0.50, p 0.01), which showed significant association ($p<0.05$). All thirty five positive samples for ELISA were subjected to polymerase chain reaction to detect the *B1* gene of *Toxoplasma gondii*, six (14.2%) came out positive. Toxoplasmosis poses a great threat to the foetus, and as such a routine test should be done on pregnant women in Nigeria.

Keywords: *T.gondii*, *B1 gene*, Seroprevalence, PCR, ELISA

1.0 INTRODUCTION:

Toxoplasmosis is a zoonotic parasitic disease caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite in the phylum Apicomplexa and found in many species of animals throughout the world. Approximately 30% of human population worldwide is chronically infected with *Toxoplasma gondii.* Toxoplasmosis is one of the most prevalent and widespread parasitic infections in West Africa, which is often neglected, especially in developing countries (Manuel *et al.,* 2020).

The infection caused by this organism is usually mild and asymptomatic causing cold and flu in immune competent individuals, but in up to 20% of infected persons, manifestation can lead to cervical lymphadenopathy, mononucleosis-like syndrome or inflammation. In immune compromised individuals, such as acquired immune deficiency syndrome (AIDS) patients, primary or reactivated infection can also result in encephalitis, pneumonia and inflammation of the heart muscles (Varsa *et al.,* 2021)

Humans who are accidental hosts usually acquire infection via ingestion of tissue cysts in undercooked meat, consumption of raw or uncooked foods such as vegetables and fruits and water that have been contaminated with sporulated oocysts, or vertically by transplacental transmission of tachyzoites (Fadel *et al.,* 2025).

Human infections are typically asymptomatic, but the parasite can cause deadly diseases. Only approximately 10% of infected people experience symptoms. Oocyst-induced infections are more severe than those brought on by tissue cysts because tissue cysts contain the bradyzoites that characterize the chronic phase of infection but do not cause a strong immune

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response, in contrast to oocysts that, upon ingestion, are released and transform into tachyzoites that can cause a strong immune reaction (Aghayan *et al.,* 2024)

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Zaria a big city in Kaduna state with a total area of 563km^2, a density of 730/km^2 and an estimated population of 809,652 (Zaria Population, 2025). Zaria city is blessed with farmlands where people farm during both rainy season and the dry season. And are well known for farming vegetables such as tomatoes, cabbage, onions, and carrots. Women are also engaged in selling those vegetables and, in the process, eat without proper washing or cooking consume those vegetables. There are many suya joints around where people buy stick meat referred to as " suya" some of which are not properly handled. Most houses in the area keep cats around as pets, which are one of the major reservoirs for the parasite *T. gondii*. All these are risk factors predisposing people to toxoplasmosis.

3.2 Study Design

A Convenience sampling method was used in the selection of the study population from Ahmadu Bello University Medical Centre, Gambo Sawaba General Hospital Kofan Gayan, and Major Ibrahim Bello Abdullahi Hospital Zaria, Nigeria. The study was hospital based and crossectional.

3.3 Study Population

The study population were pregnant women attending antenatal care at Ahmadu Bello University Medical Centre, Gambo Sawaba General Hospital Kofan Gayan, and Major Ibrahim Bello Abdullahi Hospital Zaria, Nigeria.

3.4 Inclusion criteria

Pregnant women attending antenatal care on their first booking in the two health facilities and who gave consent were included in the study.

3.5 Exclusion criteria

Pregnant women who did not consent or did not register with the clinics and those on subsequent visit were excluded during the study.

3.6 Ethical Approval

The Ethical Approval for the study was obtained from the Research and Ethics Committee of the Ministry of Health, Kaduna State.
Ministry of Health, Kaduna State

3.7 Sample Size

A sample size of 182 was obtained using a previous prevalence of 8.9% by Idris *et al.* (2015)

$N= Z^2 (1 - P)/d^2$

Where n = sample size,
Z = Z statistic for a level of confidence,
P = expected prevalence or proportion (8.9%)
d = precision (in proportion of one; if 5%, d = 0.05).

$N = (1.96)^2 x\ 0.89(1-0.89) \mid (0.05)^2$
$=3.8416 \times 0.11 \mid 0.0025$
$=150.437056$
$=150$

Sample size =150 samples.
For more precision and accuracy, 182 samples were collected for the study.

3.9 Sample Collection and Processing:

Blood samples of 5 milliliters were collected from the participating pregnant women using an aseptic venipuncture technique in sterile plain containers. These samples were then transported in cold packs to the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University in Zaria, Nigeria. After that, the blood samples were then centrifugation at 1000 rpm for 3 minutes to in order facilitate the separation of serum, which was subsequently stored in a refrigerator at -20°C for further analysis. A total of 182 samples were screened to detect

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IgM of *Toxoplasma gondii* using Enzyme Immunosorbent Assay (ELISA) All procedures were conducted in accordance with the manufacturer's instructions. Out of the 182 samples analyzed, 35 tested positive for *Toxoplasma gondii* IgM and were subjected for further examination aimed at detecting the *Toxoplasma gondii* B1 gene.

3.9.1 Detection of *Toxoplasma gondii* using Polymerase Chain Reaction

Only positive samples were subjected to PCR for the detection of *T. gondii* DNA. A total of thirty-five IgM positive samples were subjected to polymerase chain reaction for the detection of *T. gondii* B1 gene.

3.9.2 Extraction of DNA

Two hundred (200µl) of each serum was added into PCR tubes, 200µl of lysis buffer was then added into the tubes. The tubes were then put on a vortexing mixer for 15seconds and then incubated at room temperature of 25°C for 30minutes. Two hundred microlitre 200µl of absolute ethanol was added and centrifuged at 14000rpm for 60seconds, the supernatant was discarded. Four hundred ML 400µl of ethanol solution was added and centrifuged at 14000rpm for 60 seconds. The supernatant was discarded and centrifuged at 14000rpm for 3minutes. Thirty (30µl) of distilled water was added into the tubes and incubated at room temperature for 5minutes. After incubation, it was then centrifuged at 14000rpm for 1minute.

3.9.3 PCR Master mix preparation and condition

The PCR amplification was carried out using Qiagen Top Tap. A master mix solution was prepared containing, 12.5 µl of Qiagen Top Tap master mix, 1 µl of each primer, 5.5 µl of nuclease free water, 1.5 ul of coral load. The mixture was vortexed and transferred into applied biosystem 9700 programmed with the following cycling conditions: 45cycles of denaturation at 94°C for 1minute, annealing at 60°C for 1 min, extension at 72°C for 7 mins.

Table 1: Primers for amplification of Toxoplasma gondii B1 gene

Primer	Primer sequence	Expected amplicon size	Reference
Forward primer(5' to 3')	GGA ACT GCA TCC GTT CAT GAG	200bp	Saad *et al.* (2012)
Reverse primer(5' to 3')	TCT TTA AAG CGT TCG TGG TC		

3.9.4 Procedure for gel electrophoresis

A total of 1.5g of agarose powder was dissolved in 100mls of Tris-Acetate-EDTA buffer using microwave oven and was allowed to cool. Thereafter, five (5µl) of ethidium bromide was added and gently mixed. The mixture was poured into a casting tray already assembled with combs positioned. It was then allowed to solidify, after which the combs were removed. The casting tray was removed and immersed into the electrophoresis tank containing TAE running buffer. Ten (10)µl of the PCR product was mixed with 5µl of loading dye and then loaded into each well. The tank was then covered and connected to the power pack and the voltage was set at 75V and ran for 40minutes, the casting tray was then removed and viewed under gel documentation system connected to a computer.

4.0 RESULT

4.1 Agarose – gel plates of Amplified Polymerase Chain Reaction Product

Thirty five positive ELISA samples were subjected to PCR. Six were positive for Toxoplasma gondii B1 gene. Out of the six

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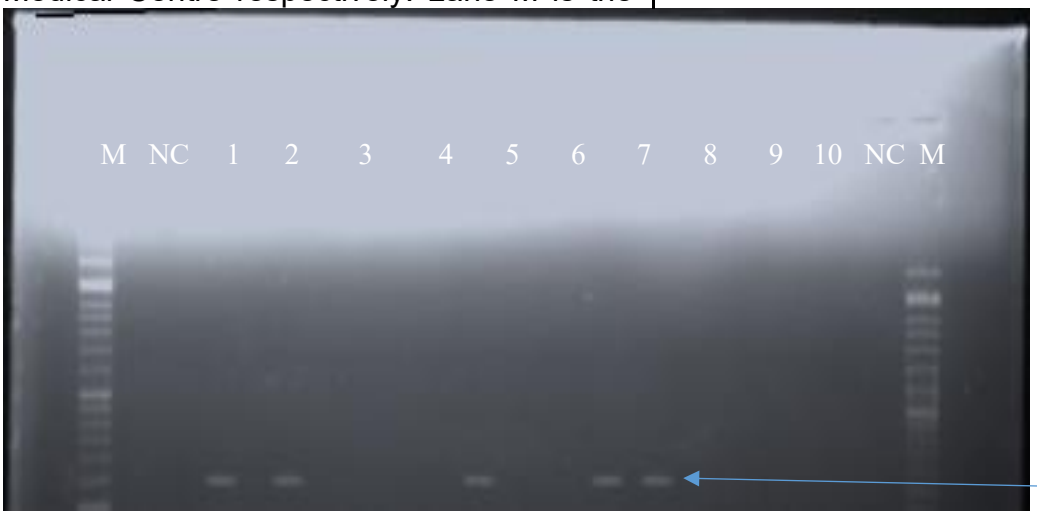
positive samples, four were from Gambo Sawaba General Hospital and one each from Major Ibrahim Bello Abdullahi Memorial Hospital and Ahmadu Bello University Medical Centre respectively. Lane M is the molecular ladder. Lane NC is the negative control. Lanes 1,2,5,7 and 8 in plate I are positive for *Toxoplasma gondii B1 gene* with an amplicon size of 200bp.



M NC 1 2 3 4 5 6 7 8 9 10 NC M

Plate I: *T. gondii B1* Gene Amplicons

KEY;

M: Molecular ladder

NC: Negative control

Lane 1: *T. gondii B1* gene from Major Ibrahim Bello Abdullahi Memorial Hospital

Lane 2, 5 and 7: *T. gondii B1* gene from Gambo Sawaba General Hospital

Lane 8: *T. gondii B1* gene from Ahmadu Bello University Medical Centre

5.0 DISCUSSION: In this study *B1* gene was detected in some of the samples. Detection of *B1* gene in the sample indicates recent infection which presents the danger of vertical transmission to the unborn baby. However out of the thirty-five ELISA positive samples only six were positive for *Toxoplasma gondii B1* gene this does not rule out the more sensitivity and specificity of PCR when compared to ELISA. ELISA has some limitations such as cross reactivity, specificity and avidity. This disparity might be due to a number of factors, including the small number of parasites circulating in the blood and the brief duration of parasitemia, a negative PCR cannot completely rule out a recent infection. Here, it is important to emphasize that the precise kinetics of parasites in humans is still unknown. Negative PCR findings can also be caused by factors such as the tiny size of the sample used for DNA extraction compared to the total amount of blood in the human body and the presence of blood constituents such as heme, hemoglobin, lactoferrin, and immunoglobulin G that may impede the PCR reaction (Fadel *et al.,* 2025). This is similar the studies of Uchida *et al.* (2025) that reported 8 positive *Toxoplasma gondii B1* samples out of 41 samples, in Brazil out of 128 samples only three where positive Colombo *et al.,* (2005) and one out of five was reported by (Rostami *et al.,* 2014)

CONCLUSION:

This study established that out of the 35 positive ELISA samples that were subjected to PCR to detect the B1 gene of *Toxoplasma gondii* only five were positive given a percentage of 14.2.

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