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# Recent Toxoplasmosis among Pregnant Women Receiving Antenatal Care at the University of Port Harcourt Teaching Hospital, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors ILO and OKO did the study design and wrote the protocol. Author ILO did the statistical analysis and literature searches while analyses of study was by author UCO. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To determine the seroprevalence of recent *Toxoplasma gondii* infection using IgG avidity Enzyme-linked immunosorbent assay (ELISA) among pregnant women in the University of Port Harcourt Teaching Hospital, Nigeria.
**Study Design:** A cross-sectional study.
**Place and Duration of Study:** Department of Medical Microbiology and Parasitology and Department of Obstetrics and Gynaecology, University of Port Harcourt Teaching Hospital (UPTH), Rivers State, Nigeria between January and June 2013.
**Methodology:** Involved two hundred and eighty-eight (288) pregnant women in their first trimester who gave informed consent. Questionnaires were administered to determine socio-demographic factors. Enzyme-linked immunosorbent assay (ELISA) was performed on all women's sera to detect

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anti-Toxoplasma Immunoglobulin G (IgG) and Immunoglobulin M (IgM) and the sero-positive samples were subjected to IgG Avidity ELISA. Data was analyzed using the statistical package Epi info version 6.04 d.

**Results:** A total of one hundred and eighty-nine (65.6%) pregnant women were seropositive for IgM and IgG among which thirty-three (11.5%) were positive for IgM only, Three (1.6%) among the one hundred and eighty-nine had low IgG avidity percentage while one hundred and eighty-six (98.4%) had high IgG avidity percentage giving a 1.04% seroprevalence of recent infection among these pregnant women.

**Conclusion:** One in every two pregnant women had been exposed; however, though only a small fraction of these were possibly recent infections, measures to prevent maternal toxoplasmosis in pregnancy as well as diagnose recent infections should be optimised due to the potential for congenital infection with its grave socio-medical implications. The IgG avidity test could therefore, be useful in avoiding unnecessary treatment for Toxoplasmosis in pregnancy.

*Keywords: Toxoplasmosis; pregnancy; recent infection; IgG avidity ELISA.*

## 1. INTRODUCTION

Toxoplasmosis caused by the protozoan parasite *Toxoplasma gondii (T. gondii),* an obligate intracellular parasite of many species of animals throughout the world, is a zoonotic disease [1].

The parasite was first discovered by Nicolle and Manceaux in 1908 in the tissues of a hamster-like rodent, *Ctenodactylus gundi*, at the Pasteur Institute in Tunis. It wasn't until 1939 however, that its medical importance was recognized when *T. gondii* was identified in tissues of a congenitally infected infant [2].

About 20% to 90% of the world's adult population in different regions is reported to have had contact with the parasite but only about 10% of infected individuals develop clinical signs and symptoms [3-5].

Toxoplasmosis presents a spectrum of clinical manifestations in humans and animals as well. It has been shown to have serious implications in pregnant women, congenitally infected fetuses and immunosuppressed persons who get infected thereby presenting a significant public health challenge [6]. It is the most common cause of intraocular inflammation in the world [3].

In the first trimester of gestation, the rate of transmission of *T. gondii* from an infected pregnant woman to her fetus is 10-15% and this may increase to 68% in the third trimester [7]. The severity of fetal disease varies inversely with the gestational age at which maternal infection occurred. This suggests that though maternal infections acquired early in pregnancy are less likely to be transmitted to the fetus, these are likely to have more severe consequences if the fetus does get infected [7]. Maternal Infection during pregnancy can result in spontaneous abortion, intra-uterine fetal death, hydrops fetalis, amniotic fluid disorders, hydrocephalus as well as neurological disorders such as chorioretinitis, blindness and mental retardation in congenitally infected neonates. Although clinical signs are not usually obvious at birth, approximately 80-85% of intra-uterine infections are likely to present with clinical manifestations later in life [7,8].

Immunosuppresion is a well documented risk for development of active toxoplasmosis or reactivation of the disease, no surprising Toxoplasmosis is a frequent cause of encephalitis in severely immunosuppressed patients with Acquired Immunodeficiency Syndrome (AIDS) [1,6]. In Port Harcourt where a high Human immunodeficiency virus prevalence rate of 7.3% was reported among pregnant women [9], it is troubling to note that immunosuppressed pregnant women could transmit infection to their babies even when they got infected prior to pregnancy [10,11].

Among the various techniques available for making a diagnosis of toxoplasmosis, the detection of *Toxoplasma*-specific antibodies using serologic techniques is the primary approach employed. The Enzyme-linked immunosorbent assay (ELISA) is one of the most commonly used serologic methods, is easy to perform and widely available [12].

A positive anti-*Toxoplasma* IgG titer indicates infection at some time however; a positive IgM result is difficult to interpret because *Toxoplasma*-specific IgM antibodies may continue to be detected by ELISA for up to 18 months after an acute infection. Various

intricacies with the interpretation of EIA results have been reported indicating that a major problem with *Toxoplasma*-specific IgM testing is lack of specificity [12]. Owing to the fact that anti-*Toxoplasma* IgM antibodies tend to persist for long periods, the time at which infection occurred may be difficult to ascertain using this since reactivation is not always accompanied by changes in antibody levels, and therefore the presence of IgM does not necessarily indicate recent infection. A negative IgM test on the other hand, essentially excludes recent infection [13].

In pregnancy however, it is important to determine the time of infection as this is a measure of the risk for congenital infection. If the patient is pregnant and IgG / IgM positive, it is recommended that the IgG avidity test which employs the Enzyme linked immunosorbent assay (ELISA) technique, should be performed [14]. *T. gondii*-specific IgG avidity is the strength with which the IgG attaches to the *Toxoplasma* antigen in the presence of urea, a protein denaturing agent which dissociates the antigen-antibody complex. It is based on the fact that IgG matures with time following primary infection. IgG produced within the first few months following primary infection exhibits low avidity, whereas that produced several months or years later exhibits high avidity [15]. The result (expressed as % avidity) reflects the extent of antigen-antibody complex dissociation caused by the denaturing agent. Several studies have shown that detection of *T. gondii*-specific IgG of low avidity reliably indicates infection within the previous eight months [16,17]. The detection of *T. gondii*-specific IgG of high avidity essentially excludes the possibility that infection occurred within the previous three to four months [18,19] therefore, a high avidity test result in the first 12 to 16 weeks of pregnancy (depends on the test kit used) basically rules out infection acquired during pregnancy [14]. IgG avidity has proven to be a powerful tool for distinguishing recent from past infection with *Toxoplasma gondii*.

This study was carried out to determine the seroprevalence of recent *Toxoplasma gondii* infection using IgG Avidity ELISA among pregnant women in Port Harcourt, Nigeria.

## 2. METHODOLOGY

A cross-sectional study carried out in 2013 to determine the seroprevalence of recent toxoplasmosis among pregnant women receiving antenatal care in the department of Obstetrics and Gynaecology of the University of Port Harcourt teaching hospital, Port Harcourt, Rivers State, Nigeria.

The Study Population was made up of pregnant women registered at the antenatal clinic whose gestational age was thirteen (13) weeks or below. They were consecutively recruited until sample size of 288 was achieved.

Five milliliters (5 ml) of venous blood was obtained using a sterile serum vacutainer needle and bottle, taken to the laboratory within one hour of collection, where tubes were centrifuged at 2500 revs per minute for 3 minutes. Clear sera was carefully collected into sterile tubes and stored frozen at -20℃ until tested.

Each sample was tested for the presence of anti-*Toxoplasma* antibodies, IgG and IgM, using commercial ELISA Kits (Diagnostics Automation Inc. / Cortez diagnostics, USA) and all IgM and IgG seropositive samples were tested for IgG avidity using the *Toxoplasma gondii* IgG Avidity kit (DRG diagnostics, Germany) following manufacturer's instructions.

For each patient's sample or control the percentage avidity (Avidity %) was calculated as the ratio between the absorbance of the well dispensed with Avidity reagent and the absorbance of the well dispensed with Washing buffer multiplied by 100:

> [Absorbance (sample or control) with Avidity reagent / Absorbance (sample or control) with washing buffer] x100 = Avidity (%)
>
> Avidity (%) > 40 = anti-*Toxoplasma* antibody with high avidity, interpreted as past infection.
>
> Avidity (%) ≤ 40 = anti-*Toxoplasma* antibody with low avidity implying acute or recent infection.

Substrate blanks and performance controls were included in all batches of testing. Their absorbance values were within the range indicated by the manufacturer, thus the results were deemed valid.

Data obtained from this study was analyzed using the statistical software package, *Epi-Info version 6.04d*, (Centres for Disease prevention and control [CDC], USA 2001). Chi-square analysis was used to determine the association

between the socio-demographic data and IgG anti-Toxoplasma seropositivity. The level of significance was set at 0.05.

## 3. RESULTS

Table 1 shows the socio-demographic distribution of the study participants. The minimum age was 22 years and maximum 46 years, with the highest number of participants, 198 (69.5%) lying between twenty-five (25) and thirty-four (34) years of age.

The minimum gestational age was four (4) weeks and the maximum was thirteen (13) weeks however, the highest number of patients was at eleven (11) weeks gestational age (Table 1).

**Table 1. Socio-demographic characteristics of participants**

| Demographic factor | Frequency N (n%) |
|---|---|
| **Age** | |
| 15-24 | 9 (3.1) |
| 25-34 | 198 (68.8) |
| 35-44 | 72 (25.0) |
| 45-54 | 6 (2.1) |
| No response | 3(1.0) |
| **Total** | 288 (100.0) |
| **Residential area** | |
| Urban | 165 (57.3) |
| Semi-Urban | 102 (35.4) |
| Rural | 21 (7.3) |
| **Total** | 288 (100.0) |
| **Educational status** | |
| Primary | 12 (4.2) |
| Secondary | 69 (23.9) |
| Tertiary | 192 (66.7) |
| No response (Blank) | 15(5.2) |
| **Total** | 288 (100.0) |

*N= Number of participants; n= Percentage value*

A total of one hundred and eighty-nine (65.6 %) samples were seropositive for IgG and/or IgM among which thirty-three (11.5%) were positive for IgM only. One hundred and twenty-three (42.4%) were positive for IgG only while thirty-three (11.5%) were positive for both IgM and IgG. Ninety-nine (34.4%) were seronegative.

Table 2 shows the analysis of the association between the socio-demographic data and anti-toxoplasma IgG seropositivity. *Seropositivity* was statistically significantly higher in the 25-34 year age group while residential area and educational status had no relationship with seropositivity.

IgG Avidity ELISA was performed on all one hundred and eighty-nine sero-positive samples. Three (1.6%) of these had low avidity percentage suggesting recent infection while the remaining one hundred and eighty-six (98.4%) had high avidity percentage indicating past infection (Table 3).

The seroprevalence of recent infection with *T. gondii* in this study, as determined by a low IgG avidity ELISA was therefore one percent (1.04%) (Fig. 1).

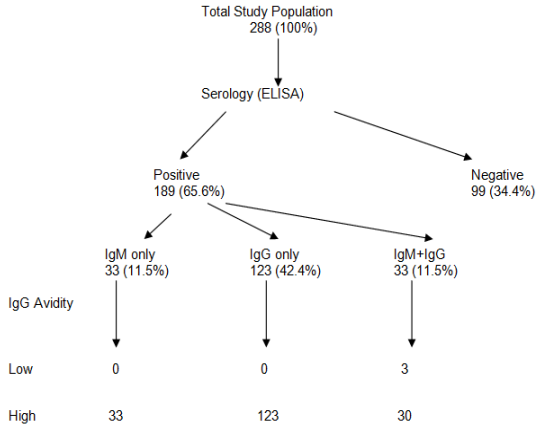


**Fig. 1. Serologic testing flowchart**

## 4. DISCUSSION

In the pregnant woman suspected to be infected with *Toxoplasma gondii*, the time of infection is very imperative in order for the managing obstetrician to take active precautions against transplacental transfer of this parasite to the fetus [20].

The seroprevalence of recent *Toxoplasma gondii* infection based solely on seropositivity for IgM in this study is 11.5%. This is similar to other studies in Nigeria and other parts of Africa [20]. However, the IgM ELISA cannot be relied on as an accurate indicator of recent infection in the pregnant woman due to the widely documented fact that anti-*Toxoplasma* antibodies of the IgM class, which ideally should indicate recent infection, tend to persist for very long periods, as much as one year or more and also due to the lack of specificity of *Toxoplasma*-specific IgM testing [22,12].

It's been noted that an estimated 1.3% of positive IgM detected during pregnancy are false positive and that as many as 20% of pregnant women

**Table 2. Association between socio-demographic data and IgG seropositivity**

| Socio-demographic factor | Serologic status | | Total | Chi-square ($\chi 2$) | *p-value* |
|---|---|---|---|---|---|
| | Positive N (%) | Negative N (%) | | | |
| **Age group** | | | | | |
| 15-24 | 3 (1.95) | 6 (4.58) | 9 (3.16) | | |
| 25-34 | 100 (64.94) | 98 (74.81) | 198 (69.47) | 17.78 | *0.001** |
| 35-44 | 51 (33.12) | 21 (16.03) | 72 (25.26) | | |
| 45-54 | 0 (0.0) | 6 (4.58) | 6 (2.11) | | |
| Total | 154 | 131 | 285 | | |
| **Residential area** | | | | | |
| Rural | 13 (8.33) | 8 (6.06) | 21 (7.29) | | |
| Semi-urban | 59 (37.82) | 43 (32.58) | 102 (35.42) | 1.767 | *0.413* |
| Urban | 84 (53.85) | 81 (61.36) | 165 (57.29) | | |
| Total | 156 | 132 | 288 | | |
| **Educational level** | | | | | |
| Primary | 6 (4.08) | 6 (4.76) | 12 (4.40) | | |
| Secondary | 38 (55.07) | 31 (24.60) | 69 (70.33) | 0.116 | *0.944* |
| Tertiary | 103 (70.07) | 89 (70.63) | 192 (70.33) | | |
| Total | 147 | 126 | 273 | | |

*N= Frequency, $\chi^2$= Chi-square, * p < 0.05 is statistically significant*

informed of a positive serologic result and its implications, will opt for a termination of pregnancy, which may lead to the abortion of many fetuses that might not be infected [23].

The IgG avidity ELISA has on the other hand, been proven by numerous researchers to be a much more reliable determinant of the time of infection [22,24]. The CDC recommends its use in the exclusion of recent infection in the pregnant woman who is found to be IgG/IgM seropositive in her first trimester of pregnancy [12].

Therefore using this technique, the prevalence of recent infection in this study as determined by a low IgG Avidity percentage was about one percent (1.04%) which is much lower than that based solely on IgM seropositivity. This prevalence is similar to findings from similar studies carried out in the United States of America and Korea [22,24]. Norway and Hungary had sero-conversion rates in pregnancy of 0.15% and 0.5% respectively [25,26]. In high risk populations however, sero-conversion rates as high as 3.5% may be observed [20].

It is worthy of note that the IgG avidity ELISA has a higher negative predictive value in that a high avidity percentage more reliably indicates that the woman was not infected in the past 5 to 8 months [15] whereas low avidity antibodies also tend to persist for much longer than expected (as much as one year or more), therefore it is recommended that the low avidity percentage result should also be interpreted with caution and requires further evaluation including the use of clinical assessment as well as Polymerase chain reaction (PCR) [22]. Therefore most of our study participants were not recently infected and as such their fetuses were not at risk for congenital transmission.

**Table 3. IgG avidity ELISA results**

| Avidity percentage | Frequency N (n%) |
|---|---|
| Low | 3(1.04) |
| High | 186(64.6) |
| **Total** | 189(65.6) |

*Key: N= Number of participants, n= Percentage value*

## 5. CONCLUSION

One in every two pregnant women had been exposed; however, though only a small fraction of these were possibly recent infections, measures to prevent maternal toxoplasmosis in pregnancy as well as diagnose recent infections should be optimised due to the potential for congenital infection with its grave socio-medical implications. The IgG avidity test could therefore, be useful in avoiding unnecessary treatment for Toxoplasmosis in pregnancy.

## CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Ethical committee of the hospital before study was commenced and written informed consent obtained from the study participants.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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