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Seroprevalence and the Associated Risk Factors of *Toxoplasma Gondii* Infection among Pregnant Women in The Middle Belt of Ghana

Derrick Adu Mensah[1, 2], Linda Batsa Debrah[1, 2], Richard Abeiku Bonney[3], Bhavana Singh[1, 4], Patience Bortie[5], Rebecca Safo[6], Clement Evans Aryee[7], Alexander Yaw Debrah[2, 8]

[1]Department of Clinical Microbiology, School of Medicine and Dentistry (KSMD), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[2]Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana
[3]Department of Health Policy, Management and Economics, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[4]Kwame Nkrumah University of Science and Technology (KNUST) Hospital, Kumasi, Ghana.
[5]Department of Development Policy, Ghana Institute of Management and Public Administration, Accra, Ghana
[6]Department of Obstetrics and Gynaecology, Korle-Bu Teaching Hospital, Accra, Ghana
[7]Goaso Municipal Hospital, Goaso, Ahafo Region, Ghana
[8]Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

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ABSTRACT

Background: Exposure to *Toxoplasma gondii* infection is prevalent in up to 90% of the world's human population. Severe infections can be fatal and deforming in neonates, with cats serving as reservoirs for *T. gondii* infections. The objective of this study was to determine the seroprevalence of *Toxoplasma gondii* infection and its associated risk factors among pregnant women in the middle belt of Ghana.
Subjects and Method: This was a cross-sectional study involving 266 pregnant women from two health facilities in the middle belt of Ghana. A structured questionnaire was administered to the participants to gather information on exposure to the associated infection risk factors and demography. In addition, 3ml of venous blood was collected from each participant for anti-*Toxoplasma gondii* IgG/IgM antibody analyses.
Results: A total of 64.3% of the study population had been exposed, whiles 26.3% were actively infected, being seropositive respectively to anti-*Toxoplasma gondii* IgG/IgM antibodies. In both districts, significant differences were recorded between anti-*T. gondii* IgG and IgM seroprevalences (95% CI= 0.00 to 0.02; p<0.001). There was no statistical difference observed between the participants' mean antibody concentration levels and age (95% CI= 0.74 to 0.85; p=0.637) as well as the stage of pregnancy (95% CI= 0.38 to 0.43; p= 0.127). Also, no strong association (OR<0.6) was found between seropositivity and the suspected risk factors assessed in the study.
Conclusion: The study showed that the seroprevalence of *T. gondii* infection among pregnant women visiting the two health facilities in the two regions was higher compared to the 30%-65% reported as the global population infection status. This study provides baseline data for future studies in other Districts and Regions in the country to ascertain the overall seroprevalence in Ghana and also push for a national programme/ policy for routine clinical screening of toxoplasmosis in pregnant women.

Keywords: seroprevalence, *Toxoplasma gondii*, pregnant women, anti-*T. gondii* IgG/IgM antibodies.

Correspondence:
Derrick Adu Mensah, Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana/Kumasi Center for Collaborative Research in Tropical Medicine, KCCR, Kumasi, Ghana. Mobile: +2332-6168-6052. Email: derrickadumensah@yahoo.com.

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BACKGROUND

Toxoplasma gondii is an obligate intracellular coccidian parasite that affects primarily members of the felid family but also most genera of warm-blooded animals, including birds and humans (Meerburg et al., 2006; Dubey and Jones, 2008; Guimarães et al., 2022). *Toxoplasma gondii* causes the disease toxoplasmosis and typically requires alternation of sexual and asexual reproductive stages in its life cycle (Hill et al., 2010). Toxoplasmosis infection in its severest form can be fatal in neonates and in immunocompromised individuals such as HIV patients and patients who have undergone organ transplantation or immunosuppressive therapies (Ayi et al., 2016). It is the most prevalent infection in humans (estimated to have infected 30–50% of the world population), more than latent tuberculosis which infects about one-third of the human population (Ayi et al., 2016).

The parasite exists in three main morphological forms: a) the first being the infective sporulated oocysts. The oocysts are remarkably stable environmentally, and are transmitted to other hosts through inadvertent ingestion; b) the trophozoite stage which is responsible for intracellular invasion; and c) the tissue cyst stage, which is either actively dividing (tachyzoites) or slowly dividing (bradyzoites) (Dubey, 2004; Giannoulis et al., 2008). T. gondii infections in humans occur through the ingestion of tissue cysts in poorly cooked meat, trans-placental transmission from mother to fetus *in-utero,* and rarely through organ transplantation and a blood transfusion from seropositive donors (Berger et al., 2009).

Generally, about 20% to 90% of the world's human adult population in different regions are reported to have had contact with the parasite. There are however lower seroprevalences in some countries especially those with quite low temperatures and humidities (Berger et al., 2009). It is reported that only about 15% of women of childbearing age are immune to toxoplasmosis (Jones et al., 2001). Women infected with *T. gondii* before being pregnant rarely transmit the infection to their fetuses. Unfortunately, women infected with *T. gondii* during pregnancy can transmit the infection across the placenta to their fetuses. The undeveloped immune system of fetuses makes them highly vulnerable when their mothers become infected for the first time during pregnancy (European Multicentre Study, 2003; Elbez-Rubinstein *et al.*, 2009). Research has shown that the risk of congenital transmission with acute maternal infection in the first trimester is the lowest (10–15%), with a maternal infection in the third trimester having the highest risk (68%) (Munoz, Liesenfeld and Heimesaat, 2011; Robert-Gangneux and Dardé, 2012). However, acquisition of the acute infection in the

first trimester by a pregnant mother has the severest complications on the fetus if transmitted (Robert-Gangneux and Dardé, 2012).

Infection acquired during the first trimester may lead to spontaneous abortion, stillbirths, mental retardation, hepatosplenomegaly, jaundice, chorioretinitis, hydrocephalus, convulsions, intracerebral calcifications and other disseminated infections, whiles infection acquired later during pregnancy is usually asymptomatic and sub-clinical in the neonate (European Multicentre Study, 2003; Ayi et al., 2009). Hydrocephalus is the least common but the most dramatic lesion of congenital toxoplasmosis, with the ocular disease being the most common sequelae of toxoplasmosis (Leung et al., 2020).

Studies on the seroprevalence of toxoplasmosis in the middle belt of Ghana are scarce. However, studies on the infection have been conducted in pregnant women in the southern part of the country (Ayeh-Kumi et al., 2010; Ayi et al., 2016; Kwofie et al., 2016). A seroprevalence of 76.0% of the anti-*Toxoplasma* antibodies was reported among pregnant women in Mamprobi Clinic in Accra, Ghana. Other similar studies in the Greater Accra Region reported overall seroprevalences which ranged from 29.7% to 92.5% (Ayeh-Kumi et al., 2010; Ayi et al., 2016; Kwofie et al., 2016).

Toxoplasmosis is a widely distributed infection that affects humans, pets, and livestock in both developing and developed countries including Ghana (Saadatnia and Golkar, 2012). Since many households in the middle belt of Ghana own pets (Arko-Mensah et al., 2000) which serve as reservoirs for *T. gondii*, there is the need to assess the prevalence of toxoplasmosis in the middle belt. Also, during pregnancy, there is the occurrence of a high cascade of complex physiological conditions that enables the mother to fully adapt to the environment. Immune-suppression is noted to be a physiological change that the body of the mother induces for the prevention of fetal rejection as the fetus is considered as an allograft. This, therefore, makes pregnant women susceptible to many opportunistic infections such as toxoplasmosis (European Multicentre Study, 2003; Munoz, Liesenfeld and Heimesaat, 2011). However, in Ghana, like most sub-Saharan African countries, there is no national programme nor policy for screening pregnant women for toxoplasmosis. This necessitated the current study in the middle belt of Ghana. This study, therefore, sought to determine the seroprevalence of *T. gondii* infection and its associated risk factors among pregnant women seeking antenatal care (ANC) at the Municipal Hospital Goaso, in the Asunafo North District of the Ahafo Region and St. Michael's Catholic Mission Hospital at Pramso in the Bosomtwe District; Ashanti Region, Ghana.

SUBJECTS AND METHOD

1. Study Design

The study takes the form of a cross-sectional screening of pregnant women at various trimesters attending antenatal care at the Municipal Hospital, Goaso in the Asunafo North district of the Ahafo Region, and the St. Michael's Catholic Mission Hospital at Pramso in the Bosomtwe District, Ashanti Region from November 2017 to June 2018. These health facilities were selected purposely because they are the district and sub-district health referral centres'— having the largest patient inflow in their respective districts with large catchment areas. Each health center has a minimum of twenty (20) pregnant women visiting the ANC unit in a week. Thus, there is a constant inflow of pregnant women within each health facility which made the sites ideal for the research objectives.

2. Population and Sample

Participants aged 16 years or more (≥16) were randomly selected and study objectives

and procedures were explained to them when they visited the hospital for their routine weekly antenatal care procedures. After every explanation, their questions and concerns were addressed satisfactorily. Informed consent forms were either signed or thumb-printed by willing participants after which closed-ended structured questionnaires were administered to gather demographic information as well as the exposure to the various risk factors from the eligible study participants. Venous blood was then collected from the median cubital superficial vein of the upper limb using sterile disposable hypodermic vacutainer needles and gel clot tubes for serological (anti-*Toxoplasma gondi*i IgG and IgM) analysis.

The recruitment involved random sampling of 146 and 120 pregnant women aged between 16 to 40 years seeking antenatal care respectively in the Asunafo North and the Bosomtwe Districts between November 2017 and June 2018. The sample size was calculated based on the estimation of a population parameter for cross-sectional studies as proposed by Charan and Biswas, in 2013 (Charan and Biswas, 2013).

Sample size (N) = $[Z^2 (P) (1-P)]/(E)^2$. Where Z= 1.96 at type I error of 5%; that is, 95% confidence interval. P= previous toxoplasmosis seroprevalence of 92.5% (0.925) as reported by Ayi et al. (2009) and E is absolute error = 5% (0.05). Using the above-stated population parameter for cross-sectional studies, the study was set out for a minimum sample size of 107 to be recruited from each study site. A 10% (of the minimum 107) participant drop or fall-out rate was also considered and that brought the minimum study number from each hospital to ~118. To be included in the study, the pregnant woman had to be 16 years or more and not anaemic (that is Hb>8.0 g/dl) since the study involved venous blood sampling. The study participants should be a resident in the respective region and should have consented to be a participate in the study.

3. Study Variables

The independent variables that were considered in the data curation were risk factors to the Toxoplasmosis infection which included the eating of vegetables, contact with or ownership of cats/ pets or the presence of cats in the participants household or immediate surroundings. Also, the participants' knowledge on the toxoplasmosis disease and the exposure to the various known transmission risk factors such as the handling and the frequency of eating meat from high transmission risk animals (pigs, sheep, and goats) and form they preferred their meat (thoroughly and/ or partially cooked). The outcome of participants' responses on the risk factors and the odds of acquiring the toxoplasmosis infection represented the dependent variables in thus study.

4. Operational Definition of Variables

Infection risk factors were defined as socio-cultural practices that predispose the pregnant woman to the toxoplasmosis infection and increase their chances of acquiring the infection.

Testing positive for the Toxoplasmosis infection was defined as having sample optical density ratios greater than or equal to 1.0 for either anti- *Toxoplasma gondii* IgG, IgM or both.

Testing negative for the Toxoplasmosis infection was defined as having sample optical density ratio less than 1.0 for either anti-*Toxoplasma gondii* IgG or IgM.

5. Study Instruments

Predefined close-ended structured questionnaires were administered by the investigators (research team) at a place far from the gathering, with participants assured of data safety and confidentiality of their responses. The questionnaires which sought to collect socio-demographic information as well as toxoplasmosis-related risk factors data were

administered in an interview format in participants' mother language (Twi/ Akan language) for easy understanding

The structured questionnaires included the eating of vegetables, determination of participants contact with or ownership of cats/ pets or the presence of cats in the participants household or immediate surroundings. Also, the participants' knowledge on the toxoplasmosis disease and the exposure to the various known transmission risk factors such as the handling and the frequency of eating meat from high transmission risk animals (pigs, sheep, and goats) and form they preferred their meat (thoroughly and/or partially cooked).

Sample collection and laboratory tests

About 3 milliliters of venous blood was collected from the median cubital superficial vein of the upper limb using a sterile disposable hypodermic vacutainer needle from each participating pregnant woman into a gel clot activated tube. One milliliter of serum was collected from the whole blood sample of each participant into 1.5ml Eppendorf tube after centrifuging at 2000g for 10 minutes without breaks and preserved at -20°C for later serological analysis at the Kumasi Center for Collaborative Research in Tropical Medicine (KCCR). The samples were analysed qualitatively against positive and negative controls for the presence of anti-*T. gondii* IgG which depicts previous or past exposure to the infection and anti-*T. gondii* IgM antibodies denoting current or present infection status using a commercially produced ELISA kit (RecombiLISA Toxo Test Kit, Fortress Diagnostics Limited, unit 2C Antrim Technology Park, BT41 1QS, UK) in accordance with manufacturer's instructions. The kit had a specificity and a sensitivity of 99% (98.3%-99.5%) and >99.9% (88.1%-100%) respectively, with an overall agreement of 99.1% (98.3%-99.5%).

Anti-*T. gondii* IgG/ IgM detection by Enzyme-Linked Immunosorbent Assay (ELISA)

Labellings on the serum samples were well designed to correspond with the sample designations on the anti-*T. gondii* IgG and IgM microtitre well plates. Following the manufacturers' instructions, 5ul each of the serum samples was loaded after 100ul of the sample diluent had been added onto the basement of the 96 wells pre-coated with the *Toxoplasma gondii* antigen. Uniformity and proper thorough mixing of samples were ensured by the rocking of the microtitre well plate for about thirty seconds (30s) after which the plates were incubated at 370C. The plates were flipped empty and washed-off with a Tris-HCL buffer in an ELISA plate washer for 5 times to take out any unbounded serum antibodies. This step was repeated. The plates were then filled with 100ul of Horse Radish Peroxidase (HRP)-anti human IgG conjugate or (HRP)-anti human IgM (depending on the test) except the blank wells. The ELISA plates were then covered and incubated for 20 minutes at 37°C. Fifty microliters (50ul) each of CPB (Citrate-Phosphate buffer) substrate A and B were added to each test well. The microtitre well plates were then covered again and incubated in the dark at 37°C for 10 minutes. The serological enzymatic reaction was stopped by the addition of 50ul of stop solution to each well. The absorbance (optical densities) of each well was read at a wavelength of 450 nm using a Thermo-Scientific ELISA plate reader against the blank well.

Antibody concentration levels

The mean antibody concentration levels (both anti-*T. gondii* IgG and IgM) of the seropositive study participants were estimated in relation to their age and gestational age. The absorbance's (optical densities) of the seropositive participants within each age group and stage of pregnancy were summed up and the average/ mean absorbance taken.

The value represented the mean antibody concentration level for the age group or the stage of pregnancy.

Assay cut-off, validation, and interpretation of results

The cut-off value was set using the formula: 0.15 + NC, where NC is the mean optical density (OD) of the negative controls, as instructed by the manufacturer. The mean OD of the negative and positive controls was 0.0025 and 1.5395 respectively. Thus, the cut-off value was 0.1525. The OD ratio of each specimen was calculated by dividing the OD value of the specimen by the cut-off value (0.1525) as was instructed by the manufacturer. The mean optical density (OD) of the Toxo IgG/IgM positive controls had to be ≥ 1.00/≥0.80 respectively whiles the mean OD of the Toxo IgG/IgM negative controls also had to be ≤ 0.10. Once these requirements were met the results were considered valid and interpreted as follows according to the manufacturers manual: positive samples were those whose specimen OD ratios were greater than or equal to 1.0 while samples with specimen OD ratio less than 1.0 were considered negative.

5. Data analysis

Collation of demographic data and participant's exposure to the suspected risk factors was performed using Microsoft excel and SPSS version 24. Test of association and means among variables were done using descriptive statistics (Pearson-Chi-square test and T-test) on GraphPad-Prism version 6.02 and Statistical Package for Social Scientists (SPSS) version 24. For non-parametric data set, analysis was done using spearman rank correlation. Statistical significance was set at $p<0.05$, with confidence interval at 95%. The prevalence odds ratio (POR), calculated using Fisher's exact test, was used to estimate the possible association between infection transmission and the potential risk factors assessed. An odds ratio of ≥0.6 was considered a strong association.

7. Research Ethics

Ethical approval was obtained from the Committee on Human Research, Publications and Ethics (CHRPE), of the School of Medicine and Dentistry of the Kwame Nkrumah University of Science and Technology, Kumasi (KNUST). The CHRPE approval code/number for the study is CHRPE/AP/-541/17. Informed consent, either signed or thumbed, was obtained from every participant before enrolling them in this study. Further permission was sought from the appropriate hospital authorities and institutions before the study commenced. The study conformed to the principles of the Declaration of Helsinki of 1975 (as revised in 1983, 2004 and 2008).

RESULTS

1. Sample Characteristics

A total of two hundred and sixty-six (266) pregnant women aged between 16 to 40 years across trimesters participated in this study. There was an even distribution of study participants in the various age groups in the two study districts (see Table 1). They comprised 146 participants from the Asunafo District (Mean age= 29.2; SD= 15.0) and 120 participants from the Bosomtwe District (Mean age= 24.0; SD= 13.4). There was no statistical difference ($p=0.151$; CI= [1.73-8.67]) in the mean ages of participants from the two districts. The number of study participants increased with age, peaking at 25 years after which there was a gradual decline till age 40 (Table 1).

The number of participants in the second trimester in Asunafo North District was more than the first and the third trimesters. However, it was not statistically different from the number of participants in the second trimester in Bosomtwe District ($p=0.066$); CI= [6.636-6.964]). None of the

participants had ever been screened for *T. gondii* infection in both Districts prior to the commencement of the study (Table 1).

More than half (56.2% and 58.3%) of the participants in both Districts have had elementary education, with few (<5%) having had tertiary education (Table 1).

Table 1. Age and stage of pregnancy distribution of study participants in the two Districts

Characteristic	Districts		Previous Toxo. screening	95% CI	p
	Asunafo North	Bosomtwe			
	Number examined (%)	Number examined (%)			
Age group (years)					
16-20	25 (17.1)	24 (20.0)	-		
21-25	55 (37.7)	42 (35.0)	-		
26-30	28 (19.2)	30 (25.0)	-		
31-35	21 (14.4)	18 (15.0)	-		
36-40	17 (11.6)	6 (5.0)	-		
Stage of pregnancy					
First trimester	36 (24.7)	23 (19.17)	-	0.13-0.31	0.128
Second trimester	61 (41.8)	42 (35.0)	-	6.64-6.96	0.066
Third trimester	49 (33.6)	55 (45.83)	-	0.01-0.04	*0.002
Total	146 (100)	120 (100)			

* Statistical significance at 95% CI

2. Serological analysis- detection of anti-*T. gondii* antibodies

The study showed that 64.3% (171/266) and 26.3% (70/266) of the study participants were seropositive for anti-*T. gondii* IgG and IgM antibodies respectively. Also, 23.3% (34/146) of the participants in the Asunafo North District and 30% (36/120) of participants from Bosomtwe District were seropositive for anti-*T. gondii* IgM whereas 67.8% (99/146) and 60% (72/120) were seropositive for anti-*T. gondii* IgG in the two districts respectively. There were significant differences recorded between the anti-*T. gondii* IgG and IgM seroprevalences in both Districts (95% CI= [0.00 to 0.02; p<0.001) (Table 2).

Most of the participants in the Asunafo North District (99/146) had significantly been exposed previously to toxoplasmosis as compared to those in the Bosomtwe District (72/120) (95% CI= [0.76 to 0.79]; p= 0.037). However, considering their current infection status (anti-*T. gondii* IgM), there was no significant difference (95% CI= 0.06 to 0.07; p=0.098). The total anti-*T. gondii* antibody seroprevalence was 78.1% (114/146) and 75% (90/120) respectively in the Asunafo North and Bosomtwe Districts and the difference was statistically not significant (95%CI= 0.023 to 0.03; p= 0.993) (Table 2).

There was an increase in the seropositivities of both anti-*T. gondii* IgM and IgG from age 16 years to 30 years. The seropositivity then declined from age 31 to 40 years in both Districts. However, concerning the stage of pregnancy, the total number of anti-*T. gondii* IgG and IgM seropositives in the study participants generally increased from the first trimester to the third trimester (Table 2).

3. Antibody concentration among participants in the Bosomtwe district

There was an increase in the mean antibody concentration levels (both anti- *T. gondii* IgG and IgM) of the seropositive participants

from 16 years to 35 years and declines at 40 (Figure 1). Considering the stage of pregnancy, the concentration of the anti- *T. gondii* IgG fairly remained the same from trimester one to three but that of anti- *T. gondii* IgM declined from trimester one through to trimester three (Figure 2). The difference in means between the concentration of antibodies and age (95% CI= 0.74 to 0.85; p= 0.637) and the stage of pregnancy (95% CI= 0.38 to 0.43; p=0.127) of the participants were however not statistically different.

Table 2. The seropositivities of anti-*T. gondii* IgG/ IgM among age groups and the stages of pregnancy in the two Districts

Characteristic	ANTIBODY SEROPOSITIVITY							
	Asunafo North District			Bosomtwe District			Total	
	IgG N (%)	IgM N (%)	Both IgG & IgM N (%)	IgG N (%)	IgM N (%)	Both IgG & IgM N (%)	IgG	IgM
Age group								
16-20	10 (12.5)	2 (13.3)	3 (15.8)	15 (14.8)	4 (22.2)	2 (11.1)	30	11
21-25	27 (33.8)	9 (60.0)	8 (42.1)	12 (22.2)	6 (33.3)	12 (66.6)	59	35
26-30	23 (28.7)	1 (6.7)	1 (5.3)	17 (31.5)	4 (22.2)	1 (5.6)	42	7
31-35	12 (15.0)	2 (13.3)	4 (21.0)	6 (11.1)	3 (16.7)	2 (11.1)	24	11
36-40	8 (10.0)	1 (6.7)	3 (15.8)	4 (7.4)	1 (5.6)	1 (5.6)	16	6
Stage of Pregnancy								
1ST trim.	22 (27.5)	5 (33.3)	5 (26.3)	11 (20.4)	4 (22.2)	2 (11.1)	40	16
2ND trim.	32 (40.0)	9 (60.0)	6 (31.6)	18 (33.3)	6 (33.3)	6 (33.3)	62	27
3RD trim.	26 (32.5)	1 (6.7)	8 (42.1)	25 (46.3)	8 (44.5)	10 (55.6)	69	27
Total	80 (100)	15 (100)	19 (100)	54 (100)	18 (100)	18 (100)	171	70

(p<0.001; CI= [0.002-0.020])- Difference between anti-*T. gondii* IgG and IgM in both Districts.
(p=0.037; CI= [0.764-0.796])- IgG seropositivity in Asunafo District as compared to Bosomtwe District.
(p=0.0985; CI= [0.065-0.069])- IgM seropositivity in Asunafo District as compared to Bosomtwe District.
(p=0.993; CI= [0.029-0.033])- Total anti-*T. gondii* seroprevalence (IgG + IgM) statistical difference in both Districts.

Figure legend

Distribution of mean antibody concentration levels against age and stage of pregnancy of seropositive pregnant women in the Bosomtwe District.

Figure 1: There was no statistical difference between the mean antibody concentration levels and age of the study participants' (95% CI= 0.74 to 0.85) p= 0.637). Figure 2*:* A similar trend was observed in the antibody concentration levels and the stage of pregnancy (95% CI= 0.38 to 0.43; p=0.127) [n(IgG) =72; n(IgM) =36].

4. Infection status and exposure to risk factors

All the study participants ate vegetables, with many of them (97.7%) steaming and/cooking them before consumption. However, more than half (51%) of the seropositive participants frequently ate vegetables. Though all participants were meat consumers with varying responses regarding the types of meat consumed, less than half of the seropositive participants (48%) frequently took in meat. The results also showed that more than half (53.4%) of the toxoplasmosis seropositive pregnant mothers (either IgG or IgM) responded in affirmative to have been exposed to cats or its excreta. There was no significant difference (p>0.05) between the seropositive participants who had been exposed and those who had not been exposed to any the risk factors assessed in the study. Also, though associations to the risk factors varied, none of them showed a strong positive correlation/ association (OR<0.50) to the Toxoplasmosis infection (Table 3).

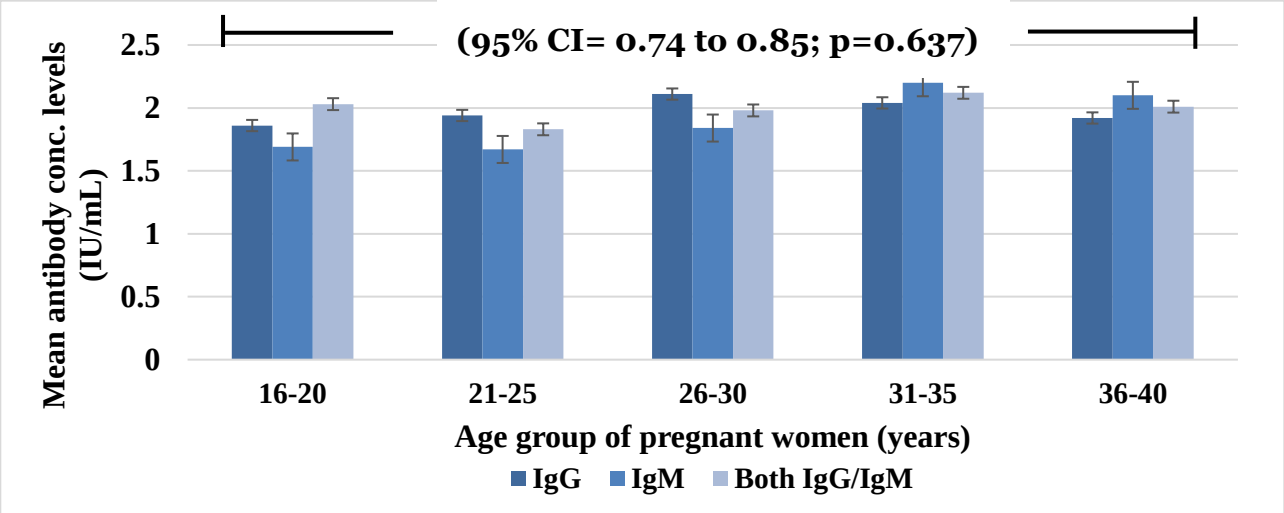

Figure 1. Distribution of mean antibody concentration levels against age of pregnancy of seropositive pregnant women in the Bosomtwe District

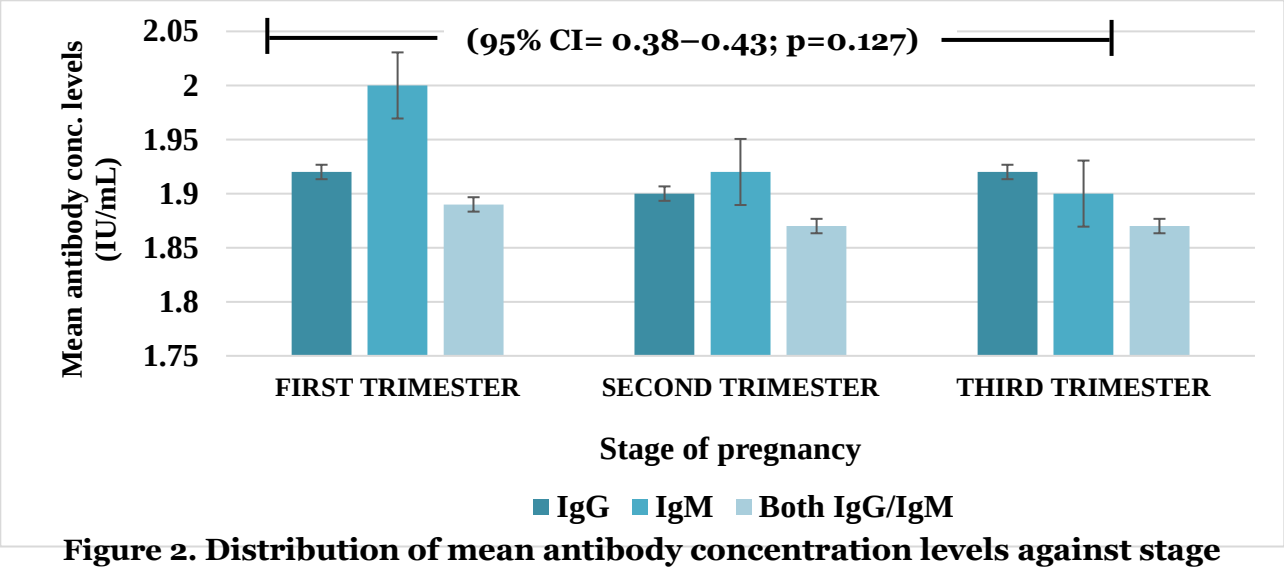

Figure 2. Distribution of mean antibody concentration levels against stage of pregnancy of seropositive pregnant women in the Bosomtwe District

DISCUSSION

Toxoplasma gondii infection in pregnant women and its transmission to the fetus continues to be the cause of tragic but preventable disease in the offspring (European Multicentre Study, 2003). The current study was carried out to determine the seroprevalence of *T. gondii* infection and the risk factors associated with it among pregnant women seeking antenatal care in the middle belt of Ghana. Pregnant women from the Asunafo North District in the Ahafo Region and the Bosomtwe District in the Ashanti Region, respectively, had seroprevalence rates of 78.1% and 75.0%, which were higher than the 30%–65% Ayeh–Kumi reported as the infection status of the global population in 2010 (Ayeh-Kumi et al., 2010). However, these were lower when compared to Ayi and her co-workers previously recorded seroprevalence of 92.5% in Ghana's Greater Accra Region (Ayi et al., 2009). The disparity in

toxoplasmosis seroprevalence estimates could be due to several factors such as geographical and climatic factors that are known to influence sero-epidemiological studies (Berger et al., 2009). Similar studies in other African countries have revealed varying seroprevalences such as 58.4 % in Tunisia, 53.6 % in Benin, and 57.9 % in Egypt (Ayi et al., 2016).

Table 3. Association of infection risk factors with *Toxoplasma gondii* seropositivity in pregnant women

Risk factor	Seropositivity		OR	95% CI	p
	Exposed (%)	Unexposed (%)			
Possession or exposure to cats	109 (53.4)	95 (46.6)	0.25	0.47-0.59	0.486
Exposure to cat faeces	109 (53.4)	95 (46.6)	0.25	0.47-0.59	0.486
Frequent intake of vegetables	104 (51.0)	100 (49.0)	0.32	0.40-0.51	0.450
Frequent intake of meat	98 (48.0)	106 (52.0)	0.47	0.15-0.23	0.108
Availability of sand box	112 (54.9)	92 (45.1)	0.57	0.07–0.08	0.074
The art of lambing	94 (46)	110 (54.0)	0.41	0.46-0.57	0.306
Handling of raw/ uncooked meat	114 (55.9)	90 (44.1)	0.19	0.38–0.43	0.228

Transmission and subsequent seropositivity to the parasite have been reported to be relatively higher in hot and humid areas such as Africa, and as a result, seroprevalences are higher in some parts of the world than others (Spalding et al., 2005; Meerburg and Kijlstra, 2009). This is due to the fact that hot, and humid climatic conditions increase the longevity and viability of *T. gondii* oocysts. In a country like Brazil which has hot and humid conditions, 51% of the population is infected, indicating a high seropositivity rate (Spalding et al., 2005). On the contrary, a moderately low seroprevalence of 18.9% has been recorded for Finland, 28% for Denmark, and 39% for the United States where the temperature and humidity are relatively low (Meerburg and Kijlstra, 2009). As a result, it is not surprising that Ghana, a tropical country with hot and humid weather, has higher seroprevalence, as reported in this and other studies (Ayi et al., 2009, 2016; Ayeh-Kumi et al., 2010).

Although there was no significant difference (95%CI= 0.74 to 0.85; p=0.637), seropositivity and antibody concentration levels were found to increase with age until 30 to 35 years, when antibody concentration began to decline (Figure 1A). This is due to the fact that as one ages, he or she becomes increasingly exposed to infection. Similarly, other studies have found that the rate of infection acquisition increases by 0.5%-1.0% per year of age, eventually reaching a peak of 23.7% during one's active years (Sakikawa et al., 2012).

Despite the fact that a statistically significant (95% CI= 0.76 to 0.79; p=0.037) greater number of Asunafo North District participants (99/146, 67.8%) had previously been exposed to the infection than those from Bosomtwe District (72/120, 60%), the number of ongoing/ current infections was not statistically different (95% CI= 0.06 to 0.07; p= 0.098). This could be due to the two regions' similar climatic conditions, despite the fact that the Ghana Statistical Service reported in their 2014 Population and Housing Census that the average humidity and temperature conditions in Ahafo Region are higher than those in Ashanti Region (Ghana Statistical Service, 2014).

The study also found significant differences (95% CI= 0.00 to 0.02; p= 0.001) in the seropositivities of anti-*T. gondii* IgGs

versus anti-*T. gondii* IgMs in both Districts (Table 2). This revealed that a higher proportion (67.8% in Asunafo North and 60% in Bosomtwe) of these pregnant women have had previous or past toxoplasma infection than those with active or ongoing infections (23.3% in Asunafo North and 30% in Bosomtwe). This could be due to the community's constant and continual exposure to the circulating parasitic oocyst, which increases the chances of infection. As a result, the majority of the study participants in both Districts may have been previously infected, as reported in a previous study elsewhere (Sroka and Szymańska, 2012).

The study recorded a seemingly high number of pregnant women with active or ongoing *T. gondii* infection (26.3%, 70/266). Being positive for IgM could indicate either a new infection or a reactivated past infection (particularly in pregnant women who tested positive for both anti-*T. gondii* IgG and IgM), which has been reported to occur in immunocompromised individuals (Liesenfeld et al., 2001). Primary congenital toxoplasmosis can occur through trans-plancental parasite transmission to the fetus, with the risk of transmission rising during gestation (Munoz et al., 2011; Robert-Gangneux and Dardé, 2012; Guimarães et al., 2022). The detection of both anti-*T. gondii* IgM and anti-*T. gondii* IgG antibodies in circulation in sero-epidemiological studies for *T. gondii* infection is always an indication of acute infection. This is due to the fact that IgM antibodies are rarely found in acquired immunity and are extremely rare in chronic infections. Furthermore, IgM antibodies wane down rapidly following a recently acquired infection (Liesenfeld et al., 2001).

In contrast to the fact that IgM antibodies wane quickly after newly acquired infection, studies have shown that IgM can persist for several months to years and may not wane, making the differential diagnoses of an acute and past infection with Toxoplasma commercial IgM diagnostic kits difficult. As a result, a previous toxoplasma infection may be incorrectly classified as acute or current infection (Dhakal et al., 2015). An avidity test is therefore done to differentiate IgM seropositive participants with true acute infection from those who may have been false anti-*T. gondii* IgM positives (Liesenfe et al., 2001). This was however not done in this study which is a notable limitation. This may have also contributed to the seemingly high anti-*T. gondii* IgM seroprevalence (26.3%) in the study participants. However, with a kit specificity and sensitivity of 99% and >99.9% respectively, these errors may be minimal. Detection of anti-*T. gondii* IgM in the majority of the pregnant women could also be attributed to some physiological changes, stressful demands, and the general hormonal imbalances associated with pregnancy that may lower their resistance to diseases (Kaaja and Greer, 2005).

However, it is unclear whether or not the high anti-*T. gondii* IgM seroprevalence observed in the pregnant women will result in congenital toxoplasmosis with the newborn babies exhibiting the clinical manifestations of toxoplasmosis. Therefore, a follow-up study on the infants is required to see if they develop any clinical symptoms of the infection. This was however not done in this study due to a lack of funding.

It has been reported in other studies that an acute maternal infection in the first trimester of the human gestation period results in a fetal transmission rate of 10-15%, rising to about 68% in the third trimester of the gestation period (Munoz et al., 2011; Robert-Gangneux and Dardé, 2012). A similar trend was observed in this study, as the total number of anti-*T. gondii* IgG and anti-*T. gondii* IgM seropositivity generally increased from the first trimester to the third trimester (Table 2). Although, there was no

statistical difference (95%CI= 0.38 to 0.43; p=0.127) in the mean antibody concentration levels from trimester one to three (Figure 1B), the babies that would be born to the anti-*T. gondii* IgM seropositive pregnant women are at a greater risk of contracting congenital toxoplasmosis, especially those in their first trimester. This is because, the anti-*T. gondii* IgM seropositive pregnant women would have carried the acute infection together with the fetus in the uterus for at least 6 months before delivery and that prolongs the time span for a probable fetal contraction of the infection (Jones et al., 2001; Dubey, 2004).

There was no strong significant association [(p>0.05) and (OR<0.6)] between seroprevalence and the various risk factors assessed in this study. Thus, the study supported previous and recent epidemiological studies that showed that cat ownership, intake of meat and vegetables, and other risk factors of *T. gondii* infection such as coming into contact with cats or its faeces were less predictive in determining the acquisition of the infection (Meerburg and Kijlstra, 2009; Sroka and Szymańska, 2012). Nevertheless, the high seroprevalence reported in this study could be due to other notable risk factors such as the carrying of oocysts from open fecal matter to food by flies and the drinking of contaminated water as observed in some outbreaks (Wallace, 1971; Bahia-Oliveira et al., 2003). Therefore, since cat feces are everywhere in environments like those where the study was conducted, one does not necessarily need to own a cat to contract the infection.

From the study, 78% and 75% of the participants from the Asunafo and the Bosomtwe District respectively were seropositive for the anti-*Toxoplasma gondii* specific antibodies and these were higher compared to the 30%-65% reported as the global population infection status.

The seemingly high anti-*T. gondii* IgM seroprevalence suggests that newborn babies that would be born to the IgM seropositive pregnant women are at risk of congenital toxoplasmosis. Clarity can however not be drawn on their congenital infectivity until a thorough follow-up screening process is done after their births. There was no association between seroprevalence and the various risk factors assessed in this study. In order to determine Ghana's overall seroprevalence, this study provides robust baseline data for subsequent studies in the other Districts and Regions. The study also provides proof in support of a national program or policy for routine clinical screening for toxoplasmosis in pregnant women which we are advocating for.

AUTHOR CONTRIBUTION

Derrick Adu Mensah, Alexander Yaw Debrah, Linda Batsa Debrah and Clement Evans Aryee conceived and designed the study. Derrick Adu Mensah and Clement Evans Aryee funded the study. Derrick Adu Mensah, Clement Evans Aryee, Patience Bortie, Rebecca Safo, Richard Abeiku Bonney, and Bhavana Singh carried out the field/ recruitment surveys. Derrick Adu Mensah, Richard Abeiku Bonney, Clement Evans Aryee, Bhavana Singh, Patience Bortie and Rebecca Safo did the data curation and formal analysis. The project administration was overseen by Alexander Yaw Debrah, Linda Batsa Debrah and Derrick Adu Mensah. Alexander Yaw Debrah, Linda Batsa Debrah, Derrick Adu Mensah and Clement Evans Aryee supervised and validated the study. The first and original manuscript draft preparation was done by Derrick Adu Mensah, Richard Abeiku Bonney and Rebecca Safo. Review and Editing of the manuscript draft was done by Alexander Yaw Debrah, Linda Batsa Debrah, Derrick Adu Mensah, Richard Abeiku Bonney and Patience Bortie. All authors read and approved the final

manuscript.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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