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Sero-prevalence of toxoplasmosis among patients visiting the Korle-Bu Teaching Hospital, Accra, Ghana

[1]*Ayeh-Kumi P. F, [2]Opoku A.G, [1]Kwakye-Nuako G, [1]Dayie N.T.K.D, [2]Asmah RH, [1]Obeng Nkrumah N, [2]Lartey M, [3]Sagoe A, [1]Attipoe MI, [1]Osafo KA, [1]Kretchy JP

[1] Department of Microbiology, University of Ghana Medical School, Korle-Bu, Ghana
[2]Department of Medical Laboratory Sciences, School of Allied Health Sciences, University of Ghana, Korle-Bu, Ghana
[3]The Central Laboratory, Korle-Bu Teaching Hospital, Accra, Ghana

*Corresponding author: payehkumi@yahoo.com

Abstract

Toxoplasmosis is a disease caused by the intracellular protozoan parasite *Toxoplasma gondii*. It affects up to about one third of the human population worldwide. Toxoplasmosis in neonates and immunocompromised patients can lead to severe disease and even death. However, there is a lack in knowledge concerning the extent of the toxoplasmosis problem in Ghana. In the present study, we determined the seroprevalence of *Toxoplasma gondii* infection among patients visiting visiting Korle-Bu Teaching Hospital using the enzyme-linked immunosorbent assay (ELISA). Of the 165 patients studied, IgG antibodies were found in 32.7% (95% CI: 25.0 – 39.3%). IgM and IgA seroprevalence were 29.7% (95% CI: 22.2 – 36.1%) respectively. There was significant association between seroprevalence of *Toxoplasma gondii* antibodies and gender ($P< 0.05$), with the male sex being at increased risk of *Toxoplasma gondii* seropositivity (OR ,95% CI: IgG - 2.78,1.34-5.82; IgM - 3.31, 1.57-6.981; IgA – 3.31, 1.57-6.981). No significant association ($P> 0.05$) was observed between the age groups and seroprevalence of *Toxoplasma gondii* antibodies. Ourstudy reveals an overall high seroprevalence of Toxoplasmosis among patients visit ing the Korle-Bu Teaching Hospital. Public campaigns may be necessary to educate the Ghanaians about ways to minimise exposure *Toxoplasma gondii.*

Keywords: Korle-Bu; Patients; Sero-prevalence; Toxoplasmosis

Abbreviations: CD 4^+_Cluster of Differentiation T helper cells; TMB_tetramethylbenzidine; KBTH_Korle-Bu Teaching Hospital

Introduction

Toxoplasmosis is a disease caused by the ubiquitous *protozoan* parasite *Toxoplasma gondii* [1, 2]. The parasite infects most warm blooded animals including humans, but the primary hosts are the organisms belonging to Felid family. Most humans are infected by ingesting tissue cyst from infected meat or oocyst from the soil or congenital transmission through the placenta [1, 2, 3] and also through water contaminated with the oocyst of the parasite [4]. In immunosuppressed patients, especially those with HIV/AIDS, Hodgkins disease or organ transplant and chemotherapy patients, toxoplasmosis is an opportunistic infection that poses significant risk. Both acute and recurrent toxoplasmosis causes severe and often fatal clinical manifest-tations [1, 3]. Latent infection can be reactivated to cause encephalitis, which can be very fatal [5]. About one-third of the world's population is estimated to carry *Toxoplasma* infection [6]. It is estimated that between 30% and 65% of all people worldwide are infected with toxoplasmosis. However, there are large variations in prevalence between and within different countries in animals and humans. The prevalence increases with age [1]. Local fauna, environmental conditions, cultural habits, and social and economic patterns may influence human prevalence: in France, for example, around 88% of the population are carriers, probably due to a high consumption of raw and lightly cooked meat [7]. In Germany, the Netherlands and Brazil, it has also been shown to have high prevalence of around 80%, over 80% and 67% respectively [8]. In Britain, about 22% are carriers and that of South Korea's rate is only 4.3%[9]. The Centre for Disease Control and Prevention noted that the overall seroprevalence in the United States as deter-mined with specimens collected by the third National Health and Nutritional Assessment Survey (NHANES III) between 1988 and 1994 was found to be 22.5%, with seroprevalence among women of childbearing age (15 to 44 years) to be 15%.[10]. West Africa might be widely endemic with human toxoplasmosis [11] even though there has been no large scale survey to establish the prevalence. This accession is supported by case reports of probable toxoplasmosis infection [12]. The prevalence of human toxoplasmosis in Ghana is unknown even though it presents a high risk of morbidity and mortality to immunosuppressed individuals and babies born to infected

women especially AIDS patients . However, a study conducted in sheep and goats gave an overall prevalence of 30.5% [13]. It is therefore imperative to find out the prevalence of *Toxoplasma gondii* infection in Ghana so that appropriate interventions can be put in place to help reduce morbidity and mortality. The study aims to determine the prevalence of *Toxoplasma gondii* infection among patients visiting the Korle-Bu Teaching Hospital (KBTH).

Materials and methods

Ethical issues

The study received ethical approval from the Protocol and Ethical Review Committee of School of Allied Health Sciences (SAHS), the College of Health Sciences and the University of Ghana. The objectives of the study were explained to individuals and those who consented were gladly recruited into the study. Names and other information collected from participants were kept confidential and anonymous.

Study Site

The present study was conducted as cross-sectional hospital based study at the Central Microbiology Laboratory of Korle-Bu Teaching hospital (KBTH). The KBTH is the largest teaching hospital in Ghana and approximately 1600-beded with intensive care units that cater for medical and trauma emergencies. It serves a pediatric and adult population of over 3 million in Greater Accra region and acts as a major referral health facility for an estimated population of 22 million Ghanaians. The Central Laboratory receives and processes over 300 specimens for parasitological examinations per day. Averagely the laboratory receives one (1) specimen, for parasitological examinations, per day from patients diagnosed with clinical symptoms suggestive of Toxoplasmosis.

Sample Size

A total of 165 patients (of all age groups) visiting KBTH between August 2007 to May 2008 were recruited for the study. One specimen per patient was examined for toxoplama antibodies. All these were patients who exhibited symptoms suggestive of toxoplasmosis of whom serum were requested by the clinicians for *Toxoplasma* analysis. Approximately, 3–5 ml of blood was collected from each of the individuals involved in the study. The serum was separated and stored at -20°C until further workup. Sera were obtained from the blood collected and tested for anti-*Toxoxplasma* sero-positivity.

ELISA assay

Immunoglobulin G (IgG) level was determined using an ELISA kit (Calbiotech company, CA, USA) according to the manufacture's instructions. The desired number of coated strips (96) was placed into the holder. A10 µl plasma was diluted with 200 µl diluent to make a 1:21 dilution of the test sample. The mixture was well vortexed. Hundred (100µl) of the diluted sera and controls were dispensed into appropriate wells. The holder was tapped to remove air bubbles from the liquid and well mixed. The sample was incubated for 20 mins at room temperature. The wells were then washed three times with 300 µl of wash buffer and blotted on an absorbance paper. A 100µl enzyme conjugate was dispensed to each well and incubated for 20 minutes at room temperature. The enzyme conjugate was removed from the wells and washed three times with 300 µl of wash buffer and blotted on absorbance paper. A substrate, 3, 3', 5, 5'-tetramethylbenzidine (TMB) (100 µl) was dispensed into the wells and incubated for 10 minutes at room temperature followed by adding 100µl of stop solution. The plate was read at an O.D of 450nm within 15minutes after addition of the stop solution. Similar procedures were used to determine IgM and IgA. The plates were read under the same condition.

Statistical analyses

Data was entered into Statistical Package for Social Scientists (SPSS), version 16. Chi-square analyses were used to evaluate significant differences in the prevalence of toxoplasma antibodies across demographic factors. *P* values < 0.05 were considered significant.

Results

A total of 165 patients were studied. Most were females (76%). The mean age of subjects was 30.16 years (median, 33; range, 8 months – 71 years).

Prevalence of Toxoplasma antibodies among the study population

The results of the ELISA assay for detecting the antibodies against *Toxoplasma. gondii* showed positive serology in 49.7% (82/165) of study subjects. Among these, 54 (32.7%) were seropositive for Ig G antibodies (95% CI: 25.0 – 39.3%). Forty-nine (29.7%) subjects were positive for IgM antibodies (95% CI: 22.2 – 36.1%). *Toxoplasma gondii* IgA antibodies were detected in 49 (29.7%) patients (95% CI: 22.2 – 36.1%).

Comparison of antibodies among the age groups

A stratified analysis by sex to evaluate its effect on the distribution of infection with *Toxoplasma gondii* is shown in Table 1. Fifty per cent of males and 27.2% of the females were positive for IgG antibodies (X^2= 7.641, *P*= 0.004). For IgM anitibodies, 50.0% of the males and 23.2% of females were positive (X^2= 10.421, *P*= 0.001). The same inclinations in IgM antibodies were observed for IgA antibodies. Overall, males were significantly at risk of *Toxoplasma gondii* seropositivity compared to females (OR, 95% CI: IgG - 2.78, 1.34-5.82); IgM - 3.31, 1.57-6.981; IgA – 3.31, 1.57-6.981).

Age related prevalence of Toxoplasma antibodies among study population

Figure 1 shows the distribution of Toxoplasma antibodies across age groups of patients.

Ten of the subjects below age 20 group tested positive to *Toxoplasma* IgG antibodies, 11 in age group 20-30 were also positive, 22 patients aged between 31- 40 were positive, 6 patients who tested positive fell within the ages of 41-50 and 5 patients who tested positive belong to age group greater than 50. The difference in IgG positivity across the various age groups was not significant (*P* value = 0. 298)

For *Toxoplasma* IgM antibody, 9 subjects in age group <20 tested positive, 12 in age group 20-30 were positive, 19 in 31-

Table 1. Seroprevalence of *Toxoplasma gondii* antibodies stratified by gender

	IgG	IgM	IgA
Males (n= 40)	50.0%	50.0%	50.0%
Females (n= 125)	27.2%	23.2%	23.2%
OR (95%CI)	2.78 (1.34-5.82)	3.31 (1.57-6.981)	3.31 (1.57-6.981)
	X^2= 7.641, *P*= 0.004	X^2= 10.421, *P*= 0.001	X^2 = 10.421, *P*= 0.001

*OR (95% CI) - Odds ratio (male to female) at 95% confidence interval
* X^2- Chi- square, *P*- value at 0.05 significance

40 age group were positive, 6 in 41-50 age group were positive and 3 in >50 age group were positive (*P* value = 0.116). Eleven (22.4%) out of the 30 subjects below the age group 20 tested positive for *Toxoplasma* IgA antibody, 11 out of 54 in age group 21-30 were positive, 19 out of 63 subjects tested positive in age group 31-40, 5 out of 9 in group 41-50 were positive to IgA antibody and 3 out of 9 in age groups <50 were positive (*P* value = 0.207). The number of subjects < 20 who tested positive for IgG, IgM and IgA ranged between 9 and 11. Eleven subjects in age group 21 - 30 tested positive for both IgG and IgA, and 12 were positive for IgM. Subjects in age group 31 - 40 recorded the highest seropositivity to the three antibodies. Nineteen subjects were positive to IgM and IgA and 20 to IgG. IgG recorded the highest positivity to *Toxoplasma* antibodies.

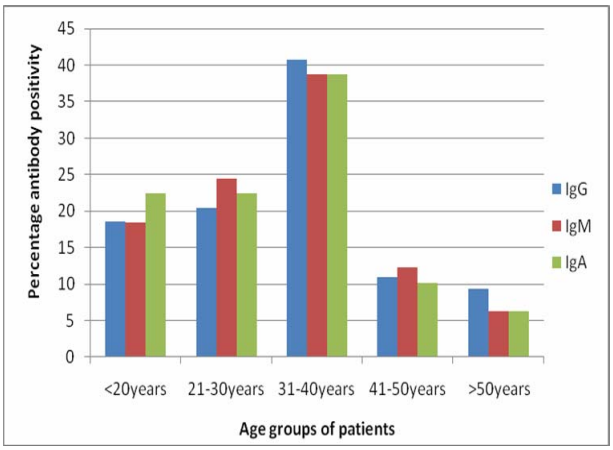

Fig 1. Prevalence of IgG, IgM and IgA across patient's age groups

Discussion

The prevalence of *Toxoplasma* anti-IgG antibodies, anti-IgM antibodies and anti-IgA was found to be 32.7%, 29.7% and 29.7% respectively. The prevalence of IgG agrees with the results from studies carried out in India [14]. But higher than that found in Britain and Korea [15] and lower than France [16] and Brazil [17]. The presence of IgG in the sera indicates the presence of chronic infection. Thus, the individuals have been exposed to the parasite but there was no evidence of acute disease. In our study, IgG antibodies were not significantly associated with age of patients (P value = 0.298). Nevertheless, adult populations of more than 50 years recorded the least prevalence of IgG cases (9%). The presence of IgG antibodies in patients' sera may also indicate latent infections, and therefore signifying the potential reoccurance of the disease condition among the older patients' population. The prevalence of IgM was found to be higher than that observed in many studies [18,19]. The presence of IgM in titres 4-8 times greater than IgG is used to diagnose acute infection [20]. In many seroepidemiological studies, there is often a marked difference between the prevalence of IgG and IgM. In this study the prevalence of IgM (29.7%) agrees with that for IgG (32.7%). The reason for this agreement may be because the population consisted of people who had shown strong clinical symptoms of toxoplasmosis so the test served to confirm doctors' diagnosis of an acute infection. IgA may be detected in the sera of acutely infected adults and congenitally infected infants, just like IgM does. The advantage of IgA is that in a number of newborns with congenital toxoplasmosis, who are negative to IgM antibodies, serological diagnosis can be established by the presence of IgA and IgG antibodies [21]. The prevalence of 29.7% observed indicates that most cases of newborns with toxoplasmosis will be detected. The high prevalence of toxoplasmosis may be due to consumption of undercooked meat, poor hygienic practises associated with food handling [22] and soil contamination in Ghana [23]. Usually it is not possible to detect any significant difference in the incidence of toxoplasmosis between gender [24], but some studies have shown higher incidence for males [3] or higher for females [25]. From the current study the seroprevalence of IgG (*P* = 0.004), IgM (*P* = 0.001) and IgG (*P* = 0.001) were statistically significant. The results suggest a higher seropositivity among females (59-63%) than males (37-40%). The distribution of toxoplasmosis is known to increase according to age [1]. However, in this study no significant association existed between the various age groups for IgG(*P* = 0.298), IgM (*P* = 0.116) , IgA (*P* = 0.207). For the women of child bearing age (21-40 years) involved in the study 61.1% were positive. This result is comparable to that published by Garcia et *al* [26] who found a seroprevalence of 70% for the women of child bearing age. This means half of the study population were infected and might have been immunized against the toxoplasmosis thus, reducing the risk of congenital infection, however the high IgA and IgM positivity also indicate a high possibility of acute infection that can result in congenital infection, with regard to association between toxoplasmosis and HIV. The patients involved in the study are from the population under the risk of HIV infection. It is estimated that 25-50% of HIV patients develop encephalitis caused by toxoplasmosis. In this study five (5) patients had been confirmed to be HIV positive, out of which three(3) test positive to IgG , IgM and IgA with IgM titre range of 42-65 iμ/ml. Another risk among the Ghanaians is the possible transmission of the parasite to patients who receive blood transfusion and various organ transplants due to the absence of screening for *Toxoplasma* antibodies. From the study it can be deduced that the prevalence rate of antibodies of *Toxoplasma gondii* in patients visiting KBTH is 32.7% for anti-IgG antibodies, 29.7% for anti-IgM antibodies and 29.7% for anti-IgA antibodies respectively. The p-values for the association between gender and prevalence of antibodies (IgG, IgM, IgA) were less than 0.05 (p<0.05), hence we conclude that the prevalence of antibodies to *Toxoplasma gondii* is dependent on gender. However, the p-value for the association between

age and prevalence was greater than 0.05 ($p>0.05$) hence the conclusion that there is no significant association between age and prevalence of *Toxoplasma gondii* antibodies. Considering the outcome and health implications of this investigation, there is the need for further studies to determine the prevalence of toxoplasmosis among groups of people such as pregnant women and women of child bearing age, people living with HIV and AIDS, blood donors and the general population. It must be mentioned however that some of the cases negative for IgM and IgA antibodies may be early infections with very low levels of antibody response. Note also that by being based on antibody detection, the assays performed in this study may have shown false negatives. Hence, seroprevalence analyses could be a low estimate of disease burden. Polymerase Chain Reaction (PCR) may be a more accurate method in delineating the infection rate.

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