



Effectiveness of SP-IPTp for Malaria and Evidence for the Need of *T. Gondii* Infection Preventive Policy during Pregnancy in Ghana

Reginald Arthur-Mensah Jnr¹, Emmanuel Awusah Blay^{2,3}, Irene Ayi^{2*}, John Larbi¹, Takashi Suzuki³ and Nobuo Ohta³

¹Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Ghana

²Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana

³Section of Environmental Parasitology, Tokyo Medical and Dental University, Bunkyo-ku Tokyo, Japan

*Corresponding author: Irene Ayi, Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana, Tel: +233-243-670-493, E-mail: layi@noguchi.ug.edu.gh

Abstract

Background: Malaria and toxoplasmosis during pregnancy are each reported to cause severe negative consequences in both mother and child. In Ghana, efforts are ongoing to control malaria from all facets but there is no such effort yet for control of toxoplasmosis. In this study, we sought to estimate the prevalence of *Plasmodium spp* and *T. gondii* infections in mothers at delivery and their neonates in a malaria holo-endemic area where Sulphadoxine-pyrimethamine intermittent preventive therapy for malaria in pregnancy (SP-IPTp) is administered and assess the possible risk of congenital *T. gondii* transmission.

Methods: A total of 185 women were recruited for this study of which 182 who delivered 186 babies voluntarily participated. Maternal and infant blood samples were obtained from the appropriate blood vessels of the expelled placenta after delivery, into clearly labeled serum separator tubes and blood blots were made on filter paper strips (Whatman No 3; GE Health Sciences, Japan). Serum obtained from blood samples were tested for anti-*T. gondii* IgG and IgM using commercial ELISA kits. Genomic DNA extracted from blood blots were amplified for detection of *T. gondii* SAG 3 gene marker and *P. falciparum* 18SrRNA gene by nested PCR. Risk factors associated with *T. gondii* and *P. falciparum* infections were assessed by responses to a structured questionnaire.

Results: An overall sero-prevalence of 45.1% (82/182) anti-*T. gondii* IgG was recorded in mothers and 22.7% (42/185) anti-*T. gondii* IgG in their babies. Parasite DNA positivity as determined by PCR was 23.1% (42/182) for *T. gondii* and 3.8% (7/183) for *P. falciparum* in mothers only. Relative risk of congenital transmission of toxoplasmosis was determined to be 103.4 (CI 95%, *p* value = 0.001).

Conclusion: The high level of exposure to *T. gondii* infections in pregnant women therefore suggests the need for establishment of an integrated management protocol during pregnancy to ensure improved quality maternal and child healthcare.

Keywords

Relative risk, Congenital toxoplasmosis, Malaria, Co-infections, Neonates

List of Abbreviations

BP: Blood Pressure; DNA: Deoxyribonucleic Acid; ELISA: Enzyme Linked Immunosorbent Assay; Hb: Haemoglobin; IPTp: Intermittent Preventive Treatment for Malaria in Pregnancy; LBW: Low Birth Weight; OD: Optical Densities; SP: Sulfadoxine-Pyrimethamine

Introduction

Systemic and opportunistic parasitic infections acquired during pregnancy such as *Plasmodium falciparum* and *Toxoplasma gondii*, respectively, have the ability to permeate the placenta and serve as the main parameter to establish congenital transmission to the foetus [1,2]. This therefore raises important public health concerns as these parasites' infections have both been reported to result in severe negative consequences in pregnancy outcomes such as intrauterine growth restrictions, intrauterine deaths, stillbirths, premature delivery, low birth weights, maternal and infant anaemia and maternal and neonatal deaths [3,4].

Toxoplasmosis in pregnancy shows varying degrees of morbidity depending on the time of infection with the rate of transmission to the foetus being 10-15% in the first trimester of gestation, which may increase to 68% in the third trimester [5]. Thus, maternal infections early in pregnancy are less likely to be transmitted to the foetus than infections later in pregnancy, but early foetal infections are likely to have more severe consequences than late infections [6]. Infections during the first trimester of pregnancy may lead to spontaneous abortion and stillbirths of the newborn while infections acquired later during pregnancy can result in chorioretinitis and mental retardation [7]. Other clinical signs of congenital transmission are low birth weights (LBW), hydrocephalus, cerebral calcifications and neurological injury [8].

In Ghana, previous data on toxoplasmosis in pregnant women indicated an overall sero-prevalence of 92.5%, comprising of 73.6% anti- *T. gondii* IgG, 64.8% IgA and 76.1% IgM, [9]. Recently, a prevalence of 29.2% by molecular diagnosis on placental tissues from mothers after delivery has been reported [10]. In a community-based

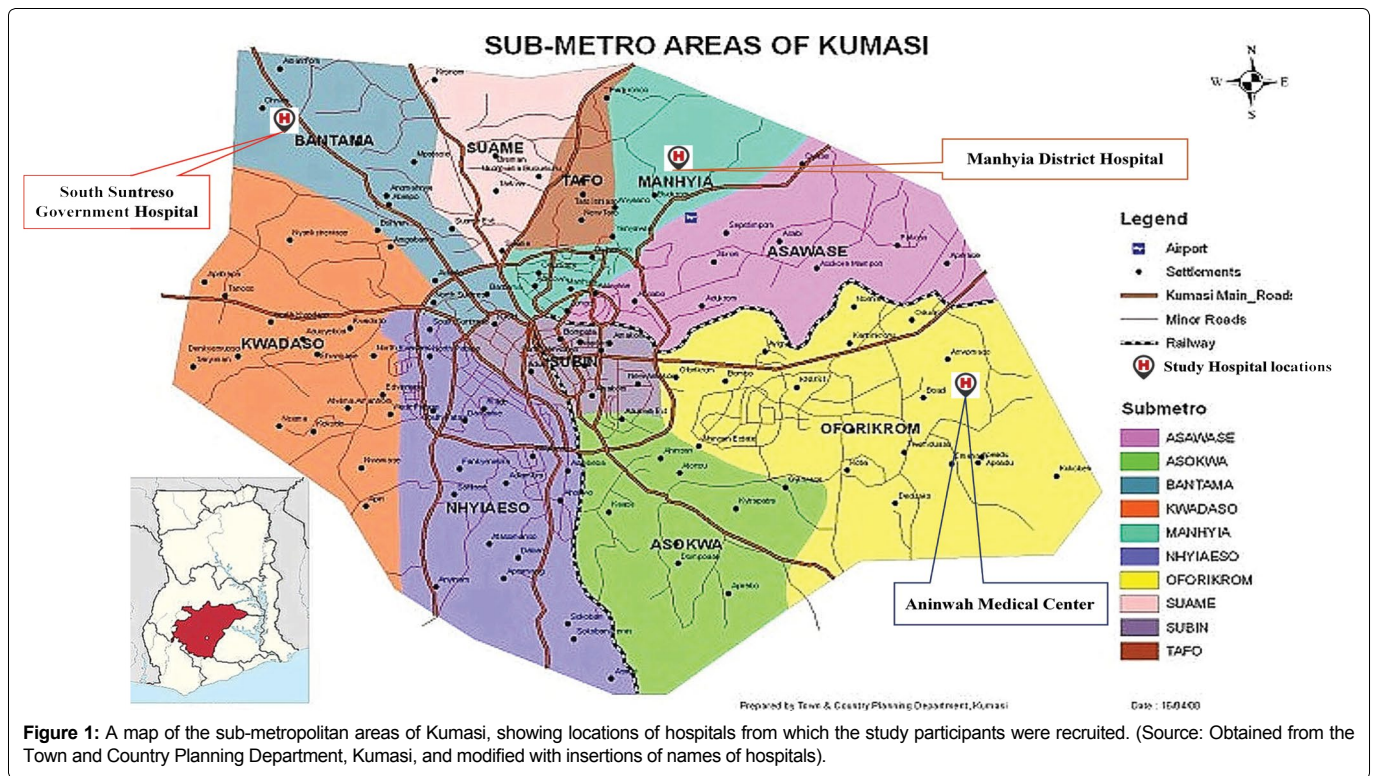


Figure 1: A map of the sub-metropolitan areas of Kumasi, showing locations of hospitals from which the study participants were recruited. (Source: Obtained from the Town and Country Planning Department, Kumasi, and modified with insertions of names of hospitals).

cross-sectional study, [11] the authors reported an overall seropositivity of anti-*T. gondii* antibodies as 85% amongst the people tested and showed no age dependence. Thus, the exposure of people to *T. gondii* infection in Ghana has been established.

Worldwide, an estimated 3.3 billion people of which 1.2 billion are at risk of being infected with *Plasmodium spp* and developing the disease [12]. In recent estimates, 198 million cases of *Plasmodium spp* infections occurred globally in 2013 (uncertainty range 124-283 million) which led to 584,000 deaths among those infected (uncertainty range 367 000-755 000). About 90% of these deaths occurred in the WHO African Region 78% of which were children less than 5 years old [12].

In Ghana, the incidence of malaria cases stood at 8.4 million in 2014 giving a nationwide prevalence of 27.5%. It accounted for nearly 30% of all out-patients department cases, 27.9% of in-patient cases and 7.2% of deaths with *P. falciparum* being the most prevalent causative factor [13]. Malaria also accounts for 13.8% of out-patients' department attendance, 10.6% of in-patients and 9.4% of deaths among pregnant women in Ghana [14]. The intermittent preventive treatment for malaria in pregnancy (IPTp) using sulfadoxine-pyrimethamine (SP) was introduced in Ghana in 2005. It is a package of administering SP four times during pregnancy from the second trimester at antenatal clinic with the initial dose at the first visit and the subsequent three doses given 4-8 weeks apart as a prophylactic. This has shown to reduce some pregnancy-related complications such as severe maternal anaemia, maternal mortality and low birth weights [15-17]. This notwithstanding, there are reports of placental malaria cases in Ghana [10] which is due to the sequestration of *P. falciparum* parasites that attach to receptors on the placenta bed and incite the release of inflammatory mediators which can affect placenta function and birth outcomes [18,19].

This study sought to determine the *T. gondii* and/or *P. falciparum* infection prevalence among women at delivery and their new born babies and the possible risk of congenital transmission.

Materials and Methods

Study area and study sites

Maternity Units of three hospitals located within Kumasi, a city in the middle belt of Ghana with perennial malaria transmission and a holo-endemicity were selected as study sites. Each Maternity

Unit has delivery wards and caters for the ante- and post-natal needs of women. They were in the Manhyia District Hospital, the South Suntreso Government Hospital and the Aninwah Medical Center located at three geographically and demographically distinct areas in the Kumasi metropolis (Figure 1).

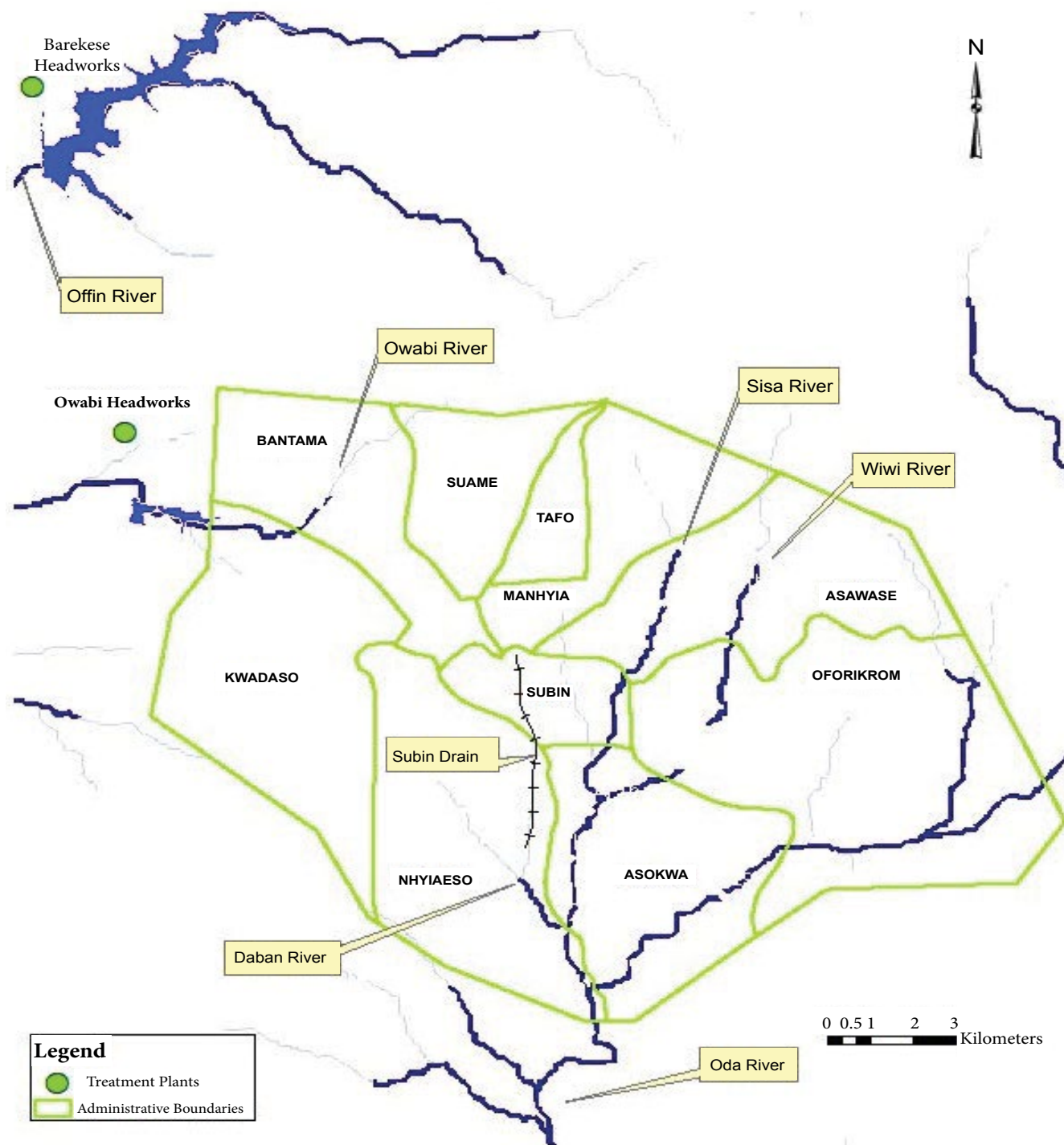
Kumasi Metropolis is one of the thirty districts in Ashanti Region and is approximately 270 km north of the national capital, Accra. The Kumasi Metropolis covers an approximate area of 254 km² and located between latitudes 6°35" and 6°4"N and longitudes 1°30" and 1°35" E with an altitude of 287 m (942 ft). The population is estimated at about 1,730,249 and represents 36.2 percent of the total population of Ashanti Region with a growth rate of 2.5% per annum. The Total Fertility Rate for the Metropolis is 2.6. The General Fertility Rate is 76.5 births per 1000 women aged 15-49 years and a Crude Birth Rate of 22.8 per 1000 population. The crude average temperature ranges from 21.5°C to 30.7°C. Annual rainfall is 625 mm with peaks in the months of June and September [20].

The Kumasi Metropolis has access to a number of surface water sources such as rivers which are fed by several tributary streams. Most of the water used in the metropolis is obtained from such rivers as the Ofin and Owabi (Figure 2).

Study design

This was a hospital-based cross-sectional study which was carried out from September 2013 to June 2014. The study involved 182 women who delivered 185 babies at the selected health facilities after written informed consent. The study details were previously explained to pregnant women in their third trimester during antenatal visits and were included in the study at delivery upon consent. Maternal and neonate blood samples were obtained from the expelled placenta post-delivery and processed for testing to detect infection with *T. gondii* and *P. falciparum*. Information on exposure to risk of infection with *Toxoplasma gondii* were obtained from participating mothers by a questionnaire guide and other *T. gondii* infection related data were sought. Information on compliance with IPTp regimen during pregnancy and birth weight of neonates were obtained from respective participants' medical records. All blood samples and data were analysed using appropriate methods in line with the objectives of the study. Test results were shared with the authorities of the hospitals from which participants were recruited for the necessary action to be taken.

Kumasi Surface Water Sources and Intake Points



© Moumie Maoulidi, MCI, Columbia University

Figure 2: A map of Kumasi Metropolitan Area showing surface water sources. (Source: <http://mci.ei.columbia.edu/millennium-cities/kumasi-ghana/kumasi-maps-and-population-data/>).

Study participants

Study participants were volunteer mothers who delivered at the selected health facilities. They were recruited based on written consent after educating them on the study objectives and details. Assuming a minimum and maximum prevalence of 10% and 90%, respectively, for either *Plasmodium spp* or *T. gondii* infections, a minimum sample size of 138 was obtained at a 95% confidence interval of width $\pm 5\%$. A 34% allowance was added to compensate for sample loss or any such eventualities. The sample size was estimated using the following formula:

$$\text{Sample size} = \frac{4(1.96) \times P(1-P)}{D^2}$$

Where P = prevalence rate, and D = the desired degree of accuracy [21].

Ethics and sampling permits

This study's protocol was reviewed and approved by the Ethical Committee on Human Research Publications and Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology (KNUST) and the Komfo Anokye Teaching Hospital (KATH), Kumasi (CHRPE/AP/113/13). Permission and approval was also obtained from the participating hospital authorities. All procedures were performed according to the guidelines for human experimentations in clinical research stated by the committee. Each

participant was required to sign or thumbprint a consent form after the study protocol, risks and benefits had been duly explained to them in a language they will understand. Participants were protected at all times and their personal information and identifiers such as name and contact details were removed prior to data analysis. Test results of the participants were shared with the respective medical authorities for appropriate action to be taken.

Maternal and neonate blood sample collection

After delivery, each placenta was collected in a kidney dish soon after its expulsion. The umbilical vein in the foeto-placental region of the placenta (foetus side) in the umbilical cord was located and 5 ml blood was drawn with a sterile disposable hypodermic syringe to represent neonate blood and dispensed into appropriately code number labelled serum separator tubes. The placenta was afterwards lightly incised in the inter-villous space of the utero-placental region (maternal side) with sterile surgical scissors and up to 5 ml blood was drawn with a fresh sterile disposable hypodermic syringe from the placental basal plate endometrial arteries to represent maternal blood. Maternal blood was dispensed into labelled serum separator tubes bearing respective mothers' codes. Care was taken to avoid any possible cross-contamination of blood samples. Clear serum was obtained from each blood sample by centrifugation at 14,000 rpm for 10 minutes. Serum samples were stored at -40°C until use. Blood blots were also made on filter paper strips (Whatman No 3, GE Health Sciences, Japan) for each blood sample. About 0.5 ml each of neonate and maternal blood samples was blotted on the filter papers, appropriately labelled and air dried thoroughly. They were placed in zip-lock bags and stored at 4°C until use [10].

Questionnaire interviews and medical data collection

Questionnaires were mostly administered to participating mothers in their preferred *lingua franca* to obtain personal and socio-

demographic information. Knowledge and exposure of participants to *T. gondii* infection risk factors were also sought. SP prophylaxis compliance during pregnancy and the determined periods of administration was also obtained by the questionnaire interview. Pre and post-delivery medical data were obtained from participants' health record book. Data obtained included Blood pressure (BP) readings, Blood haemoglobin (Hb) readings, gravidity and parity status, history of spontaneous abortions and/or still births, sex and weight of babies.

Detection of anti-*T. gondii* antibodies by enzyme linked immunosorbent assay

Serum samples were tested for the presence of anti-*T. gondii* IgG and IgM, using standard commercial 96-well ELISA Kits (CTK Biotech, Inc., San Diego, USA) following manufacturer's instructions. ELISA results were recorded using a microplate reader (XFLUOR4 v 4.51) as a measure of absorbance (Optical Densities) of the reaction intensity of *T. gondii* antigen and serum anti-*T. gondii* antibodies using a filter wavelength of 450 nm against the blank wells. Cut-off points and antibody index calculations were done according to manufacturers' recommendation to categorize seropositive and seronegative samples.

Antibody index calculations to categorize seropositive and seronegative samples, was as a measure of the specimen optical density ratios. Specimen with OD ratios ≥ 1.00 were interpreted as seropositive and specimen with OD ratios < 1.00 were interpreted as seronegative.

Genomic DNA extraction from blotted blood samples

Genomic DNA was extracted from the air dried blotted blood samples (maternal and neonate) using the Tris-EDTA buffer-based extraction method from a previously published protocol [22].

Table 1: Characteristics of participating women and neonates from health record books as categorized by study site.

Characteristics	Total number of participants, N = 182	Maternity Units in health facilities (number of participating women)		
		South Suntreso Government Hospital (n = 61)	Aninwah Medical Center (n = 50)	Manhyia District Hospital (n = 71)
Mean Age \pm SD (years)	28.00 \pm 5.52	28.18 \pm 4.84	28.66 \pm 4.72	27.38 \pm 6.44
Educational Level				
No formal Education: No. (%)	19 (10.44)	1 (1.64)	-	18 (25.35)
Primary: No. (%)	65 (35.71)	6 (9.84)	19 (38.0)	40 (56.34)
Secondary/ Vocational education (No, %)	65 (35.71)	33 (54.10)	21 (42.0)	11 (15.49)
Tertiary: No. (%)	33 (18.13)	21 (34.43)	9 (18.0)	3 (4.23)
Gravida status				
Primigravidae: No. (%)	67 (36.81)	25 (40.98)	26 (52.0)	16 (22.54)
Secundigravidae: No. (%)	40 (21.98)	15 (24.59)	8 (16.0)	17 (23.94)
Multigravidae: No. (%)	78 (42.86)	21 (34.43)	16 (32.0)	41 (57.75)
History of spontaneous abortions occurrence				
Never: No. (%)	150 (82.42)	51 (83.61)	36 (72.0)	63 (88.73)
Once: No. (%)	22 (12.09)	7 (11.48)	9 (18.0)	6 (8.45)
Twice: No. (%)	6 (3.30)	3 (4.92)	3 (6.0)	-
Thrice or more: No. (%)	4 (2.20)	-	2 (4.0)	2 (2.82)
Adherence to SP prophylaxis: No. (%)	178 (97.80)	59 (96.72)	48 (96.0)	68 (95.78)
Plasmodium spp infection during pregnancy: No. (%)	51 (28.02)	14 (22.95)	16 (32.0)	21 (29.58)
Mean Gestational age (weeks) {range}	38.35 \pm 1.41 {32.05- 44.36}	38.57 \pm 1.33 {36.2- 41.03}	38.40 \pm 1.46 {36.84- 41.31}	38.09 \pm 1.45 {32.05- 44.36}
Haemoglobin level (g/dL; median; range)	11.11 (6.4-16.7)	11.8 (9.6-16.7)	11.2 (9.2-14.2)	11.1 (6.4-15.9)
Number of babies	N = 185	n = 61	n = 50	n = 74
Sex of Babies				
Male: No. (%)	111 (60.0)	43 (70.49)	31 (62.0)	37 (50.0)
Female: No. (%)	74 (40.0)	18 (29.51)	19 (38.0)	37 (50.0)
Mean birth weight \pm SD (kgs) {range}	3.08 \pm 0.42 {2.1-4.5}	2.9 \pm 0.39 {2.3-4.1}	3.32 \pm 0.44 {2.1-4.5}	3.06 \pm 0.38 {2.1-3.85}
Mean weight of placenta \pm SD (kg)	0.24 \pm 0.12	0.27 \pm 0.077	0.29 \pm 0.079	0.24 \pm 0.12
Mean Placenta weight - to - birth ratio \pm SD	0.085 \pm 0.037	0.095 \pm 0.02	0.088 \pm 0.028	0.079 \pm 0.046
Method of Delivery				
Caesarian Section: No. (%)	17 (9.18)	3 (4.92)	13 (26)	1 (1.35)
Spontaneous Vaginal Delivery: No. (%)	168 (90.81)	58 (95.08)	37 (74)	73 (98.64)

Table 2: Prevalence of *P. falciparum* and *T. gondii* infections by ELISA and nPCR in women and their babies.

Participants	Target infection and means of testing	No. positive (%)	χ^2	<i>p</i> value McN'
Mothers (N = 182)	<i>P. falciparum</i>			
	nPCR on blood samples	7 (3.84)	156.2	< 0.001
	<i>T. gondii</i>			
	Serum anti - <i>T. gondii</i> IgG detection by ELISA	82 (45.05)	2.162	0.141
Babies (N = 185)	nPCR on blood samples	42 (23.07)	54.05	< 0.001
	<i>P. falciparum</i>			
	nPCR on Cord blood	0	N/A	N/A
	<i>T. gondii</i>			
	Serum anti - <i>T. gondii</i> IgG detection by ELISA	42 (22.70)	54.05	< 0.001

p value was calculated by McNemar test (McN) with confidence interval (CI) of 95%. *p* values less than 0.05 were considered statistically significant and are indicated in bold fonts.

Table 3: *Toxoplasma gondii* and *P. falciparum* co - infection among mothers.

		nPCR detection of <i>P. falciparum</i> DNA from maternal blood		χ^2	<i>p</i> value
		Positive (%)	Negative (%)		
<i>T. gondii</i> positivity by either nPCR or ELISA*	Positive (%)	4 (2.1)	77 (42.2)	0.484	0.486
	Negative (%)	3 (1.6)	98 (54.9)	-	-
	Total (%)	7 (3.7)	175 (96.1)	-	-

p value was calculated by Pearson's Chi square (χ^2) with confidence interval (CI) of 95% and 1 degree of freedom (df). The result is not significant at $p > 0.05$

* *Toxoplasma gondii* positivity by either nPCR or ELISA refers to women being either positive for anti - *T. gondii* IgG or nPCR detection of *T. gondii* DNA or positive for both.

Detection of *T. gondii* DNA by PCR

Previously extracted DNA was amplified using a nested PCR (nPCR) method adopted and modified from previously published protocols [23-25] (Annex).

Detection of *P. falciparum* DNA by PCR

Gene amplification for the detection of *P. falciparum* DNA involved a nested PCR method adapted and modified from a previously published protocol [26].

Data analysis

All data were analyzed using Statistical Package of Social Science (SPSS) version 20 (SPSS Inc, USA). Categorical variables were summarized as percentages and analyzed with Chi-square test to observe the differences among the various categories. Factors with *p* values < 0.05 were considered to have a statistically significant association with the infections. Difference in non-scalable variables were assessed by Mann-Whitney U rank sum with *p* values < 0.05 considered significant (CI: 95%). Associations between *P. falciparum* and *T. gondii* single- and co-infections and anaemia, pregnancy induced hypertension, LBW, still births and pre-term delivery were identified using χ^2 with confidence interval set at 95% and a margin of error of 5%.

Results

Characteristics of study participants

A total of 182 mothers aged 18-40 (mean: 28.00 \pm 5.52) years who delivered 185 live babies volunteered to participate in the study. Sex ratio of live births was 60.0% (111/185) male: 40.0% (74/185) female (Table 1). The educational background of the women varied from those who had never been to school or had any form of formal education (10.4%) to those who had attained up to tertiary level education (18.1%). For gravidity, 36.8% of women were primigravidae, 21.9% secundigravida and 42.8% multigravida. The mean gestation age was 38.35 \pm 1.41 and a range of 32.05 - 44.36 weeks. The participants had a mean haemoglobin level of 11.11 g/dL. The mean birth weight of the babies was 3.08 \pm 0.42 kg. Adherence and compliance to SP prophylaxis was 96.2% in women who had made 3 to 4 times visits to ante-natal clinic. For method of delivery, 9.18% of the women delivered through caesarian section while 90.81% had spontaneous vaginal delivery (Table 1).

In all 82.4% (150/182) of the women had no history of spontaneous abortions and/or still births whilst 12.1% (22/182)

had had one spontaneous abortion during previous pregnancy. A further 3.3% (6/182) had experienced spontaneous abortions and/or stillbirths twice and 2.2% (4/182) had had three or more of such past experiences (Table 1).

Anti-*T. gondii* antibodies sero-positivity in mothers and babies

An overall sero-prevalence of 45.1% (82/182) anti-*T. gondii* IgG was estimated by ELISA amongst the mothers and 23.1% (42/185) anti-*T. gondii* IgG amongst the babies. Anti-*T. gondii* IgM was not detected in any of the samples from mothers or their babies (Table 2). Overall sero-prevalence of anti-*T. gondii* IgG in matched mothers and babies was 21.9% (40/182).

Toxoplasma gondii DNA positivity by PCR in mothers and babies

Infection status of *T. gondii* among study participants was confirmed using the nested PCR method. Overall *T. gondii* DNA positivity was 23.1% (42/182) among the mothers. However, none of the cord blood samples for babies showed positive for *T. gondii* DNA (Table 2).

Plasmodium falciparum DNA positivity by PCR in mothers and babies

A prevalence of *P. falciparum* DNA positivity of 3.84% (7/182) was recorded for mothers whilst no *P. falciparum* DNA was detected in any of the cord blood samples for babies.

Plasmodium falciparum and *T. gondii* co-infections in mothers

Plasmodium falciparum and *T. gondii* co-infections were detected in 2.2% (4/182) mothers. However there was no statistical significance between co-infection with both *P. falciparum* and *T. gondii* and single *T. gondii* or *P. falciparum* infections. Pearson's chi-square revealed a *p* value of 0.486 which was greater than the set significance of $p < 0.05$ at 95% confidence interval (Table 3).

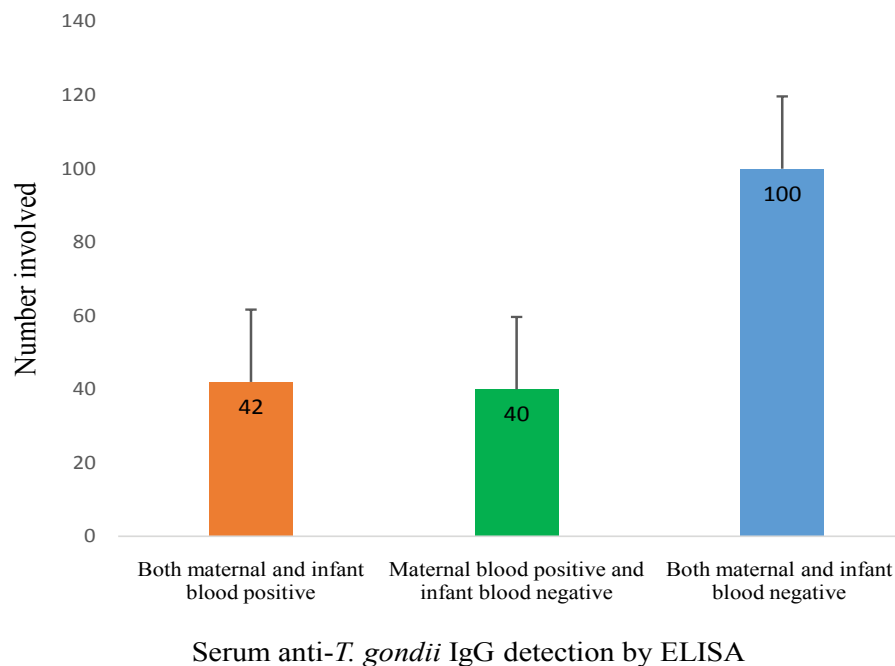
Risk of congenital transmission of *T. gondii*

Out of the 182 matched maternal and infant blood samples tested, 23.1% (42) were both positive for anti-*T. gondii* IgG whilst for another 21.9% (40), maternal blood tested positive for the same antibodies but matched infant blood samples were negative (Figure 3). The relative risk of congenital transmission of *T. gondii* (from infected or exposed mother to child) was high with a value of 103.4 at 95% CI: {1.651-2.448} and a *p* value of 0.001 which was statistically significant.

Table 4: Association of *P. falciparum* infection and adherence to Sulfadoxine Pyrimethamine (SP) prophylaxis treatment.

Diagnosis	Results	Adherence to SP prophylaxis by mothers (N =182)		χ^2	p value
		Yes	No		
Detection of <i>P. falciparum</i> by nPCR	Positive	7 (3.8%)	0	0.204	0.65
	Negative	170 (93.4%)	5 (2.7%)		
	Total (%)	177 (97.3%)	5 (2.7%)		

p value was calculated by Pearson's Chi square (χ^2) with confidence interval (CI) of 95% and 1 degree of freedom (df). The result is not significant at $p < 0.05$

**Figure 3:** Sero-prevalence of anti-*T. gondii* IgG in maternal and corresponding infant blood samples.

Prevalence of *P. falciparum* and treatment with sulfadoxine pyrimethamine

A total of 97.3% (177/182) women took all three doses of SP at the scheduled intervals under direct observation treatment (DOT) during their ante-natal visits until delivery (Table 4). The remaining 2.7% (5/182) women complained of mild allergic reactions to the drug and hence treatment was discontinued by medical officers.

Discussion

This study sought to determine the *T. gondii* and *P. falciparum* single and co-infection prevalence among mothers at delivery and their new born babies in a malaria holo-endemic area where IPTp is strictly observed. The relative risk of congenital transmission of *T. gondii* in pregnancy was also assessed. Sero-prevalence of anti-*T. gondii* IgG was generally very high in both mothers and their new born babies. However, it is relatively low compared to reports from studies conducted in Accra where sero-prevalence of anti-*T. gondii* IgG ranged from 70-90% among pregnant women [9,10]. This difference could partly be explained by geographical variation and climatic differences [27] in that there is variation in sero-prevalence across regions within a given country. This may be accounted for, among other factors such as ownership of cats or their presence in the environment [9], by the differences in climatic conditions where hotter areas are associated with higher sero-prevalence values [28]. This may be a probable reason for the high prevalence in Accra where the climatic conditions are generally hotter compared to Kumasi which falls within the forest belt. Hot weather has been found to favour the sporulation of *T. gondii* oocysts [29]. The presence of anti-*T. gondii* IgG identifies possible past *T. gondii* infections or exposure to infections in seropositive mothers. Mothers might have been either infected or exposed to the parasite in the past and presence of anti *T. gondii* antibodies could be indicative of latent infection [30]. An IgG avidity test could confirm acute infections and predict the time frame in which IgG seropositive mothers were infected [31],

however this study did not employ that method and cannot confirm acute infections or accurately determine the timeframe of past infections or exposures to *T. gondii*. Detection of anti-*T. gondii* IgG in neonates' serum indicate the transfer of maternal antibodies due to their exposure to infection. Maternal antibodies are indicators of risk of infection [32]. IgG antibodies are secreted as a monomer that is small in size and thus able to easily perfuse tissues. It is the only immunoglobulin that can pass through the human placenta thereby providing protection to the foetus *in utero*. It is also reported that predominant IgG antibodies found in newborns are IgG subclasses IgG₂ and IgG₃ against *T. gondii* antigens even though maternally transferred IgG₁ antibodies still persist in circulation [33]. The relative balance of these subclasses, in any immune complexes that form, helps determine the strength of the inflammatory processes that follow. Thus, subclass analysis of antibodies from mother to child against *T. gondii* antigens will further improve diagnosis of congenital *Toxoplasma* infection.

Anti- *T. gondii* IgM was not detected in any of the maternal and neonate sera. The presence of IgM antibodies would have been an indication of most recent infection with possible detection of circulating *T. gondii* antigens. IgM antibodies appear early in the course of an infection and is usually not expressed in acquired immunity and very rare in chronic infections [32]. Absence of anti- *T. gondii* IgM in maternal sera is suggestive of latent infections of *T. gondii*. IgM antibodies to *T. gondii* were not detected in the serum of the newborns since IgM does not cross the placenta and neonates are unable to produce their own antibodies. The detection of *T. gondii* SAG 3, a *T. gondii* heparin-binding protein that is involved in the parasite's attachment to target cells [34] are indicative of active transmission or the reactivation of latent infection [35]. In this study, *T. gondii* SAG 3 proteins were detected in the extracted genomic DNA of blood samples from 23.07% (42/182) mothers. This is an indication that the infection was acquired in the course of the pregnancy or a possible reactivation of latent infection due to immune suppression.

The low prevalence of *P. falciparum* parasitaemia observed in this study is comparable to results from authors [36] who recorded *P. falciparum* parasitaemia of 5% among 320 pregnant women who visited antenatal clinics in Accra. Low levels of *P. falciparum* parasitaemia recorded in both studies might reflect improved education on malaria prevention during pregnancy [14] and the introduction of Sulfadoxine-Pyrimethamine intermittent preventive therapy in pregnancy (SP-IPTp) in Ghana since 2005. All pregnant women who report to antenatal clinics are given at least 2 doses of SP in different gestational weeks by DOT. In the current study, 97.3% (177/182) of the mothers had at least 2 doses of SP during the period of their pregnancy. The remaining five who had discontinued treatment due to complaints of allergic reactions to SP tested negative to *P. falciparum* infection. However, low prevalence of *P. falciparum* DNA detection in maternal blood is not indicative of absence of infection because of possible sequestration of parasites in tissues such as the placenta [37]. Recent reports showed that though there was a significant low level of *Plasmodium spp* prevalence detection in peripheral blood, there was a 30 fold increase in the prevalence from diagnosis done on placental tissues from the same women [10]. Reasons adduced to this phenomenon include the increase in use and coverage of IPTp and insecticide treated bed nets during pregnancy. Moreover, the mechanism of action of SP against malaria parasites may be targeted specifically towards erythrocytic stages of the life cycle and may not affect the tissue stages.

The standard therapeutic agent for the treatment of toxoplasmosis is a combination of sulphadoxine and pyrimethamine [38]. This combination is active against the rapidly replicating tachyzoite stage of the parasite [39]. In this study, prevalence of *T. gondii* as confirmed by nPCR detection of parasite DNA using SAG3 primers which are tachyzoite-specific was 23% (42/182). This warrants a high concern if SP is the same drug used in the treatment of toxoplasmosis. Patients diagnosed with toxoplasmosis are put on a daily dosage of 3 g sulphadoxine and 50 mg pyrimethamine for about 4-6 weeks till all circulating tachyzoites is cleared but for IPTp-SP, the doses are administered intermittently in different gestational weeks. It is administered as a single-dose comprising three tablets of 500 mg sulphadoxine and 25 mg pyrimethamine. The first dose is administered at 16 weeks gestation or more, second dose is given 4 weeks after the first dose and the third dose is given 4 weeks after the second dose. This treatment regimen though effective in clearing *P. falciparum* parasitaemia might not be effective in clearing circulating *T. gondii* tachyzoites, and might account for the prevalence recorded. Moreover, SP treatment to toxoplasmosis has limited efficacy against tissue cysts [39]. Thus, *T. gondii* detection in placenta tissues of the current study participants might have yielded a high prevalence value.

Overall, 17.6% (32/182) women had had a previous history of spontaneous abortions and/or still births. Spontaneous abortions and/or still births are important in the disease conditions presented by *T. gondii*. Though history of spontaneous abortions did not show significant association with infection status, acute infections due to reactivation of latent infections of *T. gondii* as a result of immune suppression during pregnancy could account for spontaneous abortions [40].

Toxoplasma gondii and *P. falciparum* co-infections found in 2.1% (4/182) mothers could possibly be opportunistic for *T. gondii* due to immune suppression during the period of pregnancy, and systemic parasitism for *P. falciparum*. Such co-infections in this study did not seem to have any visible aggravated negative outcome on the neonates. The detection of serum anti-*T. gondii* IgG and no IgM from mothers suggests infection with *T. gondii* early in the pregnancy. Although this puts the foetus at a low risk of infection, when it does occur, it has severe consequences such as hydrocephalus which is reported in Ghana [41,42]. In view of existing evidence of toxoplasmosis in pregnancy [9,10,30,35] and findings in the current study, we suggest the development of appropriate monitoring protocols for *T. gondii* infection in women of child-bearing age and an integrated management of the infection during pregnancy to improve maternal and child health as has been established in some

temperate countries where cases are treated with pyrimethamine and sulfonamides [43]. The establishment of such a protocol in Ghana is imperative for improvement in maternal and child health care.

Conclusion

The low prevalence of *P. falciparum* parasitaemia observed in the mothers is commendable as improved education on malaria prevention during pregnancy via the SP-IPTp programme in Ghana appears to make a positive impact. SP-IPTp should be continued as it has proved efficient in the management of malaria in pregnancy in Ghana. However, this same consideration must be given to toxoplasmosis during pregnancy at antenatal clinics to minimize risk of infection and improve maternal and child health. Intensive education on awareness and preventive measures to toxoplasmosis in the general populace should be implemented and, especially, among pregnant women as has been done for malaria.

Acknowledgement

The authors are very grateful to the doctors and midwives of Maternity Units of the Manhyia District Hospital, South Suntreso Government Hospital and Aninwah Medical Center, Kumasi, for their enthusiasm and invaluable support in diverse ways. This research received partial support from the AMED/J-GRID (Japan Agency for Medical Research and Development /Japanese Initiative for Global Research Network on Infectious Diseases) Project, under the sponsorship of AMED.

References

- Fried M, Muehlenbachs A, Duffy PE (2012) Diagnosing malaria in pregnancy: an update. *Expert Rev Anti Infect Ther* 10: 1177-1187.
- Robert-Gangneux F, Murat JB, Fricker-Hidalgo H, Brenier-Pinchart MP, Gangneux JP, et al. (2011) The placenta: a main role in congenital toxoplasmosis? *Trends Parasitol* 27: 530-536.
- Rowe JA, Kyes SA, Rogerson SJ, Babiker HA, Raza A (2002) Identification of a conserved *Plasmodium falciparum* var gene implicated in malaria in pregnancy. *J Inf Dis* 185: 1207-1211.
- Abbasi M, Kowalewska-Grochowska K, Bahar MA, Kilani RT, Winkler-Lowen B, et al. (2003) Infection of placental trophoblasts by *Toxoplasma gondii*. *J Infect Dis* 188: 608-616.
- Thulliez P, Daffos F, Forrester F (1992) Diagnosis of *Toxoplasma* infection in the pregnant woman and the unborn child: Current Problems. *Scand J Infect Dis Suppl* 84: 18-22.
- Holliman RE (1995) Congenital toxoplasmosis: prevention, screening and treatment. *J Hosp Infect* 30: 179-190
- McAuley J, Boye KM, Patel D, Beckman J, Schey W, et al. (1994) Early and longitudinal evaluation of treated infants and children and untreated historical patients with congenital toxoplasmosis: the Chicago collaborative treatment trial. *Clin Infect Dis* 18: 38-72.
- Couvreur J (2004) Toxoplasmosis: A Comprehensive Clinical Guide. In: Joynson DHM, Wreghitt TG, Infection in neonates and infants. Cambridge University Press, Cambridge, UK, 254-276.
- Ayi I, Edu SAA, Apea-Kubi KA, Boamah D, Bosompem KM (2009) Sero-epidemiology of toxoplasmosis amongst pregnant women in the greater Accra region of Ghana. *Ghana Med J* 43: 107-114.
- Blay E.A, Ghansah A, Otchere J, Koku R, Kwofie KD, et al. (2015) Congenital toxoplasmosis and pregnancy malaria detection post-partum: Effective diagnosis and its implication for efficient management of congenital infection. *Parasitol Int* 64: 603-608.
- Abu EK, Boampong JN, Ayi I, Gharthey-Kwansah G, Afoakwah R, et al. (2015) Infection risk factors associated with seropositivity for *Toxoplasma gondii* in a population-based study in the Central Region, Ghana. *Epidemiol. Infect* 143: 1904-1912.
- http://www.who.int/malaria/publications/world_malaria_report_2014/en/.
- (2014) Guidelines for Malaria in Pregnancy. Ministry of Health, Ghana Health Service, M.O.H, Accra, Ghana.
- (2005) Intermittent Preventive Treatment (IPT) of Malaria in Pregnancy Training Manual for Health Providers Facilitators Guide. Ghana Health Service, 1-74.
- Radeva-Petrova D, Kayentao K, terKuile FO, Sinclair D, Garner P (2014) Drugs for preventing malaria in pregnant women in endemic areas: any drug regimen versus placebo or no treatment. *Cochrane Database Syst Rev*.

16. Kayentao K, Garner P, van Eijk AM, Naidoo I, Roper C, et al. (2013) Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: Systematic review and meta-analysis. *JAMA* 309: 594-604.
17. Garner P, Gulmezoglu AM (2003) Drugs for preventing malaria-related illness in pregnant women and death in the newborn. *Cochrane Database Sys Rev*.
18. Fried M, Duffy PE (1998) Maternal malaria and parasite adhesion. *J Mol Med* 76: 162-171.
19. Duffy PE (2001) Immunity to malaria: different host, different parasite. In: Duffy PE, Fried M, *Malaria in Pregnancy: Deadly Parasite, Susceptible Host*. Taylor & Francis, NY, USA, 71-127.
20. http://www.statsghana.gov.gh/docfiles/2010_District_Report/Ashanti/KMA.pdf.
21. Niang N (2003) Determination of sample size. *Malays J Med Sci* 10: 84-86.
22. Bereczky S, Mayrtensson A, Gil JP, Farnert A (2005) Rapid DNA extraction from archive blood spots on filter paper for genotyping of *Plasmodium falciparum*. *Am J Trop Med Hyg* 72: 249-25.
23. Prestrud KW, Asbakk K, Mørk T, Fuglei E, Tryland M, et al. (2008) Direct high-resolution genotyping of *Toxoplasma gondii* in arctic foxes (*Vulpes lagopus*) in the remote arctic Svalbard archipelago reveals widespread clonal Type II lineage. *Vet Parasitol* 158: 121-128.
24. Su C, Zhang X, Dubey JP (2006) Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. *Int J Parasitol* 36: 841-848.
25. Khan A, Su C, German M, Storch GA, Clifford D, et al. (2005) Genotyping of *Toxoplasma gondii* strains from immunocompromised patients reveals high prevalence of type I strains. *J Clin Microbiol* 43: 5881-5887.
26. Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown KN (1993) Identification of the four human malaria parasite species in field samples by polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol Biochem Parasitol* 58: 283-292.
27. Jones JL, Dargelas V, Roberts J, Press C, Remington JS, et al. (2009) Risk factors for *Toxoplasma gondii* infection in the United States. *Clin Infect Dis* 49: 878-884.
28. Nijem KI, Al-Amleh S (2009) Seroprevalence and associated risk factors of toxoplasmosis in pregnant women in Hebron district, Palestine. *East Mediterr Health J* 15: 1279-1284.
29. Kistiah KBA, Winiecka-Krusnell J, Karstaedt A, Frean J (2011) Seroprevalence of *Toxoplasma gondii* infection in HIV-positive and HIV-negative subjects in Gauteng, South Africa. *South Afr J Epidemiol Infect* 26: 225-228.
30. Ayi I, Akao N, Bosompem KM, Akafo SK, Clarke J, et al. (2005) Development of Membrane-Bases Tests for the Detection of Urinary Antigens and Antibodies in Human Toxoplasmosis: Preliminary Studies in Ghanaian Patients. *Acta Tropica* 93: 151-159.
31. Liesenfeld O, Montoya JG, Kinney S, Press C, Remington JS (2001) Effect of Testing for IgG Avidity in the Diagnosis of *Toxoplasma gondii* Infection in Pregnant Women: Experience in a US Reference Laboratory. *J Inf Dis* 183: 1248-1253.
32. Charpak Y, Nicoulet I, and Blery C (2004) Protective anti-donor IgM production after crossmatch positive liver-kidney transplantation. *Microbes infect* 315-319.
33. Buffalano W, Beghetto E, Del Pezzo M (2005) Use of recombinant antigens for early postnatal diagnosis of congenital toxoplasmosis. *J Clin Microbiol* 43: 5916-5924.
34. Jacquet A, Coulon L, De Ne've J, Daminet V, Haumont M, et al. (2001) The surface antigen SAG3 mediates the attachment of *Toxoplasma gondii* to cell-surface proteoglycans. *Mol & Biochem Parasitol* 116: 35-44.
35. Ayi I, Kwofie KD, Blay EA, Osei JH, Frempong KK, et al. (2016) Clonal types of *Toxoplasma gondii* among immune compromised and immune competent individuals in Accra, Ghana. *Parasitol Int* 65: 238-244.
36. Stephens JK, Ofori MF, Quakyi IA, Wilson ML, Akanmori BD (2014) Prevalence of peripheral blood parasitaemia, anaemia and low birthweight among pregnant women in a suburban area in coastal Ghana. *Pan Afr Med J* 17: 3.
37. Mockenhaupt FP, Ulmen U, von-Gaertner C, Bedu-Addo G, Bienzle U (2002) *J Clin Microbiol* 40: 306-308.
38. Guerina NG, Hsu HW, Meissner HC, Maguire JH, Lynfield R, et al. (1994) Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. *N Engl J Med* 330: 1858-1863.
39. Petersen E, Peyron F, Lobry JR, Musset K, Ferrandiz J, et al. (2006) Serotyping of *Toxoplasma gondii* in chronically infected pregnant women: predominance of type II in Europe and types I and III in Colombia (South America). *Microbes Infect* 8: 2333-2340.
40. Luft BJ, Remington JS (1992) *Toxoplasma* encephalitis in AIDS. *Clin Infect Dis* 15: 211-222.
41. <http://www.myjoyonline.com/news/2014/january-5th/3-donate-towards-treatment-of-baby-with-life-threatening-hydrocephalus.php>.
42. <http://www.gbcghana.com/1.8686764>.
43. Hampton MM (2015) Congenital Toxoplasmosis: A Review. *Neonatal Netw* 34: 274-278.