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Socio-demographic and Obstetric Variations of *T. gondii* and HIV-1 Co-infection among Pregnant Women in Cameroon

Lem Edith Abongwa[1*], Alberic Signang[2], Sharon Tibi[1] and Annabella Ngenwi[3]

[1]Department of Biological Sciences, Faculty of Science, University of Bamenda, BP 39, Bambili, Bamenda, N. W. Region, Cameroon.
[2]Mbingo Baptist Hospital, Cameroon Baptist Convention Health Services, BMP 41 via Bamenda, Cameroon.
[3]Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O.Box 63, Buea, South West Region, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Authors LEA, AS, ST and AN designed the study. Author LEA, AS, ST and AN managed the literature searches. Author LEA, AS and AN wrote the protocol, authors ST perform the experiment, authors LEA and AN performed the statistical analysis and managed the analyses of the study, Authors LEA, AS and AN wrote the first draft of the manuscript. All authors LEA, AS, ST and AN read and approved the final manuscript.

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ABSTRACT

Aim: This study, aimed to identify the seroprevalence of *T. gondii and* HIV-1 co-infection in pregnant women in the Northwest Region of Cameroon.
Study Design: This cross-sectional study was conducted among 606 pregnant women attending antenatal clinic in the Northwest region of Cameroon.
Place and Duration of Study: This study was carried out at the Bamenda Regional Hospital from May 2017 to December 2017.
Methodology: Venous blood samples were collected for the detection of anti-*Toxoplasma* antibodies using rapid test kits while HIV was determine using Alere Determine™HIV-1/2 test kit

**Corresponding author: E-mail: Lemedith19@gmail.com;*

and OraQuick HIV 1/2 Rapid Antibody Test kit for confirmation. Data were analyzed using SPSS version 23 statistical package. *P*-value <0.05 was considered statistically significant.
Results: The mean (SD) age was 27.3 (5.3) years. The prevalence of *T. gondii* and HIV-1 was 139 (22.9%) and 70(11.6%) respectively, while that of *T. gondii* and HIV-1 co-infection was 31(5.1%). With the exception of age group and gestational age that was significant (p<0.05) for HIV, socio-demographic and obstetrical characteristics of *T. gondii*, HIV-1 and *T. gondii* and HIV-1 co-infection prevalence did not show any significant differences (p>0.05).
Conclusions: The high prevalence of *T. gondii* and HIV-1 co-infection seen in this study demonstrates the need for routine antenatal screening for both infections. In addition, data from this study will be useful in designing control and prevention strategies against these diseases. Furthermore, the result will also be used as baseline data for further research on *T. gondii* and HIV-1 co-infection.

Keywords: Co-infection; human immunodeficiency virus; pregnant women; toxoplasmosis; Cameroon.

ABBREVIATIONS

ART : Antiretroviral therapy,
AOR : Adjusted odds ratio,
CI : Confidence interval,
HIV : Human immunodeficiency virus,
T : Toxoplasma

1. INTRODUCTION

Antenatal care (ANC) provides adequate measures against maternal-fetal transmission of several diseases, including toxoplasmosis and HIV [1,2]. Toxoplasmosis caused by *Toxoplasma gondii* is a neglected zoonotic disease and is asymptomatic [3,4]. Toxoplasmosis is prevalent worldwide whereby about one third to half of the global population is infected [5-7]. Human infections result from food borne transmission (consumption of water, raw or undercooked meat or unpasteurized milk contaminated with cyst), animal to human transmission (ingestion of oocysts through close contact with infected cats or cat's faeces), mother-to-child transmission (from an infected woman to her unborn child) and through blood transfusion and organ transplants [6-8].

Infection with *T. gondii* has severe consequences in immune compromised hosts such as pregnant women, HIV patients and patients receiving chemotherapy or immunosuppressive drugs [4, 5,9]. The prevalence of toxoplasmosis among pregnant women showed significant variation between continents and countries and ranges from 9 - 92.5% [6,8,9]. In Cameroon, the prevalence ranges from 48.5 - 70% [5,10]. This variation depends on social and cultural habits, geographic factors, individual's hygiene, route of transmission and the immune status [5,10,11]. The high prevalence in pregnant women indicates a greater probability of congenital transmission with latent infection reactivated when immunity is suppressed [5,12]. Reactivation of latent *T. gondii* infection causes severe and fatal neonatal complications such as stillbirth or abortion, anemia, petechiae due to thrombocytopenia, seizures, neurological defect (epilepsy), ocular disease (blindness, chorioretinitis, strabismus, retinochoroiditis) microcephaly, brain damage (intracranial calcifications, hydrocephalus), mental retardation, cardiac and cerebral anomalies [8,12,13]. Congenital transmission of the infection during the first trimester is critical and causes severe clinical conditions in the fetus, whereas infections during the third trimester lead to rapid transmission [4,14].

Worldwide, about 36·7 million people are infected with HIV, but very little is known about the prevalence of HIV-1 co-infection with *T gondii* parasites [15,16]. Early HIV diagnosis and interventions among pregnant women have shown to decrease the likelihood of mother to child transmission [10,17]. HIV prevalence is shown to increase among pregnant women as such screening of all women during antenatal care is important [17,18]. The prevalence of HIV among pregnant women ranges from 0.5-61.6% in other countries [11,17,19] and between 2.6 - 22.1% within other towns in Cameroon [16,20,21]. With the advent of highly active antiretroviral therapy, the rate of mother to child transmission has greatly reduced to a range of 1.4-2.5% [18].

T. gondii and HIV co-infections cause serious complications in pregnant women and pose a serious health threat [11]. Although screening practices of *T. gondii* and HIV during antenatal

care are standardized in developed countries, it is somehow limited in developing countries where the burden of *T. gondii* infection among HIV infected pregnant women is greatly felt [20]. As such screening for *T. gondii* and HIV infections among pregnant women may be an important primary prevention strategy. Studies carried out elsewhere have shown co-infection rates between 12-40.8% [9,12]. However, such data are dearth in many developing countries including Cameroon.

This study is the first study to determine the prevalence of *T. gondii* and HIV co-infection among pregnant women in the Northwest region of Cameroon. It is hoped that the outcome of this study will enable policymakers to design effective strategies for controlling and preventing the disease which in turn will curb the maternal-fetal transmission rate alongside its associated complications. In addition, it will set a base for further studies to be carried out in this area.

2. MATERIALS AND METHODS

2.1 Study site and design

This study was a hospital-based cross-sectional study conducted at the Bamenda Regional Hospital from May 2017 to December 2017. This hospital serves as a referral hospital for the entire Northwest region (NWR). The NWR is characterized by wet and hot climates which have been documented to favor *T. gondii* oocyst survival. Inhabitants in this region keep domestic animals like cats, sheep, dogs, goats, fowls that have shown to transmit the disease [4,12]. Roasted meat (beef, pork, fish, and chicken) is a common delicacy eaten by most people on a daily basis and is a medium for ingesting infectious parasites.

2.2 Sample Size Determination and Sampling Technique

The minimum sample size was calculated based on Toxoplasma morbidity using the Lorenz formula

$$N = \frac{(Z_1^{2} \; -\alpha) P(1-P)}{i^2}$$

Where,

$Z_1 - \alpha$ = the normal distribution value = 1.96

P =Relative prevalence of HIV in the region= 54.5% [5]

í = precision (sampling error) = 0.05

The minimum sample size (N) was calculated to be 382.

2.3 Data Collection

A structured closed-ended questionnaire was used to obtained information on socio-demographic and obstetric data.

2.5 Sample Collection and Processing

A total of 2ml venous blood was collected using labeled test Ethylene diamine tetra acetic (EDTA) tubes by the hospital laboratory technician and centrifuged to obtain plasma. Diagnosis of toxoplasmosis was done using the OnSite ToxoIgG/IgM rapid test (CTK Biotech Inc, USA) as per the manufacturer's procedure. This rapid test kit simultaneously detects both IgG and IgM anti-*Toxoplasma gondii* antibodies. Whereas, HIV test was done using the Alere Determine™HIV-1/2 test kit (Alere, Japan) and confirmatory test for those that were positive was done using OraQuick HIV 1/2 Rapid Antibody Test (for OraSure Technologies, Thailand) as described in the manufacturer's procedure.

2.6 Data Analysis

The data were analyzed using the SPSS statistical software package version 23. Chi-square test was used for comparison between categorical variables through cross-tabulations. P-values of < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 RESULTS

A total of 683 pregnant women were approached and, 650 provided consent for the study. Of these 606 women who had recorded HIV status were considered for the study. The age range was 14-45 years with a mean (SD) of 27.3 (5.3) years. Of the 606 participants, the age group 21-30 years 397 (65.5%) were the most represented 362 (59.7%) participants were married and 301 (49.7%) participants had attained secondary education. A greater number of them were multigravidae 381 (62.9%) and were in their third trimester of pregnancy 350(57.8%) Table 1.

3.2 Prevalence of HIV-1

The overall prevalence of HIV-1 amongst the participants was 70 (11.6%). The age group >35 years had the highest HIV-1 prevalence (18.6%), while women <21 years had the lowest prevalence (5.7%). This difference was statistically significant (p = 0.001). The youngest seropositive pregnant woman was aged 17 years and the oldest was 42 years of age. HIV prevalence in pregnant women was relatively high among married women 9(15.5%) and among women who had attended primary schools though the difference was not significant (p = 0.45). Furthermore, the prevalence of HIV-1 was significantly high (P = 0.02) among women who started antenatal care at first trimester 20(18.9) and insignificantly high (P = 0.74) among grand muiltigravidae 6(23.1) women (Table 2).

3.3 Prevalence of *T. gondii*

Considering women with either anti-Toxoplasma IgG or anti-Toxoplasma IgM or both anti-Toxoplasma IgG and IgM as positive for Toxoplasma infection, 139(22.9%) women presented with toxoplasmosis. Of the 606 participants, 135 (22.3%) were found seropositive for anti-Toxoplasma IgG antibodies, while 11(1.8%) had anti-Toxoplasma IgM. This difference was statistically significant (p=0.00). Seven (5.2%) of the women tested positive for both IgG and IgM anti-Toxoplasma. Univariate analyses of demographic and obstetrical characteristics showed no significant difference. However, the prevalence was highest among age group <21years (24.5%; P = 0.37), married women (25.9%; P = 0.46), women who had attained tertiary level of education (26.0%; P = 0.16), 1st trimester women (29.2%; P= 0.65), and primigravidae women (28.1%; P = 0.07) (Table 3)

3.4 *T. gondii* and HIV-1 and co-infection

Of the 606 women, 31(5.1%) were positive for both HIV and *T. gondii*. Co-infection rate was high among women of the age group 21-35 years (5.5%), single women 8(17.7%), women who never went to school (5.9%), women who started antenatal cars at a gestational age of < 14 weeks (8.7%) and women with more than 5 pregnancies (7.7%). This differences were however not significant p>0.05 (Table 4).

3.5 Discussion

This study is one of the few studies carried out in Cameroon to explore the seroprevalence of *T. gondii* and HIV co-infection among pregnant women in Bamenda Health District.

Table 1. General characteristic of the study participants

Demographic characteristics	Number (%)
Age group in Years	
<21	53(8.7)
21-30	397(65.5)
>30	156(25.7)
Marital status	
Single	164(27.1)
Concubine	58(9.6)
Married	362(59.7)
Widow	22(3.6)
Level Educational	
None	17(2.8)
Primary	107(17.7)
Secondary	301(49.7)
Tertiary	181(29.9)
Gestational age classification	
First (<14weeks)	106(17.5)
Second (14-28weeks)	150(24.8)
Third (>28weeks)	350(57.8)
Gravidity (number of pregnancies)	
Primigravidae (1)	199(32.8)
Multigravidae(2-4)	381(62.9)
Grandmultigravidae (>4)	26(4.3)

Despite the low HIV prevalence (4.3%) in Cameroon in 2016 [22], the prevalence of HIV (11.6%) among pregnant women was high compared to the 0.5-10.3% range reported in other countries of the world [14,17,23] and from other towns in Cameroon [20,21]. However, it was quite low compared to the 61.6% reported by Simpore et al. [11] in a study carried out in Burkina Faso.

There was no statistical significance between marriage, level of education, gravidity and HIV-1 infection ($p < 0.05$). However, the risk of contracting the disease was 78%, 8%, 14%, and 31% less likely to occur in women of the age group >30years, widows, those who attain secondary school, and women who started ANC at third trimester respectively, while the risk was 9% more likely to occur in grand multigravidae women. In this study, HIV-1 prevalence was highest among the age group >30 years contrary to the previous studies [12,23] which state that HIV-1 prevalence was high in the 21–25 years age range. Similar results have been reported in a different town in Cameroon [16]. This is most likely due to progressive increase duration of exposure to sexual activity in this age group compared to a lower age group. In addition, the majority of the women, in the >30 years age range were multigravida or grand multigravida indicating that they have been exposed more to unprotected sexual intercourse which is a risk factor for HIV infection.

As reported in other studies [19,23,24] married women had a high HIV prevalence (15.5%). However, is contrary to another study from a different town in Cameroon where single women were more infected [16]. It has been reported that susceptibility and vulnerability to HIV/AIDS are attributed to marital and family status [25]. This high HIV prevalence in the group of women is associated with the fact that married women usually have unprotected sex and in addition, it was difficult to assess information on multiple partners in these women although extramarital affairs are common in the said setting.

Data from this study showed that woman who had attained primary education had the highest HIV prevalence (13.1%) followed by those who did not go to school (11.8%). This may be attributed to lack of adequate information on the mode of transmission and prevention of HIV and other STDs. This result is similar to studies by [17,23] and contrary to other studies where women with tertiary education had a higher HIV seroprevalence [19,26].

Prevalence of HIV in this study was insignificantly high among women who were multigravida similar to report by Nayak et al. [23] and contrary to the previous study done by Patil et al. [25] where HIV was common among primigravida. The high prevalence is associated with increased risk of unprotected sexual intercourse in this group of women

The seroprevalence of *T. gondii* infection was 22.9% while seroprevalence for anti-Toxoplasma IgG and IgM, antibodies were 22.3% and 1.8% respectively. The risk of contracting *Toxoplasma* was 93%, 16%, and 37% less likely to occur in the age group >30 years, women who started ANC at second trimester and grand multigravidae women respectively, while the risk was 14% and 24% more likely to occur in women in a concubine relationship and women who had attain tertiary education respectively. The seroprevalence of *T. gondii* infection in this study was found to be lower than the 30-90% range reported in different countries [6,8,12] and was higher compared to the 5.9- 18.5% range in other studies [4,27,28]. In Cameroon, previous studies have reported a range of 54.4-77.1% [5,10,29]. The differences seen with other studies can be attributed to environmental or climatic conditions favoring the transmission and infectivity of *T. gondii* oocysts, diagnostic methods, living styles, standards of the people, sampled populations, cultural characteristics, personal hygienic practice, feeding habits and genetic background [6,10,30]. This decrease in prevalence can be as a result of the awareness that is been created from the result of previous studies.

Detection of both IgG and IgM simultaneously helps to establish the chronological status of *T. gondii* [31]. Toxoplasma IgG antibodies indicate a chronic infection while Toxoplasma IgM antibodies indicate an acute infection [8,12]. The high prevalence of Toxoplasma IgG compared to Toxoplasma IgM antibodies seen in this study have been reported elsewhere [7,30,32]. The low IgM (1.8%) antibodies indicate a low level of acute Toxoplasma infection. It has been reported that acute Toxoplasma infection is associated with a higher risk of maternal-fetal transmission [7,33]. Thus the early diagnosis of Toxo-plasmosis in pregnant mothers is of great importance for early initiation of measures and therapy that reduce the risk of transmission and possible consequence on the newborn. On the

other hand, other studies did not report the presence of *T. gondii*-specific IgM [8,11,12]. However, it has been reported that IgM antibody is usually detected within the first two weeks of infection and reduces to negligible levels within 6 months after exposure [11,12]. This accounts for the low prevalence seen in this study as most women (62.2%) came to ANC at a gestational age of >24 weeks. Nevertheless, other studies have shown that the presence of IgM may not be an acute infection since IgM can persist for prolonged times after infection [28,29]. Thus further research in this area is required to ascertain this fact.

Other studies have reported that the risk of contracting *T. gondii* infection increases with age unlike the case in this study [34,35]. Though age was not a risk factor to the T. *gondii* infection, younger women <21 years were more infected compared to older women. This result contradicts studies by many researchers [4,6,28] that identify age group> 21 years as a risk factor. In addition, the result is similar to studies by Njunda et al. [10] and Shimelis *et al.,* [36] which state that seroprevalence of *T. gondii* does not depend on age. Nevertheless, another study in Cameroon indicates that women aged between 31-35 years had a higher prevalence [10]. The variation in age classification of the different studies can also account for the variation of the results seen in the different studies. The high prevalence in younger women can be attributed to their lifestyle. It has been reported that younger people are more exposed to activities like grilled meat or fish which might be undercooked as well as raw food like fruits and salad which may be contaminated with the parasites hence increased risks of infection [12,13].

In this study no significant association was found between the seroprevalence of toxoplasmosis and educational status as opposed to a study by da Silva et al. [1] who reported low education or illiteracy as a risk factor. A similar finding was recorded by Walle et al. [31]. On the contrary women with tertiary school education which suggests a better understanding of hygiene principles had the highest prevalence of toxoplasmosis. The high prevalence in this group can be attributed with higher socioeconomic standards such as eating of raw vegetables, fruit and roasted meats which have been identified sources of disease transmission.

Table 2. Univariate analysis of HIV prevalence according to socio-demographic and obstetrical characteristics

Demographic characteristics	Number (%)	HIV neg (%)	HIV pos (%)	OR (95% CI)	P value
Age group in years					
<21	53(8.7)	50(94.3)	3(5.7)	0.22	0.001
21-30	397(65.5)	359(90.4)	38(9.6)	(1.38-3.54)	
>30	156(25.7)	127(81.4)	29(18.6)		
Marital status					
Single	164(27.1)	143(87.2)	21(12.8)	0.92	0.65
Concubine	58(9.6)	324(89.5)	38(10.5)	(0.6.3-1.34)	
Married	362(59.7)	49(84.5)	9(15.5)		
Widow	22(3.6)	20(90.9)	2(9.1)		
Level Educational					
None	17(2.8)	15(88.2)	2(11.8)	0.86	0.45
Primary	107(17.7)	93(86.9)	14(13.1)	(0.62-1.23)	
Secondary	301(49.7)	267(88.7)	34(11.3)		
Tertiary	181(29.9)	161(89.0)	20(11.0)		
Gestational age classification					
First (<14 weeks)	106(17.5)	86(81.1)	20(18.9)	0.69	0.021
Second (14-28 weeks)	150(24.8)	132(88.0)	18(12.0)	(0.51-0.97)	
Third (>28 weeks)	350(57.8)	318(90.9)	32(9.1)		
Gravidity (number of pregnancies)					
Primi gravidae (1)	199(32.8)	175(87.9)	24(12.1)	1.09	0.74
Multi gravidae(2-5)	381(62.9)	341(89.5)	40(10.5)	(0.65-1.85)	
Grand muiltigravidae (>5)	26(4.3)	20(76.9)	6(23.1)		

OR: odds ratio

Table 3. Univariate analyses of demographic and obstetrical characteristics *of T. gondii antibodies*

Characteristics	*T. gondii* negative	*T. gondii* positive	*Crude odds ratio*	95% CI	P value
Age group (Year)					
<21	40(75.5)	13(24.5)	0.83	0.56-1.25	0.37
21-30	303(76.3)	94(23.7)			
>30	124(79.5)	32(20.5)			
Marital status					
Single	135(82.3)	29(17.7)	1.14	0.81-1.59	0.46
Married	271(74.9)	91(25.1)			
Concubine	43(74.1)	15(25.9)			
Widow	18(81.8)	4(18.2)			
Level Educational					
None	14(82.4)	3(17.6)	1.24	0.91-1.70	0.16
Primary	89(83.2)	18(23.6)			
Secondary	230(76.4)	71(23.6)			
Tertiary	134(74.0)	47(26.0)			
Gestational age classification					
First (<14weeks)	75(70.8)	31(29.2)	0.94	0.69-1.26	0.65
Second (14-28 weeks)	112(74.7)	38(25.3)			
Third (>28 weeks)	280(80.0)	70(20.0)			
Gravidity					
Primigravidae (1)	143(71.9)	56(28.1)	0.63	0.37-1.04	0.07
Muiltigravidae (2-5)	301(79.0)	80(21.0)			
Grand muiltigravidae (>5)	23(88.5)	3(11.5)			

The degree of severity of the disease depends on the gestational age as severe fetal affection occurred with early gestational age infection [32]. Gestational age did not show any significant association as also reported by Frimpong et al., [4] in another study. Contrary to this study, data presented by Shao et al. [13] showed that gestational age was a significant risk factor. The highest seroprevalence of Toxoplasma antibodies (29.2%) was found in pregnant women at the first trimester is similar to the result of Alsammani [35] contrary to second and third semesters [4,24].

Despite the non-statistical significant association contrary to another study [6], data from this study showed that the risk of toxoplasmosis decreases with increase in gravidity. Primigravidae recorded the highest prevalence of 56(28.1%). This result is contrary to other studies by Awoke et al. [28] and Negero et al. [6] which state that *T. gondii* is more likely to occur in multigravidae. The likely reason for this result is that the test for *T. gondii* has been encouraged for more than 5 years in this setting. As such women with multiple pregnancies are were knowledgeable with the method of prevention than primigravidae women. Secondly, previously infected women must have been treated prior to the present pregnancy.

In this study, no significant difference was seen between seropositivity of *T. gondii* in HIV positive 31(44.3%) and negative 108(20.1%) women similar to studies in other countries [4,14,24]. The high prevalence in this group is as a result of decreased immunity which leads to reactivation of latent infection/tissue toxoplasmosis in HIV positive women [9,30]. The reason for the non-significance in this study can be as a result of the use of antiretroviral therapy (ART). ART suppresses HIV viral replication and increased $CD4^+$ T-cell counts, therefore, preventing the development of opportunistic infections. In addition, since 2012, Bamenda health district in Cameroon has been implementing the test and treat method (option B+) where all HIV pregnant

Table 4. univariate analyses of socio-demographic and obstetrical data of HIV and *T. gondii* co-infection

Demographic characteristics	Number (%)	*Co-infection*	P value
Age group (Year)			
<21	53(8.7)	1(1.9)	0.53
21-30	397(65.5)	22(5.5)	
>30	156(25.7)	8(5.1)	
Marital status			
Single	164(27.1)	8(17.7)	0.30
Concubine	362(59.7)	16(4.4)	
Married	58(9.6)	6(10.3)	
Widow	22(3.6)	1(4.5)	
Level Educational			
None	17(2.8)	1(5.9)	0.91
Primary	107(17.7)	4(3.7)	
Secondary	301(49.7)	16(5.3)	
Tertiary	181(29.9)	10(5.5)	
Gestational age classification			
First (<14weeks)	106(17.5)	9(8.5)	0.07
Second (14-28weeks)	150(24.8)	10(6.7)	
Third (>28weeks)	350(57.8)	12(3.4)	
Gravidity			
Primigravidae (1)	199(32.8)	10(5.0)	0.83
Muiltigravidae(2-5)	381(62.9)	19(5.0)	
Grandmuiltigravidae (>5)	26(4.3)	2(7.7)	

or breasting mothers are placed on ART irrespective of their $CD4^+$ T cell counts or WHO clinical stage [18]. On the other hand studies by Sitoe et al. [37] and Walle et al. [31] showed a significant difference in the prevalence rate between HIV positive and negative women.

In this study we recorded a prevalence of 5.1% co-infection rates lower than the 12- 25% range in other studies [5,12] but higher compared to the 2.1% reported by Fernandes et al. [14]. HIV and *T. gondii* co-infection rate are common in pregnant women because both pregnancy and HIV weakens the immune system that favors *T. gondii* and other opportunistic infection to occur [11,12]. Furthermore, it is more likely that these women with co-infection were recently diagnosed with HIV and were not on treatment or were newly initiated on treatment. In addition, HIV-1 and *T. gondii* co-infection could be attributed to common social lifestyle or associated risk factors common to both infections, such as exposure to sexual contacts, consumption of undercooked meat or roasted meat and raw vegetable.

4. LIMITATIONS

The present study has certain limitations that need to be taken into account. No $CD4^+$ T cells count was measured, History on ART was not taken into consideration, or the year of HIV diagnosis was not known by most women.

5. CONCLUSIONS

This study demonstrates that the prevalence of *T. gondii* infection among pregnant women is decreasing. The high prevalence of *T. gondii and* HIV co-infection among pregnant women indicates a greater probability of congenital transmission of T*. gondii*.

RECOMMENDATIONS

The high prevalence of *T. gondii and* HIV-1 co-infection indicate the need to intensify the education of the associated risk factors of both T*. gondii and* HIV-1 infections and methods of prevention. This will reduce the risk of mother to child transmission and thus prevent the consequences of toxoplasmosis and HIV in children. In addition, serological screening for *T. gondii* infection should be considered as part of an antenatal investigation during ANC follow-up.

CONSENT

Each subject gave their informed and written consents before sample collection. Participation in the study was on a voluntary basis and study

participants were free to withdraw from the study before and after collection of blood samples without losing any of the benefits they were supposed to obtain from the hospital.

ETHICAL APPROVAL

Ethical clearance and administrative authorization were obtained from the ethical review board of the delegation of Public Health Bamenda and Bamenda Regional Hospital review board.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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