



Socio-Demographic Factors and Attitudes Influencing the Seroprevalence of Toxoplasmosis among Pregnant Women in Buea, Sw Cameroon

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Authors' contributions

This work was carried out in collaboration with all authors. Author JLNN designed the work, wrote the protocol, supervised the field and laboratory work, participated in the data analysis and wrote the first draft of the manuscript. Author NJ did the laboratory work, participated in the data analysis and edited the manuscript. Author DF participated in the design of the work, the field and laboratory work and the drafting of the manuscript. Author DNN did the final editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The present study aims to determine the socio-demographic factors and attitudes influencing the seroprevalence of toxoplasmosis among pregnant women in Buea, SW Cameroon. *Toxoplasma gondii* also called *T. gondii* is derived from the Greek words toxon, plasma and gondii which mean bow, creature and the African rodent "gundi" respectively. It is a protozoan parasite that can infect all warm-blooded animals, including humans. Due to the effect of congenital toxoplasmosis on the fetus and the child after birth, the fact that routine screening for *Toxoplasma gondii* infection during

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pregnancy is oftenly not available in our health settings, it is important to examine the situation of pregnant women in Buea. This study was conducted in the Buea, found in Fako Division, South West Region of Cameroon. The study was a cross sectional study carried from March 30th to June 30th 2018 among pregnant women attending antenatal clinic (ANC) in the Solidarity Clinic, Buea. Out of the 314 participants, 48 (15.29%) of pregnant women who had cats in homes and neighborhood were found to be positive for *T. gondii*. Among these ladies who had a habit of eating raw or undercooked meat, 34 (10.83%) of them were seropositive for *T. gondii* infection. Seroprevalence among women who had contact with soil was 26 (8.28%). The study concluded that *Toxoplasma gondii* infection appears to be a public health concern and the prevalence of *T. gondii* infection in pregnant women attending antenatal in the solidarity clinic molyko is 32.5%. Although a bit lower as compared with those reported in other regions in Cameroon, prompt intervention is needed to improve on the health of mother and baby.

Keywords: Seroprevalence; *Toxoplasma gondii*; toxoplasmosis; Cameroon.

1. INTRODUCTION

Toxoplasma gondii also called *T. gondii* is a protozoan parasite that can infect all warm-blooded animals, including humans. The parasite was first described by Laveran in 1900 and was found in the liver and spleen of North African rodents (*Ctenodactylus gundii*) by Nicolle and Manceaux in 1908 [1,2]. The same year Splendore described the organism in rabbit tissue and Darling described the first case in humans [3]. In 1923 parasitic cysts were found on the retina in an 11-month-old child with congenital hydrocephaly and microphthalmia, described by the ophthalmologist Janku [3]. Levaditi suggested a possible connection between congenital hydrocephaly and the parasite and was among the first to report that tissue cysts persist for months [1,3]. Kean et al. were probably the first to describe congenital toxoplasma infection and in 1939 infection with *T. gondii* was established as a prenatal transmitted disease [4]. In 1942, Sabin described the characteristic symptoms and signs of congenital toxoplasmosis, the classic tetrad with chorioretinitis, hydrocephaly, convulsions and intracerebral calcification [5]. Cats are definitive hosts, whereas a wide range of warm blooded animals, including humans serve as intermediate hosts [6]. Toxoplasmosis is reported to be an opportunistic parasitic infection in immunocompromised individuals and may develop life threatening meningo encephalitis disease [7].

High seroprevalence of the infection has been reported among pregnant women of child-bearing age from different parts of the world [8]. The infection occurs widely and varies depending on social and cultural habits, geographic factor, climate and route of transmission especially in warm and humid areas [9,10]. *Toxoplasma*

gondii is transmitted to humans via ingestion of oocyst in water, food or soil contaminated with cat's feces or undercooked meat containing cyst [11]. Women transmit the infection via placenta to their unborn fetus. Other infection pathways are blood transfusion and organs transplantation [2]. Healthy individuals who become infected with *Toxoplasma gondii* may not developed any symptoms because their immune system prevents the parasite from causing illness.

According to Togerso and Macpherson [12], up to 95% of the population may be infected with *Toxoplasma* without developing any symptoms. When illness occurs, it is usually mild with "flu-like" symptoms that last for weeks to months. However, the parasite remains in their body in an inactive state and can become reactivated if the person becomes immune suppressed. *Toxoplasma* infection can reactivate in immune suppressed pregnant women who were infected with the parasite before their pregnancy, and this can lead to congenital infection in the unborn child. *Toxoplasma gondii* in pregnant women can result to fetal and neonatal death or various congenital defects [13]. Researchers estimate that of the over 4 million births in the United States each year, between 400 and 4000 babies are born with toxoplasmosis (congenital toxoplasmosis) [14]. This infection can be mild or severe causing stillbirth, long-term structural and neurological damage and other devastating effects [15]. Generally, if a woman becomes newly infected with toxoplasma during or just before pregnancy, she can pass the infection to her unborn child. The damage to the unborn child is often more severe in early months of the pregnancy and can lead to miscarriage, stillbirth or a child born with signs of toxoplasmosis [15,16]. The child's risk increases proportionally to the time of pregnancy. If a pregnant woman gets infected in the first trimester, the risk that

her baby will also be infected is about 15%, but if infected in the second or third trimesters her baby's risk is at 30% and 60% respectively [16]. Infants infected during pregnancy often show no symptoms at birth but may developed them later in life with potential vision loss, mental disability and seizures [1] and only about 15% of women of child bearing age are immune to toxoplasmosis according to Centre Of Disease Control and Prevention [2]. However, *Toxoplasma gondii* disseminates after infection to many organs and may lead to disease in the eyes [17,18], lymph nodes and central nervous system. Most newborns with congenitally acquired infection with *Toxoplasma gondii* are asymptomatic but clinical manifestation can develop later in life [19].

Diagnosis of infection with *Toxoplasma gondii* during pregnancy is made with the aid of serological tests, particularly the IgG avidity testing that allows for more accurate timing of maternal infection [20].

Due to the effect of congenital toxoplasmosis on the fetus and the child after birth, the fact that routine screening for *Toxoplasma gondii* infection during pregnancy is often not available in our health settings, it is important to examine the situation of pregnant women in Buea. Pregnant women are not routinely screened for Toxoplasma and predisposing factors are not often assessed in every locality despite the fact that toxoplasmosis can produce severe congenital infection with ocular and neurological damage to the infant during pregnancy. With an increasing number of studies showing the benefits of treatment in-utero [21], there is the need for prompt diagnosis and intervention. The aim of this study was to determine the predisposing factors and prevalence of toxoplasmosis in pregnant women in Buea. The rationale of the study was to that the results if well applied, could help the government to understand the burden of the disease, subsidize the cost of diagnosis and treatment among pregnant women and improve on sensitization in relation to pre-disposing factors and preventive measures.

2. MATERIALS AND METHODS

2.1 Study Area, Study Design, Study Population and Sample Size

This study was conducted in the Buea, found in Fako Division, South West Region of Cameroon. It was a hospital-based study. The hospital used

for this study was Solidarity Clinic, Molyko, which is at the heart of the University students' residential area. Buea is situated at the foot of Mount Cameroon. The climate of Buea is of the equatorial type with temperatures that range from 25-29°C annually. There are two main seasons; the rainy season and the dry season. Generally, the volcanic activities of the mountain have rendered the topography of the entire region very rugged. Buea has a cool moist equatorial type climate.

The study was a cross sectional study carried from March 30th to June 30th 2018 among pregnant women attending ANC in the Solidarity Clinic, Buea.

The sample size for this study was calculated using the formula

$$\text{Minimum sample size } (n) = z^2p(1-p)/e^2$$

Where, Z²= Standard normal variant (at 5% type 1 error<0.05) is 1.96.

P=Expected prevalence based on other studies
e=absolute error or precision.

For our study, the prevalence of Toxoplasmosis in pregnancy is estimated at 71.8% which is 0.718 as obtained by Nguetack et al. in [22].

$$\text{Sample size } (n) = (1.96)^2 \times 0.718(1-0.718) / (0.05)^2 = 300.$$

Considering that a minimum of 300 women were to be sampled, 400 consent forms were printed and issued out during the period of study, 314 women consented to participate in the study and 86 refused to sign the consent form and so were excluded from the study. All HIV positive pregnant women were excluded from the study.

2.2 Data Collection

Data were collected in 2 formats since it was a laboratory based study. Firstly structured interview questionnaires were administered in English language or in Pidgin English after the women had accepted to participate and signed the consent form. The questionnaires contained socio-demographic and risk factors. Questionnaires were meant to obtain Socio demographic data such as age, education level (illiterate, primary school, secondary school, and university), occupation, and residency. Clinical data including gestational age, blood transfusion

and behavioral data including animal contacts, raw or undercooked meat consumption, unpasteurized milk or milk products consumption, untreated water consumption, unwashed raw vegetables or fruits consumption, contact with soil (gardening or agriculture), were also obtained from all participants.

Secondly, blood samples were collected by finger pricking from the pregnant women who accepted to participate and signed the consent forms. The blood collected was processed as described by the manufacturer (ONE STEP RAPID TEST+, IVD, NIGNTOP, technology and innovation to improve the quality of life).

2.2.1 Principle of the toxoplasmosis test

The One step IgG/IgM test is a qualitative strip based immune assay for the detection of antibodies (IgG and IgM) in serum or plasma. The test device consist of a colored conjugate pad containing toxo recombinant envelope antigens conjugated with colloid gold (Toxo conjugates) and rabbit IgG gold conjugates.

A nitrocellulose membrane band containing two test bands (T1 and T2) and a control band (C band).The T1 band is pre coated with the antibody for the detection of IgM anti TOXO, T2 band is coated with antibody for the detection of IgG anti TOXO and the C band is pre coated with goat anti rabbit IgG. When an adequate sample is placed on the cassette it migrates by capillary action up the band. IgG anti toxo if present will bind to the TOXO conjugate. The immune complex is then captured by the reagent pre-coated on the T2 band forming a burgundy colored T2 band ,indicating a TOXO IgG positive and a suggesting a recent or repeat infection. IgM anti TOXO if present in the sample will bind to the TOXO conjugate. The immune complex is then captured by the reagent coated T1 forming a burgundy colored T1 indicating a fresh infection.

Absence of any T bands (T1 and T2) indicates a negative result. The test contains an internal control band the C band which should exhibit a colored ban of the immune complex of goat anti rabbit IgG/rabbit IgG- gold conjugate regardless of the colour development on any of the T bands.

2.2.2 Procedure

- The test reagent, buffer, controls and samples were allowed to reach room

temperature (15 to 30°C). The sensitivity of the test could be reduced at low temperature.

- The pouch containing the test device was brought to room temperature before opening to remove the device.
- The test device was placed on a clean and level working surface
- The dropper was held vertically and 1 drop of the specimen (blood, gotten by finger pricking) was transferred (approximately 10 ul) in the specimen well of the test device. Two drops (70 ul) of the buffer were added and the timing was started.
- The results were read after 15 mins. Test strips were discarded after 20 mins to avoid false positive results.

2.2.3 Reading and interpretation

Positive Results were indicated by a red line on the control band (C) and any of the T bands. The appearance of T1 indicates the presence of toxo specific IgM antibodies.

The appearance of T2 indicated the presence of toxo specific IgG antibodies. The appearance of both indicated the presence of both toxo specific IgG and IgM antibodies.

The test was read as negative when the red line was only at the control band and no line on any of the T bands.

The test was said to be invalid when C (control band) failed to appear. This could be because of insufficient volume of sample or incorrect technique. For such cases, the procedure was reviewed and the test was repeated.

2.3 Data Management and Data Analysis

Quantitative data from the field (questionnaires) was double-entered into a computer database designed using MS excel application and regular file backup was done to avoid any loss or tempering of data. All questionnaires were stored in a lockable drawer for confidentiality.

Data was imputed using Microsoft Excel and analyzed using SPSS (Statistical package for social science, version 20). The seroprevalence of toxoplasmosis was calculated as a proportion of serologically positive Anti-*T. gondii* samples among all samples tested at 95% confidential interval and the associated risk factors. Analysis was done to assess the association between the

characteristics of the subjects and the *T. gondii* infection. P value of less than 0.05 in the analysis was considered statistically significant. The chi square was used to test for association between the dependent variable (DV) and the independent variable (IV).

3. RESULTS

3.1 Socio-Demographic Characteristics

Three hundred and fourteen women of age range 17 – 47 years, attending ANC and consultation at the Solidarity Clinic Molyko were recruited in the study. A majority of them were between 23 and 27 years of age (27.7%). Most of them had gone to school with a majority at the university level (44.6%) and very few were illiterates (5.4%). With respect to occupation, a majority of the study population were housewives (29.6%), followed by students (28.9%), government workers (28.0%) and lastly business ladies (13.4%). This information is shown in Table 1.

3.2 Prevalence of *Toxoplasma gondii* in the Study Population

Out of 314 pregnant women in the study, 102 (32.5%) were positive for toxoplasma IgG and IgM antibodies and 212 (67.5%) were negative for toxoplasma antibodies as shown in the Fig. 1.

3.2.1 Prevalence of *Toxoplasma gondii* with respect to gestational age

This study revealed that prevalence of toxoplasmosis varied with trimester of pregnancy. The highest prevalence of 46 (14.65%) was recorded in the second trimester followed by 34 (10.83%) in the third trimester and 22 (7.01%) in the first trimester (Fig. 2) but the difference was not statistically significant ($p = 0.833$).

3.3 Factors Associated with Seropositivity

Out of the 157 participants, 48 (15.29%) of pregnant women who had cats in homes and neighborhood were found to be positive for *T. gondii*. Among these ladies who had a habit of eating raw or undercooked meat, 34 (10.83%) of them were seropositive for *T. gondii* infection. Seroprevalence among women who had contact with soil was 26 (8.28%).

The result also revealed that 6 (1.91%) of *T. gondii* positive cases were from those who had a habit of taking or drinking unpasteurized milk. Among pregnant women who had history of blood transfusion, 2 (0.64%) of them were positive for anti-*T. gondii* antibody (Table 3). On multinomial linear regression, toxoplasmosis was

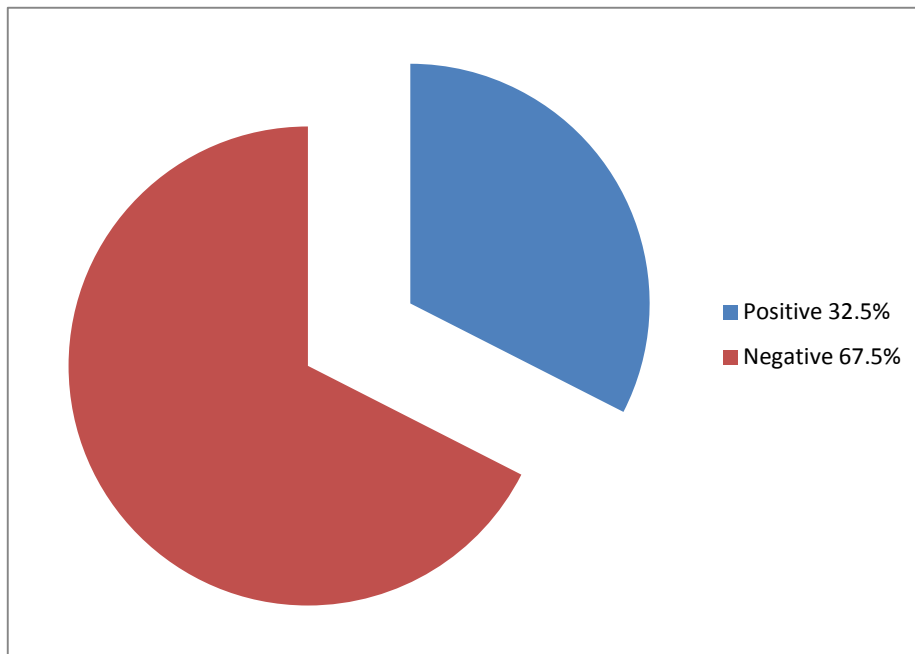


Fig. 1. Prevalence of *T. gondii* in the study population

Table 1. Socio-demographic and clinical characteristics of the study population

Variable	Frequency	Percent (%)
Age (years)		
≤ 20	70	22.3
21 - 25	87	27.7
36 - 30	74	23.6
31 - 35	36	11.5
36 - 40	29	9.2
41 - 45	18	5.7
Total	314	100
Education		
None	17	5.4
Primary	62	19.7
Secondary	95	30.3
University	140	44.6
Total	314	100
Occupation		
Government worker	88	28.0
House wife	93	29.6
Student	91	28.9
Business/ Others	42	13.4
Total	314	100
Duration of Pregnancy (trimester)		
1 – 3 months (first trimester)	78	24.8%
4- 6 months (second trimester)	138	43.9%
7- 9 months (third trimester)	98	31.2%
Total	314	100

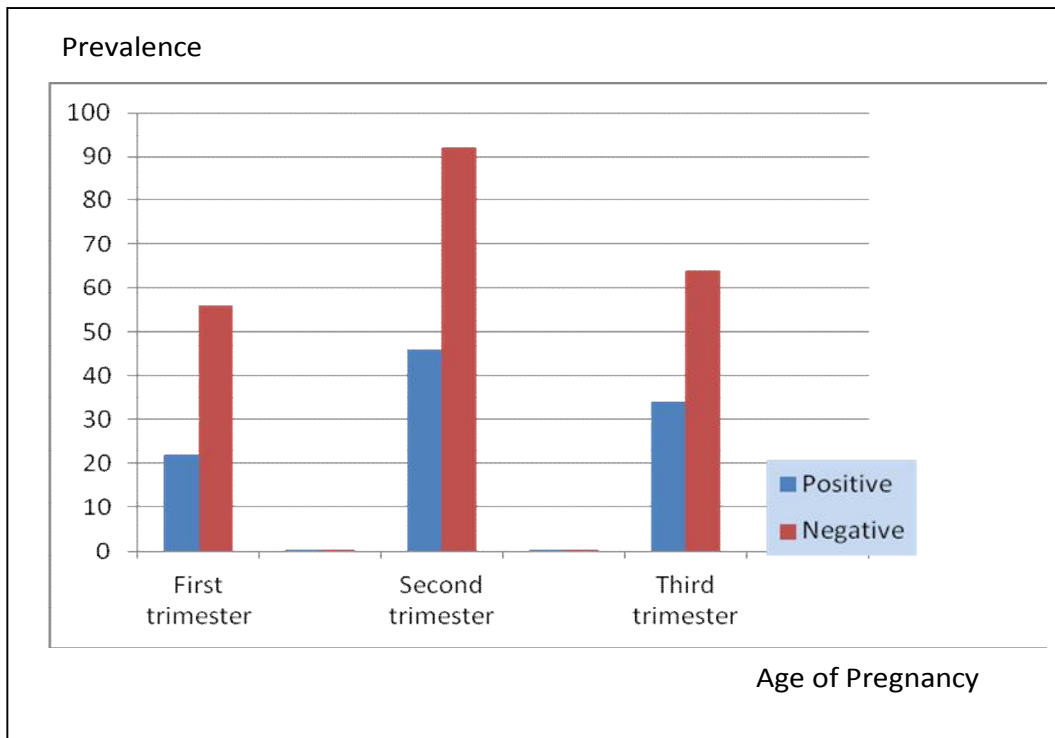


Fig. 2. Prevalence of *Toxoplasma gondii* with respect to trimester

Table 2. Factors predisposing people to infections with *T. gondii*

Variable	Responses	Total	Positive (%)	Negative	χ^2	p value
Cat owners	No	192	54 (17.20)	138(43.9)	1.235	0.266
	Yes	122	48 (15.29)	74(23.6)		
	Total	314	102 (32.5)	212(67.5)		
Contact with Soil	No	226	76 (24.20)	150(47.8)	0.804	0.374
	Yes	88	26 (8.28)	62(19.7)		
	Total	314	102 (32.5)	212(67.5)		
Consume uncooked meat	No	238	68 (21.66)	170(54.1)	4.262	0.039
	Yes	76	34 (10.83)	42(13.4)		
	Total	314	102 (32.5)	212(67.5)		
Consume unpasteurized milk	No	284	96 (30.57)	188(59.9)	3.625	0.078
	Yes	30	6 (1.91)	24(7.6)		
	Total	314	102 (32.5)	212(67.5)		
Blood transfusion	No	302	100 (31.85)	202(64.3)	1.324	0.296
	Yes	12	2 (0.01)	10(3.2)		
	Total	314	102 (32.5)	212(67.5)		

Table 3. Socio-demographic characteristics in relation to the prevalence of Toxoplasmosis

Dependent Variable: IgG						
Variable	Total	Positive (%)	Negative	χ^2	p value	
Age (years)						
≤ 20	70	16 (5.10)	54 (17.9)	4.447	0.7356	
21 – 25	87	34 (10.83)	53 (16.9)			
26 - 30	74	32 (10.19)	42 (13.4)			
31 – 35	36	6 (1.91)	30 (9.6)			
36 - 40	29	8 (2.55)	21 (6.7)			
41 - 45	18	6(1.91)	12 (3.8)			
Total	314	102 (32.5)	212 (67.5)			
Education						
None	18	8 (2.55)	10 (3.2)	1.245	0.700	
Primary	62	18 (5.73)	44 (14.1)			
Secondary	94	26 (8.28)	68 (21.7)			
University	140	50 (15.92)	90 (28.7)			
Total	314	102 (32.5)	212 (67.5)			
Occupation						
Government workers	88	30 (9.55)	58 (18.5)	8.368	0.510	
Housewives	93	44 (14.01)	49 (15.6)			
Student	91	14 (4.46)	77 (24.5)			
Business/ Others	42	14 (4.46)	28 (8.9)			
Total	314	102 (32.5)	212 (67.5)			
Duration of Pregnancy						
First trimester (1 – 3 months)	78	22 (7.01)	56 (17.8)	0.084	0.833	
Second trimester (4 – 6 months)	138	46 (14.65)	92 (29.3)			
Third trimester (7 – 9 months)	98	34 (10.83)	64 (20.4)			
Total	314	102 (32.5)	212 (67.5)			

significantly associated with the consumption of uncooked meat ($p= 0.039$), and insignificantly associated with owning a cat, contact with soil, consumption of unpasteurized milk and blood transfusion ($p> 0.05$). Cat

owners (OR; 1.54, 95% C.I.; 0.72 – 3.26) and consumers of uncooked meat (OR; 2.38, 95% C.I.; 1.04 – 5.42) were clinically more likely to suffer from toxoplasmosis (Table 2).

3.4 Prevalence of *Toxoplasma gondii* in Relation to Demographic Characteristics and Gestational age of the Study Population

Although not statistically significant, the prevalence of toxoplasmosis was highest in the age group 21 to 24 years (34, 10.83%), followed by 26 to 31 age group (32, 10.19%). The most educate group of pregnant women involved in this study (those who have acquired university education) recorded the highest prevalence of Toxoplasmosis (50, 15.92%). With respect to occupation, house wives recorded the highest prevalence and women in the second trimester of pregnancy had higher prevalence values compared to those in the first and third trimesters as seen in Table 3.

4. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion of Findings

The study revealed that the prevalence of toxoplasmosis among pregnant women was (32.5%). Considering the severe consequences that this disease has on the lives of the mother and the foetus, this prevalence is considered high. In Cameroon, and especially in Buea, diagnosis for toxoplasmosis is not done as part of routine ANC package. So many women infected go undiagnosed and untreated. As such pathological consequences resulting from this condition such as still birth and spontaneous abortions can be erroneously attributed to other pathologies such and malaria (which is endemic in the study area). In comparison with studies from other parts of Cameroon, the prevalence reported in this study was far lower than the 78.6% seroprevalence reported by Nguéfac et al. [22] in Douala, littoral region, 54.5% seroprevalence reported by Chongsi et al. [23] at Njinikom, North West, 77.1% reported among pregnant women in Yaounde by Ndumbe et al. [24] and 70% reported among pregnant women attending the Douala General Hospital by Njunda et al. [25]. The lower prevalence recorded in this study could be due to the fact that awareness (though still timid) increases as years go by. The higher prevalence in other studies could be due to the fact that they used the ELISA method of detection of *Toxoplasma* antibodies [22,26] which has been reported to have high specificity and sensitivity values [23].

This result was equally lower than the 8.6% seroprevalence reported among pregnant

women in Jimma town Ethiopia by Zemene et al. [27], 10.3% prevalence of toxoplasmosis among pregnant women in Japan by Sakikawa et al. [28] and 11% seroprevalence and congenital Toxoplasmosis among pregnant women in USA by Pappas et al. [29]. This may be due to changes in climate, cultural and ethnic practices and the difference in the diagnostic techniques used by different researchers.

In relation to pre-disposing factors, our result are similar with the 18.1% reported on seroprevalence among pregnant women in South Africa by Bessong et al. [30] and 19.1% reported on risk factors among pregnant women in Italy by Pezzoli et al. [31]. The similarities might be due to similarities in predisposing factors such as consumption of undercooked meat and vegetables and keeping of house pets especially cats.

This study indentified consumption of uncooked meat (17%) as a significant risk factors associated with Toxoplasmosis positivity in our study. This might be due to the fact that the majority of the positive cases were bellow 30 years. There is the habit of consuming meat sold by the road-side in Buea and this practice is common among young women from 18 to 30 years. Sometimes, the level of cooking is doubtful. The consumption of undercooked meat is associated with ingestion of cyst (zoites) found in the muscles of the vertebrate host (intermediate host). This constitutes one main route of transmission. The consumption of undercooked meat has also been reported as a risk factor in USA by Jones et al. [32]. This is also in line with studies carried out by Agmas, Tesfaye, et al. [33] demonstrating a significant association between sero-positivity and consumption of uncooked meat. Considering the severe consequences of Toxoplasmosis on the foetus and and the pregnant woman [34], it is necessary to take all measures to prevent transmission and treat all positive cases promptly.

Although not significant, 15.29% of the population that was positive for toxoplasmosis, owned cats (the definitive host for toxoplasmosis). This proportion of the population could have gotten the infection from infected cats that served as house pets. The emptying of litter boxes of the pets coupled with poor hygiene and ignorance in relation to the disease life cycle could be one main reason for infection transmission among pet owners.

4.2 CONCLUSION

We concluded that *Toxoplasma gondii* infection appears to be a public health concern and the prevalence of *T. gondii* infection in pregnant women attending antenatal in the Solidarity Clinic Molyko is 32.5%. Although a bit lower as compared with those reported in other regions in Cameroon, intervention is needed to improve on the health of mother and baby.

4.3 RECOMMENDATIONS

At the end of these studies, it was recommended that

1. More awareness on the source of infection, modes of transmission and prevention of *T. gondii* in pregnant women should be created through local media, churches, and mass media. Routine screening for *Toxoplasma gondii* should be part of antenatal package for every pregnant woman.
2. Reducing the cost price for the test could make it more affordable and available to the general population (that is the rich and the poor).
3. Government could revise policy and subsidize the drugs so that many more pregnant women can afford the treatment.

CONSENT

As per international standard informed and written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

Administrative and ethical clearances were obtained from the Regional Delegation of Public Health, South West, Biaka Institutional Review Board and authorization from the Director of the Clinic. Permission was also obtained from the Laboratory Head before consulting patients and collecting the samples.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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