Eyong et al. *Afr. J. Clin. Exper. Microbiol.* 2024; 25 (4): 401 – 412 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Oct 2024; Vol.25 No.4
AJCEM/2336. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Seroprevalence and risk factors of Toxoplasmosis in HIV-positive and negative patients attending the Bamenda Regional Hospital, North West Region, Cameroon

*[1]Eyong, E. E. J., [1]Elinor, T. S., and [2]Moses, S.

[1]Department of Microbiology and Parasitology, Faculty of Science, The University of Bamenda, P. O. Box 39, Bambili, North West Region, Cameroon
[2]Department of Medical Laboratory Sciences, Faculty of Health Sciences, The University of Bamenda, P. O. Box 39, Bambili, North West Region, Cameroon
*Correspondence to: joan.ebangaechi@gmail.com

Abstract:

Background: Toxoplasmosis, caused by *Toxoplasma gondii,* can infect almost all warm-blooded animals including humans. *T. gondii* is regarded as an important opportunistic pathogen that can cause infection in HIV/AIDS patients associated with high mortality. The objective of this study is to determine the prevalence and risk factors of *T. gondii* infection in HIV-positive and negative patients and establish the relationship between HIV viral load and active toxoplasmosis in the Bamenda Regional Hospital Cameroon.
Methodology: Three hundred and three (201 HIV-positive and 102 HIV-negative) participants attending the Bamenda Regional Hospital, Cameroon, were randomly recruited into the study. Well-structured questionnaires were used to obtain information on demographic characteristics and potential risk factors from each participant. Venous blood samples were collected for serological detection of toxoplasmosis using One-Step Toxo IgG/IgM rapid diagnostic test (RDT) kits, followed by confirmation of all positive samples by *Toxoplasma* IgM ELISA test. Data were analysed on SPSS version 23.0. Association of potential risk factors with seroprevalence of toxoplasmosis was done using Chi square test, and comparison of means HIV loads between participant categories was done using Mann-Whitney U-test and Kruskal-Wallis as applicable, with $p<0.05$ considered as significant level.
Results: Of the 303 participants, 93 were positive for *Toxoplasma* IgG, giving latent toxoplasmosis seroprevalence rate of 30.7%, while 2 of the 303 participants were seropositive for *Toxoplasma* IgM, indicating active toxoplasmosis in 0.7%. The seroprevalence of latent toxoplasmosis was 28.9% (n=58/201) in HIV-positive compared to 34.3% (n=35/102) in HIV-negative participants ($\chi^2=0.948$, OR=0.775, $p=0.330$), while the seroprevalence of active toxoplasmosis was 1.0% (n=2/201) in HIV-positive compared to 0% (n=0/102) in HIV-negative participants ($\chi^2=0.068$, OR=2.568, $p=0.552$). Viral load was detectable (≥ 42 viral copies/ml) in 54 of the 201 HIV-positive participants, giving an overall detectable viral load rate of 26.9%. The seroprevalence of latent toxoplasmosis was higher in HIV-positive participants with detectable viral load than those with non-detectable viral load, and the two HIV-positive patients with active toxoplasmosis had detectable viral loads. The risk factors significantly associated ($p<0.05$) with the latent toxoplasmosis were owning a cat, presence of stray cats, playing with cats, and eating soya.
Conclusion: Latent toxoplasmosis is prevalent among HIV-positive and negative patients attending the Bamenda Regional Hospital in Cameroon, with active infection only among HIV-infected patients.

Keywords: Prevalence; Toxoplasmosis; Risk factors; HIV/AIDS; Bamenda; Cameroon

Received Feb 13, 2024; Revised Aug 01, 2024; Accepted Aug 05, 2024

Séroprévalence et facteurs de risque de la Toxoplasmose chez les patients séropositifs et séronégatifs fréquentant l'Hôpital Régional de Bamenda, région du Nord-Ouest, Cameroun

*[1]Eyong, E. E. J., [1]Elinor, T. S., et [2]Moses, S.

[1]Département de Microbiologie et Parasitologie, Faculté des Sciences, Université de Bamenda, BP 39, Bambili, région du Nord-Ouest, Cameroun
[2]Département des sciences de laboratoire médical, Faculté des sciences de la santé, Université de Bamenda, BP 39, Bambili, région du Nord-Ouest, Cameroun
*Correspondance à: joan.ebangaechi@gmail.com

Résumé:

Contexte: La toxoplasmose, causée par *Toxoplasma gondii*, peut infecter presque tous les animaux à sang chaud, y compris les humains. *T. gondii* est considéré comme un pathogène opportuniste important qui peut provoquer une infection chez les patients atteints du VIH/SIDA associée à une mortalité élevée. L'objectif de cette étude est de déterminer la prévalence et les facteurs de risque de l'infection à *T. gondii* chez les patients séropositifs et séronégatifs et d'établir la relation entre la charge virale du VIH et la toxoplasmose active à l'Hôpital Régional de Bamenda, au Cameroun.
Méthodologie: Trois cent trois participants (201 séropositifs et 102 séronégatifs) fréquentant l'Hôpital Régional de Bamenda, au Cameroun, ont été recrutés au hasard pour l'étude. Des questionnaires bien structurés ont été utilisés pour obtenir des informations sur les caractéristiques démographiques et les facteurs de risque potentiels de chaque participant. Des échantillons de sang veineux ont été prélevés pour la détection sérologique de la toxoplasmose à l'aide de kits de diagnostic rapide (TDR) One-Step Toxo IgG/IgM, suivis d'une confirmation de tous les échantillons positifs par le test ELISA *Toxoplasma* IgM. Les données ont été analysées sur SPSS version 23.0. Français L'association des facteurs de risque potentiels avec la séroprévalence de la toxoplasmose a été réalisée en utilisant le test du Chi carré, et la comparaison des charges moyennes de VIH entre les catégories de participants a été effectuée en utilisant le test U de Mann-Whitney et le test de Kruskal-Wallis, selon le cas, avec $p<0,05$ considéré comme un niveau significatif.
Résultats: Sur les 303 participants, 93 étaient positifs pour *Toxoplasma* IgG, ce qui donne un taux de séroprévalence de la toxoplasmose latente de 30,7%, tandis que 2 des 303 participants étaient séropositifs pour *Toxoplasma* IgM, indiquant une toxoplasmose active dans 0,7% des cas. Français La séroprévalence de la toxoplasmose latente était de 28,9% (n=58/201) chez les participants séropositifs pour le VIH contre 34,3% (n=35/102) chez les participants séronégatifs pour le VIH (χ^2=0,948, OR=0,775, p=0,330), tandis que la séroprévalence de la toxoplasmose active était de 1,0% (n=2/201) chez les participants séropositifs pour le VIH contre 0% (n=0/102) chez les participants séronégatifs pour le VIH (χ^2=0,068, OR=2,568, p=0,552). La charge virale était détectable (≥ 42 copies virales/ml) chez 54 des 201 participants séropositifs, soit un taux global de charge virale détectable de 26,9%. La séroprévalence de la toxoplasmose latente était plus élevée chez les participants séropositifs avec une charge virale détectable que chez ceux avec une charge virale non détectable, et les deux patients séropositifs avec toxoplasmose active avaient des charges virales détectables. Les facteurs de risque significativement associés ($p<0,05$) à la toxoplasmose latente étaient la possession d'un chat, la présence de chats errants, le fait de jouer avec des chats et la consommation de soja.
Conclusion: La toxoplasmose latente est prévalente chez les patients séropositifs et séronégatifs fréquentant l'hôpital régional de Bamenda au Cameroun, l'infection active n'étant présente que chez les patients infectés par le VIH.

Mots clés: Prévalence; Toxoplasmose; Facteurs de risque; VIH/SIDA; Bamenda; Cameroun

Introduction:

Toxoplasmosis is a disease caused by the parasitic protozoan *Toxoplasma gondii* which can infect almost all warm-blooded animals including humans. It has a complex life cycle, undergoing sexual phase in the feline definitive host and asexual phase in its intermediate hosts which includes humans. Approximately 30% of the world's population is estimated to be infected with *T. gondii* (1). According to the World Health Organization (2), over 1 million cases of *Toxoplasma* infections in the European region is caused by contaminated food. The prevalence of toxoplasmosis was reported by Njunda et al., (3) in Cameroon to be 48.0%.

Humans become primarily infected by ingesting raw or undercooked meat containing viable tissue cysts or by ingesting water or food contaminated with oocysts from infected cat feces. Another route of infection is from infected mother to her fetus via placental transmission of *T. gondii*, a process known as congenital toxoplasmosis. Congenital toxoplasmosis may cause stillbirth or abortion in addition to serious damages to the fetus such as severe neurological disorders after delivery.

In healthy humans, infection with *T. gondii* is usually asymptomatic, however, infection can change into a chronic status, especially in the central nervous system of the individuals affected. In HIV/AIDS patients, a high viral load can lead to impairment of T-cell mediated immune response and this sometimes reactivate infection to cause toxoplasmic lymphadenitis, meningo encephalitis, or ocular toxoplasmosis (4).

An increased frequency of *Toxoplasma* encephalitis has been reported in AIDS patients, especially those with significant immunosuppression, when $CD4^+$ T lymphocyte cell counts are < 200 cells/µL. *T. gondii* infection is regarded as an important opportunistic pathogen that can lead to the death of AIDS patients (1). Primary infection with the parasite initially results in IgM immune response, followed by specific IgG anti-*T. gondii* response. Several studies have reported varying magnitudes of latent *T. gondii* infection in HIV-infected individuals (1,4).

The seroprevalence of *T. gondii* in HIV-infected individuals is often high in most of the reports, with substantial incidence of toxoplasmic encephalitis in AIDS patients not receiving prophylaxis. The threat that this disease poses amongst these patients was the motivation for this study. The objective was to determine the prevalence and risk factors of *T. gondii* infection in HIV-infected and HIV-negative patients and establish the

relationship between viral load in HIV-positive patients with active toxoplasmosis, with the overall aim of providing current information for effective preventive and curative management of toxoplasmosis among patients with high risk of exposure to *Toxoplasma*.

Materials and method:

Study area:

This study was carried out in the Bamenda Regional Hospital, specifically in the day hospital section in collaboration with the Biochemistry and Serology units of the hospital laboratory. Bamenda is located in the western highlands of Cameroon between latitudes $5^0$56' and $5^0$58' North of the equator and longitude $09^0$10' and 11^0 10' East of the Greenwich Meridian (5). Bamenda has an elevation of 1200-1700m above sea level and two main seasons; rainy season, which lasts for 8 months extending from March to October and the dry season from November to February (6). The average annual rainfall is 2400mm, and the average annual temperature is 23°C (7).

As of 2014 the population of Bamenda stood at 393,835 persons (5) and is comprised of high-income earners who are mostly civil servants and business men (8). There is poor town planning and housing facility. Road sides serve as disposal grounds for refuse and with these conditions many parasites and vectors proliferate (8). Most people in the locality keep pets and domestic animals like cats and dogs in free range and this condition facilitates spreading of parasites between different hosts. The Bamenda Regional Hospital was created in 1956 by Mr. Roberts, the then British High Commissioner based in Lagos Nigeria. The hospital was given the status of a third level reference health institution for the North West in 2009 to serve the population of Bamenda.

Study design:

This study was a comparative cross-sectional study of HIV/AIDS and HIV-negative participants for seroprevalence and risk factors of toxoplasmosis conducted over a period of 5 months (December 2019 to March 2020).

Ethical consideration:

Ethical clearance for this study was obtained from the ethical review board in the Faculty of Health Sciences of the University of Bamenda. An administrative clearance was obtained from the North West Regional Delegation for Public Health. An authorization to carry out research was also obtained from the General Supervisor of the Bamenda Regional Hospital. All participants enrolled in this study were treated properly and with standard of care. Each participant's information was kept confidential.

Before participating in research, all participants were appropriately informed of the objectives of the study with clear and simple explanations. Pain during collection of blood sample by venipuncture was minimized as much as possible. Sterilized syringes or vacutainer needles were used on each participant. Those who refused to participate in this study were not forced to do so. A consent form was also given to each participant which he/she voluntarily signed before participating in the study.

Sample size estimation:

The sample size was calculated using the formula described by Naing et al., (9); $n=z^2p(1-p)/d^2$, where n=sample size, z=statistic for a level of confidence (z=1.96 at 95% confidence interval), p=expected prevalence or proportion (=0.317) from a previous study by Ngobeni and Samie (10), and d=precision (=0.5). This gave a calculated sample size of 332. However, only 303 participants were recruited into the study, made of 201 HIV/AIDS patients and 102 HIV-negative patients as comparative group.

Study participants, inclusion and exclusion criteria, and sampling method:

The participants in this study included all those who came to the Day hospital during the period of the study for their viral load test. They also included all patients whose viral load results were less than 4 months old. HIV-infected patients of all ages, genders, and from different localities, who voluntarily accepted were part of the study.

HIV-negative patients who presented in the Day hospital for different medical purposes at the hospital main laboratory within the period of the study were recruited as comparative group. Excluded from the study were persons those who did not yet know their HIV status and came to the Day Hospital for HIV test. Also excluded were patients who came to collect their antiretroviral drugs but were not ready for viral load test. A simple random sampling technique was used to select the research participants into the study.

Data collection:

Structured questionnaires, comprising 15 short questions on participants exposure to some selected risk factors for toxoplasmosis transmission, were interviewer-administered by the researchers to each consenting participant. Participants' exposure to risk factors was assessed by asking questions related to their feeding and drinking habits, hygienic practices and other practices that could predispose them to infection with *Toxo-*

plasma such as playing with cats or farming practice.

Blood collection and serum extraction:

The blood sample (about 2ml) was aseptically collected by venipuncture using a vacutainer needle or sterilized syringe as described in Cheesbrough (11). Briefly, after blood collection, the tubes were allowed to stand at room temperature for 30 minutes in a well labelled tube. The blood samples were then centrifuged using a digital centrifuge (Medsor Impex) at 3000g for 2 minutes. After centrifuging, 300 µl of serum transferred into another appropriately labelled dry test tube using a micropipette. At the end of each day, the samples were stored in a refrigerator at -20°C till the day of analysis.

Detection of *Toxoplasma* antibodies by RDT:

The procedure for detecting *Toxoplasma* antibodies in the serum were performed according to the manufacturer's guide on the One-Step Toxo IgG/IgM RDT kit. The frozen serum samples were thawed and allowed to reach room temperature (25°C) and then properly mixed before testing. The test kits were also brought to room temperature before running the test. Before testing was started, a control test was done using some toxoplasmosis positive serum samples from the serology department of the hospital laboratory. This was to ensure the validity and authenticity of the test kits and to avoid faulty results at the end. The test device was placed on a clean, dry and level surface. A dropper was used to transfer a drop (about 10µl) of serum to the specimen well of the test device, 2 drops of buffer (about 80 µl) were added and the timer started. After 15 minutes, the results were read from the colored lines that appeared on the kit. No result was interpreted after 20 minutes according to the manufacturer's instruction.

If two color lines appeared (one at control region 'C' and another at test region 'G'), this indicated secondary or previous *Toxoplasma* infection. If two color lines appeared (one at control region 'C' and another at test region 'M'), this indicated primary *Toxoplasma* infection. If three color lines appeared (one at control line 'C', one at test region 'G' and one at test line 'M'), this indicated late primary or early secondary *Toxoplasma* infection. If only one-color band appeared at the control line 'C', this indicated negative test for toxoplasmosis, and if no line appeared at the different positions, it indicated an invalid result, necessitating a repeat testing.

Confirmatory detection of *Toxoplasma* antibody by ELISA:

Toxoplasma IgM antibody test was performed on all 93 samples positive for *Toxoplasma* IgG test in the RDT, using a packet of 96 ELISA kits (Erbalisa *Toxoplasma* IgM) to confirm the presence of IgM. The procedure was carried out as outlined on the ELISA kit. The desired numbers of coated wells were placed in the holder. Test samples were prepared (1:40 dilution) (negative control, positive control, and calibrators) by adding 5 µl of the sample to 200 µl of sample diluent and well mixed. The diluted sera were dispensed (100 µl), calibrators, and controls into the appropriate wells. For the reagent blank, 100 µl sample diluent was dispensed in 1A well position. The holder was tapped gently to remove air bubbles from the liquid.

The mixture was incubated at 37°C for 30 minutes. At the end of incubation period, all liquid from all wells was removed. The microtiter wells were rinsed 5 times with diluted wash buffer (1x). 100 µl of enzyme conjugate was dispensed to each well and mixed gently for 10 seconds, then incubated at 37°C for 30 minutes. After 30 minutes, the enzyme conjugate from all wells was removed and the wells rinsed and flicked 5 times with diluted wash buffer (1x). 100 µl of TMB reagent was dispensed into each well and mixed gently for 10 seconds, then incubated at 37°C for 15 minutes. 100 µl of stop solution (1N HCl) was added and mixed gently for 30 seconds to stop reaction. A complete color change from blue to yellow was ensured. The optical density (O.D) was read with a microwell reader at 450 nm within 15 minutes.

Viral load of the HIV/AIDS participants:

The most recent viral load values of the 201 HIV/AIDS participants were obtained from the registers of the Day Hospital with the full permission of the administrators.

Data analysis:

All data obtained from the study were entered into Microsoft Excel 2016 spread sheet, filtered and coded. Data were as then imported and analyzed with the Statistical Package for the Social Sciences (SPSS) version 23.0. Frequencies were calculated and the proportions in different categories including important risk factors associated with *Toxoplasma* seroprevalence were compared using the Chi-square test. The Mann-Whitney U test and Kruskal and Wallis test were used to compare differences between means in different categories. A Pearson correlation was done to determine the correlation co-

efficient. Significant levels were measured at 95% confidence level, with significant differences recorded at p <0.05.

Results:

Demographic characteristics of the study participants:

A total of 303 participants aged 2-90 years participated in the study. There were 72.9% (n=221) females and 27.1% (n=82) males. The majority of the participants were in the age group 35-49 years (43.2%, n= 131), while the age group ≤ 34years (27.1%, n=82) constituted the least number of participants. The mean age of the study participants was 42±14 years (Table 1).

Of all the participants, 72% (n=220) were from an urban settlement while 27.4% (n=83) were from rural settlement. Overall, 3.6% (n=11) of the participants had no formal education, 49.2% (n=149) attended primary education, 32.0% (n=97) had secondary education while 15.2% (n=46) had tertiary education. With respect to occupation, the participants were divided into civil servants 9.9% (n=30), self-employed (58.1%, n=176), and farmers (32.0%, n=97). With respect to marital status, 35.6% (n=108) were single while 64.4% (n=195) were married (Table 1).

A total of 201 participants (66.3%) were HIV-positive in the study while 102 were HIV-negative participants (33.7%). HIV positive participants in the study have been receiving treatment for a period ranging from 1 to 20 years and were categorised as <6 years (24.4%, n=49), 6-10 years (31.3%, n=63) and > 10 years (44.3%, n=89) as shown in Table 1.

Prevalence of *Toxoplasma* IgG and association with sociodemographic characteristics & HIV status of the study participants:

The overall prevalence of *Toxoplasma* IgG among the study participants was 30.7% (93/303). The seroprevalence was higher in males (31.7%, 26/82) than in females (30.3%, 67/221), but the difference was not statistically significant (χ^2=0.054, p=0.816) (Table 2). The prevalence was highest among participants in the age group 35-49 years (33.6%, 44/131) and lowest in those among the age group ≥50years (25.6%, 23/90) but the difference was also not statistically significant (χ^2=1.679, p=0.433). The participants who lived in rural areas had a higher prevalence of *Toxoplasma* IgG (34.9%, 29/83) than those who lived in urban areas (29.1%, 64/ 220) but the difference was not statistically significant (χ^2=0.969; p=0.325).

Table 1: Sociodemographic characteristics of the study participants at Bamenda Regional Hospital, North West Region, Cameroon

Characteristics	Category	Frequency	Percentage
Gender	Male	82	27.1
	Female	221	72.9
Age group (years)	≤ 34	82	27.1
	35-49	131	43.2
	≥ 50	90	29.7
Mean age (±SD) (years)		42 (±14)	
Residence	Urban	220	72.6
	Rural	83	27.4
HIV status	Positive	201	66.3
	Negative	102	33.7
Duration of treatment (years)	<6	49	24.4
	6-10	63	31.3
	>10	89	44.3
Educational level	No formal	11	3.6
	Primary	149	49.2
	Secondary	97	32.0
	Tertiary	46	15.2
Occupation	Civil servant	30	9.9
	Self employed	178	58.1
	Farmers	97	32.0
Marital status	Single	108	35.6
	Married	195	64.4

Table 2: Prevalence of *Toxoplasma* IgG and association with demographic characteristics and HIV status of the study participants in Bamenda Regional Hospital, Cameroon

Characteristics	Category	Number examined	Number (%) positive	Statistical parameter
Gender	Male	82	26 (31.7)	χ^2 = 0.054; *p*= 0.816
	Female	221	67 (30.3)	
Age group (years)	≤ 34	82	26 (31.7)	χ^2 = 1.679; *p*= 0.433
	35-49	131	44 (33.6)	
	≥ 50	90	23 (25.6)	
Residence	Urban	220	64 (29.1)	χ^2 = 0.969; *p*= 0.325
	Rural	83	29 (34.9)	
HIV status	Positive	201	58 (28.9)	χ^2 = 0.948; *p*= 0.330
	Negative	102	35 (34.3)	
Duration of treatment (years)	<6	49	15 (30.6)	χ^2 = 0.724; *p*= 0.696
	6-10	63	20 (31.7)	
	>10	89	23 (25.8)	
Educational level	No formal	11	4 (36.4)	χ^2 = 0.678; *p*= 0.878
	Primary	149	47 (31.5)	
	Secondary	97	30 (30.9)	
	Tertiary	46	12 (26.1)	
Occupation	Civil servant	30	9 (30.0)	χ^2 = 0.264; *p*= 0.876
	Self employed	178	56 (31.8)	
	Farmers	97	28 (28.9)	
Marital status	Single	108	32 (29.6)	χ^2 = 0.089; *p* = 0.765
	Married	195	61 (31.3)	
Total		**303**	**93 (30.7)**	

x^2=Chi-square

With respect to the level of education, the prevalence was highest among participants with no formal education (36.4%, 4/11) and lowest among those with tertiary level education (26.1%, 12/46), although the difference was not statistically significant (χ^2 =0.678; *p*=0.878). With respect to occupation, the highest prevalence of *Toxoplasma* IgG was observed among those who were self-employed (31.8%, 56/178), and lowest among farmers (28.9%, 28/97) but, there was no statistically significant difference (χ^2= 0.264; *p*=0.876). The prevalence was higher among married participants (31.3%, 61/196) than among single participants (29.6%, 32/ 108), but the difference was not statistically significant (χ^2=0.089; *p*=0.765).

The participants who were HIV-negative had higher prevalence (34.3%, 35/102) than HIV-positive ones (28.9%, 58/201), but the difference was not statistically significant (χ^2=0.948, *p*=0.330). Of the HIV-positive participants, those who had received treatment with ART for 6-10 years had the highest prevalence (31.7%, 20/63) while the lowest prevalence was amongst those who had received treatment with ART for above 10 years (25.8%, 23/89), although the difference was not statistically significant (χ^2=0.724; *p*=0.696).

Prevalence of *Toxoplasma* IgM:

For all the samples positive for IgG (RDT), an ELISA confirmatory test was done for the detection of active toxoplasmosis. Of the 303 samples by the ELISA confirmatory test, 2 were positive for *Toxoplasma* IgM giving an overall prevalence of active infection among the study participants to be 0.7% (2 of 303). The two active toxoplasmosis cases were males, both lived in urban areas, both are HIV-infected (one on ART < 6 years and the other on ART >10 years), both are self-employed, one had primary and the other had secondary education, and one is single while the other is married.

Risk factors for latent toxoplasmosis:

Of the potential risk factors for latent toxoplasmosis considered among the 303 participants, 77 (25.7%) owned cats while 226 (74.6%) did not. Of the 77 who owned cats, 8 (10.4%) had cages for their cats while 69 (89.6%) kept their cats' free range (Table 4). Two hundred and thirty (75.9%) saw stray cats while 73 (24.1%) did not see stray cats in their neighbourhood. Among the participants, 176 (58.1%) saw cats entering their homes while 127 (41.9%) did not see stray cats entering their houses.

Table 4: Bivariate analysis of the seroprevalence of potential risk factors of latent toxoplasmosis among the study participants at Bamenda Regional Hospital, Cameroon and bivariate analysis

Risk factors	**Category**	**Number examined**	**No positive for Toxo IgG**	x^2	***p* value**
Own a cat	No	226	00	4.46	0.042
	Yes	77	70		
Stray cats in the neighborhood	No	73	3	5.56	0.041
	Yes	230	90		
Cat lives in a cage	No	69	12	6.65	0.679
	Yes	8	1		
Cats entering the house	No	127	1	10.25	0.031
	Yes	176	80		
Play with/ touch cats	No	234			
	Yes	69	50	12.43	0.002
Clean cat faeces	No	201	1	12.23	0.053
	Yes	102	2		
Use gloves when cleaning	No	99	35	9.87	0.677
	Yes	3	2		
Place of disposal of feces	Bushes	80	34	9.75	0.711
	Manure	5	3		
	Latrines	17	10		
Source of water	Well	101	23	6.65	0.437
	Pipe borne	96	12		
	Both	106	2		
Covered well	Yes	37	1	2.35	0.341
	No	59	4		
	Sometimes	5	6		
Eat soya	No	56	2	14.56	0.002
	Yes	247	70		
Consume smoked meat	No	17	2	6.56	0.681
	Yes	286	13		
Eat in local restaurants	No	62	2	7.78	0.791
	Yes	241	43		
Practice farming	No	64	1	14.56	0.785
	Yes	239	35		
Wash hands with soap before eating	No	199	23	12.35	0.665
	Yes	104	2		
Drink unpasteurized milk/'folere'	No	63	1	10.25	0.561
	Yes	240	20		

Two hundred and thirty-four (77.2%) participants have the habit of playing with or touching cats while 69 (22.8%) did not. A total of 102 (33.7%) participants had cleaned cat faeces while 201 (66.3%) had never cleaned cat faeces. Of the 102 who had cleaned cat faeces, only 3 (2.9%) used gloves when cleaning, 80 (78.4%) disposed of the faeces in bushes, 5 (4.9%) used the cat faeces as manure and 17 (16.7%) disposed of the faeces in latrines (Table 4).

Among the study participants, 101 (33.3%) used water from the well for cooking and doing house chores, 96 (31.7%) used pipe borne water, and 106 (35.0%) used both well and pipe borne water for cooking. Of the 101 participants who used well water, 37 (36.6%) had their wells always covered, 59 (58.4%) had wells that are not covered and 5 (5.0%) had wells that were sometimes covered. A total of 247 (81.5%) participants consumed 'soya' and 286 (94.4%) consumed smoked meat in their homes.

Also, among the participants, 241 (79.5%) consumed food sold in local restaurants; 239 (78.9%) practiced farming; and 199 (65.7%) do not have the habit of washing hands before eating. A total of 240 participants (79.2%) consumed unpasteurised milk (locally made yoghurt) and/or 'folere' while 63 (20.8%) do not have the habit of consuming unpasteurised milk and/or 'folere'

(Table 4). From Table 4, it can be seen that factors such as owning cats, presence of cats in the neighbourhood, playing with cats and eating soya were all risk factors significantly associated with IgG seroprevalence of toxoplasmosis.

Association of viral load with sociodemographic characteristics of the HIV-infected participants:

Of the 201 HIV-positive study participants, viral load was detectable in 54, giving an overall viral load detection rate of 26.9%. The mean viral load was 3856±16968 copies/ml and ranged from 35 - 16968 copies/ml. HIV-infected males had a higher frequency of detectable viral load (28.3%, 13/46) compared to the females (26.5%, 41/156) although the difference was not statistically significant (χ^2=0.059, p=0.808). The mean viral load was however higher in females (168 copies/ml) than males (64 copies/ml), but the difference was also not statistically significant (Mann-Whitney U test, p=0.068) (Table 5).

HIV-infected participants aged less than 34 years had the highest viral load detectable rate (36.4%, 8/22) while those aged 35-49 years had the least detection rate (21.6%, 24/111), although the difference was not statistically significant (χ^2=3.606, p=0.165). On the other hand, the mean viral load was highest in those aged 50 years and above (173 viral copies/ml) and least in those aged ≤34 years old (101 viral copies/ml) although the difference was also not statistically significant (Kruskal Wallis test, p= 0.989).

With respect to residence, those resident in urban areas had higher viral load detection rate (30.0%, 39/130) compared to those residents in rural areas (21.1%, 15/71), although the difference was also not statistically significant (χ^2=1.840, p=0.175). On the contrary, the mean viral load was higher in those who resides in rural areas (161 viral particles/ml) compared to those residents in urban areas (124 viral particles/ml), although the difference was not statistically significant (Mann-Whitney U test, p=0.374).

Concerning duration of treatment, the viral load detection rate among those who had received treatment for 6-10 years (27%, 17/63) and those who had received treatment for above 10 years (27.0%, 24/89) were higher than those who had received treatment for less than 6 years (26.5%, 13/49), but the difference was not statistically significant (χ^2=0.004 p=0.998). However, the mean viral load was highest among those who had received treatment for less than 6 years (145 viral copies/ml), followed by those who had received treatment for above 10 years (131 viral copies/ml) and least in those who had received treatment for 6-10 years (127 viral copies/ml), but the difference was not statistically significant (Kruskal Wallis test, p=0.376) (Table 5).

Table 5: Association of viral load with socio-demographic characteristics of the HIV-infected study participants

Characteristics	Category	Number examined	No with detectable viral load (%)	χ^2 (p value)	GMVL (±SD)	Range	Statistical parameters
Gender	Female	155	41 (26.5)	0.059	168 (19377)	35-106000	Mann-Whitney U
	Male	46	13 (28.3)	(0.808)	64 (151)	40-541	test; p=0.068
Age (years)	≤ 34	22	8 (36.4)	3.606	101 (155)	40-511	Kruskal Wallis
	35-49	111	24 (21.6)	(0.165)	115 (12221)	40-60000	test; p=0.989
	≥ 50	68	22 (32.4)		173 (23420)	35-10600	
Residence	Urban	130	39 (30.0)	1.840	124 (19255)	35-106000	Mann-Whitney U
	Rural	71	15 (21.1)	(0.175)	161 (8994)	40-35000	test; p=0.374
Duration of treatment	<6	49	13 (26.5)	0.004	145 (29370)	40-106000	Kruskal Wallis
(years)	6-10	63	17 (27.0)	(0.998)	127 (170)	35-541	test; p=0.376
	>10	89	24 (27.0)		131 (13974)	40-6000	
Educational level	No formal	4	1 (25.0)	2.303	416	-	Kruskal Wallis
	Primary	113	31 (27.4)	(0.512)	146 (19816)	35-106000	test; p=0.599
	Secondary	67	20 (29.9)		115 (13389)	40-6000	
	Tertiary	17	2 (11.8)		83 (69)	48-145	
Occupation	Civil servant	17	1 (5.9)	4.284	145	-	Kruskal Wallis
	Self employed	111	33 (29.7)	(0.117)	168 (21495)	35-106000	test; p=0.594
	Farmers	73	20 (27.4)		90 (157)	40-500	
Marital status	Single	67	18 (26.9)	0.001	165 (14104)	40-60000	Mann-Whitney U
	Married	134	36 (26.9)	(0.999)	119 (18420)	35-106000	test; p=0.174

GMVL = Geometric mean viral load; SD = Standard deviation; x^2 = Chi square

Table 6: Prevalence of *Toxoplasma* IgG with respect to viral load.

Parameter	Category	Number examined	No positive for Toxo IgG (%)	x^2 (*p* value)	Binary logistic regression OR (CI)	*p*-value
Viral load	ND	147	39 (26.5)	1.441 (0.230)	0.7 (0.34-1.3)	0.232
	D	54	19 (35.2)		Reference	-

D= Detectable; ND = Not detectable; OR=Odds ratio; CI=Confidence interval

With respect to educational level, the viral load detection rate was highest among those with secondary level (29.9%, 20/67), followed by those with primary level (27.4%, 31/113), those with no formal education (25.0%, 1/4) and least in those with tertiary level (11.8%, 2/17), but the difference was not statistically significant (χ^2=2.303, *p*=0.512). However, the mean viral load was highest among those with no formal education (416 viral copies/ml), followed by primary level (146 viral copies/ml), then secondary level (115 viral copies/ml) and least in tertiary level (83 viral copies/ml), but the difference was not significant (Kruskal Wallis test, *p*=0.599).

Concerning occupation of the study participants, viral load detection rate was highest amongst the self-employed (29.7%, 33/111), followed by the farmers (27.4%, 20/73) and least frequent among civil servants (5.9%, 1/17), but the difference was not statistically significant (χ^2=4.284, *p*=0.17) However, the mean viral load was highest amongst the civil servants (168 viral copies/ml), followed by self-employed (145 copies/ml) and least among farmers (90 viral copies /ml) but the difference was not statistically significant (Kruskal Wallis test, *p*=0.594).

With respect to marital status, the viral load detection rate was similar among the single and married participants (26.9%) but the mean viral load was higher among the single participants (165 copies/ml) than the married participants (119 viral copies/ml). However, there was no significant difference between the mean viral loads of the two groups (Mann-Whitney U test, *p*=0.174) (Table 5).

Prevalence of *Toxoplasma* IgG with respect to viral load in HIV-infected participants:

The prevalence of *Toxoplasma* IgG was higher in participants with detectable viral load (35.2%, 19/54) than in those with non-detectable viral load (26.5%, 39/147) but there was no significant difference in the prevalence between the two groups (χ^2=1.441, *p*=0.230) (Table 6). HIV-infected participants with non-detectable viral load had lower prevalence of *Toxoplasma* IgG (OR=0.7; 95% CI=0.34-1.3) compared to those with detectable viral load, although the difference was not significant (*p*=0.232).

Discussion:

In this study, 303 (201 HIV-infected and 102 HIV-negative) participants were investigated for toxoplasmosis, and the overall prevalence for IgG antibodies (latent infection) was 30.7% while that of IgM (active infection) was 0.7%. The IgG prevalence was slightly lower in HIV-infected (28.9%) compared to HIV-negative (34.3%) patients, but the difference was not statistically significant (x^2=0.948, *p*=0.330). This finding is similar to the one reported by Ngobeni and Samie (10) in South Africa but lower than the prevalence reported by Amivi and Mlatovi (12).

A comparison of this prevalence with those of other studies in Cameroon shows a drastic drop in toxoplasmosis prevalence. For example, a study by Jules et al., (13) reported toxoplasmosis prevalence of 64.7% and 20.2% for IgG and IgM respectively in HIV/AIDS patients in Cameroon. This difference in prevalence could be due to the fact that highly active antiretroviral therapy (HAART) against HIV infection has been advocated to improve the immune status of patients thus reducing the incidence of opportunistic infections (14). Also, in some HIV/AIDS patients, the failing immune system may not produce detectable amounts of antibodies anymore, hence may account for a low seroprevalence in the sample. Another possible reason for the difference in prevalence could be the fact that there is increase awareness of toxoplasmosis and its risk factors among these patients and has led to individual prevention to exposure to these factors such as avoiding consumption of poorly cooked meat, improved personal hygiene, avoiding cats and dogs.

The slightly lower prevalence of latent toxoplasmosis in HIV-infected patient (28.9%) compared to HIV-negative patients (34.3%) could be due to the fact that HIV/AIDS patients are conscious of their immune compromised state and therefore live a careful lifestyle compared to HIV negative people who may live a more carefree life which exposes them to infection with toxoplasmosis. This observation is contrary to studies by Ngobeni and Samie (10) and Muluye et al., (15) who reported a higher prevalence of

Toxoplasma IgG among HIV/ AIDS patients than among HIV negative participants. However, the seroprevalence observed in our study did not differ greatly from previous study by Nazer et al., (16) and Mansouri et al., (17) among the general population. These results revealed that the HIV infection might not have increased the risk for latent toxoplasmosis. Another probable reason is that the patients mainly acquired this infection during childhood, adolescence or at any other time before the HIV infection.

In this study, the prevalence of *Toxoplasma* IgG was highest among participants in the age group 35-49 years (33.6%) and lowest in those among the age group ≥ 50 years (25.6%). This is slightly different from the observation by Nazer et al., (16), who reported that the overall seroprevalence was highest in the 46–60year age group (51.8%). A study by Zhang et al., (18) showed the highest prevalence of disease in 3rd and 4th decades of life. Nonetheless, Walle et al. (19) reported the highest prevalence rate in 21–30 years age group.

The participants who lived in rural areas had a higher prevalence (34.9%) than those who lived in urban areas though the difference was not statistically significant. A possible reason for this could be the fact that people in rural areas live in relatively poor hygienic conditions and drink water mostly from streams, springs or wells which could be contaminated with *T. gondii* oocysts. A study by Ngobenie and Samie (10) and Amivi and Mlatovi (12) also revealed that prevailing conditions in underdeveloped areas are responsible for increased risk of *Toxoplasma* infection. Residency in rural areas has a great influence on *Toxoplasma* seropositivity for African populations. In Egypt it was found that living in a rural area was an independent predictor of toxoplasmosis seropositivity (20). This finding is supported by studies from Saudi Arabia (21,22). African countries showed similar results indicating that rural living significantly increases seropositivity of *T. gondii*. In Italy, higher associations were also reported for living in rural areas (23).

Concerning the level of education, the prevalence of *T. gondii* IgG was highest among participants with no formal education (36.4%) and lowest among those with tertiary level of education (26.1%). Having no formal education in this part of Cameroon might means that ignorance may make individual live a care-free life, without cognizance of exposure to environmental diseases. This might be a reason for the higher prevalence amongst those with no formal education.

With respect to the occupation, the highest prevalence was observed among those who were self-employed (31.8%), and the lowest prevalence was among the farmers (28.9%). It could be expected that prevalence should be higher among farmers than among those in any other occupation because, from this study, 78.4% of participants who cleaned cat faeces disposed of the faeces in bushes and 4.9% used cat faeces as manure. This makes the soil good homes for *T. gondii* cysts and predisposes farmers and soil eaters to the disease. However, the low prevalence among farmers shows that farming might not be a potential risk factor for the transmission of toxoplasmosis provided farmers protect themselves before and clean themselves properly after work in the farms or contact with soil. This observation is contrary to that reported by Alsamani (24), where the prevalence of toxoplasmosis was high among farmers. Other studies proved that farming or contact with soil were strong predictors of infectivity with *T. gondii* infection (25). The prevalence of *Toxoplasma* IgG was also higher among married participants (31.3%) than among single participants. However, no statistically significant difference was observed between occupation, educational level, marital status and residence areas and sero-reactivity to the anti-*T. gondii* antibodies.

For active infection (positive *Toxoplasma* IgM), the prevalence was 1.0% among HIV/AIDS participants but 0% among HIV-negative participants. Similar results were found in Ethiopia where Muluye et al., (15) reported high prevalence of *Toxoplasma* IgM among HIV/AIDS patients while the prevalence was lower among HIV negative participants. Immunosuppression is the most probable reason why the prevalence of active toxoplasmosis was higher in HIV/AIDS patients than in HIV-negative patients. Nazer et al., (16) reported prevalence of anti-*T. gondii* IgM antibody of 2.6% in their study, which included eight men and two women, although they observed no statistically significant difference between the gender ($p>0.05$). Also, a study from Ethiopia showed that anti-*T. gondii* IgM prevalence in HIV seropositive individuals was 10.7% (19). However, Rahimi et al., (26) reported that no person had the IgM anti-*T. gondii* antibodies in their study. Shen et al., (27) found three patients (1.2%) with the anti-*Toxoplasma* IgM antibody. The slight disparity in the reports of all these studies with ours can probably be attributed to differences in lifestyles, geographic area and climate conditions (24).

A total of 16 potential risk factors for toxoplasmosis were analyzed in this study. There was statistically significant ($p<0.05$) association of higher seroprevalence of latent toxoplasmosis in persons who owned cats, stayed in neighborhoods with stray cats, play with cats and eat soya (roadside roasted meat). The associated risk factor of owning

cats is consistent with the study of Nguemain et al., (28) who reported that owing cat is a risk factor for toxoplasmosis. Eating grilled meat in the form of soya contributes in the spread of the disease especially when the meat is not properly cooked, and this finding is consistent with the study of Condoleo et al., (29). The presence of stray cats in an environment and the aspect of persons playing with cats account as risk factors for toxoplasmosis because cats play the critical role for *T. gondii* as the final and definitive hosts that reproduce oocysts in their faeces which contaminate soil, feeds and water (30), and oocysts shed by infective cats can remain infective for up to one month.

In this study, the viral load was detectable for 54 of the 201 HIV positive participants, giving an overall viral load prevalence of 26.9%. The prevalence of *Toxoplasma* IgG was higher in participants with a detectable viral load than in those with a non-detectable viral load although there was no significant difference in the prevalence between the two groups. In this study, *T. gondii* IgM was detected in only two participants who were HIV-infected with detectable viral loads.

Summarily, we found high prevalence of *Toxoplasma* IgG antibodies in patients with high HIV viral load, and IgM was detected in only HIV-infected patients with detectable viral loads. This same observation was reported by Ngobeni and Samie (10) and by Nazer et al., (16). This could most likely be because high viral load causes the body to be more susceptible to opportunistic infections such as toxoplasmosis. It could be concluded that the patients with HIV should be considered at the high risk for toxoplasmosis especially when $CD4^+$ T-cells count fall below 100 cells/µl and viral load is above 1000 viral copies/ml (16).

Conclusion:

From our study, toxoplasmosis is prevalent among HIV-infected and HIV-negative patients attending the Bamenda Regional Hospital, Cameroon, with prevalence of latent infection of 30.7% and active transmissible infection (IgM) of 0.7% in only HIV-infected participants with detectable viral load. Apart from the immunocompromised state of these patients, which is a great risk factor for toxoplasmosis, other factors such as owning cats, presence of stray cats in the neighborhood, playing with cats, eating soya and high HIV viral loads are probable risk factors for latent toxoplasmosis among the study participants.

Based on these findings, we recommend that routine screening for *T. gondii* infection in high-risk groups such as people living with HIV/AIDS, should be considered and treatment given to patients with active infections.

Acknowledgements:

The authors are grateful to the participants who willingly consented to participate in this study. The hospital staff where blood samples were collected are appreciated for their maximum cooperation.

Contributions of authors:

EEJE and SM conceived and designed the study. EEJE and SM supervised, reviewed and provided inputs to the manuscript. All authors wrote the initiate manuscript, read and approved the final manuscript submitted for publication.

Source of funding:

The study was funded in part by Research Modernization from the Ministry of Higher Education in Cameroon.

Conflict of interest:

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References:

1. Wang, Z., Wang, S., Liu, H., et al. Prevalence and burden of *Toxoplasma gondii* infection in HIV-infected people: a systematic review and meta-analysis. Lancet HIV. 2017; 4 (4): e177-e188. doi: 10.1016/S2352-3018(17)30005-X.
2. World Health Organization. WHO estimate of food borne diseases. 2015
3. Njunda, A. L., Jules, C. N., Nsagha, D. S., Kamga, H. L., Nde, P. F., and Yugah, V. C. Seroprevalence of *Toxoplasma gondii* infection among pregnant women in Cameroon. J Publ Health Afr. 2011; 2 (2): e24 doi: 10.4081/jphia.2011.e24
4. Yeong, H. K., Ji-hoo, L., Seong-kyu, A., et al. Seroprevalence of Toxoplasmosis with ELISA and Rapid Diagnostic Test among Residents in Gyodong-do, Inchon city, Korea: A Four-Year Follow-up. Korean J Parasitol. 2017; 55 (3): 247–254. doi: 10.3347/kjp.2017.55.3.247
5. Bamenda City Council. 2014 report. en.m.wikipedia.org.
6. Antonio-Nkondjio, C., Ndo, C., Njiokou, F., et al. Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination. Parasit Vectors. 2019; 12:501.doi:10.1186/s13071-019-3753-8
7. Ndoh, M. I., Bitondo, D., and Balgah, R. A. Climate variability and change in the Bamenda highlands of North Western Cameroon: Perceptions, impacts and coping mechanisms. Br J Appl Sci Technol. 2016; 12(5): 1-18 doi: 10.9734/BJAST/2016/21818
8. Ntonifor, H. N., and Veyufambom, S. Assessing the effective use of mosquito nets in the prevention of malaria in some parts of Mezam Division, North West Region Cameroon. Malaria J. 2016; 15: doi: 10.1186/s12936-016-1419-y.
9. Naing, L., Winn, T., and Rusli, B. N. Practical issues in calculating the sample size for

prevalence studies. Arch Orofacial Sci. 2006; 1: 9-14.
10. Ngobeni, R., and Samie, A. Prevalence of *Toxoplasma gondii* IgG and IgM and associated risk factors among HIV positive and HIV negative patients in Vhembe district of South Afr J Infect Dis. 2017; 11 (2): 1-9 doi: 10.21010/ajid.v11i2.1
11. Cheesbrough, M. District laboratory practice in tropical countries. Cambridge University Press, 1: 2009: 246-251.
12. Amivi, T. B., and Mlatovi, D. Seroprevalence and Risk Factors of Toxoplasmosis in Togo. Euro Sci J. 2018; 14 (33): 1857–7881 https://doi.org/10.19044/esj.2018.v14n33p56
13. Jules, C. N., Anna, L., Nsagha, D. S., Henri, K., Patrick, E., and Che, V. B. Toxoplasma Antibodies Amongst HIV/AIDS Patients Attending the University Teaching Hospital Yaoundé, in Cameroon. Afr J Clin Exper Microbiol. 2011; 12 (3):119-123.doi:10.4314/ajcem.v12i3.6
14. Sukthana, Y., Chintana, T., Supathanapong, W., Siripanth, C., Lekkla, A., and Chiabchalard, R. Prevalence of toxoplasmosis in Selected Populations in Thailand. J Trop Med Parasitol. 2000; 23: 53-58.
15. Muluye, D., Wondimeneh, Y., Belyhun, Y., Morges, F., Endris, M., and Fered, G. Prevalence of *Toxoplasma gondii* and associated risk factors among people living with HIV at Gondar University Hospital, Northwest Ethiopia. ISRN Trop Med. 2013: 5
16. Naser, N., Arezoo, B., Alireza, J., and Farshid, B. *Toxoplasma gondii* and human immunodeficiency virus co-infection in western Iran: A cross-sectional study. Asian Pac J Trop Med. 2018; 11(1): 58-62. doi: 10.4103/1995-7645.223562
17. Mansouri, F., Hatami, H., Mahdavian, B., and Hashemian, A. H. Epidemiology of toxoplasmosis in Kermanshah province. J Kermanshah Univ Med Sci. 2003; 7 (2): e81208.
18. Zhang, Y. B., Cong, W., Li, Z. T., Bi, X. G., Xian, Y., and Wang, Y. H. Seroprevalence of *Toxoplasma gondii* infection in patients of intensive care unit in China: A hospital-based study. Biomed Res Int. 2015; 2015: 908217. doi: 10.1155/2015/908217
19. Walle, F., Kebede, N., Tsegaye, A., and Kassa, T. Seroprevalence and risk factors for Toxoplasmosis in HIV infected and non-infected individuals in Bahir Dar, Northwest Ethiopia. Parasit Vectors. 2013; 6(1): 1. doi: 10.1186/1756-3305-6-15
20. Tammam, A., Haridy, M., Abdellah, A., Ahmed, S., Fayed, H. M., and Alsammani, M. Seroepidemiology of *Toxoplasma gondii* infection in women with first trimester spontaneous miscarriage in Qena governorate, Egypt. J Clin Diagn Res. 2013; 7: 2870–2873. doi: 10.7860/JCDR/2013/6480.3780
21. Al-Qurashi, A. R., Ghandour, A. M., Obeid, O. E., Al-Mulhim, A., and Makki, S. M. Seroepidemiological study of *Toxoplasma gondii* infec tion in the human population in the Eastern region. Saudi Med J. 2001; 22(1): 3–18.
22. Al-Mohammad, H., Amin, T., Balaha, M., and Al-Moghannum, M. S. Toxoplasmosis among the pregnant women attending a Saudi maternity hospital: seroprevalence and possible risk factors. Ann Trop Med Parasitol. 2010; 104: 493–504. doi:10.1179/136485910X12786389891443
23. Thaller, R., Tammaro, F., and Pentimalli, H. Risk factors for toxoplasmosis in pregnant women in central Italy. Infez Med. 2011; 19: 241–247
24. Alsammani, M. A., Ahmed, S. R., Alsheeha, M. A., Saadia, Z., and Khairi, S. A. Co-infection with *Toxoplasma gondii* and *Clostridium perfringens* in a postpartum woman with uterine gas gangrene: a case report. J Obstet Gynaecol. 2014; 38: 1024–1027. doi:10.1111/j.1447-0756.2011.01817.x
25. Cook, A. J., Gilbert, R. E., Buffolano, W., et al. Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study: European research network on congenital toxoplasmosis. BMJ. 2000; 321: 142–147. doi: 10.1136/bmj.321.7254.142
26. Rahimi, M. T., Mahdavi, S. A., Javadian, B., Rezaei, R., Moosazadeh, M., and Khademlou, M. High seroprevalence of *Toxoplasma gondii* antibody in HIV/AIDS individuals from North of Iran. Iran J Parasitol. 2015; 10 (4): 584
27. Shen, G., Wang, X., Sun, H., and Gao, Y. Seroprevalence of *Toxoplasma gondii* infection among HIV/AIDS patients in Eastern China. Korean J Parasitol. 2016; 54(1): 93. doi: 10.3347/kjp.2016.54.1.93
28. Nguemain. N.F., Takang, W.A., Dodgima, W.P., Guebidiang, B.M., Foumane, P., and Kamga, F. H. L. Seroprevalence of *Toxoplasma gondii* infection and associated risk factors among pregnant women attending antenatal clinic at the Bamenda Regional Hospital, Cameroon. Afr J Clin Exper Microbiol. 2020; 21 (2): 123-131. doi:10.4314/ajcem.v21i2.6
29. Condoleo, R., Rinaldi, L., Sette, S., Mezher, Z. Risk assessment of human toxoplasmosis with the consumption of pork meat in Italy. Risk Anal. 2018; 38(6): 1202-1222. doi: 10.1111/risa.12934
30. Cenci-Goga, B. T., Rossitto, P. V., Sechi, P., Chenyl, M. E., Crindle, M.C., and Culler, J. S. Toxoplasmosis in animals, food and humans: an old parasite of new concern. Foodborne Pathogen Dis, 2011; 8 (7): 751-762. doi:10.1089/fpd.2010.0795