Seroprevalence of *Toxoplasma gondii* IgG and IgM Antibodies and Associated Risks Factors in Tuberculosis Patients Admitted at the Bamenda Regional Hospital, NW Cameroon

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Abstract

Background: Toxoplasmosis is a zoonotic disease with a worldwide distribution amongst warm-blooded animals and can affect anyone in contact with the parasitic oocysts or tissue cysts. Tuberculosis (TB) and *T. gondii* co-infection is a serious public health problem to the health of these patients in developing countries. We determined the seroprevalence of *Toxoplasma gondii* IgG and *IgM* antibodies and associated risk factors among newly diagnosed sputum positive pulmonary TB patients.

Materials and methods: This was a cross sectional study including 158 TB patients. Laboratory analyses were based on quantification of anti *T.gondii IgM* and *IgG an*tibodies test using sandwich ELISA. A questionnaire captured known risk factors for toxoplasmosis among participants. Risk factors for toxoplasmosis were analyzed in a binary logistic model. The results presented as odds ratios were used to assess association of toxoplasmosis and potential risk factors. A p-value < 0.05 was considered statistically significant.

Result: Of the 158 participants, 87.97% [139/158] were seropositive for *Toxoplasma gondii Ig*M and *Ig*G antibodies. Eating cat meat [P=0.009, OR=4.2498, 95%CI=1.4300-11.1997] and water source [P=0.026, OR=5.4983, CI=1.2189-23.5827], were significant risk factors associated with *T. gondii* infection. We noted a threefold increase in the risk of toxoplasmosis among TB patients having farming as occupation.

Conclusion: The prevalence of toxoplasmosis IgM and IgG antibodies among sputum positive pulmonary TB patients in the Bamenda Regional Hospital, North West Cameroon was found to be 87.97%. The principal risk factor associated with *Toxoplasma gondii* among TB patients was consumption of Cat meat and untreated water sources.

Keywords: Toxoplasmosis; Seroprevalence; IgG; IgM; Tuberculosis; Cameroon

Introduction

One third of the world population has been estimated to be infected with *Toxoplasma gondii (T. gondii)* parasite [1]. Mostly it does not cause serious illness in healthy adults, but causes severe diseases in immune-compromised patients [2]. In immune-competent individuals, effective immune response produces a balance for both parasite and host survival but does not eradicate the infection. Weakness of the host immune function may allow reactivation of actively replicating tachyzoites, sometimes and results in extensive organ damage [3]. Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide in spite of great effort for eradication. Globally, there were an estimated 9.27 million incidences of TB and new infections that occurred at a rate of one per second [4,5]. About 33% of the world population infected with Mycobacterium tuberculosis (*M. tuberculosis*) resides in developing countries including Cameroon. Cameroon is one of the countries where the prevalence of TB is still high with no prevalence since 2012. The WHO estimated in 2012 that the rate of mortality due to TB in Cameroon was 29/100,000

excluding HIV and the prevalence of all forms including HIV positive was 319/100,000 and the rate of incidence including HIV positive was 338/100,000 inhabitants. The increasing number of multi-drug resistant, extensively drug-resistant and extremely resistant strains of M. tuberculosis made the control of the tuberculosis spread more difficult [6]. Both TB and parasitic diseases are infectious diseases causing serious harm to humans with an overlap in endemic regions, which may lead to frequent co-infection in these areas. Cases of co-infection of TB with intracellular parasites were reported as with malaria [7,8], visceral leishmaniasis [9,10], and *T. gondii* [11,12]. Hwang and colleagues [13] reported a cerebral toxoplasmosis case with disseminated tuberculosis in an immune-competent patient. In the cases of co-infection, modification of the immune response was suggested [14-16]. Oxidative stress was recorded, and implicated in the pathogenesis of both TB [17,18], and toxoplasmosis [19]. The response of the immune system against infections includes the generation of reactive oxygen species (free radicals) which are toxic to human tissues and cells. However, no studies on the prevalence of *T. gondii* among TB have been conducted in our locality. The aim of this study was therefore to determine the seroprevalence of anti *T. gondii* antibodies in TB patients and the possible associated risks factors.

Materials and Methods

Study design

A cross-sectional study was carried out from October 2015 and April, 2016 at Bamenda Regional Hospital, North West Region, Cameroon.

Study site and population

The Bamenda Regional Hospital, is a public health care. It is located in Bamenda, the capital City of the North West Region of Cameroon. The hospital was opened in 1956 by Sir Roberts the then British High Commissioner based in Lagos Nigeria. It was given the status of a 3rd level reference health institution for the North West in 2009. This hospital has a TB unit and one of the two TB reference laboratories in Cameroon. It equally has a TB treatment center, HIV treatment center, and the majority of services rendered are in the domains of gynecology, orthopedics, radiology, dialyses and general surgery.

Participants to the study were TB patients admitted at the TB ward of the Bamenda Regional Hospital who duly provided consent by signing or placing their thumbprint on the consent form.

Questionnaire administration and sample collection

Participants of this study were each provided with a structured questionnaire to fill. Those who could not read were assisted to fill the questionnaires by the laboratory technician or care nurse. The questionnaires contained simple closed ended questions regarding known risk factors of Toxoplasma exposure in addition to socio-demographic information.

Five millilitres of venous blood were collected into a dry tube and allowed to clot completely before centrifugation at 3000 rpm for 15 minutes at 4°C to obtain serum. Serum was separated from the clot into tightly screwed microfuge tubes and stored at -25°C. These frozen sera were later tested for the presence of *Toxoplasma gondii* antibodies.

Detection of pulmonary tuberculosis

Diagnosis of TB was done by direct Ziehl Neelsen staining of sputum samples for detection of acid fast bacilli and Lowenstein Jensen culture for isolation of M. tuberculosis [20].

Serological test for T. gondii antibodies

The presence of *T. gondii* antibodies in the participants' sera was determined using an indirect Enzyme-Linked Immunosorbent Assay (ELISA). This was done using the AccuDiag™ Toxo IgM and IgG ELISA Kits (The Diagnostic Automation/Cortez Diagnostics, Inc. *Toxoplasma gondii* [Toxo] IgM and IgG Enzyme-Linked Immuno-sorbent Assay [ELISA]). The Toxo IgM ELISA Kits had a specificity of 100% and a sensitivity of 100%, while the Toxo IgG ELISA Kits had a specificity of 100% and a sensitivity of 95.3%. Calibrator and controls were run with each test assay. The optical densities (OD) obtained were used to calculate the cut-of calibrator value and the Immune Status Ratio (ISR). The interpretation of results was done with respect to the ISR values. For IgM, a sample with OD ≤ 0.90 was considered negative, OD ≥ 1.10 was considered positive, and OD=0.91−1.09 was considered indeterminate. For IgG, a sample with OD < 0.90 was considered negative, OD>1.10 was considered positive, and OD within 0.91–1.09 was considered indeterminate.

Ethical consideration

Ethical clearance for this study was obtained from the Ethics Review and Consultancy Committee of the Cameroon Bioethics Initiative (CAMBIN) under the reference number CBI/370/ERCC/CAMBIN of August 18th, 2015. An authorization to collect and analyze blood samples was also obtained from the Bamenda Regional Hospital Institutionalized Review Board (IRB). All participants were duly informed of the study goals, procedures, potential harm and benefits, cost as well as the finality of the study. They willingly provided informed consent either by signing or placing their thumbprint on the consent form after being satisfied with responses to all questions asked the investigator. Patient less than 21 years provided assent and consent from guardian was also sought for these patients. Information was provided in English, French or interpreted in the local dialect by a volunteer independent of the study team. Participants' blood samples and results were anonymised. Left over blood samples were destroyed according to hospital biosafety procedures.

Data management and statistical analysis

Data were entered into Microsoft Excel program and then transferred to Epi Info 3.54 statistical program. The seroprevalence of toxoplasmosis was calculated as the proportion of serologically positive anti *T. gondii* samples among all samples tested at 95% confidence interval (CI). A simple and multivariate logistic regression model was used to investigate the association between the potential risk factors for toxoplasmosis as defined by seropositivity for *Toxoplasma gondii* antibodies of any kind. Variables used in the multiple regression were selected through step-wise backward elimination using a 20% cut-of (p to remove). The strength of associations was measured using the odds ratio (OR) at 95% CI. The Chi-square test was used for group comparisons. Statistical significance was set at 5% and all the associations that showed a p < 0.05 were considered significant.

Results

Out of the 158 participants that were recruited in this study, 50.63% (80/158) were males while 49.37% (78/158) were females. The mean age of the participants was 37.07 ± 11.94 years with minimal being 11 years and the maximal 80 years. The majority of the participant was aged between 31 and 40 years (53/158, 33.54%). Amongst all the participants, 16.46% (26/158) had not attended school, while 46.84% (74/158), 29.11% (46/158) and 6.96% (11/158), had primary, secondary and higher levels of education. Only 3.16% (5/158) knew about toxoplasmosis. Those participants who lived in tile, cemented and non-cemented floor houses were 6.96% (11/158), 84.81% (134/158) and 8.23% (13/158) respectively. Among the study participants, 81.02% (128/158) eat bush meat, 15.19% (24/158) eat cat meat, 10.76% (17/158) consume raw milk, and only 3.79% (6/158) had cats in their homes. Regarding the participants residential area, 71.52% (113/158) of the study participants lived in an urban area, while 28.48% (45/158) lived in a rural area. Out of the 158 participant recruited in this study, 55.69% (88/158) were HIV-positive and 44.31% (70/158) were HIV-negative **(Table 1)**.

Table 1 General characteristic of study participants.

Characteristic	Number (%)
Age category	
<21	6(3.79%)
21-30	40(25.32%
31-40	53(33.54%)
41-50	35(22.15%)
51-60	13(8.23%)
> 60	11(6.96%)
Level of education	
None	26(16.46%)
Primary	74(46.84%)
Secindary	46(29.11%)
High education	12(7.59%)
Knowledge of toxoplasmosis	
Yes	5(3.16%)
No	153(96.84%)
House floor type	
Tile	11(6.96%)
Cemented	134(84.81%)
Non-cemented	13(8.23%)
Consumption of Bush meat	
Yes	128(81.02%)
No	30(18.98%)

Consumption of cat meat	
Yes	24(15.19%)
No	134(84.81%)
Consumption of chicken	
Yes	155(98.10%)
No	3(1.90%)
Consumption of community water	
Yes	69(43.67%)
No	89(56.33%)
Consumption of row milk	
Yes	17(10.76%)
No	141(89.24)
Residential area	
Rural	45(28.48)
Urban	113(71.52%)
Consumption of unwashed vegetables	
Yes	96(60.76%)
No	62(39.24%)
Cats at home	
Yes	6(3.79%)
No	152(96.21%)
HIV status	
Positive	88(55.69%)
Negative	70(44.31%)

Prevalence of antiToxoplasma antibodies in the study population

The combined seroprevalence of anti *T. gondii* antibodies among the 158 TB patients in our study area was calculated to be 87.97% (139/158). Among the seropositive patients, 88 were seropositive for IgG antibodies, 116 were seropositive for IgM antibodies, and 65 were seropositive for both IgG and IgM antibodies giving a prevalence of 55.70%, 73.42% and 41.14% respectively. Of the seropositive patients 47(58.75%), 61(76.25%) and 35(43.75%) males were positive for IgG, IgM, and IgM and IgG respectively while 41(52.56%), 55(70.51%) and 30(38.46%) females were positive for IgG, IgM, and IgM and IgG respectively **(Table 2)**.

Table 2 Seroprevalence of antibodies to *Toxoplasma gondii* according to sex.

Toxo Status	IgG	Male	Female	IgM	Male	Female	IgM and IgG	Male	Female
Positive	88 (55.7%)	47 (58.75%)	41 (52.56%	116 (73.42%)	61 (76.25%)	55 (70.51%)	65 (41.14%)	35 (43.75%)	30 (38.46%)
Negative	59 (37.34%)	29 (36.25%)	30 (38.46%)	30 (18.98%)	12 (15.00%)	18 (23.08%)	93 (58.86%)	45 (56.25%)	48 (61.54%)
Equivocal	11 (6.96%)	4 (5%)	7 (8.98%)	12 (7.60%)	7 (8.75%)	5 (6.41%)	-	-	-
Total	158 (100%)	80	78	158 (100%)	80	78	158	80	78

Of the 88 patients that were HIV positive, 68.18% (60/88) were seropositive for anti *T. gondii* antibodies. Among the HIV seropositive patients, 40 were seropositive for IgG antibody, 51 were seropositive for IgM antibody, and 31 were seropositive for both IgG and IgM antibodies giving a prevalence of 45.45%, 57.95% and 35.23% respectively. Of the HIV positive seropositive patients to anti *T. gondii* antibodies 24(52.17%), 25(54.35%) and 18(39.13%) males were positive for IgG, IgM, and IgM and IgG respectively while 16(38.10%), 26(61.90%) and 13(30.95%) females were positive for IgG, IgM, and IgM and IgG respectively **(Table 3)**.

Table 3 Seroprevalence of antibodies to *Toxoplasma gondii* among HIV positive.

HIV positive	IgG+	Male	Female	IgM+	Male	Female	IgM+ and IgG+	Male	Female
Positive	40 (45.45%)	24 (52.17%)	16 (38.10%)	51 (57.95%)	25 (54.35%)	26 (61.90%)	31 (35.23%)	18 (39.13%)	13 (30.95%)
Negative	48 (54.55%)	22 (47.83%)	26 (61.90%)	37 (42.05%)	21 (45.65%)	16 (38.10%)	57 (64.77%)	28 (60.87%)	29 (69.05%)
Total	88	46	42	88	46	42	88	46	42

The seroprevalence of antibodies to *T. gondii* was significantly different (P=0.002) in general study population 87.97% (139/158) than in HIV-positive 68.18% (60/88). The seroprevalence of anti *T. gondii* IgG antibody in the general study population was 55.70% (88/158), higher than the 45.45% (40/88) in HIV-positive individuals (P=0.38). Also the seroprevalence of anti *T. gondii* IgM antibody in the general study population was 73.42% (116/158), higher than in HIV-positive individuals 57.95% (51/88) (P=0.26), while the seroprevalence of anti *T. gondii* IgG and IgM in the general study population was 41.14% (65/158), higher than in HIV-positive individuals 35.23% (31/88) (P=0.54).

A total of 58.75% (47/80) males of the general study population were positive for anti *T. gondii* IgG antibody, slightly higher than 52.17% (24/46) of the HIV positive males (P=0.7), while 76.25% (61/80) males of the general study population were positive for anti *T. gondii* IgM antibody, slightly higher than 54.35% (25/46) of the HIV positive males (P=0.26). 43.75% (35/80) males were positive for anti *T. gondii* IgG and IgM antibodies, slightly higher than 39.13% (18/46) HIV positive males (P=0.7). In addition, 52.56% (41/78) females of the general study population were positive for anti *T. gondii* IgG antibody slightly higher than 38.10% (16/42) HIV positive females (P=0.3), while 70.51%(55/78) females of general study population were positive for anti *T. gondii* IgM antibody, slightly higher than 61.90% (26/42) HIV positive females (P=0.67). 38.46% (30/78) females were positive for anti *T. gondii* IgG and IgM antibodies, slightly higher than 30.95% (13/42) HIV positive females (P=0.57).

Prevalence of signs and symptoms of *Toxoplasma gondii* in the study population

Of the 139 participants that were diagnosed seropositive for anti *T. gondii* antibodies, 29.75% complained to be suffering or to have suffered from chronic headache, 28.48% of fever, 20,89% of eye disorder, 13.92% of lymph node swellings and 6.98% of Encephalitis **(Table 4)**.

Table 4 Prevalence of signs and symptoms of *Toxoplasma gondii* amongst TB population.

Signs and symptoms	Number	Proportion (%)
Chronic headache	47	29.75
Fever	45	28.48
Eye disorder	33	20.89
Lymph node swelling	22	13.92
Encephalitis	11	6.96

Risk factors for toxoplasma seropositivity

As shown in **Table 5**, simple logistic regression analysis with suspected variables indicated that the consumption of cat meat (P=0.0092) and water sources (P=0.026) were predictors of toxoplasmosis seropositivity irrespective of the antibody type. However, results of multiple logistic regression analysis with selected variables as shown in **Table 6** showed that, the was no independent predictor of toxoplasmosis seropositivity irrespective of the antibody type.

Discussion

Toxoplasmosis is a curable but potentially deadly disease [21]. Infection with the protozoan parasite *Toxoplasma gondii* has been proven to be one of the most common parasitic infections of man and other warm-blooded animals over the years [22]. This study sought to investigate Toxoplasma infection, as evidenced by anti-Toxoplasma antibodies in the serum of tuberculosis patients at the Bamenda Regional hospital in the North West Region of Cameroon. The seroprevalence of anti *T. gondii* antibodies among TB patients in this study was 87.97%. This prevalence was high compared to the 67.4% obtained among TB patients in Egypt [23], and the 25% obtained among TB patients in Sudan [24]. This high prevalence obtained in this study compared to the studies carried out in Egypt and Sudan could be due to the difference at the level of the sample size which was high in our study (154 participants) compared to that in Sudan and Egypt which had

64 and 43 participants respectively. Also, the technic used for the diagnosis (in Sudan Latex Agglutination Test [LAT] was used for the diagnosis) could have accounted for this difference. In addition, the high prevalence could be attributed to high consumption of bush meat **(Table 1)**. It has been proven that the seroprevalence rates across continents are high in humans due to animals [25], consequent oocysts increase and high consumption of unwashed vegetables as was the case in our study area. The prevalence obtained in our study was also high compared to that obtained among women of child bearing age in Njinikom by Wam and colleagues (54.5%) [26], in Central Ethiopia (85.3%) [27], among pregnant women in Yaounde (77.1%) [28], in Douala (78.6%) [29], the 71.8% observed among pregnant women in Limbe [30]. These geographic differences in prevalence rates may be explained by differences in rural/urban setting since exposure and the main sources of infection appear to be the same. This might explain differences in risk factors of Toxoplasma seropositivity as well as climatic changes. Our study population was mainly from an urban area. The seroprevalence of anti *T. gondii* IgG antibody in female patients was 52.56% (41/78), slightly lower than 58.75% (47/80) of the males though not significantly different (P=0.67). This is significantly important to public health. This result was similar to that obtained in Germany where the prevalence of toxoplasmosis among females was not different compared to males [30] and indicates that men are more prone to *T. gondii* infection than women.

The seroprevalence of anti *T. gondii* antibody in HIV-positive participants was 68.18% (60/88). This was not significantly different from the 80.61% (79/98) obtained in the HIV-negative group in this same study population (P=0.75). This result was not similar to the 47.8% (43/90) obtained among HIV/AIDS patients in Njinikom [26]. However, another study in Ethiopia showed a significantly higher prevalence of anti-*Toxoplasma gondii* antibodies (87.4% *vs.* 70.29%, P=0.003) in HIV-positive pre-antiretroviral therapy (pre-ART) individuals than in HIV-negative blood donors [31]. Of the 139 patients diagnosed positive for Toxoplasma antibodies, the most common symptoms they complained to be having or to have suffered from were chronic headache (29.75%), fever (28.48%), eye disorders (20.89%), lymph node swellings (13.92%), and encephalitis (6.96%). These results corroborate to the symptoms cited by Sarman, 2016 [32].

Univariate analysis showed that the consumption of cat meat (P=0.009), and water source (P=0.026), were significant risk factors associated with *T. gondii* infection **(Table 5)**, whereas in multivariate analysis, no independent significant risk factor was associated with *T. gondii* infection **(Table 6)**. Similar results have been observed in studies done in Mexico [33], Ethiopia [31], Sudan [34], Douala-Cameroon [35], and Thailand [36]. Although domestic cats are probably the major source of contamination [37,38], cat ownership and contact with cats were not found to be significantly associated with *T. gondii* infection [39,40]. Indeed, only 3.79% of the total study population reported having cats at home. Sporulated oocysts survive in moist soil for months to years [41,42]. This may justify why farming was found to be associated to *T. gondii* infection.

Table 5 Simple Logistic regression with suspected variables.

Variables	Groups	OR (95%) CI	P-Value
Sex	Male	1.20(0.35-1.98)	0.684
	Female		
Residential area	Rural	1.08(0.41-3.80)	0.873
	Urban		
Age group	<21	NA	0.175
	21-40		
	41-60	NA	0.294
	>60		
Level of education	Primary	1.61(0.44-5.87)	0.451
	High		
Cat ownership	Yes	541929.9	0.872
	No	(>1.0E12)	
Contact with cats	Yes	1.5920 (0.73–2.65)	0.33
	No		
Consumption of Cat meat	Yes	4.2498	0.009*
	No	(1.4300-11.1997)	
Consumption of bush meat	Yes	1.0568	0.92
	No	(0.3573-2.7689)	
Water sources	Treated	5.4983	0.026*
	Untreated	(1.2189-23.5827)	
Consumption of raw vegetables	Yes	0.6569	0.369
	No	(0.2622-1.3835)	
Occupation	Farming	2.960.65-13.42)	0.143
	Public sector	0.40(0.03-4.60)	0.445
	Business	0.60(0.11-3.13)	0.532
	Student	1.52(0.41-5.56)	0.524
	Private sector	0.82(0.32-2.08)	0.678
	Driving	1.14(0.24-5.49)	0.873
Consumption of raw milk	Yes	1.2782 (0.3058 – 4.5397)	0.724
	No		
Toxoplasma awareness	Aware	0.82(0.08-7.67)	0.085
	Unaware		
HIV Status	Positive	1.78(0.73-4.33)	0.203
	Negative		

Table 6 Multiple logistic regressions of selected variables

Variables	Groups	OR (95%)	P-Value
Consumption of cat meat	Yes		
	No	0.94	0.927
Water sources	Treated		
	Untreated	1.59	0.725

Our study however should be interpreted with some caution as it was hospital based involving only TB patients in the hospital at the time of data collection. It is likely that we may obtain different results with increased sample size as well as with TB patients sampled from the Bamenda community at large since some people might be sick but have limited funds to visit the hospital, so do so only when the situation becomes chronic.

Conclusion

Toxoplasma gondii infection appears to be a public health concern in TB population, with a global antibody seroprevalence of 87.97% among TB population. In this study, recent infections were found in 73.42% participants while 41.14% of the study population was reactivating. No significant difference (P=0.75) exist between the HIV positive and HIV negative population. Consumption of cat meat, water source and farming were observed to be risk factors for *T. gondii* infection among TB patients in the locality of Bamenda North West Region of Cameroon. Only 3.16% (5/158) of the study participants knew about toxoplasmosis. Education on toxoplasmosis in TB treatment centers and screening for recent Toxoplasma exposure may be strategies for primary prevention of toxoplasmosis and its devastating outcomes among TB patients.

Competing Interests

The authors declare that they have no conflicts of interest.

Authors' Contributions

LFS and CBT conceived the study, JNF and OGK carried out sample analysis and data collection, ECW and VPT participate in data management and statistics,

LFS and ECW drafted the manuscript. All authors reviewed the manuscript and approved the final version prior to submission.

Acknowledgements

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