



## ***Toxoplasma gondii* and Tuberculosis Co-infection among Tuberculosis patients at the Bamenda Regional Hospital, North West Region, Cameroon**

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### **Authors' contributions**

This work was carried out in collaboration between both authors. Author WMK designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author CBT managed the analyses of the study. Both authors read and approved the final manuscript.

### **Article Information**

DOI: 10.9734/AJRB/2018/v3i129812

#### Editor(s):

(1) Dr. Spirina Liudmila V, Professor, Department of Biochemistry and Molecular Biology, Siberian State Medical University, Russia.

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Complete Peer review History: <http://www.sciencedomain.org/review-history/26718>

**Original Research Article**

**Received 25 August 2018**

**Accepted 10 October 2018**

**Published 20 October 2018**

### **ABSTRACT**

**Aim:** To determine the prevalence of toxoplasmosis and tuberculosis *co-infection* among (TB) patients.

**Study Design:** A cross-sectional study was employed to gather data on 147 sputum positive TB patients.

**Place and Duration of Study:** Bamenda Regional Hospital (BRH), Tuberculosis Reference Laboratory and Treatment Centre from October 2015 to April, 2016.

**Method:** An epidemiological questionnaire was applied to gather data on positive-sputum pulmonary TB patients of both sexes on whom some risk factors for *Toxoplasma* were explored, followed by blood sampling. *T. gondii* IgM and IgG antibodies were researched in sera samples using indirect ELISA.

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**Results:** *T. gondii* antibodies prevalence amongst the TB population was 83% (122/147, 95% CI 112-130). 85.25% of the *Toxoplasma* infection in the study population were recent infections with *T.gondii* parasite while up to 53.28% of patients were reactivating, no significant difference in prevalence between HIV negative or HIV positive TB patients was established.

**Conclusion:** The high seroprevalence of toxoplasmosis among TB subjects attending treatment at the Bamenda Regional Hospital is suggestive of co-infection. Toxoplasmosis and tuberculosis co-infection are opportunistic infections for HIV/AIDS, and life threatening to TB patients and could be the reason for unexplained deaths among these patients on treatment.

**Keywords:** *Toxoplasma gondii*; co-infection; tuberculosis; seroprevalence; TB patients; BRH.

## 1. INTRODUCTION

*Mycobacterium tuberculosis* (TB) and human immune deficiency virus (HIV) infections are among the major public health problems in many parts of the world [1,2] and coupled with the emergence and resurgence of other parasitic infections, particularly toxoplasmosis, as a co-epidemic infection in sub-Saharan countries. TB is the most common opportunistic disease and a major cause of the death for those infected with HIV [3,4,5].

Tuberculosis is a prevalent infectious disease in the world, especially in developing countries where one-third of the world's population is infected with latent TB and approximately 5–10% develop active TB during their lifetime [6]. An estimative of 8.8 million new TB cases, globally, was determined in 2012. According to World Health Organization (WHO), collaborative TB/HIV activities launched since 2004 to manage the TB and HIV co-infection [7].

Toxoplasmosis, a disease caused by an obligate intracellular parasite protozoan, *Toxoplasma gondii* (*T. gondii*), affects approximately two billion people [8]. The infection in humans ranges from asymptomatic to severe, depending on the parasite strain and the immune status of the host. Most of the human cases are asymptomatic (immunocompetent persons) and infection rates in some areas are as high as 70% [9]. Toxoplasmosis can also cause severe disease in patients with immunosuppression as AIDS, cancer chemotherapy, pregnancy, TB, organ transplant patients treated with immunosuppressive drugs [10]. Nowadays, toxoplasmosis imprints itself as an opportunistic infection in TB infection [11] and the leading cause of death. Similarly, *T. gondii* infection is associated with an increased risk of complication of TB in developing countries [12,13,14].

In this way, as well as no study has been conducted in this field, this study was aimed to determine the prevalence of toxoplasmosis and tuberculosis co-infection among the TB patients at the Bamenda Regional Hospital, North West Region, Cameroon.

## 2. MATERIALS AND METHODS

### 2.1 Ethical Clearance

This study was performed following the ethical conditions and guidelines stipulated by the Ethics Review and Consultancy Committee (ERCC), of the Cameroon Bioethics Initiative (CAMBIN) under the reference number CBI/370/ERCC/CAMBIN of August 18<sup>th</sup>, 2015, and the Bamenda Regional Hospital Institutionalized Review Board (IRB) approval. Informed consent was applied and voluntariness was necessary for participation. Patients' details as well as and results were confidentially analysed. Participants reserved the right to participate or not in the study.

### 2.2 Study Design

It is cross-sectional study at the Bamenda Regional Hospital, North West Region, Cameroon located on Longitude 10.1440°E and Latitude 5.9551°N. from October 2015 to April 2016 where we consecutively sampled 147 positive-sputum pulmonary TB subjects of both sexes on whom some risk factors for *Toxoplasma* were explored. This constituted of in-patients and outpatients at the Bamenda Regional Hospital Tuberculosis treatment centre, North West Region, Cameroon.

Participants were recruited based on these inclusion criteria:

- Diagnosed and confirmed sputum positive for pulmonary TB by GeneXPERT, Culture or DNA amplification.

- Confirmed sputum positive TB patients receiving treatment at the Bamenda Regional Hospital Tuberculosis treatment centre.
- Newly confirmed cases and outpatients on treatment.
- Consent or assent was given voluntarily by the respective individual and legal representatives of minors.

Illegibility was based on the following:

- Non sputum positive for Pulmonary TB upon diagnosis by GeneXpert, Culture or DNA Amplification
- Failure to sign and return their informed consent forms within two weeks were excluded in the study.
- Minors who gave their assent and their legal representatives did not consent.
- Refusal to consent.

## 2.3 Sampling

For the study, 147 positive-sputum pulmonary TB subjects were consecutively recruited. The general prevalence of toxoplasmosis in Cameroon remains unknown thus with this study being the first on TB-TOXO co-infection where no data exist, the 77.1% prevalence of toxoplasmosis obtained among pregnant women in Yaoundé, Cameroon [15] was considered useful. The minimum required sample size was calculated using the formula below as proposed by Lwanga and Lemeshow [16]. The study sample size was calculated using the following Lorentz formula  $N = z^2 \cdot p(1-p) / d^2$ , with N as sample size, z as confidence level, p as prevalence of toxoplasmosis and d as precision. Using the prevalence of 77.1% reported by Ndumbe et al. that is,  $P = 0.77$ , with  $d = 0.05$ , we calculated a sample size of 272 individuals to provide an estimate with 95% confidence interval and 5% precision for the overall estimate of *Toxoplasma gondii* seroprevalence.

285 participants were approached for this study and only 147 provided consent to participate.

Formulated structured questionnaire (English and French) was applied to the patients. The data collected covered socio-demographic domains (age, sex, educational status, marital status, and place of residence) and *Toxoplasma* risk factors (eating of unwashed vegetables, floor type, occupation, HIV status, water source).

Blood samples (5mL) were drawn from the antecubical vein into dry tubes. Samples were centrifuged at 1000 g, 5 mins, and the sera stored in microtubes at -20°C to -23.6°C.

## 2.4 Serology

Serum samples were researched for *T. gondii* IgM and IgG antibodies using indirect ELISA TOXO reagents (DiaSource™ Toxo IgM (Diasource, Belgium) and Golden Bio Technologies IgG ELISA Kits (USA) and a Microwell reader of 450 nm (FluoroMax-4 Spectrofluorometer, HORIBA Instruments Inc. (USA).

With quality control guidelines respected, Immune Status Ratios (ISR) were then calculated and used to decide whether sample is positive or negative for both IgM and IgG.

Mean Cut off calibrator O.D = [calibrator 1 + calibrator 2]/2

ISR (IgM or IgG) = Specimen O.D. Value/Cutoff Calibrator Value

When, ISR values are  $\leq 0.90$  =Negative,  $\geq 1.10$  = Positive.

## 2.5 Data Analysis

Epi Info 7.1.5 (CDC, USA) software was used for data entry and analysis. Descriptive analysis was also added to describe the variable 'factor'. A univariate logistic regression model investigated risk factors individually for toxoplasmosis, and a multivariate logistic regression model was used to investigate the association between the potential grouped risk factors for toxoplasmosis (independent variables) and the seropositivity toxoplasmosis (dependent variables). Odds Ratio (OR) calculated at 95% confidence interval (95% CI) was added to determine the odds of association. The Chi-square test was used for group comparisons.

## 3. RESULTS AND DISCUSSION

A total of 285 eligible participants were approached in this study and 147 TB patients provided consent to participate. Of the total 147 serum samples studied, 122 (83.00%; 122/147) were seropositive for *Toxoplasma gondii* IgM and IgG antibodies (Table 1). No significant gender difference in seroprevalence was found between males (84.21%, 64/76) and females (81.69%, 58/71) (OR = 1.20, 95% CI=0.35-1.98,  $p=0.68$ ). The highest number of seropositive were

recorded in the 21-40 age group (66/87, 75.86%) and followed by the 41-60 age group with 39 seropositive cases (90.70%, 39/43) and this did not show any association to *Toxoplasma* infection. Exposure to *T. gondii* antibodies was higher among HIV positive subjects (87.14%, 61/70, OR=1.78) than in HIV sero-negative subjects though no association was established. Subjects whose occupation is farming or being a farmer had the highest exposure to *T. gondii* antibodies (92.60%, 25/27, OR=2.96) though there was no level of significance (Table 1). *T. gondii* antibody seroprevalence was higher among those who drank community borne water (86.15%) and other water sources such as streams, wells and springs (84.62%) as

compared to those who consumed treated SNEC water (80.52%).

Regarding the participants' residential area, most, 70.74% (104/147) of the study participants lived in an urban area while 29.25% (43/147) lived in a rural area. Seropositivity to *T. gondii* antibodies was slightly higher among the participants who lived in the rural areas (83.72%) than in urban area (82.70%). No significant difference exists (Table 1). *Toxoplasma* seropositivity was high among the illiterates; those without any formal education (88.00%) as compared to the literate (81.97%) with no significant difference.

**Table 1. Univariate analysis of *Toxoplasma gondii* risk factors among tuberculosis patients at the Bamenda Regional Hospital, North West Region, Cameroon**

Variables	Groups	No. tested	No. positive	Percentage (%)	OR (95%)	p-value
Gender	Male	76	64	84.21	1.20	0.68
	Female	71	58	81.69		
Residence	Rural	43	36	83.72	1.08	0.87
	Urban	104	86	82.70		
Age group(yr)	0-20	6	6	100	NA	
	21-40	87	66	78.57		
	41-60	43	39	90.70		
	>60	11	11	100		
Educational status	Illiterate	25	22	88.00	1.61	0.46
	Literate	122	100	81.97		
Occupation	Farming	27	25	92.60	2.96	0.14
	Public sector	3	2	66.67	0.40	0.44
	Business	8	6	75.00	0.60	0.53
	Student	24	21	87.50	1.52	0.52
	Private sector	42	34	80.95	0.82	0.67
	Driving	13	11	84.62	1.14	0.87
HIV STATUS	Positive	70	61	87.14	1.78	0.20
	Negative	77	61	79.22		
Water sources	SNEC	77	62	80.52	0.69	0.40
	Community	65	56	86.15	1.51	0.36
	Others	13	11	84.62	1.14	0.87
Foods Eaten	Cat meat	22	18	81.81	0.91	0.87
	Pork	129	108	83.72	1.47	0.53
	Unwashed vegetables	88	72	81.81	0.81	0.64
	Fresh milk	16	13	81.25	0.87	0.84
	Beef	119	100	84.03	1.44	0.49
<i>T. gondii</i> associated signs/symptoms	Node	30	23	76.67	0.60	0.30
	swelling	11	7	63.64	0.32	0.07
	Encephalitis	42	36	85.71	1.33	0.58
	Eye itches	50	39	78.00	0.60	0.25
	Chronic headaches	42	33	78.57	0.66	0.37
	Fever					

NA, Not applicable, SNEC=Societe Nationale Des Eaux Camerounaise, H2O= water, other water sources includes, streams, rivers, and wells

As shown in Table 1 Eye itches accompanied by tearing (85.71%) was the most prevalent *Toxoplasma* associated signs and symptoms among the TB patients studied. This was followed by fever (78.57%), chronic headaches (78.00%), lymphadenopathy (76.67%), and 63.64% cases of encephalitis no with significant difference established.

### 3.1 Prevalence of anti - *Toxoplasma* Antibodies in the Study Population

The combined seroprevalence of anti *T. gondii* antibodies among the 147 positive-sputum pulmonary TB patients in our study area was calculated to be 82.99% (122/147). Among these seropositive subjects, 104 were seropositive to IgM antibodies, 83 were seropositive for IgG antibodies, and 65 were seropositive for both IgG and IgM antibodies giving a prevalence of 70.75%, 56.46% and 44.22% respectively (Table 2).

Table 2 also illustrates that the seroprevalence of antibodies to *T. gondii* was slightly higher in HIV-positive 61/70(87.14%), than in HIV-negative 61/77(79.22%) participants ( $p > 0.05$ ). Seroprevalence of anti-*T.gondii* IgM antibody was 72.86% (51/70) in HIV-positive participants, slightly higher than in HIV-negative participants (68.83%, [53/77]). On the other side, the seroprevalence of anti-*T.gondii* IgG antibody in HIV-positive participants was 58.57% (41/70) and this was not significantly different from the 54.55% (42/77) obtained in the HIV-negative group in this same study population ( $p = 0.20$ ). There was no difference in prevalence of anti-*T.gondii* IgM and IgG antibodies among the HIV-Positive 44.29% (31/70) and HIV-Negative 44.16% (34/77) TB patients.

TB patients are particularly vulnerable to many diseases and the case of toxoplasmosis cannot be left out. TB patients in the North West Region who are interchangeably involved in one farming activity to another, constant contact with soil, reduced immunity, eating various raw foods, drinking untreated water or contact with pets, dogs and cats are, therefore, an ideal target group to investigate toxoplasmosis prevalence and disease co-infection. Data collected from these patients can thus be used to assess co-infection with other parasitic infections.

Studies focusing on *T.gondii* seroprevalence in Cameroon are few and mostly among women of childbearing age [17,18,19,20]. This study is the first conducted among sputum positive pulmonary TB patients, and indicates a high seroprevalence (83.0%) of *T. gondii* infection among TB patients. The prevalence is higher than that reported among pregnant women in some Regions of Cameroon [17,18,19] and that reported in Sudan [21]. This discrepancy in seroprevalence within Cameroon and in different countries may be due to different study groups, ethnicity, traditions, and food habits [22,23].

The univariate analyses revealed that there was no significant difference in the various *Toxoplasma* risk factors studied. However, though with no significance difference, participants' occupation as a risk factor indicated a likelihood of *T. gondii* infection where subjects whose occupations were farming, had a higher anti-*T. gondii* antibodies seroprevalence than those whose jobs were skilled e.g., teacher, nurse. These findings concur with a study in the Democratic Republic of São Tomé and Príncipe where it was reported that parents of schoolchildren who were nonskilled, farmer, and

**Table 2. seroprevalence of antibodies to *Toxoplasma gondii* in the study population and according to HIV status**

T. gondii antibodies	HIV status	Grand total
IgM <sup>+</sup>	HIV-positive 51/70(72.86%) (60.90-82.80%) HIV-negative 53/77(68.83%) (21.09-42.70%)	104/122(85.25%) (77.69-91.02%)
IgG <sup>+</sup>	HIV-positive 41/70(58.57%) (46.17-70.23%) HIV-negative 42/77(54.55%) (42.79-65.94%)	83/122(68.03%) (58.98-76.18%)
IgM <sup>+</sup> and IgG <sup>+</sup>	HIV-positive 31/70(44.29%) (32.41-56.66%) HIV-negative 34/77(44.16%) (32.84-55.93%)	65/122(53.28%) (44.03-62.36%)
IgM <sup>+</sup> or IgG <sup>+</sup>	HIV-positive 61/70(87.14%) (76.99-93.95%) HIV-negative 61/77(79.22%) (68.46-87.63%)	122/147(82.99%) (75.93-88.68%)

+ = positive: Percentages calculated with respect to totals in each sub category. For example, we found that 72.86 % of TB subjects who were HIV positive were seropositive to *T. gondii* IgG antibody.

their children had a higher probability of acquiring *T. gondii* infection than children whose parents were skilled workers, a businessperson or civil servant [24].

In this study, we observed a higher seroprevalence among 21-40 yr age group (78.57%, 66/87), though, with no difference in significance. This corroborates other findings that seroprevalence increases with age as shown in data from various studies and countries [25,26,27].

The prevalence of *Toxoplasma* infection in man and animals globally is established but the frequency of infection varies from one country to another [27,13]. This disparity in distribution is possibly due to the presence or absence of cats or dogs, climatic factors, playing in the soil, and consumption of raw or improperly cooked meat or vegetables, or unboiled water, and study populations [28,12].

In this study, the seroprevalence of toxoplasmosis at the Bamenda Regional Hospital, North West, Cameroon, was comparable in HIV-positive and HIV-negative TB patients, possibly indicating equivalent exposure to the *Toxoplasma* parasite, since both study groups were selected from similar environments. Our results are supported by other studies within Cameroon [17], and outside the country, in Northern Iran [29], where seroprevalence of toxoplasmosis was found not to be significantly different between HIV-positive and negative adults.

Our findings indicated a high seroprevalence of anti-*T.gondii* antibodies (87.14% vs 79.22%,  $p=1.78$ ) in the sera of HIV-positive TB patients than in HIV-negative TB study participants with no level of significance. This corroborates with 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 in Ethiopia [30]

In addition, there was no significant difference in the type of foods eaten to *Toxoplasma* infection even though the overall *T.gondii* seroprevalence was high. With constant water shortage, these other sources of water: streams, rivers remain the choice of most homes, a potential transmission route.

Noteworthy, is that headache or ocular discomfort and fever showed higher

seroprevalence among TB patients associated with *Toxoplasma* signs and symptoms. There is enough evidence that a high association exists between *T. gondii* infection and chronic headaches and to a lesser level ocular discomforts [31,32,33]. It is postulated that the parasite may be responsible for the neurogenic inflammation thought to cause different types of headaches [33,34]. *T. gondii* infectious forms (badyzoites, oocysts) may circulate in the blood of immunocompetent individuals and may attain every organ [35]. Their presence in blood and all bodily parts could be associated with the reactivation of the ocular disease as well as toxoplasmosis, which has been reported to contribute a significant burden to eye disease in the United States [36].

Our sample size was small and conclusions from the present study must be measured. Our study however should be interpreted with some caution since different results may be obtained with increased sample size. A large-scale study should be carried out in order to assess the prevalence of toxoplasmosis-tuberculosis co-infection at the Bamenda Regional Hospital.

#### 4. CONCLUSION

There is a high seroprevalence of *T.gondii* antibodies among TB patients at the Bamenda Regional Hospital posing itself as a severe public health concern. This high seropositivity is suggestive of comorbidity of toxoplasmosis and tuberculosis. In this study, 85.25% of the TB patients were recent infections with *T. gondii* parasite while up to 53.28% of patients were reactivating and there existed no difference in prevalence between HIV negative or HIV positive TB patients. Toxoplasmosis and tuberculosis being opportunistic infections of HIV/AIDS further worsen patient health and could be the reason for unexplained deaths among TB patients on treatment.

#### CONSENT

"All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

## ETHICAL APPROVAL

We hereby declare that all experiments have been examined and approved by the appropriate ethics committee (Ethics Review and Consultancy Committee (ERCC), of the Cameroon Bioethics Initiative (CAMBIN), Bamenda Regional Hospital Institutionalized Review Board (IRB) approval) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## ACKNOWLEDGEMENTS

We appreciate all the TB patients who participated in this study. We also thank Leo NJOCK AYUK M.D, Director of the BRH TB Treatment Centre for permission and for providing a good working environment. We thank NCHE Quinta and all the nurses of the TB centre for their assistance during sample collection.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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