

## Seroepidemiological Evaluation of *Toxoplasma gondii* Immunity and Relationship with White Blood Cells and Haemoglobin Levels Among HIV-positive and HIV-negative Subjects in the North West Region of Cameroon

Oumar Mahamat, Nosimbang Golda Pingpoh, Lem Edith Abongwa and Ntonifor Helen Ngum\*

Department of Biological Sciences, Faculty of Science, The University of Bamenda, Bambili, Cameroon

\*Corresponding Author: Ntonifor Helen Ngum, Department of Biological Sciences, Faculty of Science, The University of Bamenda, Bambili, Cameroon.

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### Abstract

**Background:** This study aimed at determining the impact of *Toxoplasma gondii* infection on certain haematological indices in HIV-infected and non-infected persons.

**Method:** The cross-sectional study was carried out between January-June 2021 at the Bamenda Regional Hospital and Bambui District Hospital in Northwest Cameroon with 320 participants. Venous blood was collected to measure haemoglobin concentrations, leucocyte, lymphocyte, and granulocyte counts and to determine the presence of anti-*T. gondii* IgG and IgM using Rapid anti-*T. gondii* test cassettes.

**Results:** The seroprevalence of Anti-*T. gondii* IgG and *T. gondii* IgM were 40% and 3.4% respectively. *T. gondii* was more prevalent in non-HIV-infected individuals (41.7%). Anti-*T. gondii* IgM antibodies were significantly higher in HIV positive ( $p = 0.035$ ). In HIV-positive participants, the highest prevalence of *T. gondii* IgM 6.5% (4/62) and *T. gondii* IgG 38.7% (24/62) was found among those aged 25-40 years, although the difference was not significant ( $p = 0.811$ ). A significant relationship ( $P = 0.039$ ) was observed between gender on the seroprevalence of anti-*T. gondii* IgG in HIV-positive individuals with males having a higher risk. Blood counts showed no significant difference ( $p > 0.05$ ) between *T. gondii* positive and negative patients though it was high in patients who were negative for *T. gondii*. On the contrary, blood cell counts were significantly lower ( $p < 0.05$ ) in HIV-positive patients with *T. gondii*.

Absolute leukocytosis (1.33%) and leukopenia (33.33%) were higher in HIV-positive patients infected with *T. gondii*. HIV participants with *Toxoplasma* had an insignificant ( $p = 0.64$ ) lower haemoglobin level (13.10 g/dl) when compared to those without *Toxoplasma* (13.44 g/dl).

**Conclusion:** *Toxoplasma gondii* is prevalent in the Northwest Region of Cameroon with a higher prevalence in HIV-non-infected individuals than HIV-infected patients. The presence of this parasite in HIV patients significantly affects the haematological indices.

**Keywords:** *Toxoplasma gondii*; HIV; IgG; IgM; Haemoglobin; White Blood Cells Bamenda

## Abbreviations

AIDS: Acquired Immune Deficiency Syndrome; CDC: Centers for Disease Control; ELISA: Enzyme-linked immune-sorbent Assay; HIV: Human immunodeficiency Virus; Ig: Immunoglobulin; SD: Standard Deviation; SPSS: Statistical Package for Social Sciences; T: *Toxoplasma*; WBC: White Blood Cell Count

## Background

Toxoplasmosis caused by *Toxoplasma gondii* an obligate intracellular coccidian parasite is a zoonotic infection distributed worldwide [1]. It is one of the most neglected tropical diseases [2] which continues to affect most vulnerable populations, particularly in low socio-economic countries [3]. Its worldwide distribution is due to the ease with which it can be transmitted between the intermediate hosts [4]. Toxoplasmosis is an opportunistic infection among populations with a compromised immune system such as Human immunodeficiency Virus (HIV) patients. The disease is a major cause of mortality among HIV patients in endemic communities and has been associated with physical and/or psychological disruptions among these patients [5]. Furthermore, infection with this parasite leads to an asymptomatic disease in immuno-competent persons, however, 10% to 20% of patients with acute infection may develop cervical lymphadenopathy and/or a flu-like illness [6]. *Toxoplasma gondii* also infects immuno-competent populations resulting in a milder or self-limiting outcome but severe morbidity related to this parasite has been reported among HIV patients. It is suspected that *T. gondii* may degenerate the immune system of these patients by affecting some immunological factors and haematological factors such as haemoglobin concentration levels, lymphocytes, platelets, granulocytes, and white blood cell counts [7,8]. Most studies of *Toxoplasma* infection in Cameroon have reported mostly on the prevalence and associated risk factors among pregnant women, women of childbearing age, or children with very little study on HIV positive patients [9-12]. However, the mechanism through which this parasite exacerbates mortalities and morbidities among HIV patients has not been addressed in Cameroon. Therefore, this study was designed to determine the impact of *T. gondii* infection on immunological and haematological parameters among HIV-positive and negative persons with an interest to address the impact of this infection on the pathological outcomes in HIV patients in Bamenda.

## Methods

### Description of the study area

The study was a hospital-based cross-sectional study conducted between January-June 2021 at the Bamenda Regional Hospital AIDS (Acquired Immune Deficiency Syndrome) treatment centre and Tubah District Hospital in the Northwest Region of Cameroon. The study site was selected to reflect the entire population of the Northwest Region. The Bamenda Regional Hospital AIDS treatment centre serves the urban population while Tubah District Hospital serves the rural population. Most of the inhabitants in these localities have cats which are the definitive hosts and rats and other rodents, chickens, dogs, pigs, goats, sheep, horses, cattle, marsupials, and humans which are intermediate hosts for the parasite (CDC, 2018).

### Ethical considerations

Ethical clearance was obtained from The University of Bamenda Institutional Review Board of the Faculty of Health Sciences (No:2020/0229H/UBa/IRB). In addition, administrative authorization was also obtained from Tubah District Hospital and The Regional Hospital of Bamenda. The Objectives of the study were explained to participants using information sheets after which they were required to sign consent forms. Only those who signed the informed consent forms took part in the study. In the case of minors (<18 years), the consent of their parents/legal guardians was obtained.

### Study population

We recruited volunteer patients already diagnosed and confirmed to be HIV positive and on treatment, and voluntary blood donors who were HIV, Hepatitis C, and Hepatitis B negatives. Only volunteers who returned their informed consent forms duly signed were recruited irrespective of their gender or age. The exclusion criteria were HIV positive patient taking chemoprophylaxis for toxoplasmosis or anyone who did not sign the informed consent form.

### Sample size

The minimum acceptable sample size was calculated using the Lorenz formula as stated below [11].

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

Where,  $Z_{1-\alpha}$  = the normal distribution value = 1.96

P = Relative prevalence of *T. gondii* in the region = 22.9% [11]

$\delta$  = precision (sampling error) = 0.05

$$N = \frac{(1.96)^2 \times (0.229)(1 - 0.229)}{(0.05)^2} = 272.$$

### Blood sample collection

Five ml of venous blood was collected from each consented participant by a trained laboratory technician in uniquely coded tubes. Of this, 3 ml and 2 ml were transferred into dry and ethylene diamine tetraacetic acid (EDTA) tubes respectively. The sample in the dry tube was kept for 30 minutes to facilitate clotting and then the clotted blood was centrifuged at 3000rpm for 5 minutes to separate the serum from blood cells. Serum was secondly aliquoted into cryotubes and stored at -20°C until used.

### Haematological analysis

Two milliliters of whole blood were used for full blood count measurement using the automated URIT 3000 haematological analyzer according to the manufacturer's instructions. The reference ranges of these haematological parameters were defined as leukocytes: 5,000 - 21,000/ $\mu$ L (age of individual: 8- 30 days); 5,500 - 18,000/ $\mu$ L (1 - 11 months); 6,000 - 17,000/ $\mu$ L (1 - 3 years); 5,500 - 15,500/ $\mu$ L (4 - 7 years); 4,500 - 13,500/ $\mu$ L (8 - 13 years); and 4,000 - 10,000/ $\mu$ L (> 13 years). Values of leukocytes above the reference range were defined as absolute leukocytosis and below as absolute leukopenia. Reference ranges for absolute number of lymphocytes: 2,000 - 8,000/ $\mu$ L (age of individual: 15 days to 5 months), 1,600 - 7,000/ $\mu$ L (6 -23 months), 1,500 - 4,500/ $\mu$ L (2 -5 years), 1,200 - 3,600/ $\mu$ L (6 - 11 years), 1,000 - 3,200/ $\mu$ L (12 - 17 years), and 1,000 - 2,900/ $\mu$ L (> 17 years). Values of lymphocytes above the reference range were defined as absolute lymphocytosis and below as absolute lymphopenia or lymphocytopenia. Reference ranges for absolute number of granulocytes: 500 - 8500/mm<sup>3</sup>. Values of granulocytes above the reference range were defined as absolute granulocytosis and below the range were considered absolute agranulocytopenia or agranulosis [13].

### Diagnosis of *Toxoplasma gondii*

Using the rapid diagnostic test kit; TOX (*Toxoplasma* IgM/G Antibody Rapid Test Kit), serum samples were used to diagnose Toxoplasmosis according to the manufacturer's protocol using the principle of immunochromatography. All positive serum samples were then used to titrate the IgG and IgM.

### *Toxoplasma gondii* enzyme-linked immune-sorbent assay (ELISA)

Serum samples were screened for anti-*T. gondii* IgG and IgM antibodies by ELISA using "*Toxoplasma* IgG and IgM" kits as described by the manufacturer [14,15]. In brief, the serum was diluted and added to the wells coated with purified antigens. IgG or IgM-specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate was added to bind to the antibody-antigen complex if present. Excess enzyme conjugate was washed off and the substrate is added. The plate was incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM-specific antibodies in the sample. Results were obtained by comparison with a cut-off value measured at 450nm and 620nm. The absorbance was then converted to standard international units/ml (IU/ml). The Ab index <0.9 was considered as not detectable for IgG or IgM antibodies while values >1.1 were considered detectable for IgG or IgM antibodies to *Toxoplasma*. Seropositivity to IgM antibodies (with or without IgG seropositivity) was indicative of acute or recent *Toxoplasma* infection while seropositivity to IgG antibodies (with seronegative IgM antibody) was indicative of chronic or latent *Toxoplasma* infection.

### Statistical analysis

Data were entered into Microsoft Excel, cleaned, and analysed using Statistical package for social sciences (SPSS) software (Version 18.0). The data with quantitative variables were expressed as a mean ( $\pm$  standard deviation [SD]) and range, whereas, qualitative variables were estimated and presented as frequencies and percentages. Pearson Chi-square and t-test were used for group comparison and association of variables. The p-value of  $\leq 0.05$  was regarded as statistically significant.

## Results

### Demographic and clinical characteristics of the study population

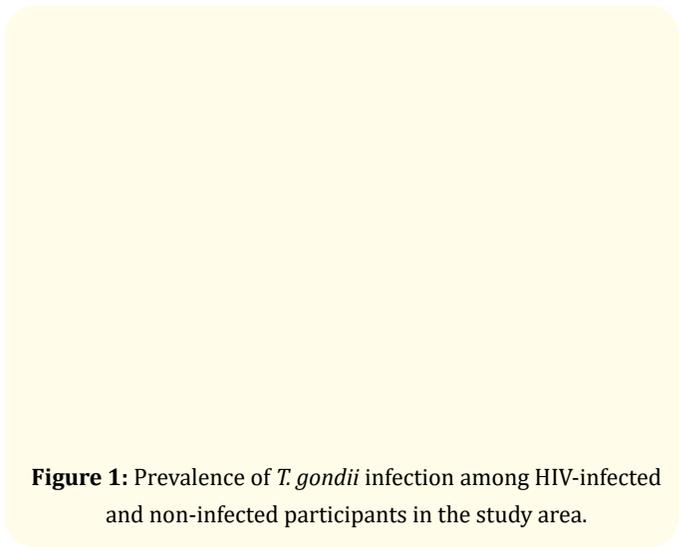
Out of the 320 participants, 63.1% (202) were females. The age range of the participants was 2- 78years with a mean  $\pm$  std error mean of  $41.9 \pm 0.86$  years. Most of the participants 82.5% (264) were between the ages of 19 and 60 years and the least were those between 11-18 years 2.2% (7) were greater than 60 years old. Equally, 60.3% (193) of the participants were HIV positive (Table 1).

Characteristics	Frequency (N = 320)	Proportion (%)
Sex		
Females	202	63.1
Males	118	36.9
Age group ( years)		
$\leq 10$	10	3.1
11 - 18	7	2.2
19 - 60	264	82.5
<b>&gt; 60</b>	39	12.2
HIV Status		
Negative	127	39.7
Positive	193	60.3

**Table 1:** Demographic characteristics of the study population.

### Seroprevalence of *Toxoplasma* IgM and IgG and HIV among the study population.

Results obtained showed that 40% (128/320) and 3.4% (11/320) of the participants had been exposed to Toxoplasmosis IgG and IgM antibodies respectively. All (100%; 11/11) seropositive cases for anti-*T. gondii* IgM antibodies were also seropositive for anti-*T. gondii* IgG antibodies. Out of the 127 HIV-negative patients, 41.7% (53/127) tested seropositive to *Toxoplasma gondii* infection while amongst the 193 HIV-positive individuals, 38.9% (75/193) tested seropositive to *T. gondii*. However, this difference was not statistically significant ( $P = 0.324$ ). On the other hand, anti-*T. gondii* IgM antibodies were significantly higher in HIV positive ( $p = 0.035$ ) while anti-*T. gondii* IgG was insignificantly higher ( $p = 0.608$ ) compared to their negative counterpart (Figure 1).



**Figure 1:** Prevalence of *T. gondii* infection among HIV-infected and non-infected participants in the study area.

### Influence of gender and age on the seropositivity of *Toxoplasma* IgM and IgG among HIV-positive and negative patients

In participants who were HIV positive, the highest prevalence of *T. gondii* IgM 6.5% (4/62) and *T. gondii* IgG 38.7% (24/62) was found among those aged between 25-40 years, while in HIV-negative participants the highest prevalence of *T. gondii* IgM was 2.6% (1/39), and *T. gondii* IgG 48.4% (15/31) was found among those >55years and those aged between 25-40 years respectively. Although, there was no significant difference  $p < 0.05$  as shown in table 2.

Variable	HIV positive		HIV Negative	
	IgG N(%)	IgM N(%)	IgG N(%)	IgM N(%)
Age group				
< 25 years	4 (33.3)	0(0.0)	12 (38.7)	0(0.0)
25-40 years	24(38.7)	4(6.5)	15 (38.5)	1(2.6)
41-55years	34(39.5)	4(4.7)	11 (42.3)	0(0.0)
>55years	13(39.4)	2(6.1)	15 (48.4)	0(0.0)
$\chi^2$	0.175	0.961	0.856	2.274
P value	0.981	0.811	0.836	0.517
Gender				
Female	44 (34.9)	5(4.0)	37 (48.7)	1(1.3)
Male	31 (46.3)	5(7.5)	16 (31.4)	0(0.0)
$\chi^2$	2.371	1.087	3.761	0.676
P value	0.083	0.297	0.039	0.411

**Table 2:** Association of *Toxoplasma gondii* IgG and IgM antibodies with age and sex among HIV-positive and negative individuals in the study area.

In figure 2, *T. gondii* antibodies were higher in females 81(40.1%) than in males and this difference was not significant (p = 0.96). However, a significant relationship was observed between gender and the seroprevalence of anti-*T. gondii* IgG in HIV-negative individuals with males having a lower prevalence of 31.4 %(16/51) than females (P = 0.039). On the contrary the prevalence of anti-*T. gondii* IgG was not significantly (p = 0.83) higher in males 46.3% (31/67) compared to females among HIV-positive patients. No significant relationship (P< 0.05) was also established between gender and seroprevalence of anti-*T. gondii* IgM in both HIV-positive and HIV-negative individuals, however, the prevalence was higher in males 7.5% (5/67) and females 1.3% (1/76) respectively (Table 2).

were negative for Toxoplasma ( $2.21 \pm 0.98 \times 10^3/\mu\text{L}$ ) compared to those positive ( $2.20 \pm \times 10^3/\mu\text{L}$ ). The number of lymphocytes was comparatively higher in HIV non-infected participants who were negative for *T. gondii* infection ( $2.35 \times 10^3/\mu\text{L}$ ) than those of HIV non-infected participants who were positive for *T. gondii* ( $2.04 \times 10^3/\mu\text{L}$ ). On the contrary in HIV-infected participants, the mean  $\pm$  SD lymphocyte count was also high in participants with *T. gondii* ( $2.10 \pm 0.81 \times 10^3/\mu\text{L}$ ) compared to those without *T. gondii* infection ( $2.04 \pm 0.76 \times 10^3/\mu\text{L}$ ). However, there was a significant difference (P = 0.016) in the number of lymphocytes count when comparing HIV-infected individuals who had lower total lymphocyte counts ( $2.07 \pm 0.78 \times 10^3/\mu\text{L}$ ) compared to HIV-infected individuals. The granulocyte means level in HIV-non-infected patients was higher ( $3.18 \times 10^3/\mu\text{L}$ ) compared to HIV-infected patients ( $2.09 \times 10^3/\mu\text{L}$ ) and this difference was highly significant (P = 0.001). On the contrary mean  $\pm$ , SD granulocyte levels were higher in HIV-infected patients ( $2.24 \pm 2.19 \times 10^3/\mu\text{L}$ ) who were positive for Toxoplasma compared to those negative ( $1.94 \pm 1.17 \times 10^3/\mu\text{L}$ ). Patients positive for Toxoplasma experienced an insignificant (t = 0.546; P = 0.460) decrease in granulocyte level compared to its negative counterpart ( $2.63 \times 10^3/\mu\text{L}$  vs  $2.64 \times 10^3/\mu\text{L}$ ) as reported in table 3.

p - Value was evaluated using an unpaired t-test.

**Figure 2:** Association between gender and *T. gondii* infection in the study area.

**Assessing white blood cell count among HIV-infected and non-infected participants with seropositivity to *Toxoplasma gondii* infection**

The range of white blood cells (WBC) among the study participants was  $1.1\text{-}23.8 \times 10^3/\mu\text{L}$  with a mean  $\pm$  SD of  $5.42 \pm 3.07 \times 10^3/\mu\text{L}$ . our data showed that total WBC was significantly (P = 0.001) higher in HIV-negative individuals ( $6.30 \times 10^3/\mu\text{L}$ ) compare to HIV-infected participants. On the contrary, the mean level of total WBC was insignificantly (t = 0.710; P = 0.40) higher in participants who were positive for *T. gondii* infection ( $5.86 \pm 3.18 \times 10^3/\mu\text{L}$ ) when compared to those negative ( $5.53 \pm 3.00 \times 10^3/\mu\text{L}$ ). The mean  $\pm$  SD of lymphocyte count was  $5.42 \pm 3.07 \times 10^3/\mu\text{L}$  and ranges from  $0.4\text{-}9.4 \times 10^3/\mu\text{L}$ . Total lymphocyte count was insignificantly (t = 0.125; P = 0.72) higher in participants who

Type of cell count	<i>T. gondii</i> status	HIV non-infected		HIV-infected patients	
		N	Mean $\pm$ SD	N	Mean $\pm$ SD
White blood cell	Negative	74	6.40 $\pm$ 4.22	118	4.67 $\pm$ 1.50
	Positive	53	6.19 $\pm$ 3.58	75	5.07 $\pm$ 2.74
	Total		6.30 $\pm$ 3.99		4.87 $\pm$ 2.09
	t value		22.642		
	P value		0.0001		
Lymphocyte	Negative	74	2.37 $\pm$ 1.240	118	2.04 $\pm$ 0.76
	Positive	53	2.30 $\pm$ 0.966	75	2.10 $\pm$ 0.81
	Total		2.34 $\pm$ 1.14		2.07 $\pm$ 0.78
	t value		5.900		
	P value		0.0160		
Granulocytes count	Negative	74	3.34 $\pm$ 3.75	118	1.94 $\pm$ 1.17
	Positive	53	3.02 $\pm$ 3.17	75	2.24 $\pm$ 2.19
	Total		3.18 $\pm$ 2.06		2.09 $\pm$ 1.65
	t value		24.62		
	P value		0.0001		

**Table 3:** Effect of *T. gondii* seropositivity on the total blood cell count in HIV-infected and non-infected individuals.

**Frequency of patients with increased or decreased blood cells in HIV-infected and non-infected patients with seropositivity to *T. gondii* infection**

Among HIV-positive patients infected with *T. gondii*, the proportions of absolute leukocytosis were 1.33% and absolute leukopenia (33.33%) compared to 0.84% leukocytosis and 30.50% leukopenia in *T. gondii* negative participants. The prevalence of absolute lymphopenia (8%) was greater in those who tested positive for *T. gondii* infection as compared to 4.23% in HIV patients who were negative for *T. gondii* infection. The absolute lymphocytosis was significantly higher (11.01%) in HIV-positive patients who tested negative for *T. gondii* than those who were positive for *T. gondii* infection. The highest prevalence of agranulosis was observed among HIV-positive participants who were *T. gondii* -positive (5.33%) as compared to that of *T. gondii*-negative patients (5.08%). Only HIV patients suffering from Toxoplasmosis had granulocytosis (1.33%).

Among HIV-negative participants, absolute leukocytosis was higher in participants with *T. gondii* infection (9.43%) compared to those without *T. gondii* (8.10%), while the prevalence of absolute leukopenia was higher in *T. gondii* positive participants (26.41%) compared to *T. gondii* negative participants (20.27%). The lymphopenia was higher in *T. gondii*-negative individuals (5.40%) compared to their *T. gondii*-positive counterparts (1.88%). Similarly, lymphocytosis was higher in *T. gondii* negative patients (21.62%) when compared to the *T. gondii*-positive patients (18.86%). Granulocytosis was also higher in *T. gondii*-negative patients (6.75%) as compared to the *T. gondii*-positive patients (5.66%), while agranulocytosis was higher in *T. gondii*-negative participants (2.70%) than that of *T. gondii*-positive patients (1.88%) as in table 4.

**Hemoglobin levels in HIV- infected and non-infected participants with seropositivity to *T. gondii* infection**

The Hb range was 6.1-26.0 g/dl with a mean ( $\pm$  SD) of 13.29(2.36) g/dl. In table 5, HIV non-infected participants, the

p - Value was evaluated by the Unpaired t-test. N\*: Number of anemia cases

<i>T. gondii</i> status	HIV non-infected patients		HIV infected patients		Total	t-value	P value
	N = 127 (N*)	Mean $\pm$ SD (% anemia cases)	N = 193 (N*)	Mean $\pm$ SD (% anemia cases)			
Negative	74 (14)	13.40 $\pm$ 2.49 (18.91)	118 (37)	13.14 $\pm$ 2.21 (31.35)	13.55 $\pm$ 2.56	0.460	0.640
Positive	53 (12)	13.70 $\pm$ 2.61 (22.64)	75 (23)	13.10 $\pm$ 2.241 (30.66)			

**Table 5:** Effect of Toxoplasmosis on the haemoglobin levels in HIV-infected and non-infected individuals in the study area.

mean value of haemoglobin level was comparatively lower in those without *T. gondii* infection (13.40 g/dl) compared to those who tested positive for *T. gondii* infection (13.75 g/dl), in the contrary, in HIV infected participants, the mean value of haemoglobin was higher in people who tested negative for *T. gondii* (13.14 g/dl) than in people who tested positive for *T. gondii* infection (13.10 g/dl). However, this difference was not significant (P = 0.908). Taking the group of participants who tested negative for *T. gondii* infection, the mean haemoglobin level (13.27  $\pm$  2.32 g/dl) was lower compared to those who tested positive (13.40  $\pm$  2.43 g/dl) although the difference was not statistically significant (t = 0.007; P = 0.930).

p - Value was evaluated by using Unpaired t-test. N\*: Number of anemia cases

Type of cell count	<i>T. gondii</i> status	HIV non-infected		HIV-infected patients	
		N	Mean $\pm$ SD	N	Mean $\pm$ SD
White blood cell	Negative	74	6.40 $\pm$ 4.22	118	4.67 $\pm$ 1.50
	Positive	53	6.19 $\pm$ 3.58	75	5.07 $\pm$ 2.74
	Total		6.30 $\pm$ 3.99		4.87 $\pm$ 2.09
	t value		22.642		
	P value		0.0001		
Lymphocyte	Negative	74	2.37 $\pm$ 1.240	118	2.04 $\pm$ 0.76
	Positive	53	2.30 $\pm$ 0.966	75	2.10 $\pm$ 0.81
	Total		2.34 $\pm$ 1.14		2.07 $\pm$ 0.78
	t value		5.900		
	P value		0.0160		
Granulocytes count	Negative	74	3.34 $\pm$ 3.75	118	1.94 $\pm$ 1.17
	Positive	53	3.02 $\pm$ 3.17	75	2.24 $\pm$ 2.19
	Total		3.18 $\pm$ 2.06		2.09 $\pm$ 1.65
	t value		24.62		
	P value		0.0001		

**Table 3:** Effect of *T. gondii* seropositivity on the total blood cell count in HIV-infected and non-infected individuals.

## Discussion

This study aimed at determining the seroprevalence of *T. gondii* infection and the impact of this parasite on some blood parameters among HIV patients attending the Bamenda regional hospital and the Bambui District hospital. The results showed that 40 % and 3.4% of the study population had been exposed to *T. gondii* IgG and IgM infection respectively. The Low IgM seropositivity rate seen in this study is consistent with the low rates reported in other studies [8,9,16,17]. The seroprevalence of IgM antibodies indicates an acute or recent infection while the seroprevalence of IgG antibodies indicates chronic or latent *Toxoplasma* infection [17-19]. The low prevalence rate recorded in this study is lower compared to the results of a similar study elsewhere in Cameroon that reported 69.9% seroprevalence of *T. gondii* infection among HIV patients [20]. This variation in results could be related to differences in the target population, the positive impact of the increased community sensitization, campaigns about HIV and related opportunistic infections, and methodological differences which include the choice of test kits.

Although HIV patients were less (38.9%) exposed to *Toxoplasma* infection compared to HIV-negative patients (41.7%). These observations are not in line with the outcome of a study conducted in Cameroon by Wam., *et al.* [10] that reported an increase in seroprevalence of *Toxoplasma* infection among HIV patients compared to HIV-negative patients. Many factors could account for the outcome of the results in this study. All the enrolled HIV patients attending the treatment centres in Bamenda are subjected to regular morbidity control against opportunistic infections such as toxoplasmosis. In addition, this low seroprevalence in HIV patients could be explained by the fact that the weakened immune system may not be efficient in producing or maintaining detectable levels of antibodies thus antibody detecting tools may be a less sensitive indicator of infection in this group accounting for the lower seroprevalence of toxoplasmosis in the HIV-positive samples. Furthermore, we also observed that 8.6% of the population suffered active *Toxoplasma* infection as measured by the specific anti-toxoplasma IgM rapid test. These findings are lower as compared to some studies that reported 16.9% active *Toxoplasma* infection among HIV patients in Cameroon [9] and elsewhere (9.7%) in Iran [21]. Improved personal hygiene and sanitation among HIV patients through sensitization campaigns and scaling up of community awareness about opportunistic infections associated

with HIV, the availability of anti-protozoan chemotherapy against *Toxoplasma* infection could explain this low trend in the active cases of *toxoplasma* infection among HIV patients in Bamenda.

Age and sex did not influence the seropositivity of *Toxoplasma* IgG/IgM levels in the population. Our study reported a higher *Toxoplasma* IgG prevalence in females and a high IgM prevalence in males. This report is contrary to reports from other countries which state that both IgG and IgM antibodies were higher in females [17,22]. Our data revealed that HIV patients that were 25 years and older were significantly exposed to *T. gondii* infection (as indicated by the increased levels of *Toxoplasma* IgG) compared to their non-infected counterparts. This was contrary to the seropositivity status of *Toxoplasma* IgM where none of the age groups recorded significant levels of this antibody within the population and between HIV patients and non-HIV infected participants. This could be linked to the fact that HIV patients generally have weakened immune systems that expose them to the risk of contracting *T. gondii* infection. This risk further increases with the attitude (sexuality, eating habits, and interaction with pets) and activities (farming, cooking) of this active population that expose them to *T. gondii* infection. In addition, immunity to parasitic infections increases over time in immune-competent persons and this possibly explains why participants with competent immune systems (non-HIV patients) in this study that were exposed to *T. gondii* infection alone recorded lower levels of toxoplasma IgG. Generally, the risk of *T. gondii* infection and positivity of *Toxoplasma* IgM/IgG was not gender or age-dependent hence populations with equal risk exposure could be positive or negative for anti-toxoplasma antibodies.

Looking at the effect of *T. gondii* infection on haematological parameters, our data showed that WBC, lymphocytes, granulocytes, and haemoglobin levels were insignificantly higher in negative patients. Similar to a report from another study by Abogdzo., *et al.* [17]. This insignificant difference is because haematological parameters might sometimes remain stable in times of infection [23]. This low level is because *T. gondii* is capable of infecting virtually any nucleated cell including immature red blood cells. It could also be that *T. gondii* exits the cell by exerting tension on the host cell membrane, causing the cell to rupture or by disrupting the cytoskeleton which results in cell lysis [24-26]. However, further investigation is needed to effusively clarify the links between toxoplasmosis and blood cell count.

Lymphocytes and granulocytes are components of the WBC that promote host defense. Comparing blood cell counts between HIV individuals who were seropositive or seronegative for *T. gondii* showed that decreased blood values were seen in those who were both positive for HIV and *T. gondii* infection. Similar findings have been reported in other studies [7,8,27] but contrary to that of [23] who stated that Hb values increases in a patient with toxoplasmosis. The most probable reason is that in immunocompetent individuals, *T. gondii* infection is generally asymptomatic and cannot be detected early enough to commence treatment [7]. Furthermore, it is worth mentioning that *T. gondii* decreases WBCs which are important factors that control the natural and acquired immunity response of an individual.

We also observed that patients infected with *T. gondii* alone were more vulnerable to suffering leukocytosis (increase in leucocytes) while leucopenia was common among patients infected with *Toxoplasma* and HIV. This probably suggests that concomitant infection of patients with *T. gondii* and HIV results to decrease levels of lymphocytes and this may be due to the high potential of HIV in decreasing host levels of leucocytes, especially during coinfection. In addition, participants that were negative for HIV and *T. gondii* had higher lymphocyte levels contrary to HIV patients infected with *T. gondii* which recorded lower levels of lymphocytes thus making them more vulnerable to lymphopenia. Normally infection with *T. gondii* in a healthy person is self-limiting meaning an immunocompetent individual produces higher levels of lymphocytes to fight the protozoan. But this is not the case in an immunocompromised person such as HIV individuals where the virus attacks the lymphocytes thus decreasing their levels and making the patient more vulnerable to *Toxoplasma* infection. This could be because the *T. gondii*/host immune potential in increasing the lymphocyte levels in a patient already infected with HIV is lower.

In this study, lower haemoglobin levels were among patients that were infected with *Toxoplasma* alone than in non-HIV and non-*Toxoplasma* infected participants. The implication of *T. gondii* infection with anemia has not been studied in humans. However, some studies in animal models have associated *T. gondii* infection with haemolytic anaemia [28] as a result of decreased erythropoiesis and reduced survival time of RBCs. Anaemia was however not observed in the patients in our study population.

## Conclusion

The seroprevalence of *T. gondii* IgG was high, especially amongst the HIV non-infected participants who might be acting as reservoirs in the populations, and so there is a need for the government to establish enlightenment and prevention programs for the population especially those at risk of infection.

## Availability of Data and Material

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

## Conflict of Interest

The authors have no competing interests to declare.

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Not applicable.

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## Ethics Approval and Consent to Participate

All methods used in this study were carried out following relevant guidelines and regulations. All experimental protocols were approved by the study protocol and were reviewed and approved by The University of Bamenda Institutional Review Board of the Faculty of Health Sciences (No:2020/0229H/UBa/IRB). Written informed consent was obtained from all subjects and/or their legal guardian(s).

## Consent for Publication

Participants gave their consent for the publication of result without being identified.

## Authors' Contributions

OM, NGP, and NHN Conceived and designed the experiments. NGP enrolled and performed the experiments. OM, NGP, LEA, and NHN contributed to data management, analysis, and interpretation. All author collaborated on the scientific writing of the manuscript. OM, NHN and LEA reviewed the analyses and the final version of the manuscript. All authors read and approved the manuscript.

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