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Research Article

Immune responses in toxoplasmosis and malaria co-infections among residents of rural communities in South-Western, Nigeria

Awobode HO[1*], Efenovwe MO[1], Anumudu CI[2]

[1]Department of Zoology, University of Ibadan, Parasitology Unit, Ibadan, Nigeria

[2]Department of Zoology, University of Ibadan, Cellular Parasitology Program, Ibadan, Nigeria

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ABSTRACT

Background: Malaria and toxoplasmosis utilize similar known cellular and biochemical pathways to modulate immune responses. Co-infections with malaria and toxoplasmosis occur in malaria endemic regions but not much is known about immune response modulation in these co-infections. The study aimed to assess the prevalence of *Toxoplasma gondii* and *Plasmodium* co-infections and associated risk factors, as well as cytokine profiles in a population living in Akinyele Local Government Area, Ibadan, Oyo State, Nigeria.

Method: In a cross-sectional survey, blood from 192 volunteers was screened for *Plasmodium falciparum*, *Toxoplasma gondii* antibodies (IgG and IgM) and cytokine (IL2, IL6, IL10 and IL12) levels. Packed Cell Volume (PCV) and epidemiological factors associated with toxoplasmosis and malaria were also determined.

Result: Out of the 192 participants, 27.1% and 8.9% tested positive for *T. gondii* IgG and IgM antibodies, while malaria was prevalent in 72.4% of participants. Co-infection of *T. gondii* and *Plasmod*ium was observed in 20.4% of participants. Malaria prevalence was highest in individuals below 20 years of age, while toxoplasmosis was most prevalent in 51-60 year olds. Toxoplasma sero-positivity, malaria prevalence and *Plasmodium* intensity were significantly higher (P<0.05) in females. High Toxoplasma IgG was associated (P<0.05) with increased *Plasmodium* intensity and high IgM with decreased intensity (P>0.05). Environmental factors, such as the presence of cats, were significantly associated with *T. gondii* IgG seropositivity. IL-2 and IL-6 were significantly lower (P<0.05) with *Toxoplasma gondii* (IgM) while IL-10 levels were lower with malaria infections and chronic (IgG) *T. gondii* co-infection, although this was not statistically significant (P>0.05).

Conclusion: Active *Toxoplasma gondii* co-infection with malaria may suppress malaria pathology while *Plasmodium* and chronic *T. gondii* co-infection may lead to increased production of IL-2 which may facilitate malaria parasite clearance. The study also found that IL-12 may be a potential biomarker for disease severity and response to treatment in co-infected individuals.

Keywords: Malaria, Toxoplasmosis, Cytokines, Co-infection

INTRODUCTION

Toxoplasma and *Plasmodium* are obligate, intracellular, apicomplexan parasites and are two of the most prevalent and successful parasites worldwide (Bargieri et al., 2013; Onkoba, et al., 2015). Both parasites harness similar cellular and biochemical pathways for their pathology, nutrition, metabolism, host cell invasion and immune modulation (Frolich, et al., 2012; Butler, et al., 2013). Genetic analyses revealed that one third of the amino acid sequences of the inner membrane complex from the parasite *Plasmodium* berghei is similar to that of *Toxoplasma gondii* (Lee, et al., 2019).

Toxoplasmosis is the zoonotic disease, caused by *Toxoplasma gondii*. Felids are the only definitive hosts of *T. gondii*, while humans and other vertebrate animals serve as intermediate hosts (Ekanem, et al., 2018). Toxoplasmosis is most prevalent in warm and humid areas (Studenicova, et. al., 2006). Infection with Toxoplasma in humans may occur through ingestion of tissue cysts from undercooked or raw meat such as pork and lamb (Dubey, 2010; Dehkordi et. al., 2013) or direct contact with infected cats and pets (Dubey and Prowell, 2013). Congenital infection may also occur during pregnancy following maternal

[*]**Corresponding author.** Awobode HO, E-mail: awobodet@gmail.com

infection. Prevalence of toxoplasmosis ranges between 30% and 60% in developed and developing communities (Daryani, et al., 2014). The parasite disseminates within the host's body and affects lymph nodes, eyes, central nervous system, and other tissues (Nunura, et al., 2010). It also has unfavourable effects on the reproductive capacity of men and women (Sarkar, et. al., 2012).

Malaria is caused by a protozoan parasite, *Plasmodium* spp and transmitted through the bite of infected female adult Anopheles mosquitoes. Of the *Plasmodium* species known to infect humans, P. falciparum is the most virulent and accounts for over 90% of human malaria (Beier et al., 1999). Malaria remains a global burden causing approximately 584,000 deaths among an estimated 198 million cases annually (Bwanika, et al., 2018). In 2015, malaria was the leading cause of death in Nigeria, causing approximately 192,284 deaths (WHO, 2015). The World Malaria report 2018 states that malaria is the most serious parasitic disease in terms of associated burdens and public health importance, with 300,500 million cases and more than a million deaths each yearly (WHO, 2018). It was stated in the World Malaria Report 2022 that Nigeria currently accounts for the highest malaria death worldwide (WHO, 2022), Infection with *T. gondii* and *Plasmodium* activates host innate phagocytic cells to secrete several cytokines (Baccarella et al., 2013; Koblansky, et al., 2013).

Cytokines such as IL-2, IL-12, IL-6, IL-10 are secreted in response to *T. gondii* infections (Suzuki et al., 2011). In malaria, the balance between pro and anti-inflammatory cytokines ensures an appropriate immune response. Specifically, malaria infection is associated with elevated levels of pro-inflammatory cytokines such as interferon-γ (IFN-γ), IL-12, increased levels of CD4+ Th1 cells, $CD8^+$ T cells, NK cells and the stimulation of pro-inflammatory classically activated (M1) macrophages (Perez-Maliah and Langhorne, 2015). Toxoplasmosis and malaria co-infections is assumed to occur frequently in malaria endemic regions since parasites can comfortably co-exist within a single host; and mosquito vectors of *Plasmodium* and sporulated oocyst of *T. gondii* thrive in similar environmental conditions (Oyeyemi et al., 2020; Okunlola and Oyeyemi, 2020). Co-infection with both parasites is expected to cause more deleterious effect on the host, due to their effects on hosts' haematological parameters (Cumber, et al., 2016) and modulation of the clinical outcomes associated with both diseases, resulting from competitive interaction by both parasites (Ibrahim, et al., 2020).

While there are several reports on the epidemiology of single infection of P. falciparum and *T. gondii*, very few reports are available on co-infection of the two parasites. Presently, only three countries in Sub-Saharan Africa have epidemiological data on co-infection of malaria and toxoplasmosis; Cameroon (Achonduh-Atijegbe et al. 2016; Cumber, et al. 2016), Ghana (Blay et al. 2015; Arthur-Mensah et al. 2018) and Sudan Salih et al. 2014).

In this study, the prevalence of *T. gondii* and *Plasmodium* co-infections, including risk factor assessment of infections in Akinyele Local Government Area, Ibadan, Oyo State was determined. The concentrations of pro-inflammatory cytokines (IL-2 and IL-12) and anti-inflammatory cytokines (IL-6 and IL-10) in plasma samples of individuals infected with both *T. gondii* and *Plasmodium* were also determined.

MATERIALS AND METHODS

Study area/study design

The cross-sectional study was conducted in three communities in Akinyele LGA, Oyo State, Nigeria with 192 consenting individuals enrolled into the study. Standardised pre-tested questionnaires were administered to obtain information on socio-demographics, knowledge, attitude and practice regarding the diseases.

Sample collection

Blood (5 ml and 2 ml) was collected from adults and children respectively. Plasma was separated by centrifugation at 3,000 rpm for 5 minutes and aliquoted into Eppendorf tubes; whole blood and plasma were stored at -80^{o}C until further analysis was carried out. Packed cell volumes were determined for each sample after centrifuging blood in heparinized micro-haematocrit capillary tubes.

Identification of malaria parasites and determination of parasitaemia

Thin and thick blood films were made, and slides viewed at × 100 magnification. Malaria parasitaemia was calculated as the percentage of infected red blood cells in 1,000 red blood cells. Individuals were categorised as having mild, moderate or severe malaria according to the Revised WHO criteria (2000). Revised WHO criteria (2000) which referred to mild malaria as parasitaemia up to 1% or 40,000 trophozoites/µl of blood, moderate parasitaemia as 1-5% or 40,000 to 200,000 trophozoites/µl of blood and severe malaria as parasitaemia>5% or >200,000 trophozoites/µl of blood. Parasitaemia was determined calculating

(Number of Infected Red blood cells)/ (Total Number of Red blood cells counted) × 100

.

Detection of *T. gondii* specific IgG and IgM Antibodies

Plasma were analysed by Enzyme Linked Immunosorbent Assay (ELISA) for *T. gondii* specific IgG and IgM antibodies. Ninety-six well microtitre plates were coated (100 µl/well) with *Toxoplasma gondii* Microneme Protein 3 (MIC3) antigen (Cd Biosciences; U.S.A) diluted to 2.5 µg/ml in coating buffer (0.1 M carbonate buffer, pH 9.6) and incubated overnight at 4^{o}C. The antigen was aspirated off and plates blocked with 200 µl/well of 2% skimmed milk in PBS (blocking buffer) for 1 hour at room temperature. Each plasma sample was diluted (1:160) in blocking buffer and added to duplicate wells (100 µl/well). Plates were incubated at room temperature for 2 hours.

The plates were washed five times and 100 µl rabbit anti-human IgG (Sigma); and mouse anti-human IgM (Bethyl laboratories, U.S.A) HRP conjugate both diluted at 1:2000 was added to wells on separate plates prepared accordingly, and incubated at room temperature for 1 hour.

The plates were washed again and 100 µl of freshly prepared 40% ortho-Phenylenediamine Dihydrochloride (OPD; Sigma) was added to each well. The reaction was incubated in the dark for 30 minutes and terminated with 0.2 M sulphuric acid. The Optical Density (OD) for each well was determined on a VersaMax TM ELISA reader at 492 nm.

Measurement of cytokines

Plasma concentrations of two pro-inflammatory (IL-2 and IL-12) and two anti-inflammatory (IL-6 andIL-10) cytokines were measured using ELISA kits (MABTECH, Stockholm, Sweden) The wells of the microtitre plates were coated (100 µl/well) with monoclonal antibodies diluted to 1 µg/ml in PBS coating buffer (pH 7.4) and incubated overnight at 4^{o}C. Unbound monoclonal antibodies were poured off and plates blocked (200 µl/well) with 2% BSA in PBS (blocking buffer) and incubated for 1 hour at room temperature. Plates were flipped dry. Standards were serially diluted in incubation buffer (1% BSA in PBS-0.05%Tween 20), plasma were diluted 1:200 in incubation buffer.

Standards and samples were loaded onto plates (100 µl/well) and incubated for 2 hours at room temperature. The plates were washed and 100 µl/well of biotin (0.25 µg/ml in incubation buffer) was added to plates and incubated for 1 hour at room temperature. The plates were washed and 100 µl/well of streptavidin HRP (Horseradish Peroxidase) conjugate diluted 1:2000 in incubation buffer was added to the wells; plates were incubated for 1 hour at room temperature.

Washing was repeated and freshly prepared 0.4 mg/ml OPD (Sigma) containing 30% H_2O_2 was added (100 µl/well) to each well. The reaction was allowed to develop in the dark for 30 minutes and stopped by adding 50 µl/well sulphuric acid. Absorbance was measured at 492 nm.

Data analysis

Data was analysed using the SPSS software package version 20 (SPSS, Inc., Chicago, IL, USA). *Chi square* (χ^2) test was used to determine association between categorical variables. Differences between means were tested using Independent Samples T-test and One-way Analysis of Variance (ANOVA). Statistical significance was considered at $P<0.05$. Cytokine concentration (pg/ml) was extrapolated from standard curves obtained from cytokine standards using Graphpad prism version 8.

RESULTS

Out of the 192 volunteers investigated for the presence of *T. gondii* antibodies, a total of 52 (27.1%) and 17 (8.9%) participants were positive for *T. gondii* IgG and IgM antibodies respectively. Additionally, 5 (2.6%) showed evidence of both IgG and IgM antibodies. Malaria was prevalent in 139 (72.4%.) of the study participants. Figure 1 displays the seroprevalence of *T. gondii*, as well as the overview of the prevalence of malaria, and *T. gondii* seroprevalence in the study. The mean absorbance values for ELISA ranged from 0.02–2.42 and 0.16–2.69 for IgG and IgM respectively. Samples with mean absorbance values ≥ 0.41 and 0.52 were considered positive for IgG and IgM respectively. These values were equivalent to the mean absorbance +2S. D+0.05, estimated error of the ELISA reader (Table 1).

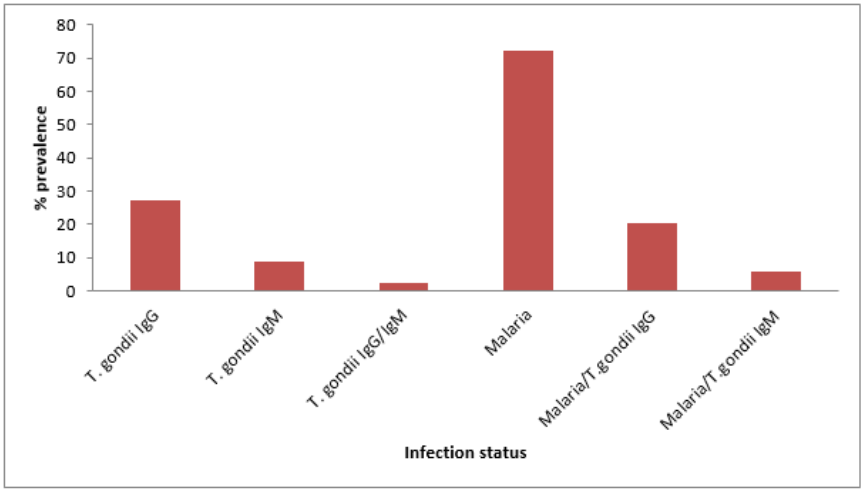

Figure 1. Overview of the prevalence of malaria and toxoplasmosis in the study.

Table 1. Sero-Prevalence of *T. gondii* in the gender.

Gender	No examined	No infected	% seropositive
Males	61	13	21.3
Females	106	33	31.1
TOTAL	167	46	27.5

T. gondii IgG seroprevalence was significantly higher (P<0.05) in females (33.0%) than in males (26.5%), while Malaria prevalence was significantly higher (P<0.05) in females (98/124; 79.0%) than in males (42/68; 61.8%).

There was a significant association (P<0.05) between malaria and age of participants: with higher malaria prevalence in the 0-20-year age group as seen in Figure 2. In contrast, *T. gondii* IgG and IgM seroprevalence showed no significant association (P>0.05) with age.

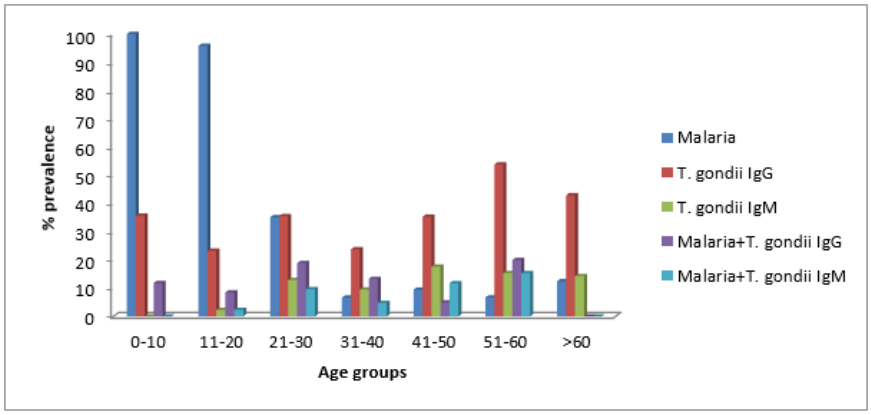

Figure 2. Overall prevalence of malaria and *Toxoplasma gondii* antibodies in the age groups.

Table 2 shows that the presence of cats in the environment was significantly associated (P<0.05) with *T. gondii* (IgG) seropositivity, but no association (P>0.05) with *T. gondii* IgM. History of miscarriage, educational, marital and occupational status had no significant (P>0.05) association with seropositivity. Drinking water source was not a significant risk factor for infection, although 51.5% of *T. gondii* IgG seropositive individuals sourced their drinking water from wells. Table 3 shows the environmental and behavioral factors associated with malaria prevalence, significantly (P<0.05) lower malaria prevalence was recorded in individuals who used insecticide treated nets. Other factors such as presence of stagnant water, use of window nets, presence of bushes around residences and use of insecticide spray did not significantly (P>0.05) influence malaria prevalence in the study area.

Table 2. Exposure related seroprevalence of Toxoplasmosis in the study population.

Risk factors	Response	Frequency (%)	% positive	P-value
Presence of cats in the vicinity	Yes	35.3	40.7	0.013*
	No	64.7	20.4	
Presence of other animals around the house	Yes	73.1	29.5	0.556
	No	26.9	22.2	
Source of drinking water	Well	51.5	27.9	0.691
	Borehole	27.5	28.3	
	Others	21	25.7	
Consumption of undercooked meat	Yes	19.8	24.2	0.789
	No	80.2	28.4	
Consumption of raw vegetables and/or salad?	Yes	57.5	28.1	0.984
	No	42.5	26.8	

Washing of fruits before eating	Yes	89.8	27.3	1
	No	10.2	29.4	
Drinking unpasteurized milk.	Yes	65.9	30	0.421
	No	34.1	22.8	
History of miscarriage (s)	Yes	27.9	35.3	1
	No	72.1	36.4	
History of blood transfusion	Yes	12	30	0.901
	No	88	22.6	
*Statistically significant at (P<0.05)				

Table 3. Environmental and behavioral characteristics of the study population in relation to malaria prevalence.

Risk factors	Response	Frequency (%)	% positive	P-value
Presence of bushes around the house	Yes	46.88	75.55	0.701
	No	53.12	37.25	
Presence of stagnant water	Yes	25	81.25	0.413
	No	75	70.13	
Use of insecticide treated nets	Yes	13.02	12	0.016*
	No	85.93	83.03	
Use of insecticide spray	Yes	6.15	19.35	0.624
	No	83.85	83.23	
Use of window nets	Yes	22.4	40.58	0.083
	No	77.6	77.14	
*Statistically significant at (P<0.05)				

Co-infection of malaria with toxoplasmosis

Toxoplasma antibodies (IgG) and malaria co-infection was recorded in 39 (20.4%) individuals; 15 (38.5%) females and 24 (61.5%) males, although this was not statistically significant (P>0.05). Toxoplasma IgM antibodies and malaria was recorded in 3 (5.76%) participants, and there was no record of malaria in individuals with antibodies to both *T. gondii* IgG and IgM.

Malaria parasite intensity

Overall mean malaria parasite intensity was 1.47 ± 1.82, (Table 4), females had significantly higher (P<0.05) intensity than males. Severe malaria (parasitaemia >5%) was observed in 10% of infected individuals. Significantly higher (P<0.05) mean malaria parasite intensity was observed in *T. gondii* and *Plasmodium* co-infections.

Table 4. *Plasmodium* intensity and hematological parameters stratified by gender.

Parameters	Males	Females	Total	P-value
Plasmodium intensity (Mean ± S.D)	1.13 ± 1.63	1.80 ± 2.0	1.47 ± 1.82	P<0.05
PCV% (Mean ± S.D)	44.04 ± 7.72	41.63 ± 7.30	42.84 ± 7.51	P<0.05

The overall mean PCV, which is a measure of the proportion of red blood cells in the study population was 42.84 ± 7.51, with males having a significantly higher (P<0.05) PCV (44.04 ± 7.72) than females (41.63 ± 7.30). Significantly lower (P=0.028) PCV (41.71 ± 7.90) was observed in individuals with malaria parasite compared to uninfected individuals (44.58 ± 7.53) The mean PCV for the *T. gondii* seropositive group was 42.40 ± 6.07, while the mean PCV for the seronegative group was 42.38 ± 8.06. Statistical analysis revealed no significant difference in PCV levels between *T. gondii* seropositive and seronegative groups (P>0.05). Furthermore, *T. gondii* co-infection did not have a significant effect on PCV levels (P>0.05).

Cytokine levels in the study population

Significantly lower IL-2 and IL-6 were observed in individuals (P<0.05) with malaria and *Toxoplasma gondii* (IgM) (Figure 3), while IL-10 levels were lower in participants with malaria and chronic (IgG) *T. gondii* co-infections, although this was not statistically significant (P>0.05). Also, IL-2 was found to be

higher (P>0.05) in individuals with malaria and *T. gondii* IgG co-infection compared to those with single infections of either malaria or *T. gondii* Table 5 presents data showing cytokine levels in relation to malaria severity, IL-6 increased significantly (P<0.05) with malaria severity and IL-2 was lowest in severe malaria cases (Table 6).

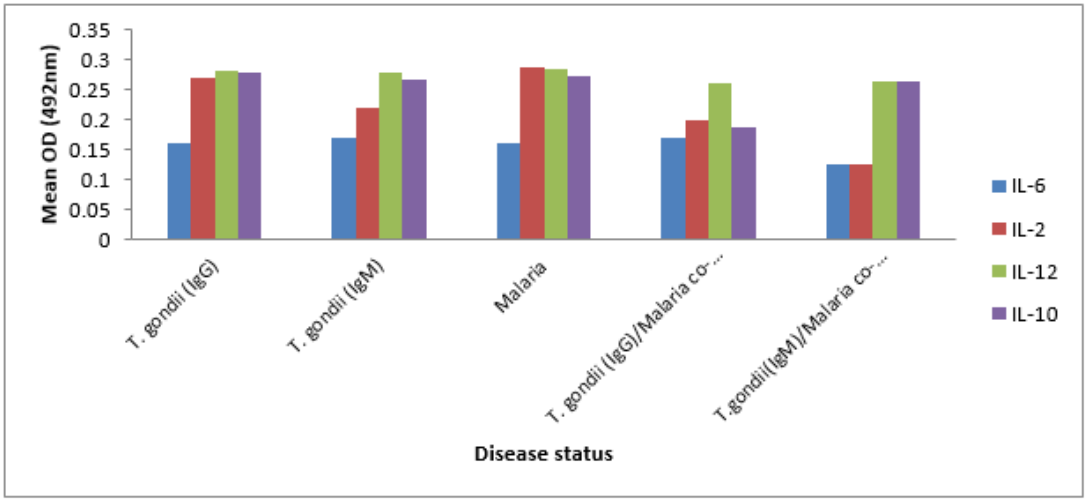

Figure 3. Cytokine levels of infected individuals in the study.

Table 5. Cytokine levels in Toxoplasmosis co-infection with malaria severity.

Infection status	**Mild malaria**		**Moderate malaria**		**Severe malaria**		
Cytokines (Pg/ml)	*T. gondii* IgG+	*T. gondii* IgM+	*T. gondii* IgG+	*T. gondii* IgM+	*T. gondii* IgG+	*T. gondii* IgM+	P-value
IL-2 (Mean ± S.E.)	21.30 ± 2.50	15.24 ± 3.11	19.59 ± 7.62	15.89 ± 2.54	19.97 ± 7.80	15.72 ± 4.90	0.409
IL-6 (Mean ± S.E.)	17.82 ± 3.43	15.13 ± 0.91	17.91 ± 13.36	17.74 ± 4.30	18.28 ± 3.22	20.17 ± 0.09	0.853
IL-10 (Mean ± S.E.)	45.28 ± 7.80	42.93 ± 15.32	41.71 ± 12.74	47.62 ± 15.30	37.91 ± 11.02	34.57 ± 1.17	0.048*
IL-12 (Mean ± S.E.)	34.14 ± 0.07*	38.50 ± 0.19	40.43 ± 0.21	44.28 ± 0.04	50.62 ± 0.32	39.08 ± 1.24	0.038*

Table 6. Association between cytokine levels and malaria severity.

Malaria status	**Mild**	**Moderate**	**Severe**	**Negative**	**P-value**
IL-2 (Mean ± S.E.)	17.99 ± 0.03	18.48 ± 0.03	14.42 ± 0.11	18.53 ± 0.07	0.127
IL-6 (Mean ± S.E.)	16.80 ± 0.01	16.20 ± 0.007	18.79 ± 0.008	15.55 ± 0.004	0.038*
IL-10 (Mean ± S.E.)	23.61 ± 0.013	37.20 ± 0.015	40.76 ± 0.049	35.43 ± 0.22	0.577
IL-12 (Mean ± S.E.)	55.81 ± 0.02	59.20 ± 0.023	61.50 ± 0.049	51.80 ± 0.222	0.115
Significant at P<0.05					

DISCUSSION

This study provides serologic and epidemiological data, as well as cytokine profiles, as measures of immune responses to single and co-infections of malaria and toxoplasmosis in Akinyele LGA, Oyo State, Nigeria.

These findings highlight the prevalence of *T. gondii* exposure and recent infections among volunteers in our study. The detection of IgG antibodies suggests previous exposure to the parasite, whereas the presence of IgM antibodies indicates recent or ongoing infection. The subset of individuals with both IgG and IgM antibodies may represent an active infection stage. These results contribute to our understanding of *T. gondii* epidemiology and its effects on human health.

The prevalence of toxoplasmosis and malaria coinfection can be explained by the fact that both parasites are endemic in Nigeria. The coexistence of both parasites in this region is enhanced by the ability of mosquito vectors of *Plasmodium* and *T. gondii* sporulated oocysts to thrive under similar environmental

conditions (Okunlola and Oyeyemi, 2020; Oyeyemi et al., 2020). Both parasites also thrive in areas with large numbers of poor and underserved populations, where there is a lack of portable water supply, proper sewage treatment infrastructure, and access to healthcare (Cumber, et al., 2016; El Bissati et al., 2018; Chimezie, 2020).

The seroprevalence of 27.5% and 8.98% recorded for *T. gondii* IgG and IgM antibodies, respectively, is indicative of an ongoing transmission of the parasite in the study area, probably resulting from the repeated exposure of these individuals to *T. gondii* bradyzoites through contaminated food or oocysts in the environment. IgM antibodies are produced in the first week after infection and their presence indicates recent exposure to the parasite (Wana et al., 2020). The production of IgG antibodies peak within two or three months of infection. As a result of the persistence of latent cysts in immuneprivileged organs, IgG remains at a plateau for about six months before gradually decreasing until the end of the infected subject's life (Robert-Gangneux and Dardé, 2012; Villard, et al., 2016). Thus, IgG seropositivity indicates the chronic stage of infection resulting from older infections or previous exposure to the pathogen (Casartelli-Alves et al., 2014). However, the presence of both *T. gondii* IgG and IgM antibodies in 2.6% of individuals may indicate an evolving infection or re-infection, or an old infection switching from acute (IgM) to chronic (IgG) stage; a phenomenon known as antibody class switching (Guemgne, et al., 2019; Obijiaku, et al., 2019).

The significant association ($P<0.05$) between having cats around houses and the presence of *T. gondii* IgG antibodies further confirms the importance of cats in the transmission of toxoplasmosis. Cats are the definitive hosts for *T. gondii*, and they play an important role in transmission by excreting *T. gondii* oocysts into the environment, which contaminate soil, food, or water (Dubey and Prowell, 2013). The association between cats and *T. gondii* infection has been previously reported (Amany et al., 2012; Uttah et al., 2013; Awobode et al., 2014). The high toxoplasmosis prevalence observed in individuals drinking well water could be a result of contamination of well water by oocysts from runoffs, making these water-ready sources of infection, as also reported by Jones and Dubey (2010). The high toxoplasmosis prevalence observed in individuals drinking well water is consistent with reports from other studies (Awoke et al., 2015; Ishaku et al., 2009).

The significantly higher IgM observed in females indicates that females in our study area have a higher risk of contracting *T. gondii* infection than males, which may be due to increased exposure to *T. gondii* risk factors as a result of their daily activities; a similar higher frequency in females compared to males has been reported by other researchers (Anuradha and Preethi, 2014, Modrek, et al., 2015; Anvari, et al., (2019). They attributed this trend to the fact that females spend a lot of time doing domestic activities such as taking care of household pets, gardening, cleaning, cooking, handling raw meat, and washing vegetables and fruits.

Malaria infection was very high in this study, similar to the high malaria prevalence reported by Anumudu et al. (2007). From the data obtained from the questionnaire, most respondents did not observe malaria preventive measures such as the use of mosquito nets, which could be responsible for the high prevalence of malaria recorded. Previous studies have shown that populations that adhere to malaria preventive measures such as the use of long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) would have low malaria prevalence (Sam-Wobo, et al., 2014; Animut and Negash, 2018; Okpokor, et al., 2020; Nyasa, et al., (2021).

The high malaria prevalence recorded among the 21-30 age group may be because this age group is more involved in outdoor activities, and they frequently seek temporary employment, which mostly involves farming and other outdoor activities, making them more predisposed to mosquito bites. Similar results have been reported by Obasi et al. (2012). Another reason for the high prevalence observed in this age group may be the result of increased immunity, as immunity to malaria is said to build up after years of sustained exposure (Farrington et al., 2017). This build-up of immunity with age may also be responsible for the low prevalence observed among individuals older than 40 years.

Haematological abnormalities such as anaemia are hallmarks of P. falciparum malaria. The significantly low PCV observed in individuals with malarial parasites is attributed to haemolysis of parasitized red blood cells induced by P. falciparum as suggested by Bashawri, et al., (2002). Our findings demonstrate that there was no significant difference in PCV between T. gondii-seropositive and-seronegative individuals. *T. gondii* co-infection with malaria did not exert a significant effect on PCV levels. These results suggest that *T. gondii* seropositivity and coinfection may not directly influence PCV levels.

Cytokines, including IL-2 and IL-6, play critical roles in immune regulation and inflammation (Liu, et al., 2021). Lower levels of these cytokines in co-infected individuals in this study might suggest a reduced inflammatory response compared to individuals with single infections. Also, since excessive inflammation contribute to tissue damage and more severe symptoms, so the lower levels of IL-2 and IL-6 in co-infected individuals might indicate a dampened immune response, which could potentially result in milder disease manifestations

The importance of IL-10 as a crucial immunoregulator is evident in various pathogenic infections, including malaria and *T. gondii*. Low IL-10 levels have been suggested to impair the ability of the immune system to regulate the excessive Th1 and $CD8^+$ T cell responses that are responsible for much of the immunopathology associated with infections (Couper, et al., 2008). Consequently, results from this study where individuals co-infected with malaria and *T. gondii* (IgG) exhibited low IL-10 levels suggest that there may be an increased risk of developing severe immunopathology during chronic *T. gondii* co-infection with malaria.

The high IL-2 levels observed in single infections of malaria and *T. gondii* is suggestive of an enhanced regulatory function of IL-2, leading to better-regulated immune responses and reduced risk of immunopathology in these single infections. IL-2 is a key

cytokine that plays a critical role in the activation and proliferation of T cells, particularly $CD4^+$ T cells, which are important in controlling malaria and toxoplasmosis (Villegas, et al., 2002; Kurup, et al., 2019).

These results suggest that chronic (IgG) infection with *T. gondii* does not affect IL-12 concentration. IL-12 concentrations were similar in individuals with chronic (IgG) toxoplasmosis and individuals with chronic toxoplasmosis and malaria co-infection (Settles et al., 2014). IL-12 is an immunoregulatory cytokine that orchestrates the production of interferon gamma (IFN-γ) in immune cells. IFN-γ is a critical component of host resistance to *T. gondii* (Fereig, et al., 2022)

Our results suggest that the immune response to malaria and toxoplasmosis co-infection is complex and may be influenced by disease severity. Even though chronic (IgG) infection with *T. gondii* did not affect the IL-12 concentrations. However, the finding that IL-12 levels were lower in mild malaria with toxoplasmosis co-infection suggests that IL-12 may be a potential biomarker for disease severity and response to treatment. This result is consistent with the finding that chronic *T. gondii* infection elicits a strong and protective Th1 immune response characterized by IFN-γ, IL-12, and $IgG2^a$ antibodies (Ahmed et al., 2017). This is consistent with the fact that IL-12 is involved in the pathogenesis of severe malaria. (Chen, et al., 2000)

CONCLUSION

We have provided data on prevalence and cytokine response of malaria and toxoplasmosis co-infection in the study area. This study highlights the critical role of IL-10 as a key immunoregulator in pathogenic infections such as malaria and *T. gondii*. Our findings also suggest that chronic (IgG) infection with *T. gondii* may not significantly affect IL-12 concentrations, while IL-12 levels may serve as a potential biomarker for disease severity and treatment response in malaria and toxoplasmosis co-infection. However, further research is warranted to elucidate the precise mechanisms and therapeutic implications of these cytokines in the context of co-infections. It is also expedient to intensify public health awareness on preventive measures regarding toxoplasmosis and malaria. Rapid screening tests for toxoplasmosis should be made available and accessible in primary health centres.

ETHICAL APPROVAL

Approval for the study was obtained from the Research Ethical Review Committee of the Oyo State Ministry of Health. Permission was also obtained from the Primary Health Care Co-ordinator of the Local Government. Written informed consent was obtained from adult or parents of children participants.

CONFLICT OF INTEREST

The authors declare that they have no conflicting interests.

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