Original Research Paper

Seroprevalence of *Toxoplasma gondii* Antibodies and Associated Risk Factors among School Children in Parts of Kaduna State, Nigeria

ABSTRACT

Aims: the study was aimed at determining the seroprevalence of *Toxoplasma gondii* and associated risk factors among school children in parts of Kaduna state.
Study design: cross sectional community based study.
Place and Duration of Study: Kaduna state from February to September, 2016.
Methodology: A total of 300 blood samples were collected from school children in three Local Governments Areas (LGA) of Kaduna state namely: Soba, Kauru and Giwa LGA. The sera were screened for *Toxoplasma gondii* IgM and IgG antibodies by Enzyme Linked Immunosorbent Assay (ELISA).
Results: Of 300 blood samples screened, seropositivity for *Toxoplasma* IgM antibody was 108 (36.0%). Fifty (16.67%) of the tested seropositive for the *Toxoplasma* IgG antibody and 24 (8.0%) tested seropositive for *Toxoplasma* IgG+IgM antibodies. Children in Soba Local Government Area had the highest seroprevalence of *Toxoplasma gondii* IgM (72%), followed by Giwa LGA (27%). The least seroprevalence was recorded in Kauru LGA (9%). The highest seroprevalence of *Toxoplasma gondii* IgG was found among children in Kauru 21 (21%), followed by Soba 15 (15%) and finally Giwa 14 (14%). IgM seroprevalence of 63.44%, 29.79% and 10.61% were recorded among school children between the age groups 7-10, 11-14 and 15-18 years respectively, whereas *T. gondii* IgG seroprevalence was recorded in 29.03%, 13.48% and 6.06% children between the age groups 7-10, 11-14 and 15-18years respectively. A lower seroprevalence of *Toxoplasma gondii* antibodies was recorded among the female children (IgM = 34.9% and IgG = 16%) compared to the male (IgM = 37.4% and IgG = 17.6%) the difference observed was not statistically significant. In this study, presence of cats at home, contact with cat and contact with cat litter were estimated to be risk factors associated with *T. gondii* infection.
Conclusion: Hand washing before eating, hand washing after playing and drinking of treated water (not well water) were estimated to be protective factors associated with *T. gondii* infection.

Keywords: Toxoplasma gondii, risk factors, IgM, IgG, ELISA, Children

1. INTRODUCTION

Toxoplasma gondii is an obligate intracellular apicomplexan that can infect virtually all **warm blooded**warm-blooded animals. The definitive hosts of *Toxoplasma gondii* are felids. Toxoplasmosis is a zoonotic parasitic infection caused by *Toxoplasma gondii* [1]. In man, *Toxoplasma* infection is acquired mainly by ingestion of tissue cysts of the parasite in raw or undercooked meat, by ingestion of parasite oocysts in cat faeces that contaminate soil, vegetables and other food sources and transplacentally from infected mothers to their infants [1,2].

The infection has a worldwide distribution, approximately one third of humans have been exposed to this parasite, but the seroprevalence varies considerably between countries and population groups [3]. Seroprevalence of *T. gondii* have been shown to vary from 0% in Eskimos to 93% in Ghana. These rates have been reported to be 23% in Nigeria, 70% in Indonesia, 81%in Ethiopia, 52% in Brazil, 10.8% in the U.S., and 13.2% in Korea [4]. Although different populations were targeted in

these studies and different immunological assays were used, the widespread prevalence of this infection is indisputable.

All mammals, including humans and birds are usually intermediate hosts, whereas Felidae (cats) can serve as intermediate as well as definitive hosts and are the only animals that pass oocyst in their faeces. Sheep and goat meat are also sources of toxoplasmosis [5].

The life cycle of *T. gondii* can be broadly summarized in two components: a sexual component that occurs only within cats (wild or domestic feline) and asexual component that occurs within virtually all warm blooded animals including humans, cats and birds. *Toxoplasma gondii* can theoretically be passed between intermediate hosts indefinitely via a cycle of consumption of tissue cysts in meat [6].

Toxoplasma gondii enters through the intestinal epithelium, spreading to tissues and breaking through biological barriers, such as the placenta and haematocephaly barriers [7], reaching immunologically-deprived sites where the parasite can cause even more severe pathologies, such as disseminated congenital toxoplasmosis [8], acute neurological complications in immunologically-compromised individuals [9] and ocular pathologies in healthy individuals [10]. Although the severity of the fetal illness is inversely proportional to gestational age at which maternal infection occurs, the vertical transmission rate is directly proportional to the stage of pregnancy of the mother when acquiring the infection for the first time [11].

The parasite reaches the foetus transplacentally, causing various degrees of damage. Depending on the virulence of the parasite, the immune response of the mother and the trimester infection it may result in foetal death or in severe clinical symptoms [12]. It can also be acquired during the birth of normal children and later presents as retinochoroiditis alterations, provoking mental and psychomotor disorders [13].

Approximately 80% of children diagnosed with sub-clinical toxoplasmic infection present ocular sequels at some point in their lives. Lesions on the retina are the most frequent sequels, and they can be easily detected during ophthalmological examinations. These signs indicate that neurological symptoms are possibly involved [14].

The prevalence of *T. gondii,* risk factors and of previous infections varies from one country to another [15]. Congenital toxoplasmosis occurs almost exclusively as a result of primary maternal infection during pregnancy. Rarely, reactivation of infection in immune-compromised women during pregnancy can result in congenital toxoplasmosis [12].

Most maternal infections are asymptomatic or they result in mild illnesses [16,17]. Congenital toxoplasmosis may present as a mild or severe neonatal disease, with onset during the first few months of life, or with sequels or relapse of a previously undiagnosed infection at any time during infancy, or sensorineural hearing loss (SNHL) later in life [18].

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Kaduna State. Kaduna state is found in northern Nigeria and lies between $10^0$31'23"N and $7^0$26'25"E. The state is made up of three senatorial districts: North, Central and South. Three local government areas, one from each of the three senatorial districts were selected namely: Soba from north, Giwa from central and Kauru from south. Two schools each from the three selected Local Government Areas namely Soba, Kauru and Giwa were used.

2.2 Study Design

A cross sectional study. The children were selected randomly.

2.3 Study Population and Data collection

The study population comprised of primary school children between the ages of 7 – 18 years in parts of Kaduna State (Giwa, Kauru and Soba Local Government Area). Structured questionnaire was administered during sample collection to obtain information on the socio-demographic factors (such as age and gender) and risk factors (contact with cat and its litter and poor hand washing habit) associated with *Toxoplasma gondii* infection in children. The pupils were assisted in filling the questionnaires with all the information well explained to them.

2.4 Ethical Approval

Permit and Ethical approval for the study were obtained from the Kaduna State Ministry of Education and Health respectively.
Informed consent were obtained from the parents / guardians of the children before samples were collected

2.5 Sample Collection

A total of 300 blood samples were collected from 300 pupils in plain bottles by venepuncture. The blood was allowed to stand at room temperature for 2-3 hours to clot effectively. Samples were then centrifuged at 1500 rpm for 10 minutes to separate serum. Sera samples were then kept at – 20^0C until assayed.

2.5 Specimen Analysis using Enzyme Linked Immunosorbent Assay (ELISA)

Sera samples were screened for the presence of *Toxoplasma gondii* IgM and IgG antibodies using Enzyme Linked Immunosorbent Assay-ELISA (Toxo IgM (Catalog No. 9071-11) and IgM (Catalog No. 9072-11) ELISA kit) following the manufacturer's instruction (Diagnostic Automation/Cortez Diagnostics, Inc, CA USA).

2.6 Procedure of ELISA

The test sera, calibrator and control sera were diluted 1:81 for IgM and 1:21 for IgG in serum diluent (provided in the kit) and mixed well. To the individual antigen coated wells, 100µl of the appropriate diluted calibrator, controls and patient sera were added into the appropriate wells. Then 100µl of serum diluents were added to the blank well. The plates were incubated at room temperature for 30 minutes for IgM and 25 minutes for IgG. After incubation, the liquid was aspirated out of the well and then washed with the diluted wash buffer. The washing was done 3 times.

The wash buffer was completely removed from the wells after the last washing step and then blot dried. Then 100µl of conjugate was added to each well and incubated at room temperature (25-28^0C) for 30 minutes for IgM and 25 minutes for IgG. The washing step was repeated and then 100µl of chromogen/substrate solution was added to each well and incubated at room temperature or 15 minutes. The reaction was stopped by the addition 100µl of stop solution (1N H_2SO_4) and the developed colour was read on an ELISA plate reader at an absorbance of 450 nm.

2.7 Result interpretation

A negative IgM and a positive IgG antibody test essentially excludes acute infection while a positive IgG test with a negative IgM indicates the patient was previously infected with *Toxoplasma gondii* and that infection occurs more than a year ago. A positive IgM and IgG antibodies results suggest that the patient is acutely infected with *Toxoplasma gondii.*

2.8 Data analysis

Chi square (χ^2) using quickcal statistical package (graphpad.com), Odds ratio at 95% confidence interval was employed to interpret the results at P value ≤ 0.05.

3. RESULTS

The overall seroprevalence of *Toxoplasma gondii* antibodies among school children in Kaduna State is shown in Figure 1. Of the total 300 sera collected from school children in some parts of Kaduna State, 108 (36.0%) were seropositive for *Toxoplasma gondii* IgM antibody, while 50 (16.67%) of the 300 serum samples were seropositive for *Toxoplasma gondii* IgG antibody as determined by ELISA.

Seroprevalence of *Toxoplasma gondii* IgM and IgG antibody in relation to the Local Government Areas (LGA) is shown in Figure 2. Children from Soba LGA shows the highest seroprevalence of *Toxoplasma gondii* IgM 72 (72%), followed by children from Giwa LGA 27 (27%) and then children from Kauru LGA 9 (9%). The highest seroprevalence of *Toxoplasma gondii* IgG was found among children in Kauru 21 (21%), followed by children from Soba 15 (15%) and then children from Giwa 14 (14%). The difference observed in the seroprevalence of toxoplasmosis among children in the three different local government areas was statistically significant for IgM $p < 0.0001$ but not for IgG $p = 0.3563$ (IgM: $\chi^2 = 91.406$, df = 2, $p < 0.0001$ IgG: $\chi^2 = 2.064$, df = 2, $p = 0.3563$).

Figure 3 showed the number of children that are seropositive to only IgM, only IgG and those positive to both antibodies. A total of 84 (28%) children were IgM seropositive, 26 (8.6%) were IgG seropositive while 24 were seropositive to both antibodies.

The seroprevalence of *T. gondii* antibodies with regards to age group distribution is presented in Table 1. IgM seroprevalence of 63.44%, 29.79% and 10.61% was observed among children between the age groups 7-10, 11-14 and 15-18 years respectively. The seroprevalence rate of *T. gondii* IgG was found to be 29.03%, 13.48% and 6.06% among children between the age groups 7-10, 11-14 and 15-18years respectively. The difference observed in the seroprevalence between the different age groups was statistically significant for both IgM and IgG ($p < 0.05$).

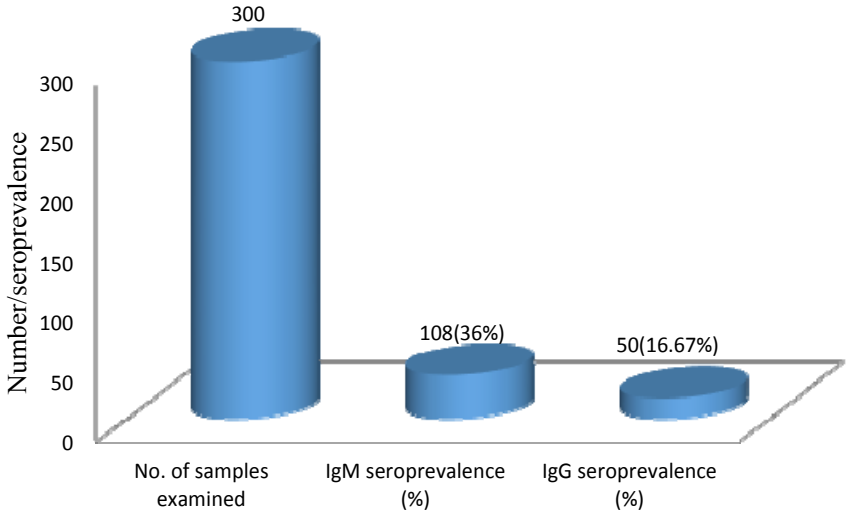

Figure 1: Overall seroprevalence of *Toxoplasma gondii* IgM and IgG antibodies among school children in parts of Kaduna state.

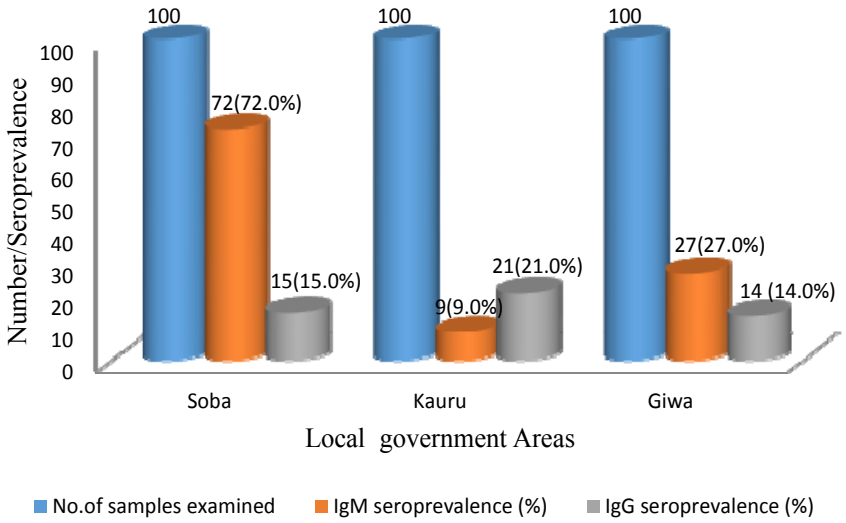

Figure 2: Seroprevalence of *Toxoplasma gondii* IgM and IgG antibodies among school children in the three local government areas.
IgM: χ^2 = 91.406, df = 2, P < 0.0001 IgG: χ^2 = 2.064, df = 2, P = 0.3563

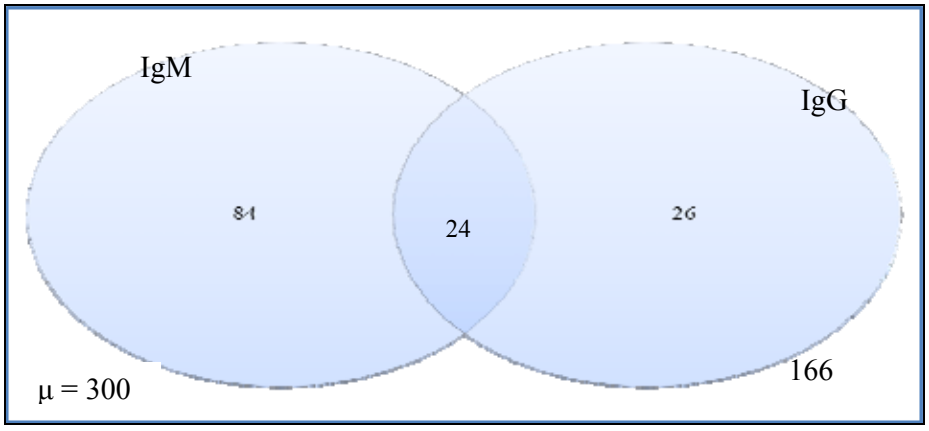

Figure 3: Venn diagram showing comparative seropositivity of *T. gondii* IgM and IgG antibodies among school children

Table 1: Seroprevalence of *Toxoplasma gondii* IgM and IgG antibodies among school children according to age group.

Age group (years)	No. examined	IgM		IgG	
		Number positive	Seroprevalence	Number positive	Seroprevalence
7-10	93	59	63.44%	27	29.03%
11-14	141	42	29.79%	19	13.48%
15-18	66	7	10.61%	4	6.06%

IgM: χ^2 = 51.229, df = 2, *P* = 0.0001 IgG: χ^2 = 16.618, df = 2, *P* = 0.0002

Seroprevalence of *Toxoplasma gondii* IgM and IgG antibodies according to gender is shown in Table 2. A slightly lower seroprevalence of *Toxoplasma gondii* antibodies was observed among the female (IgM = 34.9% and IgG = 16%) compared to the male (IgM = 37.4% and IgG = 17.6%). The difference observed was not statistically significant for both IgM and IgG (*P* > 0.005).

Table 3 shows the seroprevalence of *Toxoplasma gondii* IgM antibody among school children in relation to risk factors and Odds ratio (OR) with 95% confidence intervals (CI).
In this study, presence of cats at home (OR=1.36, 95% CI=0.77-2.42, *P*=0.2927), contact with cat (OR=1.21, 95% CI=0.76-1.95, *P*=0.4232) and contact with cat litter (OR=4.63 95% CI=2.40-8.94, *P*=0.0001) were estimated to be risk factors as associated with *T. gondii* IgM seropositivity.

Hand washing before eating (OR=0.83, 95% CI=0.44-1.57, *P*=0.5729), hand washing after playing (OR=0.85, 95%CI=0.52-1.38, *P*=0.5061), and drinking of treated water (not well water) (OR=0.76, 95%CI=0.33-1.78, *P*=0.5303) were not estimated to be risk factors associated with *T. gondii* IgM seropositivity.

Surprisingly, eating of raw or undercooked meat (OR=0.86, 95%CI=0.54-1.39, *P*=0.5440) and playing with soil (OR=0.67, 95%CI=0.41-1.11, *P*=0.1221) were estimated to be protective factors also.

Table 2: Seroprevalence of *Toxoplasma gondii* IgM and IgG antibodies according to gender.

Gender	No. examined	IgM		IgG	
		Number positive	Seroprevalence	Number positive	Seroprevalence
Male	169	59	34.9%	27	16.0%
Female	131	49	37.4%	23	17.6%

IgM: χ^2 = 0.199, df = 1, *P* = 0.6554 IgG: χ^2 = 0.133, df = 1, *P* = 0.7155

Table 4 shows Seroprevalence of *Toxoplasma gondii* IgG antibody among school children in relation to risk factors. Odds ratio value up to unit one indicate association between the risk factor and toxoplasmosis.

The presence of cats at home (OR=1.79, 95% CI=0.80-4.02, *P*=0.1592) and contact with cat litter (OR=1.88, 95%CI=0.90-3.92, *P*=0.0948) were determined to be risk factors associated with *T. gondii* IgG seropositivity. Drinking of treated water (OR=4.92, 95% CI=0.65-37.31, *P*=0.1231) and hand washing before eating (OR=5.41, 95% CI=1.27-23.08, *P*=0.0225) were also estimated to be a risk factor associated with *T. gondii* IgG seropositivity

Hand washing after playing (OR=0.74, 95%CI=0.39-1.42, *P*=0.3661), contact with cat (OR=0.75, 95% CI=0.40-1.38, *P*=0.3521), eating of under-cooked meat (OR=0.61, 95% CI=0.33-1.12, *P*=0.1112) and playing with soil(OR=0.75, 95% CI=0.39-1.44, *P*=0.3885) were estimated to be protective factors associated with *T. gondii* IgG seropositivity.

Table 3: Risk factors associated with seroprevalence of *Toxoplasma gondii* IgM antibody among school children in three local government areas in Kaduna State.

Risk factors	Number examined	*T. gondii* IgM seropositive		OR (95% CI)	*P* value
		Number positive (%)			
Cat at home					
Yes	221	86	(38.9)	1.36 (0.77-2.42)	0.2927
No	69	22	(31.9)		
Contact with cats					
Yes	138	53	(38.4)	1.21 (0.76-1.95)	0.4232
No	162	55	(34.0)		
Hand washing before eating					
No	48	19	(39.6)	0.83 (0.44-1.57)	0.5729
Yes	252	89	(35.2)		
Hand washing after playing					
Yes	187	70	(37.4)	0.85 (0.52-1.38)	0.5061
No	113	38	(33.6)		
Eating of raw or undercooked meat					
Yes	157	54	(34.4)	1.18 (0.54-1.39)	0.5440
No	143	54	(37.8)		
Playing with soil					
Yes	106	32	(30.2)	0.67 (0.41-1.11)	0.1221
No	194	76	(39.2)		
Contact with cat litter					
Yes	48	32	(66.7)	4.63 (2.40-8.94)	<0.0001
No	252	76	(30.2)		
Source of drinking water					
Well	278	98	(35.3)	0.76 (0.33-1.78)	0.5303
Tap (water board)	18	9	(50.0)		
Borehole	2	0	(0.00)		
Sachet water	2	1	(50.0)		

Table 4: Risk factors associated with seroprevalence of *Toxoplasma gondii* IgG antibody among school children in relation to risk factors.

Risk factors	Number examined	*T. gondii* IgG seropositive		OR (95% CI)	*p* value
		Number positive	(%)		
Cat at home					
Yes	221	42	(19.0)	1.79 (0.80-4.02)	0.1592
No	69	8	(11.6)		
Contact with cats					
Yes	138	20	(14.5)	0.75 (0.40-1.38)	0.3521
No	162	30	(18.5)		
Hand washing before eating					
No	48	2	(41.7)	5.41 (1.27-23.08)	0.0225
Yes	252	48	(19.1)		
Hand washing after playing					
No	187	34	(18.2)	0.74 (0.39-1.42)	0.3661
Yes	113	16	(14.2)		
Eating of under-cooked meat					
Yes	157	21	(13.4)	0.61 (0.33-1.12)	0.1112
No	143	29	(20.3)		
Playing with soil					
Yes	106	15	(14.2)	0.75 (0.39-1.44)	0.3885
No	94	35	(18.0)		
Contact with cat litter					
Yes	48	12	(25.0)	1.88 (0.90-3.92)	0.0948
No	252	38	(15.1)		
Source of drinking water					
Well	278	49	(17.6)	4.92 (0.65-37.31)	0.1231
Tap (water board)	18	1	(5.6)		
Borehole	2	0	(0.0)		
Sachet water	2	0	(0.0)		

DISCUSSION

The high seroprevalence observed in this study can be attributed to exposure of most of the children in this study to cats which is a risk factor for the transmission of toxoplasmosis. The overall seroprevalence of *T. gondii* antibody (44.67%) among school children observed in parts of Kaduna state in this study is higher than 24.0% reported by Gyang *et al.* [4] in Lagos city among school children and 15.13% reported by Meng *et al.* [19] among children in Shandong and Jilin provinces, China.

However a higher seroprevalence (63.1%) was reported by Fan *et al.* [20] among Primary school children in the capital areas of Democratic Republic of São Tomé and Príncipe, West Africa. The differences observed in the seroprevalence may be due to differences in serologic technique used (ELISA was used in this study however Gyang *et al*. [4] and Fan *et al.* [20] used Latex Agglutination while Meng *et al.* [19] used Enzyme Immuno-Assay), ethnicity, traditional culture, geographical conditions, dietary habit, source of drinking water and cat rearing habit of the study population. The discrepancy in seroprevalence of *T. gondii* IgM (36.0%) and IgG (16.67%) observed in this study is an indication that most of the children are having acute infection. Increasing number of pet cats and stray cats might have contributed to the high rates of acute *T. gondii* infection observed in this study. This seroprevalence is higher than IgM seroprevalence of 2.0% and IgG seroprevalence of 13.13% reported by Meng *et al.* [19] in China. This difference can be attributed to difference in geographical location, dietary habit and cat rearing habit.

Variation of *Toxoplasma gondii* seroprevalence variation among different Local Government Area may be due to difference in eating habit, cat rearing habits and decline in the survival of shed oocysts and sporulation at low temperatures and drought, which may be the cause of different seroprevalence observed among the local government in this study.

The differences observed may also be due to differences in geographical conditions, dietary habit, source of drinking water, hand washing habit, cat rearing habit and age of the participants.

In this study, the highest seroprevalence of IgM and IgG was observed among children within the age group 7-10 years (IgM = 63.44% ; IgG = 29.03%) followed by age group 11-14 (IgM = 29.79% ; IgG = 13.48%)and finally 15-18 years old (IgM = 10.61% ; IgG = 6.06%) hence a decline in seroprevalence with increase in age. This may be due the fact that the older children seem to be less exposed to the risk factors based on their questionnaire responses. This finding is in disagreement with the finding of Gyang *et al*. [4] in Lagos, Fan *et al.* [21] in Taiwan and Onadeko *et al.* [22] in Ibadan. Where an increase in seroprevalence of *T. gondii* antibody with increase in age was reported. The finding is however in agreement with finding of Fan *et al.* [20].

There was no significant difference in seroprevalence of *T. gondii* among the different gender. This might be due to the fact that both genders might have acquired the infection at the same rate and the same routes infection of *T. gondii* via faecal-oral route through frequent contact with cat, raising of cats, playing with soil, eating raw/undercooked meats, and drinking contaminated water. This finding is in agreement with the finding of Gyang *et al.* [4] in Lagos city, Fan *et al.* [20] in Democratic Republic of São Tomé and Príncipe, West Africa and Fan *et al.* [20] in China. Generally, gender difference is usually not recorded in *T. gondii* seroprevalence [1].

In this study, among the risk factors analyzed, presence of cats at home (OR=1.36, 95% CI=0.77-2.42, *P*=0.2927), contact with cat (OR=1.21, 95%CI=0.76-1.95, *P*=0.4232) and contact with cat litter (OR=4.63 95%CI=2.40-8.94, *P*=0.0001) were found to be the common risk factors associated with *T. gondii* IgM seropositivity. Cat ownership, Contact with domestic cats and their litter are among the generally accepted risk factors for infection with toxoplasmosis.

Hand washing before eating (OR=0.83, 95%CI=0.44-1.57, *P*=0.5729), hand washing after playing (OR=0.85, 95%CI=0.52-1.38, *P*=0.5061), and drinking of treated water (not well water) (OR=0.76, 95%CI=0.33-1.78, *P*=0.5303) were estimated to be protective factors associated with *T. gondii* IgM seropositivity. Personal hygiene and drinking of treated water are among the measures of preventing toxoplasmosis.

The consumption of under-cooked meat and playing with soil did not seem to be a significant risk factor in this study because meat is traditionally well cooked. So also from the questionnaire response, most of the children wash their hands after playing with soil. However, eating raw or undercooked meat can serve as a source of transmission of infection, although it was not statistically significant in this study.

In relation to IgG seropositivity, among the risk factors analyzed, presence of cats at home (OR=1.79, 95% CI=0.80-4.02, *P*=0.1592) and contact with cat litter (OR=1.88, 95%CI=0.90-3.92, *P*=0.0948) were estimated to be risk factors associated with *T. gondii* IgG seropositivity. Whereas hand washing after playing (OR=0.74, 95%CI=0.39-1.42, *P*=0.3661) was estimated to be a protective factor associated with *T. gondii* IgG seropositivity.

It seemed likely that exposure to cat faeces or contact with soil or water contaminated by *Toxoplasma* oocysts was one of the most important factors associated with *Toxoplasma* infection in the childhood life due to children living and playing very close to the soil and water. Such a route of transmission would explain the similar incidences of seropositivity between boys and girls [21].

4. CONCLUSION

It can be concluded from the present findings that a high seroprevalence of *T. gondii* (44.7%) was recorded among the study population in the study area. Out of all the socio-demographic and risk factors considered in this study, only age, contact with cat litter and hand washing before eating were found to be associated with *T. gondii* infection among the study subjects.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of these findings

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