



Molecular Detection of *Toxoplasma gondii* among Children Attending Selected Schools in Kaduna State, Nigeria

¹*Lawal, S.M., ²Shitu, S. and ¹Hussaini, I.M.

¹ Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria

² Scientific and Industrial Research Department, National Research Institute for Chemical Technology, Zaria.

Corresponding Author: lawalshitu@gmail.com

Abstract

Toxoplasmosis is a zoonotic infection of both humans and animals and has a worldwide distribution. A total of 300 blood samples were collected from school children in Kaduna state. The sera were screened for Toxoplasma gondii IgM and IgG antibodies by Enzyme Linked Immunosorbent Assay (ELISA). The seropositive samples were screened for T. gondii specific B1 gene. Out of the 300 blood samples screened for Toxoplasma IgM antibody, 108 (36.0%) were positive while 50 (16.67%) were seropositive for the Toxoplasma IgG antibody. So also 24 (8.0%) samples were seropositive for Toxoplasma IgG+IgM antibodies. B1 gene specific for T. gondii was detected by PCR in the ELISA positive samples. This confirms the presence of the parasite among the school children in Kaduna state.

Keywords: *Toxoplasma gondii, antibodies, Kaduna, PCR, ELISA, children, school.*

INTRODUCTION

Toxoplasma gondii, an obligate intracellular apicomplexan, can infect virtually all warm blooded animals. Felids serve as the definitive hosts of *Toxoplasma gondii*. It is a zoonotic parasitic infection caused by *Toxoplasma gondii* (Montoya and Liesenfeld, 2004).

All mammals, including humans as well as 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 (Sevgiliet al., 2005).

Toxoplasma gondii infection causes asymptomatic lifelong latent infection in majority of healthy non-pregnant adults (Montoya and Liesenfeld, 2004; Sukthana, 2006). Human infection may be asymptomatic for life unless if immunosuppression occurs (Dubey, 2008). Primary infection of toxoplasmosis in immunocompetent patients may be asymptomatic or associated with self limited symptoms such as fever, malaise, and during pregnancy is frequently associated with transmission of *T. gondii* to the fetus, resulting in congenital infections. In immunocompromised patients, *T. gondii* infection causes severe manifestations, including splenomegaly, chorioretinitis, pneumonitis, encephalitis, multisystem organ failure, and even death (Montoya, 2002). In patients infected with Human Immunodeficiency Virus (HIV), more than 90% of *Toxoplasma* encephalitis cases involve reactivation of a latent infection. This variability is related to

various factors such as, age, socio-cultural, nutritional habits and contact with animals (Mahmood et al., 2013).

Among pregnant women, 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, resulting in foetal death or in severe clinical symptoms. It can also be acquired during the birth of normal children and later presents as retinochoroiditis alterations, provoking mental and psychomotor disorders (Spalding et al., 2003).

Exposure of susceptible individuals to toxoplasmosis may not necessarily lead to infection. Infection will occur if the stage of the parasite, the route of infection, the virulence of the strains and the infectious dose have occurred in the right individual. Infectivity is greatest from oocyst, then tissue cyst, and least from tachyzoites. For most acquired *Toxoplasma* infection, the route of infection is faecal-oral by ingestion (Chemohet al., 2016).

Approximately 80% of children diagnosed with sub-clinical *Toxoplasma* 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 in ophthalmological examinations. These signs indicate that neurological symptoms are possibly involved (Chemohet al., 2016).

Although toxoplasmosis is a cosmopolitan infection, the disease appears to be overshadowed in the tropics by other endemic diseases such as malaria and HIV (Fan et al., 2001).

Upon acquiring *T. gondii* infection, immunocompetent adults and children are usually asymptomatic or have spontaneously resolved symptoms such as fever, malaise, and lymphadenopathy indicating thus, a symptomless latent infection (Montoya and Liesenfeld, 2004). *T. gondii* causes severe encephalitis via the acute infection or reactivation of latent infection (Hung *et al.*, 2007, Innes, 2010).

Serological studies in humans indicate that toxoplasmosis is endemic in most parts of Nigeria, with seroprevalence ranging from 22% to 78% (Kamaniet *al.*, 2009). Most of the cases of severe primary toxoplasmosis were reported in French-Guiana in immunocompetent adults. These cases were associated with the consumption of game (Carme *et al.*, 2002). In Brazil, there is also a report of an immunocompetent individual who developed pneumonia caused by *T. gondii* (Leal *et al.*, 2007). Ocular lesions are also frequent in immunocompetent individuals with *T. gondii* infection.

Routine screening for toxoplasmosis involves detection of IgM and IgG antibodies in the patient's serum (Montoya and Liesenfeld, 2004). In the antibody response to *Toxoplasma* infection, IgM antibodies are detected within a few days to one week of infection and disappear generally after three to five months. The IgG antibodies are detected within one to two weeks of infection, reaching a peak after four months and then declining to lower levels and remaining positive for the remainder of the individual's life. A negative IgM antibody test essentially excludes acute infection while a positive IgG test with a negative IgM indicates chronic infection (Montoya and Liesenfeld, 2004; Sukthana, 2006).

In developed countries, the prevalence of *Toxoplasma* infection among primary school children (PSC) was low with a range of 0.0% to 11.0% in Japan and Ireland, respectively (Suzuki *et al.*, 1988). In Asia, East Europe, and Latin American, the seroprevalence in Iran, Indonesia, Slovak, and Brazil was high, ranging from 20.9% to 68.4% (Konishiet *al.*, 2000). In Africa, studies concerning the seroprevalence of *T. gondii* infection in PSC have been limited; the seroprevalence of *T. gondii* infection in PSC was 37.5%, 36.3%, and 0.0% in Somalia (East Africa), Madagascar (off the Southeastern coast of Africa) and Swaziland (in Southern Africa) respectively (Liao *et al.*, 2009).

Felids play a vital role in the transmission of *T. gondii* as the only definitive hosts of the parasite. Infected cats are considered to be a potential threat to public health because they can shed and excrete environmentally resistant

oocysts in their feces (Dubey, 2010; Wu *et al.*, 2011). Domestic cats are one of the closest companions of humans. Contact with cats has not been found to be a risk factor so much as contact with cat feces or soil that cats have defecated in.

Routine screening for toxoplasmosis involves detection of IgM and IgG antibodies in the patient's serum. In the antibody response to *Toxoplasma* infection, IgM antibodies are detected within a few days to one week of infection and disappear generally after three to five months. The IgG antibodies are detected within one to two weeks of infection, reaching a peak after four months and then declining to lower levels and remaining positive for the remainder of the individual's life. A negative IgM antibody test essentially excludes acute infection while a positive IgG test with a negative IgM indicates chronic infection (Montoya and Liesenfeld, 2004). The study was carried out to molecularly detect *Toxoplasma gondii* among children attending selected schools in Kaduna State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Kaduna State. Kaduna state is located in northern Nigeria and lies between 10°31'23"N and 7°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 Kuru from south. Two primary schools each from the three selected Local Governments Areas namely Soba, Kuru and Giwa were used.

Study Design

A cross sectional study involving male and female primary schoolchildren between the ages of 7 - 18 years in the three local governments was carried out.

Ethical Approval and Consent

Permit and Ethical approval for the study were obtained from the Kaduna State Ministry of Education and Health respectively (Ref. No. of ethical letter: MOH/ADM/744/VOL.1/334). Consent of the school authority, children and the parents were obtained.

Sample Collection

A total of 300 blood samples were collected from 300 pupils in plain bottle containers 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°C until assayed

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 kit (TOXO IgM and TOXO IgG ELISA kit manufactured by Diagnostic Automation/Cortez Diagnostic Inc) following the manufacturer's instruction.

Procedure for 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 in to the appropriate wells. Then 100µl of serum diluents was 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°C) 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 H₂SO₄) and the developed colour was read on an ELISA plate reader at an absorbance of 450 nm.

Result Interpretation

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

Molecular Characterization of *Toxoplasma gondii*

DNA extraction

Extraction of DNA was carried out by using DNA extraction kit in accordance with the manufacturer's directions. About 5 ml of the blood from each pupil was used for the DNA extraction.

Amplification procedures

The amplification reactions was performed with 50-µl reaction mixtures containing the following: 100 pmol of each primer, 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 200 µM concentrations of each deoxynucleoside triphosphate, and 2.5 U of *Taq* DNA polymerase. A total of 2.0 µg of genomic DNA was used for blood samples. After initial denaturation of the DNA at 94°C for 4 min, 55 cycles was ran, as follows: 94°C for 1 min, 42°C for 30 s, and 72°C for 2 min. The final extension step continued for an additional 10 min at 72°C. For the nested PCR, the amplification reactions was performed in 50-µl reaction mixtures containing the following: 30 pmol of each primer, 100 µM concentrations of each deoxynucleoside triphosphate, 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.02% gelatin, and 2.5 U of *Taq* DNA polymerase (Perkin-Elmer). A total of 20 µl of a 1:200 dilution of the first PCR product was used for the second amplification. The reactions were ran in a Perkin-Elmer thermocycler. After initial denaturation of the DNA at 94°C for 4 min, 17 cycles were ran, as follows: 94°C for 1 min 20 s, 53°C for 2 min, and 72°C for 2 min and 30 s. The final extension step was continued for an additional 5 min at 72°C.

After amplification, an aliquot of 25 µl from each reaction mixture was ran on a 3.0% electrophoresis-grade agarose gel in 1× TBE buffer (0.09 M Tris-borate, 0.002 M EDTA), and DNA was stained with ethidium bromide (50 µg/ml). Bands were visualized under UV illumination.

Table 1: Primers used for molecular detection of *T. gondii* gene B1 and the molecular frequency.

B1 gene	Prime pair	Primer Sequence	Size (bp)	References
(P1 and P3)	F	5'-TGCATAGGTTGCAGTCACTG-3'	133	Burg, 1989
	R	5'-TCTTTAAAGCGTTCGTGGTC-3'		
(P1 and P2)	F	5'-TGCATAGGTTGCAGTCACTG-3'	97	Burg, 1989
	R	5'-GGCGACCAATCTGCAATACAC-3'		

F = Forward primer

R = Reverse primer

RESULTS

Seroprevalence of *Toxoplasma gondii* IgM and IgG Antibody

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.

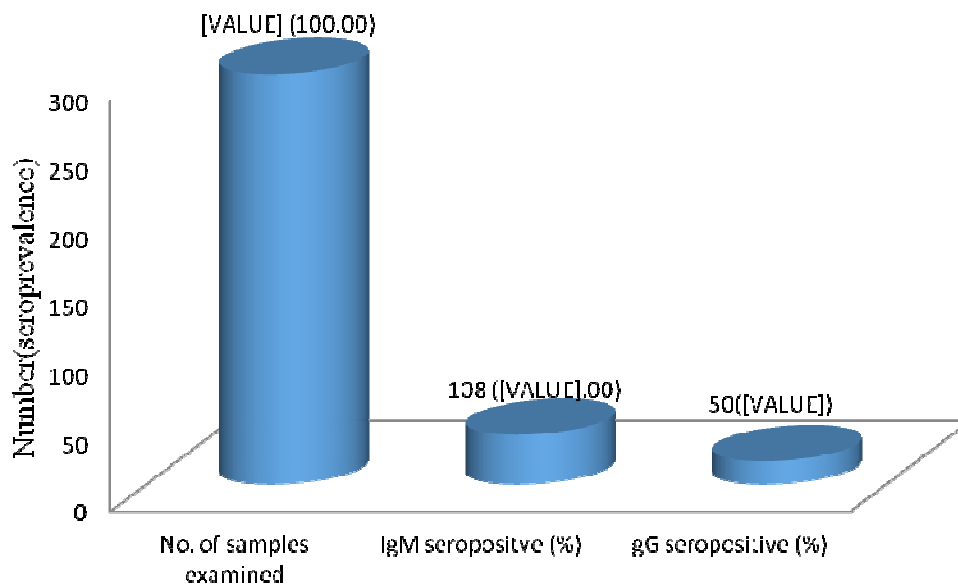


Figure 1: Overall seroprevalence of *Toxoplasma gondii* IgM and IgG antibody among school children in parts of Kaduna state.

Figure 2 show 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.

Plate I shows the gel electrophoresis result of the PCR product using pair of primer specific gene sequence of *Toxoplasma gondii*. Samples 1, 2, 3, 4, 6, 7, 8, 10, 12 and 13 shows band at 97bp which corresponds to the internal fragment of the repetitive B1 gene of *Toxoplasma gondii*.



Figure 2: Venn diagram showing comparative seropositivity of *T. gondii* IgM and IgG among school children

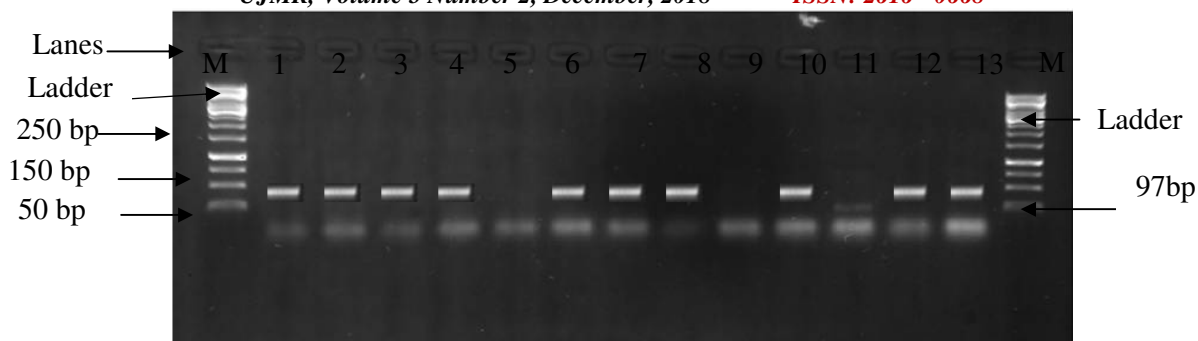


Plate I: Gel electrophoresis result of the PCR products using pair of primer specific gene sequence of *Toxoplasma gondii*. Lane M 50 bp DNA ladder and lanes 1-13 PCR amplicons

DISCUSSION

Of the 300 serum samples screened for *T. gondii* antibody, 134 (44.7%) were positive for *T. gondii* (either IgG, IgM or both) as determined by ELISA. A total of 108 sera were IgM seropositive while 50 were IgG seropositive as determined by ELISA giving seroprevalences of 36.0% and 16.67% respectively. 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 in 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 Gyanget *al.* (2015) in a similar survey conducted in Lagos city among school children. It is also higher 15.13% reported by Menget *al.* (2015) among children in Shandong and Jilin provinces, China.

However a higher seroprevalence (63.1%) was reported by Fan *et al.* (2012) 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, Fan *et al.* (2012) and Gyanget *al.* (2015) used Latex Agglutination while Menget *al.* (2015) used Enzyme Immuno-Assay. 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. This seroprevalence is higher than IgM seroprevalence of 2.0% and IgG seroprevalence of 13.13% reported by Menget *al.* (2015) in China. This difference can be attributed to difference in geographical location, dietary habit and cat rearing habit.

Nested PCR was used to detect *T. gondii* specific B1 gene using specific primers in those samples that were IgG and IgM seropositive. Of the 13 samples tested, the B1 gene was amplified in 10 (76.92%) cases by PCR.

Lower percentages of detection of *T. gondii* B1 gene in blood sample 41.0%, 53.3% and 69% were recorded by Bin Dajem and Almushait (2012) in pregnant Saudi women from the Aseer region, Saudi Arabia; Bouet *al.* (1999) in immunocompetent patients with ocular toxoplasmosis and Dupouy-Camet *et al.* (1993) in Human Immunodeficiency Virus (HIV) positive patients with cerebral toxoplasmosis respectively.

CONCLUSION

It can be concluded from the findings of this study that a high seroprevalence of *T. gondii* (44.7%) was recorded among the study population in the study area. PCR detection of B1 gene (97bp) confirmed the presence of *T. gondii* in the IgM-IgG seropositive samples.

REFERENCES

- Bin Dajem, S.M. and Almushait, M.A. (2012). Detection of *Toxoplasma gondii* DNA by PCR in blood samples collected from pregnant Saudi women from the Aseer region, Saudi Arabia. *Annals of Saudi Medicine*, 32(5):507-512
- Bou, G., Figueroa, M.S., Martí-Belda, P., Navas, E. and Guerrero, A. (1999). Value of PCR for Detection of *Toxoplasma gondii* in Aqueous Humor and Blood Samples from Immunocompetent Patients with Ocular Toxoplasmosis. *Journal of Clinical Microbiology*, 37(11):3465-3468.
- Burg, J. L., Grover, C. M., Pouletty, P. and Boothroyd, J. C. (1989). Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *Journal of Clinical Microbiology*, 27:1787-1792.
- Carme, B., Bissuel, F., Ajzenberg, D., Bouyne, R., Aznar, C., Demar, M., Bichat, S., Louvel, D., Bourbigot, A.M., Peneau, C., Neron, P. and Dardé, M.L. (2002). Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. *Journal of Clinical Microbiology*, 40:4037-4044.

- Chemoh, W., Sawangjaroen, N., Nissapatorn, V. and Sermwittayawong, N. (2016). Molecular investigation on the occurrence of *Toxoplasma gondii* oocysts in cat feces using TOX-element and ITS-1 region targets. *The Veterinary Journal*, doi: 10.1016/j.tvjl.2016.05.018
- Dubey, J. P. (2008). The history of *Toxoplasma gondii* the first 100 years. *Journal of Eukaryotic Microbiology*, 55(6):467-475
- Dubey, J.P. (2010). *Toxoplasmosis of Animals and Humans*, second edition, Boca Raton, Florida: CRC Press; 313.
- Dupouy-Camet, J., de Souza, S. L., Maslo, C., Paugam, A., Saimot, A., Benarous, R., Tourte-Schaefer, C. and Derouin F. (1993). Detection of *Toxoplasma gondii* in venous blood from AIDS patients by polymerase chain reaction. *Journal of Clinical Microbiology*, 31:1866-1869.
- Fan, C.K., Liao, C.W., Kao, T.C., Lu, J.L. and Su, K.E. (2001). *Toxoplasma gondii* infection: relationship between seroprevalence and risk factors among inhabitants in two offshore islands from Taiwan. *Acta Medica Okayama*, 55:301-308.
- Fan, C.K., Lee, L.W., Liao, C.W., Huang, Y.C., Lee, Y.L., Chang, Y.T., Ramos José da Costa, A.S., Gil, V.Chi, L.H., Nara, H., Tsubouchi, A. and Akinwale, A.K. (2012). *Toxoplasma gondii* infection: relationship between seroprevalence and risk factors among primary school children in the capital areas of Democratic Republic of São Tomé and Príncipe, West Africa. *Parasites & Vectors*, 5:(141)1-7.
- Gyang, V. P., Akinwale, O.P., Lee, Y.L., Chuang, T.W., Orok, A., Ajibaye, O., Liao, C.W., Cheng, P.C., Chou, C.M., Huang, Y.C., Fan, K.H., and Fan, C.K. (2015). *Toxoplasma gondii* infection: seroprevalence and associated risk factors among primary school children in Lagos City, Southern Nigeria. *Revista da Sociedade Brasileira de Medicina Tropical*, 48(1):56-63.
- Hung, C.C., Fan, C.K., Su, K.E., Sung, F.C., Chiou, H.Y., Gil, V., Reis da Conceicao dos, Ferreira M, de Carvalho J.M., Cruz, C., Lin, Y.K., Tseng, L.F., Sao, K.Y., Chang, W.C., Lan, H.S. and Chou, S.H. (2007). Serological screening and toxoplasmosis exposure factors among pregnant women in the Democratic Republic of Sao Tome and Principe. *Transaction of the Royal Society of Tropical Medicine and Hygiene*. 101:134-139.
- Innes, E.A. (2010). A brief history and overview of *Toxoplasma gondii*. *Zoonosis and Public Health*, 57:1-7.
- Kamani, J., Mani, A.U., Egwu, G.O. and Kumshe, H.A. (2009). Seroprevalence of human infection with *Toxoplasma gondii* and the associated risk factors, in Maiduguri, Borno state, Nigeria. *Annals of Tropical Medical Parasitology*, 103(4):317-321.
- Konishi, E., Houki, Y., Harano, K., Mibawani, R.S., Marsudi, D., Alibasah, S. and Dachlan, Y.P. (2000). High prevalence of antibody to *Toxoplasma gondii* among humans in Surabaya, Indonesia. *Journal of Infectious Disease*, 53:238-241.
- Leal, F.E., Cavazzana, C.L., De Andrade, H.F., Galisteo, A.J., De Mendonça, J.S., Kallas, E.G. (2007). *Toxoplasma gondii* pneumonia in immunocompetent subjects: case report and review. *Clinical Infectious Disease*, 44:62-66.
- Liao, C.W., Lee, Y.L., Sukati, H., D'lamini, P., Huang, Y.C., Chiu, C.J., Liu, Y.H., Chou, C.M., Chiu, W.T., Du, W.Y., Hung, C.C., Chan, H.C., Chu, B., Cheng, H.C., Su, J., Tu, C.C., Cheng, C.Y. and Fan, C.K. (2009). Seroprevalence of *Toxoplasma gondii* infection among children in Swaziland, southern Africa. *Annals of Tropical Medical Parasitology*, 103:731-736.
- Mahmood, S.H., AL-Qadhi, B.N. and Zghair, K.H. (2013). Prevalence of Toxoplasmosis of Males Blood Donors in Baghdad. *Iraqi Journal of Science*, 54(4):832-841
- Meng, Q.F., You, H.L., Zhou, N., Dong, W., Wang, W.L., Wang, W.L. and Conge, W. (2015). Seroprevalence of *Toxoplasma gondii* antibodies and associated risk factors among children in Shandong and Jilin provinces, China. *International Journal of Infectious Diseases*, 30:33-35
- Montoya, J.G. (2002). Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *Journal of Infectious Diseases*, 185(Suppl 1):S73-82.
- Montoya, J. G. and O. Liesenfeld. (2004). Toxoplasmosis. *Lancet*. 363:1965-1976.
- Sevgili, M. C. B., Nalbantoglu, S. and Vatansever, Z. (2005). Determination of Seropositivity for *Toxoplasma gondii* in sheep in Sanliurfa Province, Turkey. *Journal of Veterinary Animal Science*, 29:107-111.
- Spalding, S.M., Amendoeira, M.R.R., Ribeiro, L.C., et al. (2003). Estudo prospectivo de gestantes e seus bebês com risco de transmissão de toxoplasmose congênita em município do Rio Grande do Sul. *Revista da Sociedade Brasileira de Medicina Tropical*, 36:483-91.
- Sukthana, Y. (2006). Toxoplasmosis: beyond animals to humans. *Trends Parasitology*, 22(3):137-142. doi: 10.1016/j.pt.2006.01.007
- Suzuki, H., Aso, T., Yamamoto, Y. and Matsumoto, K. (1988). Seroprevalence of *Toxoplasma* infection in two islands of Nagasaki by ELISA. *Tropical Medicine*, 30:129-139.
- Wu, S.M., Zhu, X.Q., Zhou, D.H., Fu, B.Q., Chen, J. and Yang, J.F. (2011). Seroprevalence of *Toxoplasma gondii* infection in household and stray cats in Lanzhou, Northwest China. *Parasitology and Vectors*, 4:214.