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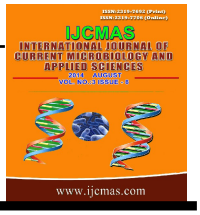

Original Research Article

Watching of *Toxoplasma gondii* antibodies among peoples in Kirkuk Province from 1993 to 2012 by using different serological tests

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Keywords

Toxoplasma, abortion, Congenital abnormality, still birth. ELISA

A B S T R A C T

Toxoplasma gondii is the most common microorganism which cause different sequela during pregnancy such as congenital aplasia, hydrops fetalis, abortion and other congenital anomalies in fetus (Such as neuro- ophthalmic complications). The aim of this study were to assess prevalence of toxoplasmosis among different groups of populations in Kirkuk Province while the second aim is to determine relationship between distribution of toxoplasmosis in relation to gender, periods of gestation, abortion, active , and protective toxoplasma antibodies, residency and type of serological tests. A total of 3264 venous blood were drawn from different groups of population for detecting antibodies specific to toxoplasmosis by using direct agglutination test (DAT toxo test) as screen test and using semi-quantitative micro-titration test(MTT) for detecting active toxoplasmosis, that compared with Elisa IgM Kit. While Toxoplasma IgG antibodies were tested using 2-mercapto-ethanol test (2.me .test) , and Elisa IgG Kit. The overall *Toxoplasma* rate was 31.35 % .High rate of toxoplasmosis 63.80%was recorded in 2005 compare to 17.59 % in 2012 P<0.05. According to occupations and population groups the all rate was divided in to the following rate : 35.59 % , 24.04 % ,23.07%, 22.85%, 22.44 % , 18.75 % ,17.39 %,15.78 % 15.38 %,15.10 and 11.11 % in house wives, butchers, barbers, medical staff, veterinaries, tanning workers, epileptic patients , still birth, neonates, donated blood and food handler respectively P <0.05..Relationship between *Toxoplasma* and patient residency not significant. *Toxoplasma* antibody detections by using the following tests :DAT ,MTT,2-m.e.test and ELISA IgM , IgG tests reveal 31.25 % ,8.52 %,2.46%,1.77 % 2.40 % respectively ,P<0.05.

Introduction

Toxoplasmosis is the most widespread zoonosis and an important human disease particularly in children where it can cause visual and neurological impairment and mental retardation (Adesiyun et al.,2007). It is able to infect, or be present in the highest number of host species any warm-blooded animal may act as an intermediate host, and oocysts may be transported by invertebrates (Paul et al.,2001).Toxoplasmosis mostly increased with age, education, crowding, sanitary, habits, socio economic, ethnic

considerations, undercooked meats, animal contacts including cat the final host (Al-Muhaymen and Ahmed,2011).Prevalence of toxoplasmosis in the world was studied in different parts, the high rate was recorded among pregnant women in France, and the rate is ranged from 87 % in 1984 to 81-85 % in 1999 (Ljugstrom et al.,1995) and (Heybas et al.,2003) . In Arab countries toxoplasmosis rates were fluctuated, the high rate 58.4 % was recorded in Tunisia by(Desmonts and Couvrew,1984). In Iraq several studies were carried on in relation to toxoplasmosis and most of these studies were detect toxoplasmosis by using serological methods and few attempts were forwarded for toxoplasma isolation in Kirkuk and Mosul by (Al-Attar , 2000) and (Al-Ubaydie , 2004).

An accurate estimation in consider to the prevalence of toxoplasmosis in Kirkuk province is absent, except the earliest study which was done in 1992 by (Kadir et al.,1992), who found24.2 % of *Toxoplasma* antibodies in sera of women. An idea of watching *Toxoplasma* infection among Kirkuk community was created after the war of 1991 upon Iraq which followed by economic sanction against Iraq that lead to lack of medicine specially anti toxoplasma drugs, controversy had role in visible increasing in the rate of abortions among women and congenital abnormalities, so this study was conducted to exposure the light on the prevalence of toxoplasmosis in Kirkuk province using different serological methods from 1993 to 2012.

Materials and Methods

A prospective study was based on testing sera from different groups of peoples who were more suspected for acquiring the infection from nature or due to their jobs, congenitally from mothers and chronic diseases like epileptic patients.

Sample collection

From the period of 1st January 1992 to 31th December 2012, a total of 3264 venous blood samples were drawn from peoples attending Hospitals and private clinics and laboratories in Kirkuk Province .Then age group of peoples enrolling the study ranged from neonate (one day after delivery) to 41 years and over. Prior to this process, special questionnaire form for each individual was prepared including complete information, then after venous blood were centrifuged for five minutes using 3000 rpm, clear sera were separated and kept in -20 C till to examination.

Procedures

The kits for direct toxoplasma antibody test (DAT) were obtained from different purchase sources (Linear, Biokit & Bio-megrhib companies in USA, Spain andTunisia respectively. The procedure was done according to (Mustafa, 2000), the test is screen test for toxoplasmosis. Semi-quantitative micro-titration test (MTT) which described by (Al-jubori,2005) was used for each positive sample to detect active toxoplasmosis according to cut off the test, which is started from 1/32 IU/ml. Also ELISA kits including IgM and IgG were applied for samples testing for *Toxoplasma* antibodies from 2005 to 2012(Al-Ajeel, 2003). *Toxoplasma* IgG was determined according to (Salamn, 2007) compared by sera treatment with 2-mercpto-ethanol according to(Othman,2004).

Statistical Analysis

All data were tabulated in special file of personal computer, source of variances were obtained by using SPSS compact disc, differences accepted under $0.05P < 0.05$

Results and Discussion

By examining the total 3264 sera of different groups of peoples in Kirkuk province, the overall rate of toxoplasmosis was 31.35 % . The relationship between *Toxoplasma* antibodies distribution and according to years of the study was significant ($p<0.05$), especially in 2005,via which high rate of *Toxoplasma* sero-positive was 63.80% in compare to 17.59 % in 2012, (Table 1).

Considering *Toxoplasma* distribution according to patients occupation, growing, some diseases; the higher rates of toxoplasmosis were recorded in the following groups: House wife 36.59 %, butchers 24.07 % followed by 23.07, 22.85, 22.44,18.75 and 17.39 %%,in sera of barbers, medical staff, veterinarians, tanning workers and epileptic patients respectively. While slightly lower rates 15.78 %,15.38%,15.10% and lower rate 11.11 % were recorded in sera of still birth, neonates ,blood donors and food handlers respectively, $P<0.05$ (Table 2).

According to gender the frequency of toxoplasmosis was obviously higher in sera of females 38.28 % compare to 16.62% in sera of males, $P<0.05$ (Table 3).

Frequency of *Toxoplasma* antibodies according to age show the following high rates, 36.58 % and 35.09 % among peoples aging from 25 to 35 and 16 to 25 years respectively, meanwhile low rate 12.72 % was recorded among patients aging over than 41 years ,$P<0.05$.Table 4.

Table 5 is showing the distribution of *Toxoplasma* seropositive according to gestational periods, through which high rate of toxoplasmosis 84.44 % was recorded in third trimester followed by 83.65 and70.25 % in second and first trimesters respectively ($P<0.05$).Also the relationship between period of gestations and women number abortions was significant($p<0.05$), via which high rate of abortion 29.74 % was recorded in sera of women in first period of gestation .$P<0.05$.

compare between active toxoplasmosis in pregnant women and protective *Toxoplasma* antibody levels (IgG) in non-pregnant women and to observe sera conversion (rising titers) by using semi-quantitative micro titration(MTT) test the following results were obtained ,from a total of 381 pregnant women only288 sera were positive for *Toxoplasma* antibodies, when it was tested by MTT it reveal *Toxoplasm*a IgM positivity in 38 and 30 sera with the following dilutions 1:32 and 1:64 respectively, compare to 8 and 10 sera of non-pregnant women with 1:32 and 1:64 respectively .$P<0.05$ (table 6)

Considering patients residency and *Toxoplasma* antibodies distribution, table 7 is exerting 164 sera positive for toxoplasmosis in urban area compare to 64 sera positive in rural area ($P>0.05$). Table7.
Assessments of *Toxoplasma* antibodies according to laboratory tests, the all rate of positive rate 31.03% was retested by using MTT(specific for IgM), 2.m.e test(Specific for IgG), ELISA IgMand ELISA IgG as shown in (table 8) below and reveal the following rates:9.12% compare to 1.77 % positive rate for *Toxoplasma* IgM antibodies by using Elisa-IgM kit ($p<0.05$).While checking of *Toxoplasma* IgG antibodies using 2-mercapto-ethanol show 2.63 % compare to 2.57 % using Elisa-IgG Kit ,($P>0.05$).Table 8.

The result of the present study is a confirmatory to the importance of toxoplasmosis in Iraq specially when it will

be compare with that recorded by (Naizi,1976) in Baghdad ,how found the rate 44%, and with rates 24%, and 40.6% in Mosul by (Al-Ubaidy, 2004) and (Abdul-Ridha,2000) respectively. Also its close to that recorded by (Salman,2007) in Kirkuk, while the rate is higher than that recorded by (Mustafa, 2000) and (Salman et al.,2003) in the same province, whom they record 24% & 28 % respectively and with 16.90 % that recorded in Tikrit by (Othman,2004) ,. The result of the present study is disagree with that recorded by (Kasim, 2013) and(Tewfik,2013) in Kirkuk also, they found the following rates respectively (91.6% and 48.9 %). The variances in the results may be attributed to differences in the number of the specimens, type of method, source and type of *Toxoplasma* kits or due to large number of specimens included the present study. The reasons for obtaining the high rate in the present study are factorial, most like to be due to exposure of Iraq to several wars and economic sanction which affects nutrition controversy that had role in diminishing the immune state against the infectious agents including toxoplasma parasite. In addition the shortage of insecticides (quality & quantity) which enhance transmission of the infective stage (oocyst) mechanically by the means of wings, legs antenna of flies, fleas and mosquitoes .

The high incidence of *Toxoplasma* in house wives 36.59% reflects contamination of houses thresholds with cat feces (oocyst), in addition to fact that women are more contact to oocyst through vegetable, meat , so predication of toxoplasmosis is higher than in other group of peoples . To confirm this hypothesis, comparative studies on childcare and toxoplasmosis infection are necessary, in order to provide preventive measures (Gilbert and Peckham, 2002).

Blood transfusion is an important process for life saving, especially when the donated blood is free from pathogens, but *Toxoplasma* rate 15.10 % in the present study may reflect poor hygienic condition and low level of sanitation in addition to un controlling the movement of cats in houses, the importance of this subject may be explain by the possibility of transporting of *Toxoplasma* during the rupture of cysts contain the bradyzoites or by the proliferate stage the tachyzoites (Al-Attar,2000).Our result is close to that recorded by (Ismaiel, 2012) and not agree with 7.14 % recorded in Mexico by(Cosme,2007) .The high rates of *Toxoplasma* IgM antibodies in sera of neonates, epileptic children and still birth are indicating to women acquiring infection during gestations.

Regarding the gender, the finding of high rate of *Toxoplasma* antibodies in females than in males is in agreement with that recorded by (Hodkova et al.,2007) as a results of physiological and anatomical differences between males and females which enhance the appearance of clinical features in infant after delivery or during pregnancy as abortion(Muhammad etal,2010).

Concerning frequency of *Toxoplasma* antibodies according to ages,16.16 % of neonates and babies positive for *Toxoplasma* IgM antibodies this result is critical because most of them will undergo postnatal complains such as deafness and blurry vision at 4 or 8 years(Markel and Voge, 2006).While high rates of toxoplasmosis among peoples aging from 15 to 36 years is agree with that recorded in Nepal by (Acharya et al.,2014) the reason may be attributed to fact, that peoples in this age group may consume more vegetables and undercooked meat (Al-Attar,2000) .also this result is more critical to women because 1/3

of study populations in the present study are women, whom they were in childbearing or conceiving and they were more susceptible for abortion due to toxoplasmosis than women 41 years and over .This result is agree with that recorded by (Ahmed, 2008) in Tikrit province.

The rate of sero-positivity in non-pregnant women 33.67% means, that they are possess protective antibodies against toxoplasma, while the rate 75.59 % in pregnant women is highly significant and required follow up by using other techniques like IFAT or toxoplasma IgG avidity test, also watching of rising titer specially 1/16and 1/32 IU/ml is an important step to recognize and detect primary infection or to exclude reactivation of toxoplasmosis (Hany, 2009). The result of the present study is agree with that recorded in Ethiopia by (Xabier et al.,2007) and within the same province.

Acquiring of toxoplasma infection during pregnancy especially in third gestational period had role in the outcome of pregnancy as congenital abnormalities like choir-retinitis, deafness or other central nervous system involvement (Dubey,2008) , also the occurrence of abortion in third and second trimester, the result of high incidence of *Toxoplasma* antibodies in first trimester of pregnancy is agree with that recorded by (Othman,2004)but it is disagree with that recorded by (Al-Jubori,2005) in the same province who recorded high rate of *Toxoplasma antibodies* during first trimester of pregnancy.

Distribution of cats in nature had strong role in spreading the infective stage (oocyst) to environment , but as keeping it in houses as domestic animal especially in urban area can leads to more exposure to toxoplasmosis, this can explain why the rate of toxoplasmosis in the present study is high in sera of people from urban area than in rural area in spite of the relationship was not significant (Salman, 2007).

Diversity of serological tests used in this study is vital and benefit for detecting the *Toxoplasma* cases, clinical classification (acute or chronic) and watching of rising titers of *Toxoplasma* antibodies or observing of sero-conversion. From the results of the present study, it seems that DAT is screen test for detecting *Toxoplasma* antibodies ,while Elisa IgM had priority to MTT for detecting recent or acute toxoplasmosis, the explain to that may be due to use of solid phase antigen of *Toxoplasm gondii* in Elisa technique that react with specific toxoplasma IgM antibodies ,while in MTT some technical errors such as adjusting dilutions, latex antigen particles and others had role in interfering the results(Al-Jubori,2005).Elisa IgG and 2-m.e. test had less value for detecting chronic or latent toxoplasmosis. From other view, *Toxoplasma* IgG antibodies detecting by two described tests with low rates are so important in highlighting the degree of *Toxoplasma* effect on public health in Kirkuk Province and weak immunization against previous toxoplasmosis.

From the result of the study, it can be concluded , that toxoplasmosis among peoples in Kirkuk province is still high from 1993 to 2012 , watching of rising titer, follow up of positive cases are recommended especially in gestational periods of pregnancy of women to avoid loss of babies . Also toxoplasmosis should be taking in consider especially during blood transfusion process and toxoplasma test might to be added to other protective tests of blood pints in blood banks.

Table.1 Frequency of Sero-positive *Toxoplasma gondii* antibodies according to years.

Years	Total number examined	Number positive	Percentages positive
1993	163	72	44.17
1994	178	74	41.57
1995	160	56	35.00
1996	172	47	27.32
1997	110	36	32.72
1998	185	55	29.72
1999	142	51	35.91
2000	153	39	25.49
2001	179	69	38.50
2002	182	71	39.01
2003	193	79	40.93
2004	257	66	25.68
2005	105	67	63.80 *
2006	116	41	35.34
2007	206	47	22.81
2008	219	46	21.00
2009	189	44	23.28
2010	102	20	19.60
2011	107	24	22.22
2012	108	19	17.59
Total	3264	1013	31.03

*P<0.05

Table.2 Toxoplasmosis according to the different community groups .

Community groups	Total number examined	Sample positive	Percentages
House wife	2372	868	36.59 *
Donated blood	437	66	15.10
Food handler	162	18	11.11
Veterinaries	49	11	22.44
Butchers	54	13	24.07
barbers	39	9	23.07
Tanning workers	48	9	18.75
Medical staff	35	8	22.85
Neonates	26	4	15.38
Epilepsy	23	4	17.39
Still birth	19	3	15.78
Total	3264	1013	31.03

* P<0.05

Table.3 Distribution of sera -positive toxoplasma antibodies according to gender

Gender	Total No. examined	NO.+ve	Percentage +ve
female	2392	868	38.28 *
male	872	145	16.62
Total	3264	1013	31.03

***P<0.05**

Table.4 Distribution of sera- positive toxoplasma according to ages

Age group in years	**Number examined**	**Number positive**	**Percentage positive.**
1day to 1year**	42	7	16.66
2 to 15	109	27	24.77
16 to 25	1456	511	35.09
26 to35	1129	413	36.58 *
36 to 40	418	41	13.92
41 and above	110	14	12.72
Total	3264	1013	31.03

***P<0.05 ** neonates and stillbirth**

Table.5 Frequency of sera-positive Toxoplasma antibodies among pregnant women according to gestations

Gestational periods	**Total number examined**	**Number positive**	**Percentage positive**	**Number of women with abortion**	**percentages**
first	232	163	70.25	69* **	29.74
second	104	87	83.65	17	16.34
third	45	38	84.44**	* 7	15.55
Total	381	288	75.59	93	24.40

** , *** P<0.05 Total number of women exam: 868. * referring to preterm delivery

Table.6 Toxoplasma antibody distribution between pregnant and non- pregnant women using semi-quantitative micro-titration test(MTT) and ELISA IgM positive

Women	**Number examined**	**Number positive**	**Percentage positive**	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/64**
Pregnant	381	288	75.59 *	49	58	61	52	38	30
Non pregnant	487	164	33.67	62	38	26	20	8	10
Total	868	452	52.07	111	96	87	72	46	40

* P<0.05

Table.7 Distribution of sera positive toxoplasma antibodies according to residency

Residency	Total No. examined	NO. positive	Percentages +ve
Urban area	405	164	40.49
Rural area	157	64	40.76
Total	562	228	40.56

* P>0.05

Assessment of toxoplasma antibodies according to laboratory tests

Tests	NO.+ve	% +ve	No. -ve	% -ve	
DAT	1096	33.57 *	2168	66.434	*P<0.05
MTT	298	9.12 **	2966	90.88	**P<0.05
2.m.e test	86	2.63 ***	3180	97.47	***P>0.05
Elisa IgM	62	1.89 **	3202	98.11	
Elisa IgG	84	2.57***	3180	97.43	

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References

Abdul-Ridah, R.R.(2000) Biochemical changes in aborted toxoplasmosis seropositive women. M.Sc. thesis Al-Mustanseryia Univ.

Acharya1, D.; Shrestha, A.; Bogati, B.; Khanal, K.; Shrestha, S.;Gyawali, P.,2014. Serological screening of TORCH agents as an etiology of spontaneous abortion in Dhulikhel hospital, Nepal . American Journal of Biomedical and Life Sciences, 2(2): 34-39.

Adesiyun, A.A., Gooding, R., Ganta, K.,Seepersadsingh, N.and Ramsewak, S.(2007).Congenital toxoplasmosis in two health institutions in Trinida .West Indian Medical Journal.;56(2):166-70.

Ahmed, Z.Y.(2008).Isolation and diagnosis of Toxoplasmosis among women with abortions attending Tikrit Teaching Hospital .M.Sc .thesis .College of Education.Tikrit University.

Ajeel,N.F.(2003).Biochemical and immunological tests for detecting Toxoplasma antibodies among women in Tikrit city.M.Sc.thesis.College of Medicine Tikrit University.

Al-Attar, Sh. A.(2000) . Epidemiological study of toxoplasmosis in Kirkuk city. M.Sc. thesis, College of Education, Tikrit Univ.

Al- Jubori, A.M. (2005) .Serological study of toxoplasmosis in Kirkuk province.M.Sc. thesis. College of Technology. Baghdad. Iraq.

Al-Muhaymen, N., and Ahmed,

M.A.(2012).VEGF expression in trophoblastic tissue of women with spontaneous miscarriage and infected by *Toxoplasma gondii* Insitu hybridizations .Iraqi Journal of Biotechnology11(2):492-502.

Al-Timimi,R.L.(2004) Detecting of Toxoplasmosis among different groups of aborted women during gestational & age of pregnancy. Diploma thesis, college of medical &health technology, Baghdad Iraq.

Al-Ubaydi GT.(2004) Toxoplasmosis in pregnant women& its relation with some parameters. M.Sc . thesis college of science, Mosul University.2004.

Bourtabine, A., Siala, E., Chahand, M.K., Aunon, K. and Ben-Esmail R.(2001) Sero- epidemiologic profile of toxoplasmosis in northern Tunisia. Parasit. 8(1_): 61-66.

Cosme,A.L, Miguel, F, Suarez, M Martínez-García S.(2007). Sero-epidemiology of infection with *Toxoplasma gondii* in healthy blood donors of Durango, Mexico.BMC.Infectiuos diseases.7:11-15.

Desmonts, G. and Couvrew,J(1974) Congenital toxoplasmosis. A prospective study of 378 pregnancies. New Engl. J. Med., 290: 1110-1116, 1974.

Desmonts, G. and Couvrew,J.(1984) Congenital toxoplasmosis, Prospective study of the outcome of pregnancy in 542 women with toxoplasmosis Acquiring during pregnancy . Annals of pediatric (Paris); 31: 805.

Heybas, S.M., and Al- Mutawa, S.A.(2003) Immuno pathogenesis of Toxoplasmosis . Clinical Experimental Medicine.; 3(2):89-105.

Gilbert,R.E. and Peckham,C.S.(2002) Congenital toxoplasmosis in the United Kingdom: to screen or not to screen? Journal of Medical Screening;9:135-143.

Hodková, H., Kolbeková, P., Skallová, A., Lindová, J.and, Flegr, J.(2007). Higher perceived dominance in Toxoplasma infected men--a new evidence for role of increased level of testosterone in toxoplasmosis-associated changes in human behavior. Neurology and Endocrinology 28(2):110-114.

Ismaiel, M S.(2012). Seroprevalence study of toxoplasmsosis in human and domestic animals in Al-Faloja-Al-Anbar province-Iraq. M.Sc. thesis. College of Scince Tikrit University.

Kadir,M.A., Abdulatief, B.M., and Jaftan A. Prevalence of toxoplamosis among Women in kirkuk . Iraqi journal of Faculty of Medicine Baghdad..; 34(3):279_285 .

Kasim,T.N.(2013).Efficacy of some laboratory methods in detecting protozoan parasites in Kirkuk city M.sc. thesis. College of Science, Kirkuk University.

Ljugstrom, L. I., Gille, E., Nokes J., Linders E and Forsgren M.(1995) Sero-epidemiology of *Toxoplasma gondii* among pregnant women in different parts of Sweden. European Journal of Epidemiology.;11:149-156.

Othman,N F.(2004).Sero-prevalence study of Toxoplasma gondii among pregnant women in Kirkuk city.M.Sc.thesis.College of Medicine Tikrit University. Mohammad,H.I.; Amin, T.T.; Balaha, M.H.; Moghannum, M.S.,2010. Toxoplasmosis among the pregnant women attending a Saudi maternity hospital: sero-prevalence and possible risk factors. Ann Trop Med

Parasitol,104:493–504.

Mostafa, W.Gh.(2000). Biochemical profile of pregnant women with toxoplasmosisin Al-Tammime province. PDCLI.College of Medicine Tikrit Univ.

Niazi, A. D.(1976). Prevalence of toxoplasmosis in Iraq. Bulletin of endemic disease .;17 (1— 4) :3-35.

Paul,M.,Petersen,E., and Szczcapa,J.(2001).Prevalence of Congenital *Toxoplasma gondii* Infection among new-borns from the poznan´ region of Poland: Validation of a new combined Enzyme Immunoassay for *Toxoplasma gondii*-specific IgA and IgM antibodies. J Clin.Microbiol.;May :1912-1916.

Salman,Y.G., Abdulah EKh., Fathel, S.S. and Ghaffor D.A.(2003). Epidemiological study of toxoplasmosis among pregnant women in Kirkuk city. Dissertation Technical College .Kirkuk

Salman, Y. J.(2007) Serological cross reaction among some causative agents of women abortions (*Toxoplasma gondii*, *Cytomegalovirus* and *Rubella* virus), with the incidence of Hepatitis virus (B and C). Tikrit journal of pharmacological science;3(2): 8-14.

Segundo, G.R., Silva, D.A ., Minea, J.R. and Ferreira,M.S.(2004)Congenital toxoplasmosis inUberlandin,Barazil. Journal of pediatric.;50 (1) :50-53.

Tewfik,S.K.(2013).Some immunological aspects of toxoplasmosis among women with abortion and congenital abnormalities .M.Sc. thesis. College of Medicine-Tikrit University.

Wilson, M. and McAuley,J.M.Toxoplasmosis: In: Murrary R.R .Manual of clinical microbiology.7th ed.ASM Washington, DC. 1999:1374-82.

Xabier,G.,Nuriligen,M.,Hiwot,A.G.Hailu, A.,Siassay,Y.,Gatachew,E. and Formmel,.D.(2007). Sero-epidemiological study of Toxoplasma gondii infection in Ethiopia .Journal Ethiopia Medicine,31:201-208.