Seroprevalence of Toxoplasma gondii antibodies among farmers in Erbil Government by using enzyme linked immunosorbent assay (ELISA)

Sarmad Nagemaldeen Mageed
Department of Medical Microbiology
Faculty of Science and Health
Koya University

Abstract

Toxoplasmosis is a parasitic and a universal zoonotic disease caused by *Toxoplasma gondii* parasite. The research aimed to determining the prevalence of *Toxoplasma gondii* infection among local farmers in 5 villages belongs to Erbil governorate/ Kurdistan region during the beginning of November 2014 to the beginning of March 2015. Prevalence of previous/recent *T. gondii* infection was assessed among farmers (men and women) through using an enzyme-linked immunosorbent assay (ELISA) for the presence of recent specific anti-*T. gondii* IgM antibodies, and previous history of infection through IgG antibodies. Sample collection were supported by taking personal information about individual health history through a specified designed questionnaire. The study examined 113 individuals for both IgG and IgM, including 91 females and 22 males. The results showed that 35 (31%) individuals were positive for IgG, 30 (28.5%) females and 5 (2.5%) males, and 78 (69%) individuals were negative for IgG, 61 (54%) females and 17 (15%) males. While, the results showed that only one female was positive (0.97) for IgM, and 112 individuals were negative for IgM, 90 (79.6%) females and 22 (19.4%) males. The results showed that toxoplasmosis is prevalent among female and male farmers in Erbil government and can be consider as a serious risk on their life.

Keywords: Toxoplasma gondii, ELISA, IgG and IgM, and Erbil government.

Introduction

Toxoplasmosis is an important zoonotic cosmopolitan parasitic disease arising from infection with the cat-borne apicomplexan, coccidian protozoan *Toxoplasma gondii*, an obligate intracellular parasite that forms cysts in mammalian tissues throughout the body (Dumètre and Dardé, 2003, Kravetz and Federman, 2002, Rorman et al., 2006). *Toxoplasma gondii* has cosmopolitan transmission, acting as an important pathogen and an opportunistic parasite that infects humans and nearly all other warm-blooded animals such as mammals and birds all over the world (Dubey et al., 1998, Lehmann et al., 2006, Sibley and Ajioka, 2008, Dubey and Beattie, 1988a).

The only known definitive hosts of *T. gondii* are domestic and wild felids, in which sexual reproduction is occur (Frenkel et al., 1970), and hence cats play a major role in *T. gondii* epidemiology, constituting the only known source of environmental contamination with the infective oocyst stage (Miller et al., 1972). And thus human communities that come into contact with cats expose to a high risk of the spread of infection (McAllister, 2005).

T. gondii has a worldwide distribution in human populations infecting up to one third of the global population and a wide range of other mammalian and avian species (Miller et al., 1972, Dubey and Beattie, 1988b, Tenter et al., 2000, Hill and Dubey, 2002, McAllister, 2005, Sukthana, 2006). Toxoplasmosis is a major public health problem, with a high socioeconomic impact in terms of human suffering including the cost of caring for sick, mentally retarded and blind children (Roberts et al., 1994).

Seroprevalence of *T. gondii* infection in man rises with age and it does not vary greatly between sexes (Montoya and Remington, 2000). Toxoplasmosis's prevalence significantly increases with age in which the highest seropositivity rate, 35.4% was found among pregnant women in an age group of 35 to 44 years old in Slovakia (Studeničová et al., 2006).

The most important channels for transmission to humans are by ingestion of food or water contaminated with oocysts shed by cats, by eating undercooked or raw meat containing infective tissue cysts and via transplacental transfer, notably when the mother becomes infected for the first time during pregnancy (Dumètre and Dardé, 2003, Dubey and Beattie, 1988b, Tenter et al., 2000, McAllister, 2005, Kravetz and Federman, 2005, Wallace, 1973, Aramini et al., 1999, Sroka et al., 2006). Human infection with *T. gondii* is a huge challenge for which there is no effective treatment.

Objectives: As in many cities throughout the world, Erbil in Iraq has a significant number of cats that has considerable possible knock-on effects for toxoplasmosis and human health, which have multiplied and colonized rapidly around food and water resources, mainly in rural but also in urban areas.
The research aimed to investigate the prevalence range of infections as a result of *T. gondii* parasite among local male and female farmers using Enzyme Linked Immunosorbent Assay (ELISA) test.

Material & Methods

Sampling area

Blood samples were collected randomly from 113 local farmer individuals (91 females and 22 males). The samples were collected over four months during the period from the beginning of November 2014 to the beginning of March 2015 by many visits to five villages located within TaqTaq and Koya cities that are belonging to Erbil government. The visits were also supported by preparing a questionnaire to extract some personal information from those farmers regarding their age, the history of any persisting diseases, and whether they are regularly going to nearest hospitals for treatment purposes.

Blood samples collection

5ml of venous blood was taken from each farmer (Male and Female) in a disposable syringes. Blood samples then were transferred into 10ml disposable EDTA free sterile tubes and put it in incubator at 37 ºC or room temperature for clotting. The blood samples were then centrifuged at 5000rpm for 10 minutes to separate the serum. The serum then were transferred into new disposable sterile tubes and labelled with the name of the person and the age and divided into six age groups. All isolated sera were kept at -80°C until used to detect anti- Toxo IgG and IgM by Elisa tests.

Serological tests for detection of *T. gondii* infection

Enzyme-linked Immunosorbent assay (ELISA) as one of the few reliable methods was used to examine the sera of all cases for the detection of anti-*Toxoplasma* antibodies, the tests were carried out at Koya University Genome Centre. Serum samples were brought from the -80°C freezer and let to reach 37°C by put it in the incubator before proceed to perform the test. In the ELISA test, soluble antigen is coated to microtiter plates and serum sample is added to form an antigen– antibody complex (if specific antibodies are present). A secondary enzyme-linked antibody specific to the host species was added to detect antigen–antibody complex. An enzyme conjugation then added to the secondary antibodies and read by an ELISA reader.
This assay was performed by using two types of kits (Rapid Labs Ltd, United Kingdom). One type for detection of IgG and another type for detection of IgM specific antibodies against *T. gondii* antigens in the patient's serum. Used ELISA device was from (BioTek Instruments, Italy).

Detection of IgG and IgM titers in all samples were analyzed for *T. gondii* by the titer of IgG and IgM antibodies using ELISA kit as described by Rapid Labs Ltd, United Kingdom. The optical densities (OD) of the samples were measured at 450/630nm using the OD value of the blank well to correct all the OD reading from test wells (Rapid Labs Ltd, United Kingdom).

Results

The study included serum examination of 113 individuals (91 females and 22 males) for anti-*Toxoplasma* IgG and IgM. The overall percentage of positive reactions to *T. gondii* IgG showed in (Table 1). The table showed that among 113 examined individuals (91 females and 22 males), the results of (31%) of them (28.5% females and 2.5% males) were positive for anti-*Toxoplasma* IgG, while 78 (69%) individuals were negative to IgG, 61 (54%) females and 17 (15%) males.

The most frequent age groups for positive reactions to IgG were among those of 41-50, and then 16-25 years old and it represents 8.85% and 7.96% of the total number of seropositive for IgG group, respectively (Table 1).

The samples of each group of seropositive and seronegative IgG were divided into two groups of males and females with their percentages and eventually the total percentage of the females and males groups according to the age groups.

The rate of seroprevalence of IgG was higher among females comparing to males in both groups of seropositive and seronegative (Table 2). The highest rate of IgG seropositive among females were showed by the 26-30 age group by recording 7 (53.8%) out of total number of 7 of the positive samples of this age group, while there was no record for any positive samples among males in this age group.

The second highest rate of IgG seropositive among females were showed by the 41-50 age group in which 9 (42.8%) out of 10 of the positive samples of this age group were females, which is out of the total number of 30 of IgG seropositive females, whereas only one out of 10 of total number of the same age group at 4.8% was male (Table 2).

The same status of female predominance are applied for the age groups 31-35 and 36-40 at (5 out of 5) and (4 out of 4) at the percentages of (27.8%) and (23.5%) respectively. At the age group of ≤ 16-25 the total number of 9 of the IgG seropositive were divided into 5 females and 4 males at 12.8% and 10.3% respectively. The noticeable group was the > 50 age group by showing zero case of IgG seropositive among both males and females (Table 2).

In term of the IgG seronegative samples, the group aged > 50 years old took the lion share prevalence rate of the total number of negative samples for both females (80%) and males (20%).

On the same line, groups aged 31-35 and 16-25 years old occupied the second and third highest rate of IgG seronegative (66.6%) and (58.9%) respectively, among females. While males of age group (16-25) years old were on the second highest rate (17.9%) and of age group (36-40) at the third highest rate (17.6%) of IgG (Table 2).

Table 1. Seroprevalence of anti-toxoplasma IgG in relation to participants' age.

Age groups (Years)	IgG positive		IgG Negative	
	Number	%	Number	%
≤ 16-25	9	7.96	30	26.55
26-30	7	6.2	6	5.31
31-35	5	4.42	13	11.5
36-40	4	3.54	13	11.5
41-50	10	8.85	11	9.73
> 50	0	0.00	5	4.42
Total	35	31%	78	69%

Table 2. Seroprevalence of anti-toxoplasma IgG in relation to participants' age and gender.

Age groups (Years)	IgG Positive						IgG Negative					
	No.	Gender				Total %	No.	Gender				Total %
		M	%	F	%			M	%	F	%	
≤ 16-25	9	4	10.3	5	12.8	23.1	30	7	17.9	23	58.9	76.8
26-30	7	0	0.0	7	53.8	53.8	6	2	15.3	4	30.7	46
31-35	5	0	0.0	5	27.8	27.8	13	1	5.5	12	66.6	72.1
36-40	4	0	0.0	4	23.5	23.5	13	3	17.6	10	58.8	76.4
41-50	10	1	4.8	9	42.8	47.6	11	3	14.2	8	38	52.2
> 50	0	0	0.0	0	0.0	0.00	5	1	20	4	80	100
Totals	35	5	2.5	30	28.5	31	78	17	15	61	55	69

On the same context, in term of the IgM seropositive and seronegative samples, there was only one (4.7%) positive IgM reaction of a female aged 45 years in the age group 41-50 years old out of 113 (0.9%) examined serum samples, whereas 112 (99.1%) samples of both sexes were negative to IgM, 90 (79.6%) females and 22 (19.4%) males (Table 3).

Table 3. Seroprevalence of anti-toxoplasma IgM in relation to participants' age and gender.

Age groups (Years)	IgM positive						IgM Negative					
	No.	Gender				Total %	No.	Gender				Total %
		M	%	F	%			M	%	F	%	
≤ 16-25	0	0	0.0	0	0.0	0.00	39	11	28	28	71.8	99.8
26-30	0	0	0.0	0	0.0	0.00	13	2	15	11	84.6	99.6
31-35	0	0	0.0	0	0.0	0.00	18	1	5.5	17	94.4	99.9
36-40	0	0	0.0	0	0.0	0.00	17	3	17	14	82	99
41-50	1	0	0.0	1	4.7	4.7	20	3	14	17	81	99.7
> 50	0	0	0.0	0	0.0	0.00	5	2	40	3	60	100
Totals	1	0	0.0	1	0.8	0.9	112	22	19.4	90.4	79.6	99.1

Discussion

The results showed and confirmed, based on the serological tests, our expectations that *Toxoplasma* gondii infection is prevalent among local farmers and a substantial proportion of those farmers showed evidence of earlier infection (IgG), whilst only one case sample had evidence of current infection (IgM). The occurring of infections in all age stages throughout the life of adults were also confirmed by the rising prevalence of IgG positive tests with increasing age.

According to age, the results showed that the highest rate of infection was among age group (26-30) age group by recording (53.8%) of the total number of examined individuals having toxoplasmosis infection (Table 2). Results were in agreement with Khoshnaw (2011), who showed the highest percentage of toxoplasmosis infection in Erbil-Iraqi Kurdistan was among women at age group of 26-30 years, because it is the most age of workers go outside home.

Results were in agreement with Al- Bajalan (2015) (Al-Bajalan et al., 2015) who showed the highest percentage of toxoplasmosis infection at age group of 46-50 years. Although, our results showed that 26-30 age group were also recorded slightly higher percentage of infection compared to 41-50 age group, but the number of infected individual was higher in the 41-50 age group in agreement with the findings of Al- Bajalan (2015).

Results were in agreement with Abdul-Aziz (2014) who revealed an increase in the prevalence of toxoplasmosis infection at age group 43-60 years in hemodialysis patients in Baghdad. In term of *Toxoplasma* seropositivity among women in Erbil-Iraqi Kurdistan, the highest rate was recorded at age group 47-57 years old (Hamad and Kadir, 2013).

The findings of our investigation of having higher percentage of infections with increasing age can be as a result of longer exposure time, which end in most humans acquire infections (Ashraf and Abdul-Haleem, 2010).

At a time where many studies were in disagreement with Siddiqui et al. (2014), who stated that among pregnant women in India, the highest prevalence of infection was at age group 21-30 years, our results were harmonious with these findings by showing the highest percentage of Toxoplasma infections at age group 26-30 years (Table 2).

Similar study were performed in Erbil city, Iraq, was highly compatible with our findings by observing the highest toxoplasmosis infections in women at aged between 26 and 30 years (Khoshnaw, 2011). The reason for the disease's prevalence at this age group may return to their personal daily activities and the possibility of having contact with one of the many infection routes (Tawfiq, 2013).

On the other hand, according to previous studies performed by Al-Shua'aibi (2012) (Al - shua aibi, 2012) in Iraq; Muluye et al. (2013) (Muluye et al., 2013) among people living in Northwest Ethiopia; Ghana; Chiang et al. (2014) (Chiang et al., 2014) in Taiwan, showed that there were no significant difference between infection with toxoplasmosis and individual age.

In contrast, different results can be achieved from different serological tests due to the differences of inherent sensitivity between these serological tests, the use of kits from different sources by researchers and their changeable conditions, time of studies, laboratories conditions. What is more, the results can be affected by different factors such as the type of exposure to the parasite, genetic background of the host and even the parasite, and the type and degree of immune response probably triggered by the parasite, due to differences in study region and sample population (Opsteegh, 2011, Djakovic, 2012, Ferreira et al., 2014, Abbas, 2014).

Other factors that could have influence on the serological results may include cultural levels, feeding habits in each country and different geographical areas within one country, dissimilar behavioural and nutritional patterns of life, as well as among ethnic groups living in certain areas, climate condition, and possessing of pets especially cats (2009, Abdi et al., 2008, AL-Mayahi, 2011, Akhlaghi et al., 2014).

In term of occupation, various studies revealed significant relation between infection and working in gardening and farming (Jafari et al., 2012). Occupational exposure with the gardening soil has significant association with toxoplasmosis (Malarvizhi et al., 2012, Senthamarai et al., 2013). In Ethiopia, a study performed by Kudakwashe and Yesuf (2014) (Kudakwashe and Yesuf, 2014) showed that 87.5% of farmers were tested positive to toxoplasmosis, whereas only 25% of merchants had the same positive results. Possessing a garden or working in the yard increases the chances of oocyst transmission by soil contact (Jones et al., 2006).

The risk for *T. gondii* infection increased with age and is more prevalent in populations that have low educational level, lived in crowded conditions, and those who worked in jobs related with soil (Jones et al., 2001). Inhalation or ingesting oocyst-contaminated dust particles is another route for human infection by toxoplasmosis (Dubey et al., 2009).

Many other factors have contribution for the high seroprevalence of the infection including inadequate hygiene, nutritional habits and proper factors of climate for sporulation and survival of oocysts in the environment. Countries are varies in their relatively important risk factors because of the differences in cultural patterns and climatic factors affecting the survival of oocysts.

Moreover, sample size, studied area and population, age, sensitivities of serological techniques employed, cat densities in such areas and contaminated food and water

with cat's oocysts and geographical variability may consider for some of the differences in the reported seroprevalence (Gebremedhin et al., 2013).

Conclusion

The study can be considered as an attempt to analyse the trends in prevalence among farmer group of the community, as an initial step in a longer-term investigation of the role of domestic animals in general, and feral cats in particular, in the transmission of toxoplasmosis infections among the farmers and general population in Kurdistan region and the associated risks to human health in general. Based on the outcomes of this investigation we can conclude that females were more sensitive than males to the infection. On the same context, our findings showed that acquiring infections is increase with age which could be as a result of longer exposure time, which end in most humans acquire infections.

References

2009. Leaf-nosed bat. *Encyclopædia Britannica.* Encyclopædia Britannica Online.

ABBAS, H. H., ALASADIY, Y.D.K., AL- TEMIMI, M.B.S 2014. Detection Toxoplasma gondii by latex and ELISA test in abortive women in Al Muthanna province. *J. Inter. Acad. Res. Multidisciplin,* 1(12)**,** 308 316.

ABDI, J., SHOJAEE, S., MIRZAEE, A. & KESHAVARZ, H. 2008. Seroprevalence of toxoplasmosis in pregnant women in Ilam province, Iran. *Iranian Journal of Parasitology,* 3**,** 34-37.

AKHLAGHI, L., GHASEMI, A., HADIGHI, R. & TABATABAIE, F. 2014. Study of seroprevalence and risk factors for Toxoplasma gondii among pregnant women in Karaj township of Alborz province [2013]. *J. Entomol. Zool. Stud,* 2)**,** 217 219.

AL-BAJALAN, R. R., AL-NASIRI, F. S. & MAHMOOD, S. M. 2015. Detection Toxoplasma gondii by Latex and ELISA Test in Infertile and Fertile Men in Kalar City, Kurdistan Region, Iraq. *Int. J. Curr. Microbiol. App. Sci,* 4**,** 570-585.

AL-MAYAHI, J. 2011. *Epidemiological study on Toxoplasma gondii in aborted women in Kut city.* M. Sc. Thesis. College of Science. University of Baghdad. pp: 125.

AL - SHUA AIBI, M. M. M. 2012. Immunology, molecular and epidemiology study of Toxoplasma gondii in specimens of Al-Anbar province. *Ph. D. Thesis, College of Education, University of Tikrit***,** 134.

ARAMINI, J. J., STEPHEN, C., DUBEY, J., ENGELSTOFT, C., SCHWANTJE, H. & RIBBLE, C. 1999. Potential contamination of drinking water with Toxoplasma gondii oocysts. *Epidemiology and infection,* 122**,** 305-315.

ASHRAF, M. & ABDUL-HALEEM, S. 2010. Seroprevalence Of Anti Toxoplasma gondii IgG and IgM Among Pregnant Women in Sana'a Capital and Capital Trusteeship. *Scientific Journal of King Faisal University (Basic and applied Sciences),* 11**,** 1431.

CHIANG, T.-Y., KUO, M.-C., CHEN, C.-H., YANG, J.-Y., KAO, C.-F., JI, D.-D. & FANG, C.-T. 2014. Risk factors for acute Toxoplasma gondii diseases in Taiwan: a population-based case-control study. *PloS one,* 9.

DJAKOVIC, O. D. 2012. Toxoplasmosis recent advances. *InTech publ., Rijeka, Croatia***,** 198.

DUBEY, J. & BEATTIE, C. 1988a. *Toxoplasmosis of animals and man*, CRC Press, Inc.

DUBEY, J., LINDSAY, D. S. & LAPPIN, M. R. 2009. Toxoplasmosis and other intestinal coccidial infections in cats and dogs. *Veterinary Clinics of North America: small animal practice,* 39**,** 1009-1034.

DUBEY, J. P. & BEATTIE, C. 1988b. *Toxoplasmosis of animals and man*, CRC Press, Inc.

DUBEY, J. P., LINDSAY, D. S. & SPEER, C. A. 1998. Structures of Toxoplasma gondii Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. *CLINICAL MICROBIOLOGY REVIEWS,* 11**,** 267-299.

DUMETRE, A. & DARDE, M.-L. 2003. How to detect Toxoplasma gondii oocysts in environmental samples? *FEMS microbiology reviews,* 27**,** 651-661.

FERREIRA, A., DE MATTOS, C., FREDERICO, F., MEIRA, C., ALMEIDA, G., NAKASHIMA, F., BERNARDO, C., PEREIRA-CHIOCCOLA, V. & DE MATTOS, L. 2014. Risk factors for ocular toxoplasmosis in Brazil. *Epidemiology and infection,* 142**,** 142-148.

FRENKEL, J., DUBEY, J. & MILLER, N. 1970. Toxoplasma gondii in cats: fecal stages identified as coccidian oocysts. *Science,* 167**,** 893-896.

GEBREMEDHIN, E. Z., ABEBE, A. H., TESSEMA, T. S., TULLU, K. D., MEDHIN, G., VITALE, M., DI MARCO, V., COX, E. & DORNY, P. 2013. Seroepidemiology of Toxoplasma gondii infection in women of child-bearing age in central Ethiopia. *BMC infectious diseases,* 13**,** 101.

HAMAD, N. R. & KADIR, M. A. 2013. Prevalence and comparison between the efficacy of different techniques for diagnosis of Toxoplasma gondii among women in erbil province-Iraqi Kurdistan. *European Scientific Journal,* 9(21).

HILL, D. & DUBEY, J. 2002. Toxoplasma gondii: transmission, diagnosis and prevention. *Clinical microbiology and infection,* 8**,** 634-640.

JAFARI, R., SADAGHIAN, M. & SAFARI, M. 2012. seroprevalence of Toxoplasma gondii infection and related risk factors in Tabriz City, Iran, 2008. *Journal of research in health sciences,* 12**,** 119-121.

JONES, J. L., KRUSZON-MORAN, D., WILSON, M., MCQUILLAN, G., NAVIN, T. & MCAULEY, J. B. 2001. Toxoplasma gondii infection in the United States: seroprevalence and risk factors. *American journal of epidemiology,* 154**,** 357-365.

JONES, J. L., MUCCIOLI, C., BELFORT JR, R., HOLLAND, G. N., ROBERTS, J. M. & SILVEIRA, C. 2006. Recently acquired Toxoplasma gondii infection, Brazil. *Emerging infectious diseases,* 12**,** 582.

KHOSHNAW, L. S. H. 2011. Detection of Toxoplasma gondii of abortedwomen in Erbil city serological, molecular (PCR) and mouse inoculation. *M. Sc. Thesis, College of Science, University of Tikrit***,** 68 Pp.

KRAVETZ, J. D. & FEDERMAN, D. G. 2002. Cat-associated zoonoses. *Archives of Internal Medicine,* 162**,** 1945-1952.

KRAVETZ, J. D. & FEDERMAN, D. G. 2005. Toxoplasmosis in pregnancy. *The American journal of medicine,* 118**,** 212-216.

KUDAKWASHE, M. & YESUF, K. M. 2014. Application of Binary Logistic Regression in Assessing Risk Factors Affecting the Prevalence of Toxoplasmosis. *American Journal of Applied Mathematics and Statistics,* 2**,** 357-363.

LEHMANN, T., MARCET, P. L., GRAHAM, D. H., DAHL, E. R. & DUBEY, J. 2006. Globalization and the population structure of Toxoplasma gondii. *Proceedings of the National Academy of Sciences,* 103**,** 11423-11428.

MALARVIZHI, A., VISWANATHAN, T., LAVANYA, V., ARUL SHEEDA MALAR, S. & MOORTHY, K. 2012. Seroprevalence of Toxoplasma gondii in pregnant women. *J Public Health Epidemiol,* 4**,** 170-177.

MCALLISTER, M. M. 2005. A decade of discoveries in veterinary protozoology changes our concept of "subclinical" toxoplasmosis. *Veterinary Parasitology,* 132**,** 241-247.

MILLER, N. L., FRENKEL, J. & DUBEY, J. 1972. Oral infections with Toxoplasma cysts and oocysts in felines, other mammals, and in birds. *The Journal of parasitology***,** 928-937.

MULUYE, D., WONDIMENEH, Y., BELYHUN, Y., MOGES, F., ENDRIS, M., FEREDE, G., YITAYEW, G. & NEGESE, D. 2013. Prevalence of Toxoplasma gondii and

associated risk factors among people living with HIV at Gondar University Hospital, Northwest Ethiopia. *ISRN Tropical Medicine,* 2013.

OPSTEEGH, M. 2011. Toxoplasma gondii in animal reservoirs and the environment.

ROBERTS, T., MURRELL, K. & MARKS, S. 1994. Economic losses caused by foodborne parasitic diseases. *Parasitology Today,* 10**,** 419-423.

RORMAN, E., ZAMIR, C. S., RILKIS, I. & BEN-DAVID, H. 2006. Congenital toxoplasmosis—prenatal aspects of Toxoplasma gondii infection. *Reproductive toxicology,* 21**,** 458-472.

SENTHAMARAI, S., SIVASANKARI, S., SANDHYA, B., KUMUDAVATHI MSANITHA, C. & AMSHAVATHANI, S. 2013. Seroprevalence of toxoplasmosis in pregnant women with bad obstetrics history in a tertiary care hospital, kanchipuram-a pilot study. *Disease,* 3**,** 29-32.

SIBLEY, L. D. & AJIOKA, J. W. 2008. Population structure of Toxoplasma gondii: clonal expansion driven by infrequent recombination and selective sweeps. *Annu. Rev. Microbiol.,* 62**,** 329-351.

SROKA, J., WÓJCIK-FATLA, A. & DUTKIEWICZ, J. 2006. Occurrence of Toxoplasma gondii in water from wells located on farms. *Annals of Agricultural and Environmental Medicine,* 13**,** 169.

STUDENIČOVá, C., BENČAIOVá, G. & HOLKOVá, R. 2006. Seroprevalence of Toxoplasma gondii antibodies in a healthy population from Slovakia. *European journal of internal medicine,* 17**,** 470-473.

SUKTHANA, Y. 2006. Toxoplasmosis: beyond animals to humans. *Trends in parasitology,* 22**,** 137-142.

TAWFIQ, S. K. 2013. Some immunological aspects of toxoplasmosis in pregnant and repeated aborted women. *M. Sc. Thesis, College of Medicine, University of Tikrit***,** 138 Pp. .

TENTER, A. M., HECKEROTH, A. R. & WEISS, L. M. 2000. Toxoplasma gondii: from animals to humans. *International journal for parasitology,* 30**,** 1217-1258.

WALLACE, G. D. 1973. The role of the cat in the natural history of Toxoplasma gondii. *The American journal of tropical medicine and hygiene,* 22**,** 313.