

Original Article

Toxoplasmosis Seroepidemiology in Serum of Suspected Patients Attending Medical Lab, in 2013

Ali Fattahi Bafghi¹, Roya Anvari², Mohammad Hossein Anvari^{1*}

¹ Department of Medical Parasitology and Mycology, school of medicine, Shahid Sadoughi University of medical sciences, Yazd, Iran.

² Student of lab technology, school of paramedical, Shahid Sadoughi University of medical sciences, Yazd, Iran.

Received: 2015/5/1

Accepted: 2015/6/14

Abstract:

Introduction: Toxoplasmosis is Zoonoses among humans and animals with cosmopolitan distribution. Acquired form of the disease often has no symptoms or discomfort of swollen lymph nodes and associated Chorioretinitis. The congenital form of the disease is delivered via the placenta from mother to fetus. Congenital infection may cause abortion or damage to the central nervous system and eye disorders. The aim of this study was to determine toxoplasmosis Seroepidemiology in serum of suspected patients referring to medical lab, in 2013.

Materials & Methods: This study was cross-sectional. After physical examination, of the total cases 712 were diagnosed with suspected toxoplasmosis infection, and were referred for evaluation of serological diagnostic laboratories. Serum samples were collected from patients' in the laboratory using kits of anti-Toxoplasma gondii and Chorus Toxoplasma IgG, IgM antibodies were tested by ELISA method. Data on age, sex and time of the visit and laboratory test results were recorded in the Czech list, and then were analyzed using SPSS software.

Results: From 712 sera tested, 649 (91.2%) were female while 63 (8.8%) were male. 171 (24.3%) of the antibody IgG and 25 (3.5% in terms of IgM in serum were positive. In sex-wise distributed groups 159 female (93%) and 12 male (7%) tested positive for IgG. and, 24 female (96%) and 1 male (4%) were IgM positive. Most positive tests (9.5%) were observed in the group aged over 50 years. whereas The lowest percentage of positive tests were in the group with age less than 20 years (20%) and the highest was observed in patients above 60 years (8/47 percent).

Conclusion: As a general conclusion, it can be stated that the frequency of specific IgM and IgG antibodies in toxoplasmosis, in the suspected-to-have toxoplasmosis and control groups were not statistically significant. Also, we can conclude that abortion is involved in the development of chronic toxoplasmosis.

Keywords: Toxoplasmosis, Seroepidemiology, IgG, IgM, Medical lab.

*Corresponding author; Tel:+989131545131 E-mail: hosein_anvari@ssu.ac.ir

Introduction

Toxoplasmosis is caused by an Apicomplexa protozoan parasite, called *Toxoplasma gondii*. That cats act as its definitive host, and warm-blooded animals as intermediate hosts. Humans get infected with *Toxoplasma gondii* by ingestion of raw or undercooked meat, or cat-shed oocytes via contaminated soil, food and water; or congenitally by transplacental transmission of Tachyzoite^[1]. Its prevalence is with up to 30% of the world's human population affected by this parasite and the third main cause of food-related deaths in the United States of America. In Iran, the rate of toxoplasmosis, in 2008, was 40.7% for Isfahan, 44.2% for Lorestan, and 34.2% for Bandar-e-Abbas^[2, 3, 4]. It is a major opportunistic pathogen in hosts with suppressed immune system. Infection merely occurs via oral routes. (intake of food, water or soil that is contaminated with oocysts shed by cats, or by eating undercooked or raw meat containing tissue cysts)^[5, 6, 7].

Toxoplasmosis commonly causes mild symptoms in immunocompetent individuals; whereas, it can be fatal to Immunocompromised patients. Acute toxoplasmosis in the pregnancy period can lead to abortion, neonatal death, and poor growth or delivery before time^[8, 9, 10]. Histopathological assessment and serological procedures, including the dye test (DT), indirect fluorescent antibodies (IFA), modified agglutination test (MAT), and enzyme-linked immunosorbent assay (ELISA) is generally used for the diagnosis of *Toxoplasma gondii* infection.

The aim of this study was to determine toxoplasmosis seroepidemiology in serum of suspected patients referred to the medical lab.

Materials & Methods

The present cross-sectional study was conducted in Yazd city and the participants were patients visited Yazd medical laboratory for antenatal follow up or medication and blood samples were collected from patients from January to December 2014. Blood samples (5 ml) were collected by using sterile plain Vacutainer tubes and were left overnight at room temperature to allow clotting and were further centrifuged at 3000 rpm for 10 minutes. The sera were then collected in Eppendorf tubes and stored at 4°C for 48-78 hours until transported in an ice box to the Microbiology laboratory of the School of medicine and stored at - 20°C until tested. Sera were analyzed for the presence of IgG and IgM antibodies against *T. gondii* by the indirect enzyme linked immunosorbent assay (ELISA) kit conducted according to the manufacturer's instructions. The kit has reported sensitivity and specificity of 98% and 99%, respectively. The optical densities of wells were measured by a photometer at a wavelength of 450 nm. Comparisons among the experimental groups were done by Chi-Square test using SPSS software program. The upper level of significance was chosen as ($P < 0.05$).

Results

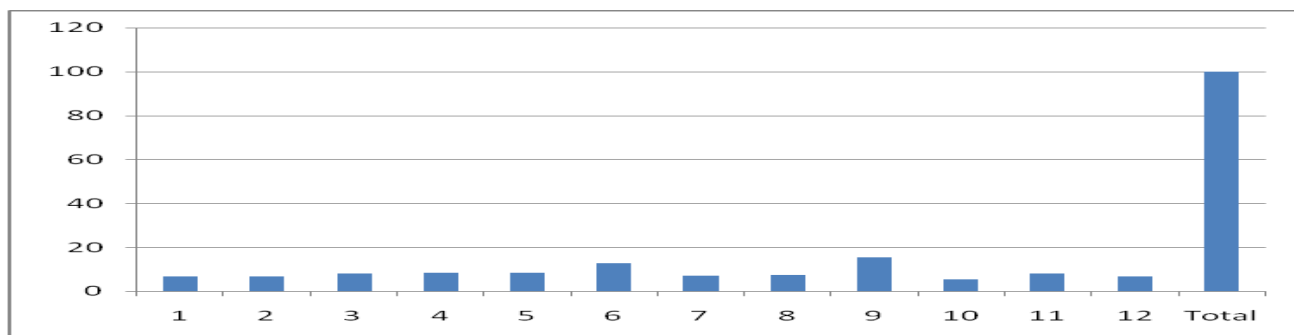
Of the 712 participants, 68 (8.8%) were female while 649(91.2%) were male. Prevalence of *Toxoplasma gondii*, was estimated on monthly basis. the current result revealed, the lowest

prevalence for the month of December with (39)5.5% and the highest for the month of November with 110 (15.2%) cases and no Significant difference was observed among suspected to TG and without TG and months ($p=0.548$) (Table and Fig I)

Table I. Toxoplasmosis seroepidemiology in serum of suspected patients referring to medical Lab, according to sex.

Month	Pos-IgG		Neg-IgG		Sus-IgG		Pos-IgM		Neg-IgM		
	N	%	N	%	N	%	N	%	N	%	
1	11	23.4	36	76.6	0	0	2	4.3	45	95.7	
2	10	21.7	36	78.3	0	0	2	4.3	44	93.6	
3	12	21.7	43	78.2	0	0	3	5.4	53	94.6	
4	11	18.3	46	76.7	3	5	2	3.3	58	96.7	
5	16	27.6	41	70.7	1	1.7	0	0	59	100	
6	24	27	63	70.8	2	2.2	4	4.4	86	95.6	
7	13	26.5	36	73.5	0	0	1	2	48	98	
8	17	33.3	34	66.7	0	0	3	5.9	48	94.1	
9	25	22.9	81	74.3	3	2.8	4	3.6	103	93.6	
10	14	36.8	24	63.2	0	0	1	2.6	37	94.9	
11	10	17.9	45	80.4	1	1.8	0	0	55	100	
12	8	17	38	80.9	1	2.1	3	6.4	44	96.4	
Total	171	24.3	523	74.2	11	1.6	25	3.5	680	95.8	
Pearson Chi-Square Test						P=0.548					
Pearson Chi-Square Test						P=0.548					

Fig I. Toxoplasmosis seroepidemiology in serum of suspected patients referring to medical Lab, according to month

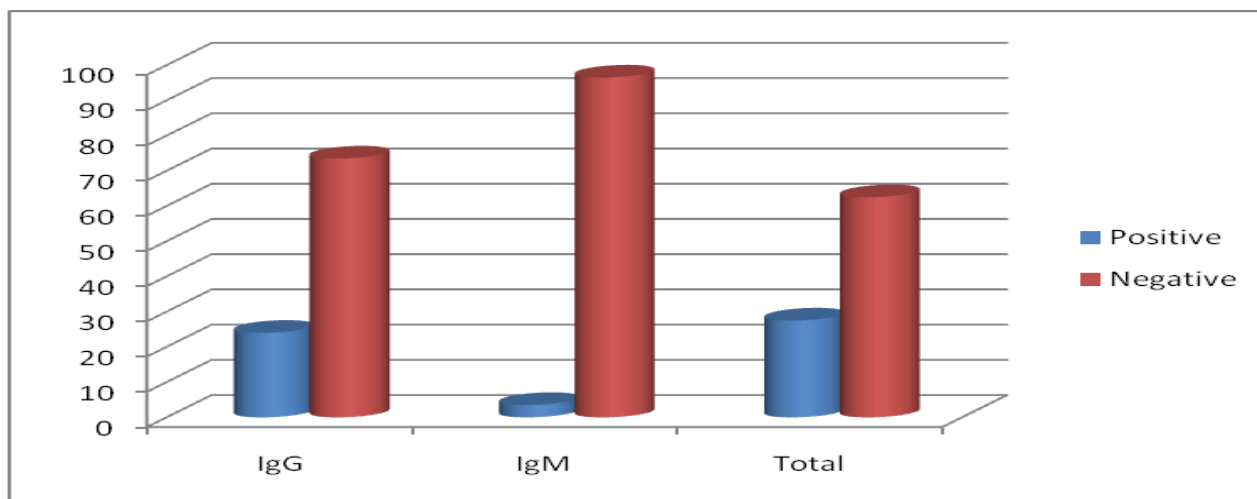


Chi-Squire ($p > 0.05$)

In accordance to the type of Immunoglobulin, our result revealed that: 196 (27.5%) cases seropositive 516 (62.5%) seronegative, 171(24%) seropositive to IgG and 523(62.5) seronegative to

IgG, 25(3.5%) seropositive to IgM and 680(96.5%) cases seronegative to IgM, and Significant difference were observed between Toxoplasmosis and type of immunoglobulin ($p = 0.723$) (Fig II).

Fig II. Toxoplasmosis seroepidemiology in serum of suspected patients referring to medical Lab, according to type of Immunoglobulin



Chi-Squire ($p < 0.05$)

According to age-wise distribution, our result showed: 5 cases (9.3%) seropositive to IgG in the group of 0-9 year, 46 cases(82.2%) seronegative to IgG and 3 cases (3.6%) were susceptible to IgG, and in the group of 10-19 years 9 cases (10.5%)

seropositive to IgG, 77 cases(89.5%) seronegative to IgG and 0 cases (0%) were susceptible to IgG. in the group of 20-29 years 82 cases (27.7%) seropositive to IgG, 275 cases(76.2%) seronegative to IgG and 4 cases (1.1%) were susceptible to IgG,

In the group of 30-39 years 49 cases (31.4%) seropositive to IgG, 104 cases(66.7%) seronegative to IgG and 3 cases (1.9%) were susceptible to IgG, in the group of 40-49 year 21 cases (56.8%) seropositive to IgG, 16 cases(43.2%) seronegative to IgG and 0 cases (0%) were susceptible to IgG, in the group of >50 year 5 case (45.5%) seropositive to IgM, 15 cases (9.1%) seronegative to IgG and of total 171 cases(24.3%) seropositive to IgG, 523 (74.2%)were seronegative to IgG and 11 cases (1.6%) were susceptible to IgG and Significant difference was seen between Toxoplasmosis and the type of immunoglobulin ($p<0.05$).(Table II).

In the group of 0-9 years 1 case (1.9%) seropositive to IgM, 53 cases(98.12%) seronegative to IgM and 03 cases (0%) were susceptible to IgM, in the group of 10-19 year 2 cases (2.35%) seropositive to IgM, 84cases(97.7%) seronegative to IgM and 0 cases (0%) were susceptible to IgM, in the group of 20-29 year 11 cases (3%) seropositive to IgM, 350 cases(96.2%) seronegative to IgM and 3 cases (0.8%) were susceptible to IgG, in the group of 30-39 year 8 cases (5.1%) seropositive to IgM, 148 cases(93.7%) seronegative to IgM and 2 cases (1.3%) were susceptible to IgM, in the group of 40-49 year 3 cases (8.1%) seropositive to IgM, 34 cases(91.9%) seronegative to IgM and 0 cases

(0%) were susceptible to IgM, in the group of >50 year 0 case (0%) seropositive to IgM, 11 cases (100%) seronegative to IgM and of total 171 cases(24.3%) seropositive to IgM, 523 cases(74.2%) seronegative to IgM and 11 cases (1.6%) were susceptible to IgM and there was a Significant difference between Toxoplasmosis and the type of immunoglobulin ($P=0.000$). (Table II).

According to sex, our result showed: in the group of male 19 cases (19%) seropositive to IgG, 48 cases (76.2.2%) seronegative to IgG and 3 cases (4.8%) were susceptible to IgG, whereas in the group of female 159 cases (24.8%) seropositive to IgG, 475 cases (74%) seronegative to IgG and 8 cases (1.2%) were susceptible to IgG and a significant difference was observed between Toxoplasmosis and sex of the patient ($p<0.05$) (Table III). in the group of male 1 case (1.6) was seropositive to IgM, 62 cases (94.8%) seronegative to IgM and 0 cases (0%) were susceptible to IgM, in the group of female 24 cases (3.7) seropositive to IgM, 618 cases (59.5%) seronegative to IgM and 5 cases (0.7%) were susceptible to IgM and no significant difference was seen between Toxoplasmosis and sex of the patient($P=0.069$) (table III)

Table II. Toxoplasmosis seroepidemiology in serum of suspected patients referring to medical Lab, according to age.

Age Groups	IgG			IgM			Suspective					
	Positive		Negative		Positive		Negative		Suspective			
	N	%	N	%	N	%	N	%	N	%		
0-9	5	9.3	46	85.2	3	5.6	1	1.9	53	98.1	0	0
10-19	9	10.5	77	89.5	0	0	2	2.3	84	97.7	0	0
20-29	82	27.7	275	76.2	4	1.1	11	3	350	96.2	3	.8
30-39	49	31.4	104	66.7	3	1.9	8	5.1	148	93.7	2	1.3
40-49	21	56.8	16	43.2	0	0	3	8.1	34	91.9	0	0
>50	5	45.5	5	45.5	1	9.1	0	0	11	100	0	0
Total	171	24.3	523	74.2	11	1.6	25	3.5	680	95.8	5	7

Pearson Chi-Squire test P=0.000 Pearson Chi-Squire test P=0.000

Table III. Toxoplasmosis seroepidemiology in serum of suspected patients referring to medical lab, according to sex.

Sex	Pos-IgG		Neg-IgG		Sus-IgG		Pos-IgM		Neg-IgM		Sus-IgM	
	N	%	N	%	N	%	N	%	N	%	N	%
Male	19	19	48	76.2	3	4.8	1	1.6	62	98.4	0	0
Female	159	24.8	475	74	8	1.2	24	3.7	618	59.5	5	0.8
Total	171	24.3	523	74.2	11	1.6	25	3.5	680	95.8	5	0.7

Pearson Chi-Squire Test P=0.069

Discussion

Toxoplasmosis is a wide-spreading zoonotic disease found all over the world. Infection with *Toxoplasma gondii* during pregnancy could cause drastic sequelae in the fetus. Toxoplasmosis generally causes mild symptoms in immunocompetent individuals; while, it could be lethal in immunocompromised patients. IgM and

IgG are the two specific antibodies that are present in the sera, and reveal the stage and kind of infection to *Toxoplasma gondii*. It has been shown that IgM antibodies appear earlier and decrease more quickly than IgG antibodies and are frequently the first class of antibodies detected after primary infection [11, 12, 13]. There was a statistical significant association between the seropositivity of *Toxoplasma* antibodies and some

risk factors. We observed a significant association between the seropositivity of *Toxoplasma* antibodies and abortion history [14, 15, 16]. However, in the meantime, epidemiological studies also gained more importance. The emergence of *Toxoplasma* as a waterborne disease in several countries has stimulated environmental research. An ecological and integrated approach was developed for a better understanding of the complex circulation of *Toxoplasma* amongst its multiple hosts and the environment. And its risk factors for human infection [17, 18, 19]. We now know that this unique species is not totally identical around the world. The pathogenic role associated with some of these genotypes has been well studied in experimental models. These models allowed the detection of virulence-associated genes. although it is difficult to formally establish the role of infecting genotype in human toxoplasmosis due to the difficulties in isolating strains from patients and other pathogenesis-associated factors, such as host immune status and genetic background, it appears now that the

References

1. Ebrahimzadeh A, Mohammadi S, Salimi-Khorashad A, et al. Seroprevalence of Toxoplasmosis among pregnant women referring to the reference laboratory of Zahedan, Iran Zahedan Journal of Research in Medical Sciences. 2013;15(12):32–5.
2. Fayer R, Dubey JP, Lindsay DS. Zoonotic protozoa: from land to sea. Trends in Parasitology 2004;20(11):531-536.
3. Heidari H, Gharekhani J, Tavosidana G. Role of toxoplasmosis in abortion of ewes in western Iran: A serological study. Scientia Parasitology. 2013;14:99–103.
4. Eskandarian AA, Jafarnezghad GA, Akbarib M. Seroprevalence of *Toxoplasma*-specific antibodies in patients suspected to have active toxoplasmosis: A cross-sectional survey, Advance Biomedical Research. 2014; 3(1): 236.
5. Gautam B, Singh G, Singh S. Virtual screening of threonine synthase as a target for antimicrobial resistance in *Toxoplasma gondii*. Elixir Appl Biology. 2012;48:9542–5.

description of clinical aspects of toxoplasmosis must be unraveled in the light of *Toxoplasma* genotypes and of their geographical distribution [20, 21].

Conclusion

The high seroprevalence of *Toxoplasma gondii* infection in the current study suggests the need for preventive measurements, to study identified risk factors, in order to reduce associated morbidities and mortalities. The results of the present study help to alert the public health delivery system of the country to undertake large scale studies and uncover the economic and health impacts and formulate guidelines and policies leading to mitigation of the potentially devastating outcomes of these Zoonoses.

Acknowledgements

We are grateful to Mrs Mahin Ghafourzadeh the expert of laboratory technology department, school of Paramedical, Shahid Sadoughi University of Medical Sciences for their sincere cooperation.

6. Pratama DA, Artama WT. Analysis of *Toxoplasma gondii* Repeat Region 529 bp (NCBI Acc. No. AF146527) as a probe candidate for molecular diagnosis of toxoplasmosis. *Indonesian Journal of Biotechnology*. 2013;14:1124–1131.
7. Uttah E, Ogban E, Okonofua C. Toxoplasmosis: A global infection, so widespread, so neglected. *International Journal of Scientific and Research Publications*. 2013;3:1–6.
8. Edwards JF, Dubey JP. *Toxoplasma gondii* abortion storm in sheep on a Texas farm and isolation of mouse virulent atypical genotype *T. gondii* from an aborted lamb from a chronically infected ewe. *Veterinary Parasitology*. 2013;192:129–36.
9. Heidari H, Gharekhani J, Tavosidana G. Role of toxoplasmosis in abortion of ewes in western Iran: A serological study. *Scientia Parasitology*. 2013;14:99–103.
10. Habibi G, Imani A, Gholami M, et al. Detection and identification of *Toxoplasma gondii* type one infection in sheep aborted fetuses in Qazvin province of Iran. *Iranian Journal Parasitology*. 2012;7:64–72.
11. Pfaff AW, Abou-Bacar A, Letscher-Bru V, et al. Cellular and molecular physiopathology of congenital toxoplasmosis: the dual role of IFN-gamma. *Parasitology*. 2007;134(13):1895–1902.
12. Villena I, Ancelle T, Delmas C, et al. Toxosurv network and National Reference Centre for Toxoplasmosis. Congenital toxoplasmosis in France in 2007: first results from a national surveillance system. *Euro Surveill*. 2010;15(25):pii=19600.
13. Robert-Gangneux F, Dardé ML. Epidemiology of and Diagnostic Strategies for Toxoplasmosis. *Clinical Microbiology Review*. 2012;25(2):264-296 .
14. Jones JL, Dargelas V, Roberts J, et al. Risk factors for *Toxoplasma gondii* infection in the United States. *Clinical Infection Disease*. 2009;49(6):878–884.
15. Pfefferkorn ER. Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. *Proceedings of the National Academy of Sciences of the United States of America*. 1984;81(3):908–912.
16. Ryning FW, McLeod R, Maddox JC, et al. Probable transmission of *Toxoplasma gondii* by organ transplantation. *Annals of Internal Medicine*. 1979;90(1):47-9.
17. Derouin F, Pelloux H. Prevention of toxoplasmosis in transplant patients. *Clinical Microbiology and Infection* 2008;14:1089–1101
18. Gourishankar S1, Doucette K, Fenton J, et al. The use of donor and recipient screening for *Toxoplasma* in the era of universal trimethoprim sulfamethoxazole prophylaxis. *Transplantation*. 2008;85(7):980–5.
19. Kijlstra A, Jongert E. *Toxoplasma*-safe meat: close to reality?. *Trends Parasitology*. 2009;25(1):18–22.
20. Hill D, Coss C, Dubey JP, et al. Identification of a sporozoite-specific antigen from *Toxoplasma gondii*. *Journal of Parasitology*. 2011;7(2):328–337.
21. Hill DE, Benedetto MC, Coss C, et al. Effects of time and temperature on the viability of *Toxoplasma gondii* tissue cysts in enhanced pork loin. *Journal Food Protection*. 2006;69:1961–1965.