



MOLECULAR DIAGNOSIS FOR SURFACE ANTIGENS GENES (SAG1, SAG2, & SAG3) OF TOXOPLASMOSIS IN BASRAH PROVINCE-IRAQ

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Article Received on 30/10/2018

Article Revised on 20/11/2018

Article Accepted on 10/12/2018

ABSTRACT

The current study was done in Basrah province during the period from January 2017 to September 2017. The results show that 8 (68.5%) were positive by conventional PCR for SAG1 gene and 12 (17.1%), 13 (25.7%) were positive by nested-PCR for SAG2, SAG3 genes respectively, whereas 4 (20%) of negative latex (control group) was positive by PCR for SAG1 gene only. No statistically significant difference between PCR positive for SAG1 and gender but there are significant role with age for same gene, also there are a relationship between infection with toxoplasmosis and time of abortion in aborted women, no positive case were seen in 3th trimester of pregnancy in all genes.

KEYWORDS: *Toxoplasma*, surface antigen, SAG1, SAG2, SAG3, Basrah, Iraq.

INTRODUCTION

Toxoplasmosis is a disease caused by an obligate intracellular protozoan parasite called *Toxoplasma gondii* which appears to have broad host specificity and capable of infecting warm-blood animal including human.^[1] Felines act as intermediate and definitive hosts, all non-feline vertebrates including humans serve as intermediate hosts.^[2] There were several virulence factors that make *T. gondii* one of the most successful parasitic protozoa, one of these are surface antigens (SAG1, SAG2, SAG3 and SAG4) and they are very interesting in recent years, some researchers have used this surface antigens as a modified molecules for vaccine^[3], other used them as recombinant antigens for serodiagnosis to detection the antibody IgM and IgG by ELISA because they are the main target of the host immune response and they have no cross-reactivity with protein from other microorganisms.^[4, 5, 6]

Nowadays, PCR is the most important and sensitive tool in diagnosis of toxoplasmosis infection and genotyping in clinical samples of human, and main attention has been attracting toward the surface antigen (SAG) of the parasite in both tachyzoite and bradyzoite stages named as SAG1, SAG2, SAG3 & SAG4.^[7, 8, 9, 10] These antigens are important in determine the acute infection also its consider immune target which provides a strong immune response against the invasive or act as an adhesion during host-cell attachment through is binding to proteoglycans.^[3] The aims of this study were detection the prevalence of toxoplasmosis among random samples

(female, male, children) by using conventional and nested-PCR for genes which encodes for surface antigen (SAG1, SAG2, SAG3) and determination the relationship between toxoplasmosis with age, gender and state of women (aborted, un aborted, un married).

MATERIALS AND METHODS

Regions, time and location of Study

A total of 200 venous blood samples were collected from different groups of patients whom retreated Basra laboratories during January 2017 to September 2017. The work was done in unit of PCR lab in Biology department/ College of Education for Pure Sciences / University of Basra.

Collection of blood samples

Different group of patients including of 76 of aborted women, 37 of unaborting women, 21 of un married women, 44 men and 14 child (<10 years). For each patient complete information were taken using questionnaire, patients ages ranged from two up to 40 years. Four ml of venous blood has divided into two volumes, 2 ml drawn into gel tube containing clot activator for serum separate. Gel tubes containing blood sample were left for about 10 minutes, then transported into centrifuge (fixed on 3000 rpm for about 5 minutes) to use in subsequent latex test, 2 ml of whole blood drawn into EDTA vial then kept in 20- C for longer period to use in subsequent molecular studies. All samples were tested by latex agglutination test then

chosen the positive latex cases only and 15 negative samples as control in molecular study.

Genomic DNA extraction

The DNA was extracted from the positive cases (70 blood samples) of latex test and 15 negative latex as control using genomic DNA extraction kit (Geneaid, USA) with accordance to the manufacturer's instructions.

Detection of *T. gondii* by PCR

Polymerase Chain Reaction (PCR) used to amplified the gene *SAG1* for DNA genomic of *T. gondii* which extracted from whole blood sample by standard PCR for 30 cycles with using specific primes which obtained from Bioneer company as SAG1F (5'-TTGCCGCGCCCACTG ATG-3') and SAG1R (5'-CGCGACAC AAGCTGCGATAG-3').^[11] Nested-PCR using to detection the *SAG2* gene using two separately amplified by standard PCR for 40 cycles with external primers SAG2a (5'-GCTA CCTCGAACAGGAACAC-3') and SAG2aR- (5'-GCATCAACAGTCTTCGTTGC-3') at 62°C annealing temperature, the resulting amplification products were diluted 1/10 in DDW, and a second amplification of 40 cycles was performed with the internal primers SAG2b F (5'-GAAATGTTTCAGGTTGCTGC-3') and SAG2b (5'-GCAAG AGCGAACTGAACAC-3') R by using 1 µl of the diluted product as the template.^[12] Also *SAG3* gene was amplified by standard PCR for 35 cycles with external primers SAG3aF (5'-CAACTCTCACCATTCCACCC-3'), SAG3aR (5'-GCGCGTTGTTAGAC AAGACA-3') at 60°C and a second amplification of 35 cycles was performed with the internal primers SAG3bF, (5'-TCTTGTCGGGTGTTCACTCA-3'), SAG3bF, (5'-CACAAG GAGACCGAGAAGGA-3') by using 1 µl of first amplified product as the template^[13] then the products of all genes were electrophoresed on 2% agarose gel.

Statistical analysis

The data obtained was analyzed using Chi-square test to find out the significant differences among the data, the differences were recorded as significant whenever the probability (p) was less than 0.05.

RESULT

PCR of Toxoplasmosis for *SAG1*, *SAG2* and *SAG3* genes

The amplification of *SAG1* gene by conventional PCR showed products at 913 bp in agarose gel electrophoresis analysis (Fig.1) and the separate amplification of *SAG2* gene by n-PCR showed products at 241 bp, whereas the amplification of *SAG3* gene showed products at 290 bp in first amplification, and 241 bp in second amplification (Fig.1).

Out of 70 positive latex patients, 48 (68.5%) were positive by conventional PCR for *SAG1* gene and 12 (17.1%), 13 (25.7%), were positive by nested-PCR for *SAG2*, *SAG3* genes respectively, whereas 4 (20%) of negative latex (control group) was positive by PCR for *SAG1* gene only (Table1).

According to the gender, the high percent of positive *SAG1*, *SAG2* and *SAG3* genes were seen in female but no significant effect ($P < 0.05$) was observed, whereas result revealed that there was a positive relation between the age group and *SAG1* gene (Table 2).

The high percent of *SAG1* gene positive was seen in un aborted women whereas the high percent for *SAG2*, *SAG3* gene were seen in aborted women with significant effect, despite recording the highest percentage of *SAG1* gene positive in un aborted women but there are no significant effect ($P < 0.05$). (Table 3).

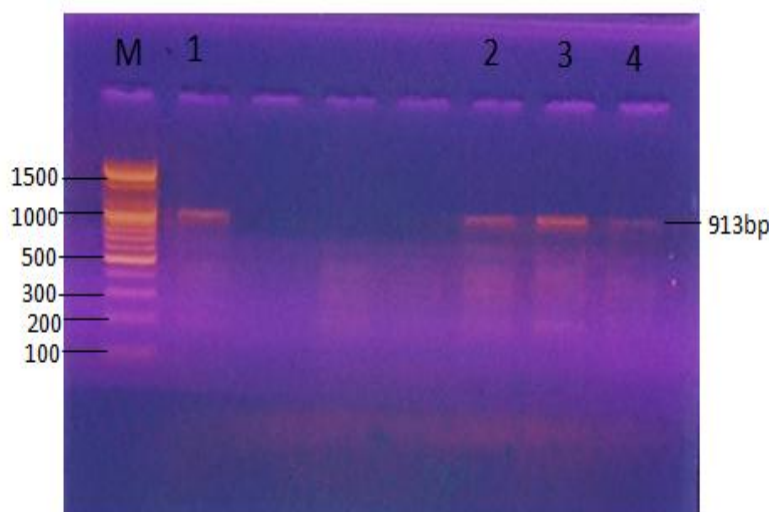


Figure 1: Agarose gel electrophoresis analysis of *SAG1* gene
M. 100 bp marker, 1,2,3,4, product 913 bp.

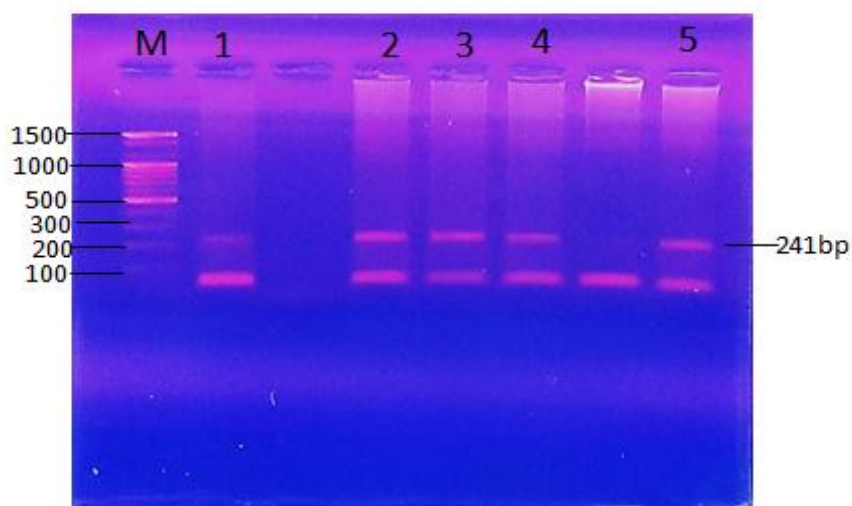


Figure 2: Agarose gel electrophoresis analysis of SAG2 gene
M. 100 bp marker, 1,2,3,4,5, product 241 bp.

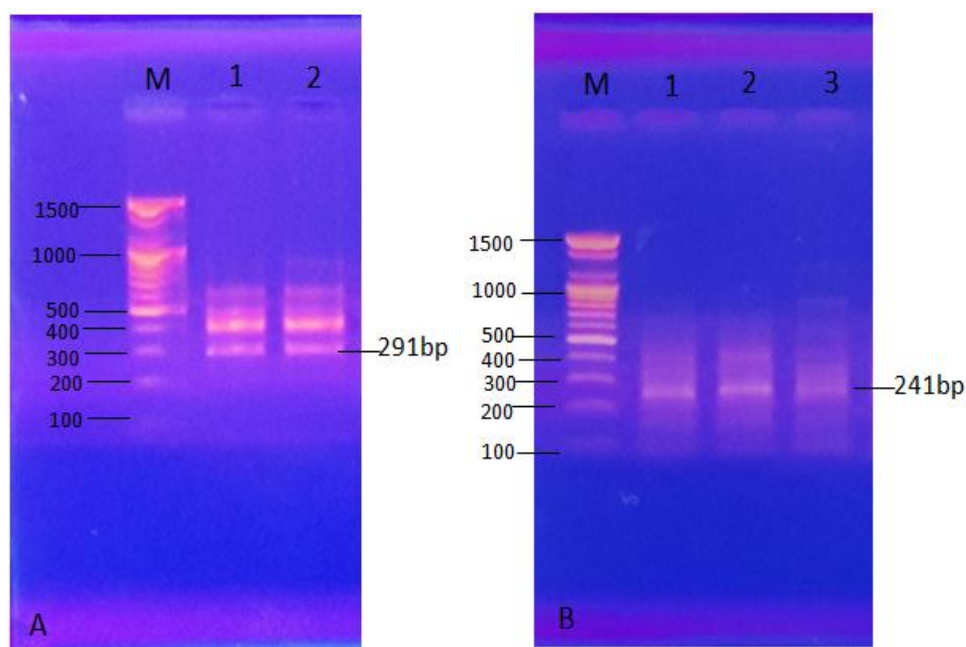


Figure 3. Agarose gel electrophoresis analysis of SAG3 gene PCR amplification products

A. First amplification (M. 100 bp marker, 1,2, product 291 bp .

B. second amplification (M. 100 bp marker, 1,2,3, product 241 bp.

Table 1: Prevalence of PCR positive cases of toxoplasmosis in patients according to the genes (SAG1, SAG2, SAG3).

Genes	Patients			Control		
	No.	Ve+	%	No.	Ve+	%
SAG1	70	48	68.5	15	3	20
SAG2	70	12	17.1	15	0	0
SAG3	70	13	25.7	15	0	0
(P ≤ 0.05)						

Table 2: Prevalence of PCR Positive cases of toxoplasmosis in patients according to the Age and gender).

		SAG1			SAG2			SAG3		
		No.	Ve+	%	No.	Ve+	%	No.	Ve+	%
Genders	Female	47	35	74.4	47	10	21.2	47	14	29.7
	Male	17	10	58.8	17	1	5.8	17	3	17.6
	children	6	3	50	6	1	16.6	6	1	16.6
	Total	70	48	68.5	70	12	17.1	70	18	25.7
Age groups years	<10	6	3	50	6	1	16.6	6	1	16.6
	11-20	13	8	61.5	13	1	7.6	13	4	30.7
	21-30	22	18	81.8	22	5	22.7	22	4	18.1
	31-40	29	19	65.5	29	5	17.2	29	9	31
		(P ≤ 0.05)								

Table 3: Prevalence of PCR Positive cases of Toxoplasmosis in female and relation with aborted, un aborted and un married.

State of female	SAG1			SAG2			SAG3		
	No.	Ve+	%	No.	Ve+	%	No.	Ve+	%
Aborted	29	21	72.4	29	9	31	29	11	37.9
Un aborted	11	9	81.8	11	0	0	11	2	18.1
Un married	7	5	71.4	7	1	14.2	7	1	14.2
Total	47	35	74.4	47	10	21.2	47	14	29.7
(P ≤ 0.05)									

Table 4: Relationship between number and time of abortion and toxoplasmosis.

		SAG1			SAG2			SAG3		
		No.	Ve+	%	No.	Ve+	%	No.	Ve+	%
Number of abortion	single	12	7	58.3	12	2	16.6	12	3	25
	Double	7	7	100	7	2	28.5	7	3	42.8
	triple	7	5	71.4	7	4	57.1	7	2	28.5
	>3	3	2	66.6	3	1	33.3	3	3	100
	Total	29	21	72.4	29	9	31	29	11	37.9
Time of abortions	1 st	62	28	45.1	28	8	28.5	28	11	39.2
	2 nd	12	4	33.3	4	1	25	4	3	75
	3th	5	0	0	0	0	0	0	0	0
(P ≤ 0.05)										

The highest percent of SAG1 gene positive (100%) was seen in women with double abortions, and the highest percent of SAG2 and SAG3 genes positive were seen in women with triple and more than three abortions with significant effect ($P < 0.05$) for all genes, according to the time of abortion, the highest percent of SAG1 and SAG2 genes positive were seen in women whom abortion in first trimester of pregnancy with no significant effect, whereas the highest percent of SAG3 gene positive was seen in women whom abortion in 2th trimester of pregnancy, no positive case were seen in 3th trimester of pregnancy in all test (Table 4).

DISCUSSION

The conversion between tachyzoite and bradyzoite forms is associated with morphological and molecular biological changes, including stage-specific gene expression and alterations in metabolism^[14] so some genes are specific in tachyzoite stage like SAG1 gene and another is specific in bradyzoite stage such as SAG4 gene^[15], others are expression in both stage like SAG2^[16] and SAG3 gene.^[17] The use of molecular diagnostics for these antigens are most effective, not only to detect the

parasite but also to determine the acute infection and the techniques which is used to detect these surface antigens is PCR which is used to detect DNA of *T. gondii* in various biological samples and has showed higher sensitivity and specificity in diagnosis compared to serological tests.^[18,19]

The results showed that the SAG1 gene was the most prevalence (50.7%) in molecular diagnosis of the acute infection of toxoplasmosis in blood samples, followed by SAG3 then SAG2 genes (19.8 %, 16.6 %) respectively, and the positive case of these genes mean that the patients have acute stage of toxoplasmosis. Recorded of SAG1 gene as a most gene prevalence in this study comparison with SAG2 and SAG3 may be due SAG1 antigen constitutes the most abundant and predominant antigen in surface of *Toxoplasma*.^[20]

Recording of highest prevalence in female is agree with other previous studies^[21] found that *T. gondii* in female was highest compared with male in some Iraqi individuals. In Baghdad, no difference in the rate of infection between male and female.^[22] But

seroprevalence of toxoplasmosis in females was more than male^[23], the positive cases of Real-Time PCR of SAG1 in female were significantly higher than those of male.^[24] But its disagreement with^[25] whom recorded *Toxoplasma* in male highest than female, this difference variation due to several factors such as sex differences in immune responses, social activities and different occupational, and differences in exposure of toxoplasmosis infection.^[26]

The variation between adults and young human beings in deferent country may be due to the difference in environmental conditions, different nutrition, behavioral patterns of life immune status of the persons, socio-cultural differences, and topography which expose the population to the infective form of parasite in different ages.^[27]

It seem that the highest positive PCR for surface antigen was seen in 21-30 age group by SAG1 and SAG2 genes, the study was in disagreement with some local studies such as^[28] which recorded highest percentage of toxoplasmosis was seen in^[30-39] category of women suspected infection by this parasite, researcher recorded the highest percentage of infection in^[30-40] age group.^[22, 29, 30, 31] The varying results from those regions may be due to differences in patients number, type samples, demographics, history and used techniques.

It is appeared that all groups of women are susceptible to *T. gondii* infection and the risk of this parasite is not limited to women with a history of abortion, but even to women who are not abortions for the chance of subsequent abortion, also the present study demonstrate that the parasite can be dangerous on un married women this agree^[29] in Kirkuk province who recorded the highest positive toxoplasmosis in unmarried women compared with married women.

REFERENCES

1. Dubey, JP. *Toxoplasmosis of Animals and Humans*. CRC Press, Boca Raton, Florida, 2016.
2. Sudan V, Jaiswa, AK, Shanker, D. Recnt trends in the diagnosis of toxoplasmosis. *Clin. Rev. Opin*, 2013; 5: 11-17.
3. Liu K, Zhang D, Wei Q, Li B, Li G, Yu H. Biological role of surface *Toxoplasma gondii* antigen in development of vaccine. *World J of Gastroenterol*, 2006; 12: 2363-2368.
4. Kazemi B, Maghen L, Bandehpour M, Shahabi S, Haghighi A. Gene cloning of P43 surface protein of *Toxoplasma gondii* tachyzoite and bradyzoite (SAG3). *Gene Ther. Mol. Biol*, 2007; 11: 113-116.
5. Khanaliha K, Motazedian M, Kazemi B, Shahrian B, Bandwhpour M, Sharifniya Z. Evaluation of recombinant SAG1, SAG2, and SAG3 antigens for serodiagnosis of toxoplasmosis. *Korean J. Parasitol*, 2014; 52: 137-142.
6. Sudan V, Tewari AK, Singh H. Serodiagnosis of *Toxoplasma gondii* infection in bovines from Kerala, India using a recombinant surface antigen 1 ELISA. *Biologicals*, 2015; 43: 250-255.
7. Lekutis C, Ferguson DJ, Boothroyd JC. *Toxoplasma gondii*: identification of a developmentally regulated family of genes related to SAG2. *Exp. Parasitol*, 2000; 96: 89-96.
8. He XL, Grigg ME, Boothroyd JC, Garcia KC. Structure of the immunodominant surface antigen from the *Toxoplasma gondii* SRS superfamily. *Nat. Struct. Biol*, 2002; 9: 606-611.
9. Kim SK, Boothroyd JC. Stage-specific expression of surface antigens by *Toxoplasma gondii* as a mechanism to facilitate parasite persistence. *J. Immunol*, 2005; 174: 8038-8048.
10. Weiss LM, Kim K. *Toxoplasma Gondii*: The Model Apicomplexan. *Perspectives and Methods*, 2011.
11. Contini C, Cultrera R, Seraceni S, Segala D, Romani R, Fainardi E, Cinque P, Lazzarin A, Delia S. The role of stage specific oligonucleotide primers in providing effective laboratory support for the molecular diagnosis of reactivated *Toxoplasma gondii* encephalitis in patients with AIDS. *J. Med. Microbiol*, 2002; 51: 879-890.
12. Howe D, K, Honore S, Derouin F, Sibley LD. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. *J. Clin. Microbiol*, 1997; 35: 1411-1414.
13. Grigg ME, Ganatra J, Boothroyd JC, Margolis TP. Unusual abundance of atypical strains associated with human ocular toxoplasmosis. *F. Infect. Dis*, 2001; 184: 633-639.
14. Mesquita RT, Ziegler AP, Hiramoto RM, Vidal JE, Pereira-Chiocola VL. Real-time quantitative PCR in cerebral toxoplasmosis diagnosis of Brazilian human immunodeficiency virus-infected patients. *J. Med. Microbiol*, 2010; 59: 641-647.
15. Ferragut CO, Soete M, Engels A, Samyn B, Loyens A, Beeumen JV, Camus D, Dubremetz JF. Molecular cloning of the *Toxoplasma gondii* SAG4 gene encoding an 18 kDa bradyzoite specific surface protein. *Mol Biochem Parasitol*, 1996; 82: 237-244.
16. Saeij J, Arrizabalaga G, Boothroyd J. A Cluster of Four Surface Antigen Genes Specifically Expressed in Bradyzoites, SAG2CDXY, Plays an Important Role in *Toxoplasma gondii* Persistence. *Infection and immunity*, 2008; 76: 2402-2410.
17. Khanaliha K, Motazedian MH, Sarkari B, Bandehpour M, Sharifnia Z, Kazemi B. Expression and purification of P43 *Toxoplasma gondii* surface antigen. *Iranian J. Parasitol*, 2012; 48-53.
18. Olariu TR, Remington JS, Montoya JG. Polymerase chain reaction in cerebrospinal fluid for the diagnosis of congenital toxoplasmosis. *Pediatr. Infect. Dis. J*, 2014; 33: 566-570.
19. Robert-Gangneuxa, F. & Belaza, S. *Molecular diagnosis of toxoplasmosis in immunocompromised patients.*, Wolters Kluwer Health, Inc, 2016; 29: 330-339.
20. Hartati S, Kusumawati A, Wuryastuti H, Widada JS. Primary structure of mature SAG1 gene of an

- Indonesian *Toxoplasma gondii* and comparison with other strains. J. Vet. Sci, 2006; 7: 263–270.
21. Saleh MA. Determination of antibodies (IgG, IgM) against *Toxoplasma gondii* in some Iraqi individuals by using ELISA technique. Baghdad Sci. J, 2011; 8: 940-946.
 22. Khalil HI, Merdaw MA, Abdullah AM, El-Hashimi WK, Al-Bashier NM, Mohemmad HJ. Estimation of *Toxoplasma gondii* infection by serological and immunohistochemical methods in Baghdad City-Iraq. Inter. J. Advan. Res, 2016; 4: 272-278.
 23. Al-Mosawi RA. Diagnostic and epidemiological study of *Toxoplasma gondii* for students of Thi-Qar University by ELISA and Real-Time PCR techniques. Thesis. M. Sc. College of Science, 2014.
 24. Zainodini N, Zare-bidaki M, Abdollahi S, Afrooz M, Ziaali N, Ebrahimian M, & Kazemi M. Molecular and Serological Detection of Acute and Latent Toxoplasmosis Using Real-Time PCR and ELISA Techniques in Blood Donors of Rafsanjan City, Iran 2013. Iran J Parasitol, 2014; 9: 336–341.
 25. Manji ZF, Al-Hamairy AK. Seroepidemiological and molecular study of Toxoplasmosis in the Babylon Province, Iraq. Res. J of Pharmaceutical, Biological and Chemical, 2015; 6: 1106-1114.
 26. Klein SL, Flanagan KL. Sex differences in immune responses. Nature Reviews Immunology, 2016; 16: 626–638.
 27. Akhlaghi L, Shirbazou S, Maleki F, Keyghobadi A, Tabaraei Y, Tabatabaie F. Seroepidemiology of Toxoplasma Infection in Pregnant Women in Qom Province, Iran. Life Sci. J, 2013; 10: 322-325.
 28. Aais NN. Genotyping analysis to determine the lineages types of *Toxoplasma gondii* with study of autoantibodies production. PhD. Thesis, college of scienc, Kuffa university, 2000; 200.
 29. Obaid HM. Serological and microscopical detection of *Toxoplasma gondii* in Kirkuk city- Iraq. Diyala J for pure Sciences, 2014; 10: 59-73.
 30. Al-Bajalan RR, Al-Nasiri FS, Mohmood SM. Detection *Toxoplasma gondii* by Latex and ELISA test in infertile and fertile men in Kalar City, Kurdistan region, Iraq. (2015). Int. J. Curr. Microbiol. App. Sci, 2015; 4: 570-585.
 31. Naser MA. Immunological and epidemiological study of toxoplasmosis among miscarriage women and cats in Basrah province. MSc. Thesis, college of veterinary medicine, university of Basrah, 2015; 106.