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Immunological and Molecular Diagnosis of *T. gondii* Infection among Aborted Women in Sana'a Capital and Capital Trusteeship, Yemen

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ABSTRACT

This study was conducted to evaluate polymerase chain reaction (PCR) assay to determine recent infections with Toxoplasma gondii in pregnant women. T. gondii DNA was detected by using the B1 gene as a target for amplification which is highly specific for T. gondii. The overall prevalence of Toxoplasma gondii infection was: PCR was positive in 32 subjects (8.0%) and 2 of controls (2.0%), 44 (11%) had positive IgM and 1.0 (1.0%) of controls had positive for IgM, whereas IgG antibody was positive in 176 (44%) compared to 29 (29%) was positive in the control group. There was no significant association between positivity PCR and abortion whereas there was a significant association between IgM positive and abortion. There was a significant association between eating raw or undercooked meat, contact with cats, contact with soil, and having domestic animals and positive IgM ELISA and PCR positive. There was no significant relationship between age, residency, educational level and either ELISA or PCR. This study showed that the use of PCR for a confirmatory test to detect primary acute toxoplasmosis in pregnant women is very important. It demonstrates the possibility of defining and selecting the high-risk cases for mother-to-child transmission of infection by combining specific serology and PCR tests to formulate a specific approach.

Keywords: PCR, ELISA, IgM, IgG, *T. gondii*, Abortion

INTRODUCTION

Toxoplasmosis is one of the most common parasitic zoonotic diseases worldwide, it is considered one of the widest spread parasite in the world causing abortion. It is an intracellular protozoan that infects humans and other warm-blooded mammals [1,2]. Infection is acquired by ingestion of viable tissue cysts in meat or oocysts excreted by cats that contaminate food or water [3]. Primary maternal infection during pregnancy is frequently associated with the transmission of *T. gondii* to the fetus [4]. Transplacental transmission of *Toxoplasma gondii* from infected pregnant women to the unborn results in fetal damage to a degree depending on the gestational age [5]. Early first trimester maternal infections are less likely to result in congenital infection but the squeal is more severe [6].

Cats are the primary source of infection to human hosts, although contact with raw meat, especially lamb, is a more significant source of human infections in some countries. Fecal contamination of hands is a significant risk factor [7]. *Toxoplasma gondii* was first described in 1908 by Nicole and Manceaux working in North Africa and by Splendora working in Brazil [8].

The species designation originated from the name of the North African rodent *Ctenodactylus gondii* from which this parasite was isolated. The genus name was derived from the Greek work toxon, meaning (bow) and referring to the crescent shape of the organism. Toxoplasma belongs to the Phylum Apicomplexa, which consists of intracellular parasites that have a characteristically polarized cell structure and a complex cytoskeletal and organelles arrangement at their apical end [8,9].

MATERIALS AND METHODS

A total of 400 aborted women attending to the Department of Obstetrics and Gynecology of the major gynecological centers of Al-Kuwait University Hospital, Al-Jumhory Hospital, Al-Thawrah General Hospital, Al-Sabine Hospital, Al-Mutwakil Specialist Hospital, Al-Om Hospital, King Specialist hospital and 2 private clinics during 2010-2012 were the subject of this study and informed consent was obtained from all the study groups. Women ranged from 15-44 years, were enrolled in the current study and were further classified into 3 categories:

- Group A: One abortion women (n=225)
- Group B: Recurrent spontaneous abortion (RSA) women (n=175)
- Group C: Control (successful pregnancy) (n=100)

Also, the study group was classified according to the gestational age when the abortion happened, to the first trimester (n=197 women); second trimester (n=203); C=100. From each patient, control blood samples were collected and serum was separated for the estimation of antibodies against *T. gondii* infection, EDTA samples were used for DNA determination by PCR.

Study Groups

Group I (Study group): Women with abortion were included in the study, and other causes of abortion were excluded. This group comprised of 400 cases selected randomly from patients attending Obstetrics and Gynecology, complaining of abortion. They were collected through 2 years from 2010-2012.

Group II (Control group): This group included 100 healthy females, with normal pregnancy, matched for age, residency and socioeconomic status.

Methods

Enzyme-Linked Immuno-Sorbent Assay (ELISA): ELISA was used according to the instruction for the detection of Toxoplasma/IgM/IgG (DRG, Inc., U.S.A) and results were registered as mean optical density (OD) readings.

DNA extraction (PCR amplifications): Extraction of DNA: DNA was extracted from blood samples which were spotted into filter paper No. 3, using a Phenol chloroform isoamyl (PCI) following the manufacturer's instructions for DNA purification from spotted blood in filter paper [10]. Final pellets were resuspended in 30 µL of TE buffer (10 mMTris, 1 mM EDTA, pH 7.2) and stored at -20°C until used.

Nested PCR assay: The nested PCR was performed on all DNA samples to amplify a fragment from the B1 gene, which is present in 35 copies and is conserved in the *T. gondii* genome, as described by Burg, et al., [11].

First cycle: The primers used in the first round of the PCR (inner primers) were (5'-TGCATAG GTT-GCAGTCACTG-3' and (5'-GGCGACC-AATGTGC-GAATAGACC-3'), which correspond to nucleotides 757-776 and 853-831, respectively at 197 bp.

Second cycle: The primers used in the second round (outer primers) (5'-GGAACTGCATCCGTTCATGAG-3'), and (5'-TCT-TTAAAGCGTTCGTGGTC-3'), which correspond to nucleotides 694-714 and 887-868, respectively at 96 bp [3].

For the first round, in a 0.5 ml PCR tube the following solutions were placed in a total volume of 20 µl: 2.0 of 10 × PCR buffer (10 Mm tris-Hcl, ph 8.3, 50 mMKcl), 0.2 of 10 mM dNTPs (Sigma), 0.8 of 50 mM $Mgcl^2$, 1.0 of each 10 µM B1 gene outer primers, 0.25 µl of 5 U/µlTaq DNA polymerase (Sigma), 9.75 µl of ddH_2o, and 5 µl of DNA sample.

For the second round, in a 0.5 ml PCR tube the following solutions were placed in a total volume of 20 µl : 2.0 of 10x PCR buffer (10 Mm tris-Hcl, ph 8.3, 50 mMKcl), 0.2 of 10mM dNTPs (Sigma), 0.8 of 50 mM $Mgcl^2$, 1.0 of each 10 µM B1 gene outer primers, 0.2 µl of 5 U/µlTaq DNA polymerase (Sigma), 13.8 µl of ddH_2o, and 1.0 µl of DNA sample [3].

The amplification was performed in Hybaid thermal cycler PCR. The cycling conditions for both PCRs were 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 90 seconds, and 72°C for one minute, and a final extension at 72°C for 10 minutes. One negative control and one positive control were included for every 23 samples in each PCR. The positive controls were loaded last to avoid contamination of the sample. To guarantee the reliability of the results and detect any possible contamination, all samples were processed in duplicate. The test result was considered positive if the amplified DNA fragment was clearly visible in both samples. About 5 µl of the PCR product was subjected to electrophoresis on a 1.2% agarose gel stained with ethidium bromide [11].

Statistical Analysis

Statistical analysis was done by computer using SPSS version 13.0. Comparison between various variables was done using Chi-square (χ^2) test (significant if $p<0.05$), McNemar test, the correlation between variables was done using Spearman correlation.

RESULTS

The current study was conducted on 400 aborted women as a case study and 100 healthy women who have had clinically normal ones with previous normal full-term deliveries as a controlled study. These women ages ranged from 15-45 years with a mean age of 28.7. From September 2010-2014, blood samples of 400 aborted women and 100 healthy women who attended major Sana'a hospitals were analyzed for *T. gondii* IgG, IgM antibody using ELISA method and confirmation by nested PCR. The general characteristics of the total of 400 studied aborted women are shown in Table 1.

Table 1 Demographic data of subjects included in this study

Characters		Sample number (N=400)	Percentage (%)
Age group (years)	15-20	39	9.75%
	21-26	193	48.25%
	27-32	158	39.50%
	33-38	7	1.75%
	39-44	3	0.75%
Residence	Rural	211	52.75%
	Urban	189	47.25%
Educational Levels	Illiterate	213	53.25%
	Education	187	46.75%
Trimester of abortion	First	197	49.25%
	Second	203	51.75%
No. of abortion	One	225	56.25%
	Recurrent	175	43.75%
Socioeconomic level	low	224	56.00%
	medium	108	27.00%
	high	68	17.00%
Occupation of respondent	Housewife	248	62.00%
	Student	96	24.00%
	Employee	56	14.00%

Prevalence of Latent Toxoplasmosis using IgG

A total of 400 serum samples from Sana'a city and Sana'a trusteeship aborted women were tested by using ELISA IgG. The results showed in Figure 1 indicated that the total prevalence rate of 176 (44%) of women showed latent infection by *T. gondii*.

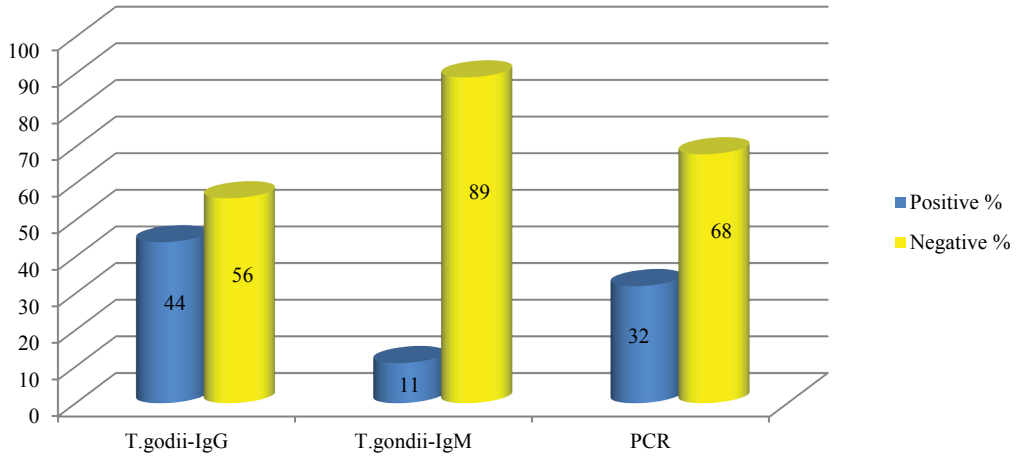

Figure 1 Prevalence of *T. gondii* IgM, IgG antibodies by using ELISA compared to positive by PCR among aborted women in Sana'a, Yemen

Detection of Acute Toxoplasmosis

Detection using ELISA IgM: A total of 400 serum samples from Sana'a city and Sana'a trusteeship aborted women were detected by using ELISA IgM. Prevalence of acute toxoplasmosis was 44 (11.0%) (Figure 1).

Confirmation of Acute Toxoplasmosis

A total of 400 cases were tested. For confirmation of these cases, PCR confirmation test was done as shown in Figure 1.

There was a significant association between seropositive for *T. gondii* IgM and abortion (χ^2=6.87, p<0.05), whereas there was no significant association between seropositive for *T. gondii* IgG, the positivity of *T. gondii* by PCR and abortion (p>0.05) (Table 2).

Table 2 *T. gondii* and abortion by using ELISA IgG, IgM, and PCR

Serological Marker		Case		Control		χ^2	p-value
		No.	%	No.	%		
IgG	Positive	176	44%	29	29%	2.9	>0.05
	Negative	224	56%	71	71%		
	Total	400	100%	100	100%		
IgM	Positive	44	11%	1	1%	6.78	<0.05*
	Negative	356	89%	99	99%		
	Total	400	100%	100	100%		
PCR	Positive	32	8%	2	2%	2.29	>0.05
	Negative	368	92%	98	98%		
	Total	400	100%	100	100%		
*: statistical significant							

There was no great difference in the prevalence rates among the 3 levels of socio-economic status, there was no statistically significant difference between 3 levels on IgM, IgG antibodies for *T. gondii*, also by PCR (p>0.05) (Figure 2). Also, there was a statistically insignificant difference in the infection according to the occupation of the respondent (p>0.05) (Figure 3).

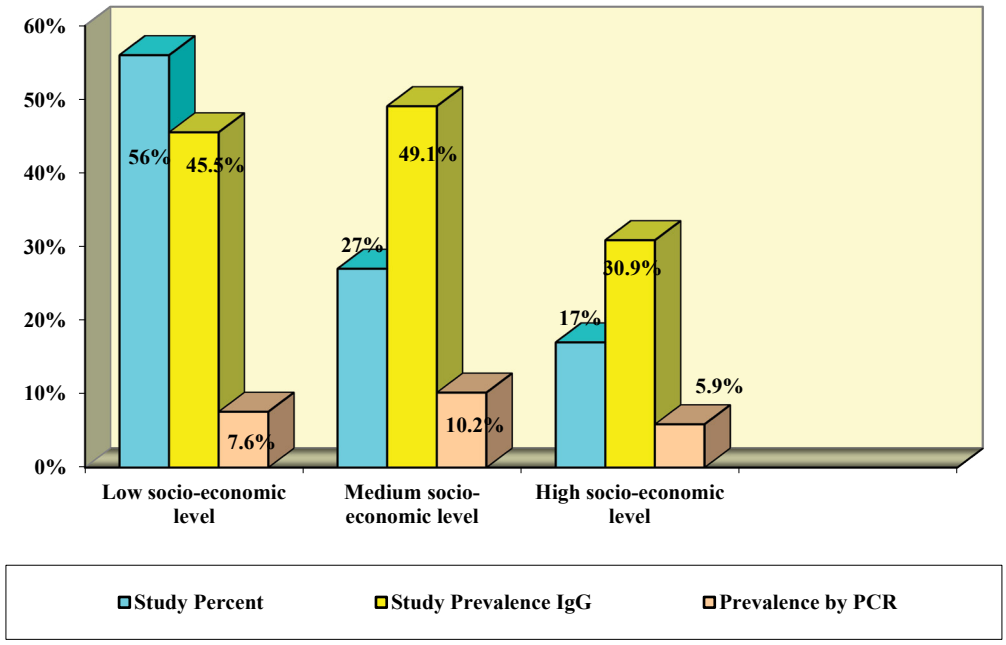

Figure 2 Distribution and prevalence of *T. gondii* according to the socioeconomic status of the respondents

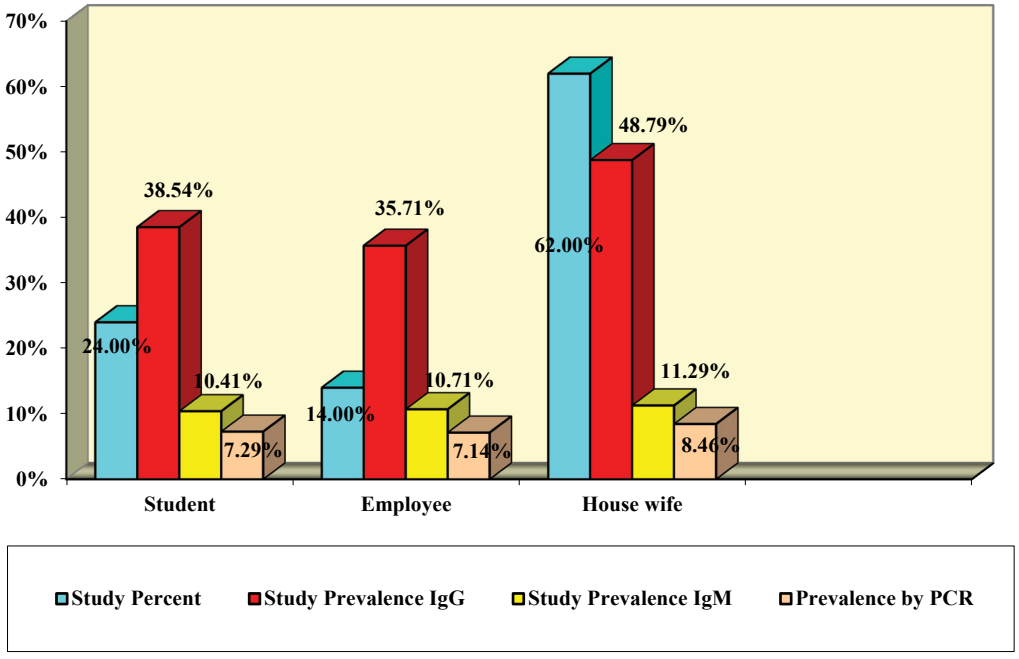

Figure 3 Distribution and prevalence of *T. gondii* according to the occupation of the respondents

There was a significant difference in the infection of *T. gondii* IgM between 3 groups: (A) One abortion; (B) Recurrent abortion; (C) Control ($p<0.05$) (Table 3).

Table 3 Distribution and prevalence of *Toxoplasma gondii* infection in three studied groups (A=one abortion; B=recurrent abortion; C=control) by ELISA (IgG, IgM) and PCR

Variable	Result	Groups			Total (N=500)	χ^2	p-value
		A (n=225)	B (n=175)	C (n=100)			
		No (%)	No (%)	No (%)			
Anti-*T. gondii* IgG	Negative	190	153	99	442	0.97	>0.05
	Equivocal	6	7	0	13		
	Positive	29 (12.90%)	15 (8.57%)	1.0 (1.00%)	45 (9.00%)		
Anti-*T. gondii IgM*	Negative	127	90	67	284	9.27	<0.05[a]
	Equivocal	5	2	4	11		
	Positive	93 (41.30%)	83 (47.40%)	29 (29.00%)	205		
PCR	Negative	205	163	98	356	3.80	>0.05
	Positive	20 (8.88%)	12 (6.85%)	2 (2.00%)	44		
a: marginally significant							

The prevalence of *T. gondii* infection in the first, second-trimester abortion and control were compared. The distribution and prevalence of IgG, IgM, and PCR are shown in Table 4. There was a significant difference in the infection by *T. gondii* IgM between 1st (first), 2nd (second) trimester of abortion compared to control group (χ^2=8.2, p<0.05) (Table 4).

Table 4 Comparison between *Toxoplasma gondii* antibodies IgM, IgG and positivity of Toxoplasmosis by PCR in relation to gestational age abortion (first, second) trimester and control

Variable	positive					Sig. between groups		
	Group	N=400	(%)	χ^2	p-value	groups	χ^2	p-value
Anti-*T.* gondii IgM	1st	197	17%	8.20	<0.05*	1st-2nd	1.55	>0.05
	2nd	203	27%			1st-C		<0.05*
	C	100	1%			2nd-2C		<0.05*
Anti-*T.* gondii IgG	1st	197	89%	2.68	>0.05	1st-2nd	5.24	>0.05
	2nd	203	87%			1st-C		>0.06
	C	100	29%			2nd-2C		>0.07
PCR result	1st	197	14%	3.58	>0.05	1st-2nd	8.20	>0.08
	2nd	203	18%			1st-C		>0.09
	C	100	2%			2nd-2C		>0.10
*: statistical significant								

Toxoplasmosis and Risk Factors

The influence of some risk factors on the prevalence of the toxoplasmosis was considered in this study, which includes: cat exposure (the presence of cats or other animals means either it inside the house, around the house, or no present), consuming raw or undercooked meat, exposure to soil, drinking unpasteurized milk, having domestic animals. There was a significant association between cat exposure, drink of unpasteurized milk, exposure to soil, have domestic animals, exposure to soil and infection with toxoplasmosis (acute and chronic) Table 5.

Table 5 *T. gondii* IgG, IgM and PCR result in relation to influence risk factors

Risk factor		Total	IgG positive (%)	χ^2	p-value	IgM positive (%)	χ^2	p-value	PCR positive (%)	χ^2	p-value
Cat exposure	Yes	329	48.32%	4.37	<0.05*	12.15%	1.40	>0.05	8.81%	7.61	<0.05*
	No	71	26.70%			5.63%			4.22%		
Consuming raw or uncooked meat	Yes	122	52.45%	2.25	>0.05	15.57%	19.70	<0.05*	13.11%	5.12	<0.05*
	No	278	40.20%			8.99%			5.75%		
Contact to soil	Yes	190	57.89%	5.19	<0.05*	14.20%	4.30	<0.05*	11.57%	4.79	<0.05*
	No	210	31.42%			8.10%			4.61%		
Drinking unpasteurized milk	Yes	156	60.89%	5.78	<0.05*	10.25%	4.19	<0.05*	7.69%	0.81	>0.05
	No	244	33.10%			11.47%			8.19%		
Having domestic animals	yes	223	46.63%	0.94	>0.05	13.45%	0.90	>0.05	11.65%	4.09	<0.05*
	No	177	40.67%			7.91%			3.38%		
*: statistical significant											

Correlation between ELISA and PCR Test for Determination of *T. gondii*

Correlation between ELISA IgM, IgG and PCR test in study group: There was a significant difference between ELISA IgM and PCR tests (χ^2=5.54, p=0.021, p<0.05) (Table 6). Also, there was a highly statistically significant difference between ELISA IgG and PCR tests (χ^2=123, p=0.0001, p<0.05) (Table 7).

Table 6 Correlation between ELISA IgM and PCR test in the study group

PCR test	ELISA-IgM			McNemar	p-value
	Positive	Negative	Total		
Positive	25	7	32	5.54	<0.05*
Negative	19	349	368		
Total	44	356	400		
*: Statistical significant					

Table 7 Correlation between ELISA IgG and PCR test in the study group

	ELISA-IgG			McNemar	p-value
PCR test	Positive	Negative	Total		
Positive	20	12	32	123	0.0001 <0.05*
Negative	156	212	368		
Total	176	224	400		
*: Statistical significant					

PCR Findings

Polymerase Chain Reaction (PCR) Test: Application of PCR test in study group revealed positivity percentage of 8.0% (32 out of 400), while 2% (2 out of 100) of healthy women (control group) were positive. No significant difference was found between the 2 groups (χ^2=2.29, p>0.05).

Amplification of the *T. gondii* B1 gene in the study group compared to the control group: Amplification of *T. gondii* B1 gene in the study group is illustrated in Figures 3 and 4. To avoid contamination in all PCR tests applied, positive control (*T. gondii*), and negative control was used, the first one was represented by a sample which was blank (no DNA). Samples 6, 8, 13, were positive by PCR when amplified, they obtained the expected band (194 bp), whereas samples 2, 3, 4, 5, 7, 9, 10, 11, 12 were negative.

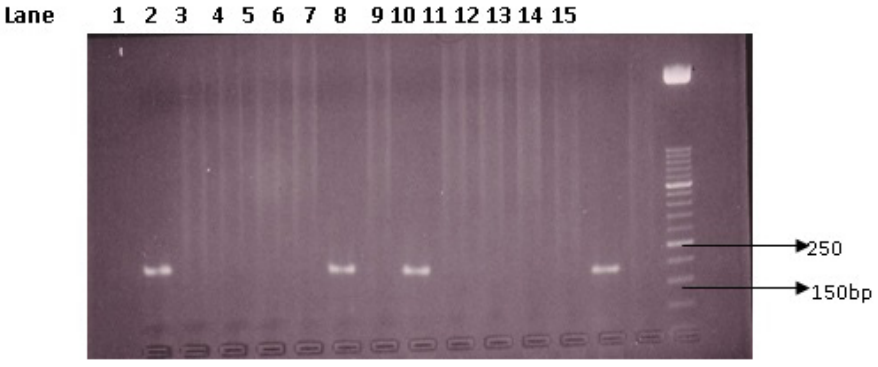

Figure 1 Amplification of *T. gondii* B1 gene in study group Lane 15: 50 bp DNA markers. Lane 14: Negative control. Lane 6, 8, 13: Positive samples. Lane 2, 3, 4, 5, 7, 9, 10, 11, 12: Negative samples, Lane 1: Positive control

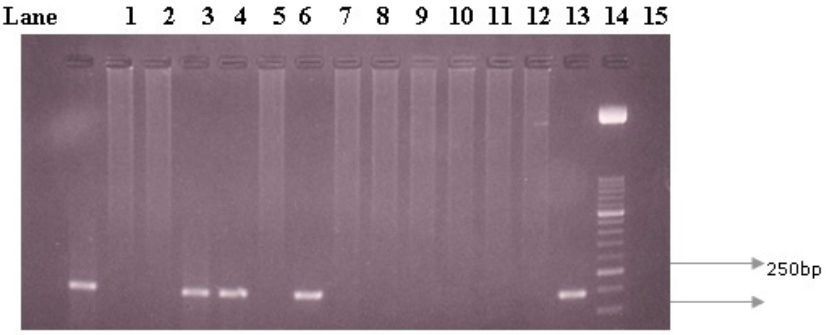

Figure 2 Amplification of *T. gondii* B1 gene in study group. Lane 15: 50-bp DNA marker. Lane 14: Positive control. Lane 13: Negative control. Lane 2, 3, 6, 8, 9, 10, 11, 12: Samples negative Lane 1, 4, 5, 7: Positive samples

Nested PCR: To confirm *T. gondii* infection, all positive samples of the first amplification were re-amplified using nested PCR on *T. gondii* B1 gene (Figure 3). They all obtained the expected fragment of 97 bp. Control positive and 2 control negatives were used so as to avoid contamination.

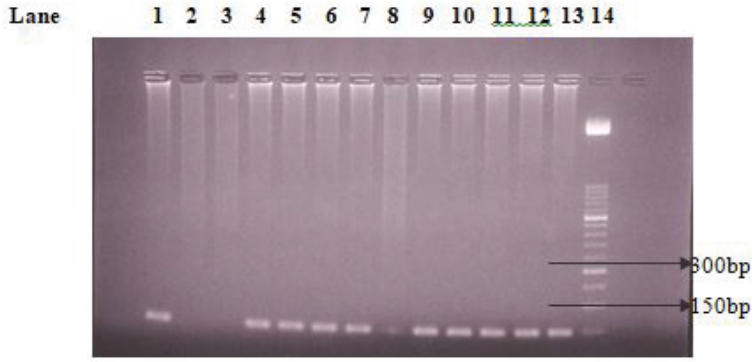

Figure 3 Nested PCR on B1 gene. Lane 1: positive control. Lane 2: negative control. Lane 3: negative control. Lane 4-13: some positive samples. Lane 14: 50-bp DNA marker

DISCUSSION

Toxoplasmosis is a serious health problem in various countries. It has a worldwide geographical distribution. Although *T. gondii* infections are asymptomatic in almost all cases, they can cause congenital toxoplasmosis in infants and acute infections in immunosuppressed patients. Toxoplasmosis can lead to a single pregnancy loss, and there are no confirmed studies to suggest that specific infections will lead to recurrent pregnancy loss in humans [12,13]. Fetal toxoplasmosis remains a significant disease as a result of acute parasitic infection in mothers not previously infected; consequences of infection are most severe if it occurs during the first trimester [14].

The current study reflects the prevalence of *T. gondii* infection among aborted women by ELISA (IgM and IgG) and confirmed the result by PCR.

The importance of the current study is related to: firstly, this is the first study pick which was done on the prevalence of *T. gondii* among aborted women in Yemen. Secondly, our study used a confirmatory test to confirm *T. gondii* infection by PCR, which was not used before, also evaluation of environmental and behavioral factors that may influence the infection rate in Yemen was done.

The present study revealed the following findings:

1. PCR was positive in 32 subjects (8.0%), and 2 of controls (2%)
2. Showed that 44 subjects (11%) had positive IgM and 1.0 of controls (1.0%) had a positive result for IgM
3. Showed that 176 subjects (44%) had positive IgG and 29 of controls (29%) had positive IgG (Figure 1)

4. Showed that there was a significant association between IgM seropositive and abortion (Table 2)
5. Showed that there was a significant association between IgM seropositive and 3 groups A, B, and C, (Table 3), also there was a significant association between IgM seropositive and trimester of abortion (Table 4)
6. Showed no significant association between socioeconomic status, occupation of respondents and positive ELISA or PCR (Figures 2 and 3)
7. Showed significant association between eating raw meat, contact with cats, contact with soil, having domestic animals, drinking of unpasteurized milk and positive ELISA or PCR (Table 5), the current study recorded no significant relation between residency, educational level and either ELISA or PCR

The recent study was partially asymptotic to Al-Nahari, et al., in Sana'a capital and Capital Trusteeship, Yemen, who found that the prevalence of *T. gondii* IgM and IgG were 11.88% and 41.90% respectively [15]. Our results were lower than that reported by Kotina E, among pregnant women who had a history of abortion in Sana'a, Yemen which was 55.5% for anti-IgG and 16.7% for anti-IgM [16].

Our findings were higher than those reported by Ghazi, et al., [17]. Conversely showed the highest positive reactions for *T. gondii* antibodies, in another countries/region in women or general people or another animal as reported by Decavalas, et al., [18], in Greece 52.3%, higher prevalence rates in some Arab countries like Kuwait (58.2%) [19].

The variation of the prevalence of *T. gondii* antibodies in Sana'a capital and Capital Trusteeship, may be due to socioeconomic and behavior differences, or it is reasonable to assume that animals their living in the first floor from Yemeni homes, to contact with cats, as well as much women working in breeding animals and milking it in their homes, drinking unpasteurized milk, contact with soil which play a roles in acquiring the disease and hence different serological patterns appear [20]. It is also known that various serological test may produce different results because of the inherent sensitivity differences between serological tests [21].

Regional variations in the incidence of *T. gondii* infection rates from one country to another or even within the same country has been well documented. This variation has been attributed to climate, cultural differences regarding hygienic and feeding habits. The frequency of stray cats in a humid rainy climate favoring the survival of oocyst has contributed to the high *T. gondii* prevalence in Central America [22]. Stray cats are widely spread in Sana'a city, the cold and humidity or rainfall weather conditions are ideal for oocyst survival, compared to hot and dry environmental conditions, which are in favor of higher prevalence. Farming and animal rearing are also common [23].

Many studies were done worldwide looking for the correlation between *T. gondii* infection and abortion. Compared to studies done in Yemen our findings agree with Hassan who found that there was a statistical association between women who had a history of abortion and *T. gondii* IgM antibody seropositivity [24].

As regards the age of aborted women with *T. gondii*; our study showed no significant correlation between the age and the positivity of PCR or ELISA, although there was a slightly increase in the older age group. Our results agree with Kotina but disagree with Remington, et al.; Al-Shaebi; Al-Nahari; and Hassan [15,16,22,24,25].

The present study agrees with Hassan; Al-Shaebi; Elamin, et al.; Naga, et al.; Jenum, et al., [24-28], whom reported that the incidence of *T. gondii* for women living in urban areas was not significantly different from that living in rural areas, also the incidence of *T. gondii* for uneducated women was not significantly different from that educated, although there was a slight increase in the infection among uneducated women.

The current study showed that the socioeconomic level didn't play a role in the infection of *T. gondii*, although there was a slight increase rate in the low socioeconomic level. Our results were supported by Elamin, in contrast to Al-Nahari in Yemen [15,26], who reported that the infection with *T. gondii* increased among pregnant of low socio-economic level, he attributed their results to that, lower classes were drinking unfiltered water contaminated with *T. gondii* oocysts.

In the current study, there was a significant difference, in the serum level of *T. gondii* specific IgM among the 3 investigated patients groups (A-one abortion B-recurrent abortion C-control). In Yemen, Hassan showed that there was a significant difference. Our results agree with Mohymenet, al., [29].

Recent study showed that there was a significant difference in the serum level of *T. gondii* IgM in relation to trimester

of abortion (first and second) compared to control, there was a significant difference in the serum level IgM between groups first compared to control and second trimester compared to control, whereas there was no significant difference between first and second trimester. It has been proposed that during pregnancy, the systemic maternal immune response is biased in favor of Th2 cytokine [29,30].

In the current study, the risk factor strongly associated with acute infection in PCR-positive women in contact with cats, contact with soil, consuming raw or uncooked meat, having domestic animals. Naga, et al., [27], reported that the risk factor strongly associated with acute infection in PCR-positive women in contact with soil, although eating raw meats and contact with cats are also associated risk factors. Nimriet, al., found that the risk factors strongly associated with acute infection in the IgG-positive and PCR-positive women were eating raw meat and contact with soil [31]. Cook, et al., reported that risk factors most strongly predictive of acute infection in pregnant women were eating undercooked lamb, beef or game and contact with soil, and that contact with cats was not a risk factor [32].

The current study reported that, 56.8% of IgM positive were PCR positive, our results are slightly lower than the result reported by Vado-Solís, et al., in Mexico who reported that 65% of IgM positive were PCR positive [33]; whereas our result disagrees with Elamin who found that all IgM positive were PCR positive [26]; Khalil found that, all IgM positive were negative PCR among aborted women in Gazira.

In the recent study, most samples negative with ELISA IgG test in the study group, were negative by PCR 94.7%. The findings partially agree with Vado-Solís, et al., Elamin, et al., who found that all IgG negative samples of pregnant women were negative by PCR [26,33]. These results could partially be explained by the presence of a well-developed humoral immune response among those women, as it was found to attenuate or kill the circulating parasites rapidly, but the genetic components of the cells are not immediately destroyed [34].

CONCLUSION

The current study showed the need for a confirmatory test to determine acute toxoplasmosis in pregnant women. Confirmatory testing for on-going or recent Toxoplasma infection with the ELISA IgG/ IgM antibody test in pregnant women has the potential to decrease the need for follow-up sera and for unnecessary therapeutic interventions in pregnant women. It demonstrates the possibility of defining and selecting the high-risk cases for mother-to-child transmission of infection by combining specific serology and PCR tests to formulate a specific approach.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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