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Prevalence of *Toxoplasma gondii* in Women Population of Rafha City, Saudi Arabia

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ABSTRACT

Toxoplasmosis, a disease caused by *Toxoplasma gondii* parasite, is one of the most worldwide parasitic infections of warm-blooded animals including man. In most adults it does not cause serious illness, but it can cause serious health problems in infants, pregnant women and immune compromised patients. Abortion is one of the most serious outcomes in pregnant women. In this cross-sectional study, a total of 162 women of child bearing age were included from Rafha city of Saudi Arabia. Demographic data was recorded. Specific *Toxoplasma gondii* IgG and IgM were detected in serum by ELISA method. *Toxoplasma gondii* DNA was detected using PCR. Most women were in the 20-29 years age group (48%), followed by 30-39 years (33%) and >40 years (14%). Overall prevalence of *Toxoplasma gondii* IgG was seen in 12%, with significantly more in women with age more than 30 years as compared to those with age less than 30 years (19% vs 8%; p=0.038). The percentage of IgG seroprevalence was higher in women who ever got pregnant as compared to those who were never pregnant (13% vs 5%). The percentage was also higher in women with direct contact to soil (14% vs 5%). Only one sample was PCR positive. The results show that overall prevalence of *Toxoplasma gondii* infection in young women of Rafha city is low as compared to other regions of Saudi Arabia.

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Authors' Contribution
AA contributed in designing the study, sample collection, lab work, acquisition, analysis and interpretation of data and drafting the article. KM and FT contributed in conception and designing the study and drafting and revising the article.

Key words
***Toxoplasma gondii*, Seroprevalence, IgG, IgM, ELISA, Polymerase chain reaction.**

INTRODUCTION

Toxoplasma gondii is a single-celled, obligate intracellular protozoan parasite that causes the disease toxoplasmosis (Dardé *et al*., 2011). *T. gondii* can infect virtually all warm-blooded animals (Skariah *et al*., 2010), but domestic cats are the main hosts (CDC, 2016). *Toxoplasmosis* is one of the most common infections in developed countries; more than 60 million people in the United States may be infected with the *Toxoplasma* parasite (Cook *et al*., 2000; Jones *et al*., 2001). Among the infections causing food-borne deaths, Toxoplasmosis stands third after salmonellosis and listeriosis (Dubey and Jones, 2008). It has been estimated that 30–50% of the world population has been exposed to the parasite and a huge fraction may be chronically infected (Flegr *et al*., 2014; Pappas *et al*., 2009).

The lifecycle of *T. gondii* consists of two phases. The sexual phase takes place only within cats (felids, wild or domestic). The asexual phase can take place in all warm-blooded animals which are defined as intermediate hosts (Dubey and Jones, 2008; Hill and Dubey, 2002). In the epithelial cells of cat, the parasite sexual produces millions of thick-walled oocysts which are released in the feces (Dubey, 2009). When a warm-blooded animal ingests these oocytes, the sporozoites are released. These sporozoites differentiate into active and quickly proliferating tachyzoites (Dubey *et al*., 2011). Under the influence of the host's immune system, the tachyzoites are converted to bradyzoites which form clusters. These clusters are called tissue cysts (Weiss and Kim, 2011). Tissue cysts are mostly observed in the brain, the eyes, and striated muscle (including the heart) approximately 7–10 days after initial infection (Dubey *et al*., 1998). The cysts can persist for the life time of the infected host (Weiss and Kim, 2011).

A *Toxoplasma* infection can occur by consuming undercooked, contaminated meat, food and water (Jones and Dubey, 2012). The parasite may also be transmitted from mother to child; receiving an infected organ transplant or infected blood via transfusion (Paquet and Yudin, 2013).

Among the infected people, very few have mild, flu-like symptoms; majority of infected people do not show any observable symptoms. However, in infants, pregnant women and immune compromised patients the infection can cause serious health problems (Paquet and Yudin, 2013). If pregnant mother is infected, the parasite can move to unborn fetus via the placenta resulting in congenital

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toxoplasmosis. This can result in with fetal death and abortion. In infants, congenital toxoplasmosis is associated with neurologic defects, neurocognitive defects, and chorioretinitis resulting in serious symptoms like blindness or mental disability (Torgerson and Mastroiacovo, 2013).

MATERIALS AND METHODS

Ethics statement

Present study was approved by the Ethics Committee of Northern Border University, ArAr, Kingdom of Saudi Arabia (Approval No. 3-11-1436-5). The samples were collected strictly according to the recommendations of the committee. Informed consents were taken from each participant.

Collection of samples

The present study was carried out at faculty of pharmacy, Northern Border University Rafha branch. A total of 162 females of child bearing age (16-45) were included in this study. Samples were collected from Rafha Central Hospital. Blood (1 to 2 mL) and urine samples collected in appropriate containers. The demographic data was recorded on prescribed forms.

Serological detection of T. gondii *infection*

The serum IgM and IgG antibody (Ab) levels against *T. gondii* antigens were measured using the UDI (United Diagnostic Industries, KSA) *Toxoplasma IgM ELISA* kit for direct IgM antibody detection (REF EM127) and *Toxoplasma IgG ELISA* kit for direct IgG antibody detection (REF EG127). Both the kits are based on the classical ELISA technique. The manufacturer's recommended protocols were followed. Serum/plasma samples were diluted to a ratio of 1:101 with sample diluent buffer. Blank and calibrators were also run along with samples. For both IgG and IgM ELISA assays, optical density (OD) was measured at 450 nm (reference filter 620 nm). The ratios between the OD value of unknown samples and that of calibrator were calculated. Any sample with the ratio equal or more than 1.100 was considered positive. On the other hand, if the ration was less than 0.900, the sample was supposed to be negative.

PCR detection of T. gondii *infection*

Isolation of DNA

The genomic DNA from blood samples were extracted using the solution based blood DNA preparation kit (Jena Biosciences, Germany, Cat# PP205S) according to the manufacturers' instructions. The DNA pellet was dissolved in 70 µl of DNA Hydration Solution and stored at -20 ^{0}C.

For DNA extraction from urine, the urine samples were first centrifuged at 5000 rpm for 10 min to pellet down the parasite. Almost all the supernatant was discarded with only 300 µl of residual liquid, mixed vigorously with pipette and used for DNA extraction using above procedure.

PCR amplification

Two PCR approaches were used to amplify target; single PCR targeting B1 gene (GenBank accession number AF179871); Nested PCR targeting RE region (GenBank accession number AF146527). The oligonucleotide primers used for the amplification of the B1 and RE PCR are shown in Table I.

Positive control

ABI *Toxoplasma gondii* (RH strain) quantitated DNA (Catalog #: 08-948-250) was used as positive control. According to the manufacturers descriptions, this positive control contained 16000 copies of *Toxoplasma gondii* (RH strain) genomic DNA per microliter. A total of 8 fresh serial dilutions were made containing 16000 (provided), 1600, 160, 16, 8, 4, 2 and 1 copy per microliter in distilled water immediately before use.

Table I.- The oligonucleotide primers used for the PCR amplification.

Gene		Primer sequence	Product size
B1 gene			
	B1F	5'–CTCAAGGAGGACTGGCAACC-3'	250 bp
	B1R	5'–GCCTCTCGACCGGAACTTTT-3'	
RE region			
First Round	REF	5'-GATGAAGGCGACGGTGAGGA–3'	267 bp
	RER	5'-CGCTGCAGACACAGTGCATCT–3'	
Second Round	F3	5'-GAAGCGACGAGAGTCGGAGA – 3'	205bp
	B3	5'-GATTCCTCTCCTACGCCTCCT – 3'	

For the detection of *T. gondii* DNA in this study, human blood samples were used for DNA extraction; the human genomic DNA is also extracted with the expected *T. gondii* DNA which obviously is the major part. This human DNA can affect the sensitivity and specificity of the PCR. In order to study this, about 30ng of human genomic DNA was also added to each PCR in separate PCR reactions when using the various dilutions of ABI *Toxoplasma gondii* (RH strain) quantitated DNA as positive control.

Amplification of the B1 gene

Single round amplification: In B1 PCR, a 250 base pair fragment covering *B1 gene* was amplified. The total 20µl PCR mixture contained 1X PCR buffer (Thermo Scientific) , 1.5mM $MgCl_2$, 0.2mM of each dNTPs, 0.5µM each primer (B1F, B1R, Table I), 0.5 units Taq polymerase (Thermo Scientific) and 1µL of extracted DNA. Cycling conditions for PCR were: Initial denaturation at 95°C for 5 min and then 40 cycles each of 95°C for 10 seconds, 56°C for 20 seconds and 72°C for 30 seconds, followed by a final extension at 72°C for 5 min. A known negative control contained sterile water. The positive control is described above. Five microliters of each PCR product were run on a 2% agarose gel in 1X TBE buffer to confirm the presence of PCR product.

Amplification of the RE region

Nested PCR approach was used to amplify the RE gene as follows:

First round amplification: A 267bp selected region of the RE region of the parasite was amplified in the first round. Each 20µL PCR reaction contained 1X PCR buffer (Thermo Scientific), 1.5mM $MgCl_2$ 0.2mM of each dNTPs, 0.5µM each primer (REF, RER, Table I), 0.5 units Taq polymerase (Thermo Scientific) and 1µL of extracted DNA. Cycling conditions for PCR were: Initial denaturation at 95°C for 5 min and then 35 cycles each of 95°C for 20 seconds, 56°C for 30 seconds and 72°C for 40 seconds, followed by a final extension at 72°C for 5 min. A known negative control containing sterile water and a positive sample containing *T. gondii* DNA was also amplified along with patient samples.

Second round amplification: One microliter of the first round PCR product was used as template in the second round. The 20µl PCR mixture contained 1X PCR buffer (Thermo Scientific), 1.5mM $MgCl_2$ 0.2mM of each dNTPs, 0.5µM each primer (F3, B3, Table I), 0.5 units Taq polymerase (Thermo Scientific). Cycling conditions for PCR were: Initial denaturation at 95°C for 1 min and then 40 cycles each of 95°C for 10 seconds, 60°C for 15 seconds and 72°C for 20 seconds, followed by a final extension at 72°C for 5 min. Negative and positive control PCR from first-round amplified product were used in the second round negative and positive controls respectively. The size of the second round PCR product was 205bp. Finally, 5ul of each of positive and negative controls along with unknown samples were run on 2% agarose gel containing ethidium bromide and observed under UV light. A known molecular weight 50bp DNA marker was included in each run.

Table II.- The demographic data of the cases included.

	Total (162)	IgG Positive (n=19)	IgG Negative (n=143)	*p* Value
Nationality				
Saudi (%)	124 (77)	15 (12)	109 (88)	0.79
Non-Saudi (%)	38 (23)	4 (11)	34 (89)	
Age (years)				
<30 (%)	103 (64)	8 (8)	95 (92)	0.038
>30 (%)	59 (34)	11 (19)	48 (81)	
Pregnancy				
Ever (%)	140 (86)	18 (13)	122 (87)	0.26
Never (%)	22 (14)	1 (5)	21 (95)	
Abortion (No. of women)				
Abortion (%)	30 (19)	6 (20)	24 (80)	0.12
Never (%)	132 (81)	13 (10)	119 (90)	
Water source				
Home filtered (%)	79 (49)	11 (14)	68 (86)	0.4
Bottled (%)	83 (51)	8 (10)	75 (90)	
Drinking milk				
Fresh (%)	29 (18)	1 (3)	28 (97)	0.13
Bottled (%)	133 (82)	18 (14)	115 (86)	
Type of meat consumed				
Camel (%)	53 (33)	4 (8)	49 (92)	0.38
Beef (%)	33 (20)	3 (9)	30 (91)	
Mutton (%)	73 (45)	11 (15)	62 (85)	
Non	3 (2)	1 (33)	2 (67)	
Frequency of eating meet				
Daily (%)	109 (67)	14 (13)	95 (87)	0.37
Weekly (%)	50 (31)	4 (8)	46 (92)	
Never (%)	3 (2)	1 (33)	2 (67)	
Meat preference				
Partially cooked (%)	36 (22)	2 (6)	34 (94)	0.24
Completely cooked (%)	123 (76)	16 (13)	107 (87)	
Vegetarian (%)	3 (2)	1 (33)	2 (67)	
Contact with cats				
Yes (%)	90 (56)	12 (13)	78 (87)	0.48
No (%)	72 (44)	7 (10)	65 (90)	
Direct contact with soil				
Yes (%)	88 (54)	14 (16)	74 (84)	0.08
No (%)	74 (46)	5 (7)	67 (93)	
Dusting / Cleaning at home				
Yes (%)	120 (74)	16 (13)	104 (87)	0.28
No (%)	42 (26)	3 (7)	39 (93)	

Statistical analysis

Data were recorded, validated and analyzed for average and percentage prevalence between groups. Chi square test were used as tests of significance at 5 % level of significance. The *P* values were calculated online at following website http://www.quantpsy.org/chisq/chisq.htm

RESULTS

Out of 162 women of child bearing age included in this study, 12% were IgG positive for *Toxoplasma gondii*. None of them was positive for *Toxoplasma gondii* IgM. Based upon IgG reactivity, two groups were made (Table II). Among the participants, 124 were Saudi national while 38 belonged to different nationalities including Pakistanis, Indians, and Sudanese. The prevalence of *Toxoplasma gondii* IgG among Saudi and non-Saudi women was almost the same (p=0.79). Two age groups were made and compared for the seroprevalence the IgG. It was found that the seroprevalence was significantly high in women with more than 30 years of age (p=0.038). Insignificantly higher incident was seen in women who ever got pregnant than those who were never pregnant (13% vs 5%, p=0.26). Although higher percentage of IgG seroprevalent cases was seen in women who ever had abortion than those who never experienced it, the correlation was not significant (20% vs 10v%, p=0.12). Other parameters also showed the similar correlation with seroprevalence of *T. gondii* IgG (Table II). Average number of pregnancies among seropositive women was slightly higher than the seronegative women (5.1 *vs* 4.25; p=0.5; data not shown in Table II).

Selection of the suitable PCR approach

To compare and select the better of the two, B1 and RE PCR were performed using serial dilutions of the positive control as described in methods. These dilutions were used as template to amplify a fragment of B1 gene (single round) and RE region (nested). The BI PCR was designed as a single round PCR as it is believed to be sensitive enough to detect a single copy of *T. gondii* DNA from human samples (Jones *et al*., 2000).

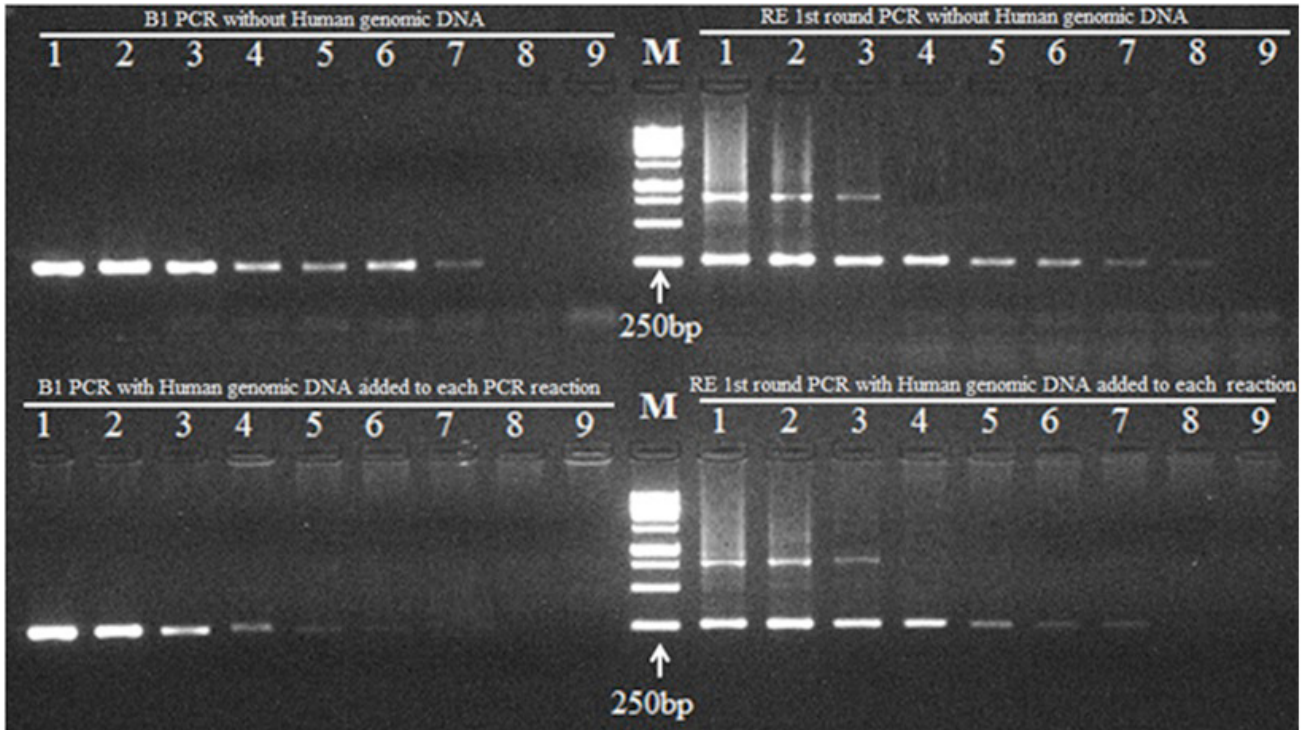

Fig. 1. PCR optimization of B1 and RE (first round) reactions. Serial dilutions of the ABI *Toxoplasma gondii* (RH strain) quantitated DNA were used for PCR in both B1 and RE reactions. Lane 1, PCR reaction using 16000 copies of *T. gondii* genomic DNA (provided by supplier); Lane 2, 1600 copies *T. gondii* genomic DNA; Lane 3, 160 copies *T. gondii* genomic DNA; Lane 4, 16 copies *T. gondii* genomic DNA; Lane 5, 8 copies *T. gondii* genomic DNA; Lane 6, 4 copies *T. gondii* genomic DNA; Lane 7, 2 copies *T. gondii* genomic DNA; Lane 8, 1 copy *T. gondii* genomic DNA; Lane 9, negative control. The PCR products of RE first round with and without human genomic DNA were used as template for RE second round PCR. M, DNA Marker (SolisBiodyne 1Kb DNA ladder, cat# DM010-R500).

Sensitivity and specificity of the B1 gene PCR

The B1 PCR showed a single amplicon of 250bp on 2% agarose gel. Using different dilutions of the positive control, this reaction was capable of detecting 2 copies and more of *T. gondii* DNA (Fig. 1). It could not however, detect single copy of the target genome. It has been estimated that one copy of *T. gondii* contains ~50fg DNA which means that B1 PCR was able to detect 100 fg of Toxoplasma DNA. It was seen that the added human genomic DNA (30ng) decreased the sensitivity of the B1 PCR to 4 copies per microliter. It did not however, affect the specificity of the B1 PCR as a single amplicon of the same size was observed in this case too.

Sensitivity and specificity of the RE PCR

First round

The RE first round showed a 266bp amplified product on 2% agarose gel. Using different dilutions of the positive control, the sensitivity of the RE first round PCR was higher than that of B1 PCR as it detected single copy of the target DNA (Fig. 1). However, at higher concentrations of the target DNA (16000, 1600 and 160copies /ul) it showed nonspecific PCR bands on agarose gel along with the specific one. The nonspecific band(s) fainted at lower concentrations. No extra efforts were done to decrease the non-specificity as it could decrease the sensitivity of the assay too. The added human genomic DNA did not affect the specificity of the RE first round PCR but it decreased the sensitivity of the assay to 2 copies per microliter (Fig. 1). This time it could not detect single copy of the target DNA.

Second round

The second round PCR amplification was done using first round amplicons as a template with and without added human genomic DNA in the first round. It is to be noted that no human genomic DNA was added in the second round. The second round PCR showed extra bands and smear in reactions with high initial copy number of the target DNA added in the first round. In case of smaller initial copy number, little or no extra bands were observed (Fig. 2). Rather, a single sharp PCR band was seen in these cases. In this way this represent a semi quantitative PCR. So no efforts were done to get rid of the extra bands/ smear. The sensitivity of this round was also higher than first round as the last dilution with single copy number (with added human genomic DNA) which was negative in the first round produced a good sharp single band in the second round.

Patient samples

The RE nested PCR approach was finally selected for the PCR amplification/detection of *Toxoplasma gondii* DNA in the samples. Known negative and positive controls were also run in each PCR batch. For positive control, the dilution with intermediate copy number (16 copies/µl) was used. Out of all 162 samples, only one was PCR positive in DNA sample extracted from blood (Fig. 3). It was however, negative in DNA extracted from urine.

DISCUSSION

The copy number (repeats) of B1 gene within genome varies from 3 to 7 (Costa and Bretagne, 2012) depending on the type of strain while previously it was estimated to be around 35 (Wahab *et al*., 2010; Correia *et al*., 2010). The copy number of RE region varies from 30 to 60 while others have reported it to be 200 to 300 (da Silva *et al*., 2011; Costa and Bretagne, 2012).

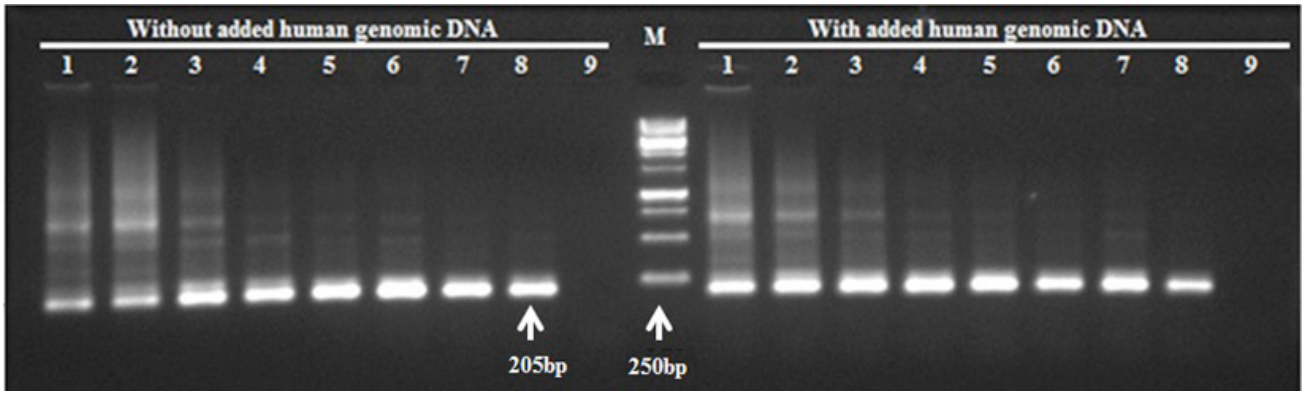

Fig. 2. The RE second round PCR products; Lanes 1 to 8 on left represent PCR amplification using first round PCR amplified without human genomic DNA; Lane 9, negative control; Lanes 1-8 on the right represent PCR amplification using first round PCR amplified with added human genomic DNA; Lane 9, negative control; M, DNA marker (SolisBiodyne 1Kb DNA ladder, cat# DM010-R500).

Table III.- *T. gondii* seroprevalence studies done in Saudi Arabia.

Research Group	Total samples	IgG +ve (%)	IgM +ve (%)	PCR +ve (%)	Region
Alghamdi *et al*. (2016)	203	32.5	6.5	22.2	Riyadh
Almogren (2011)	2176	38	zero	--	Riyadh
Bin Dajem and Almushait (2012)	137	38.6	6.5	41	Aseer
Al-Mohammad *et al*. (2010)	554	51	9	--	Al Ahsa
Aqeely *et al*. (2014)	195	20	6.2	--	Jazan
Eida (2015)	226	27	13	3.5	Jazan
Almushait *et al*. (2014)	487	38.8	6.2	--	Southwest
El-Shahawy *et al*. (2014)	96	29.2	3.1	--	Najran
Imam *et al*. (2016)	150	21	0	--	Madina

For the detection of *T. gondii*, the most frequently used sequence is the B1 gene, first identified by Burg *et al*. (1989). Although Jones *et al*. (2000) reported that the BI PCR is sensitive enough to detect a single copy of *T. gondii* DNA; our protocol detected at least 2 copies of template DNA in a single round. With Human genomic DNA added to the B1 PCR, the detection limit further increased to 4 copies of the genome which is obvious. Yamada *et al*. (2011) reported the detection limit of B1 assay in a single PCR to be 100 copy numbers repeats of B1 gene (around 20 copies of the template DNA) which decreased to 10 copy number (around 2 copies of the template DNA) in the second round (nested PCR). In a study by Kong *et al*. (2012), the detection limit of the RE nested PCR was reported to be 600fg (around 12 copies of the genome) of the template DNA. We performed much better as we were able to detect single copy of the genome using RE nested PCR. Jones *et al*. (2000) reported that the addition of human lymphocyte DNA (10–150 ng) does not inhibit the B1 PCR amplification (193-bp PCR product) from 1 ng purified *T. gondii* genomic DNA. In the current study we found that the addition of human lymphocyte DNA (30 ng) decreases the sensitivity of B1 (250bp) and RE PCR amplification (266-bp) especially from lesser amount of purified *T. gondii* genomic DNA.

The prevalence of worldwide *T. gondii* infection in human varies from region to region ranging from less than 10% to over 90% (Bouratbine *et al*., 2001; Torgerson and Mastroiacovo, 2013). In Saudi Arabia, different studies have reported the seroprevalence of *T. godii* IgG ranging from 21 to 38.6% while that of IgM ranging from zero to 09% (Table III). In the current study, the overall seroprevalence of *T. gondii* IgG was low (12%). This shows that overall prevalence of *T. gondii* infection in this part of Saudi Arabia is low. All the samples were negative for *T. gondii* IgM. This means that there was no recent infection among the cases and all the IgG positive cases acquired the infection long ago. This is also evident from the PCR results as all the IgG positive cases were negative for *T. gondii* DNA. In Saudi Arabia, two studies have reported same results for IgM (M).

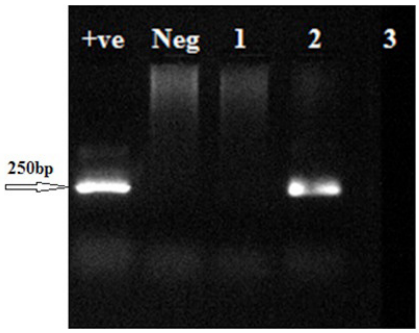

Fig. 3. The representative agarose gel electrophoresis of RE second round PCR products using patient's samples; Lanes 1 to 3, patient samples.

Higher seroprevalence of *T. gondii* IgG (> 50%) is seen in countries where people use raw or poorly cooked meat and in tropical regions with lots of cats and more favourable climate for the survival of *T. gondii* oocysts (Cook *et al*., 2000; Jones *et al*., 2001; Di Carlo *et al*., 2008). In the United States, 15% of women at child bearing age (15 to 44) have *T. gondii*, infection. The most common sources of acquiring infection are exposure to cats or soil (gardening without gloves), drinking contaminated water or consuming raw or undercooked meat (Skariah *et al*., 2010; Elmore *et al*., 2010). Transfusion or organ transplantation from an infected person can also be a source of infection (Dubey and Jones, 2008). The climate of Saudi Arabia is mostly hot and dry so it does not favour the survival of *T. gondii* oocysts. Traditionally most of the people do not like to keep pet cats. They like to eat thoroughly cooked meat. In the current study all the above mentioned factors and many more were correlated with the seroprevalence of the infection (Table II). Significantly high IgG seroprevalence

was seen in the age group >30 years. This was in accordance with a study conducted in India in 2014 in which a higher incidence (41%) of IgG positivity was seen in age group >40years (Singh *et al*., 2014). They also reported higher prevalence in married women and in people living in mud houses. In the current study, higher percentage of IgG seroprevalence was seen in women who ever got pregnant than those who were never pregnant (13% vs 5%) but this was statistically insignificant (p=0.26). The percentage of IgG positive cases gradually increased with increasing number of pregnancies (Fig. 4). In Saudi Arabia, majority of people do not live in mud houses. So, we compared seroprevalence between women who are exposed to dust/soil and those who are not. Women with direct contact to soil had higher percentage of IgG seroprevalence than those who were not (14% *vs*. 5%). Similar trend was seen in women who do regular dusting/cleaning at home. As discussed earlier, cats are considered as one of the sources of *T. gondii* infection but this was not seen in the current study.

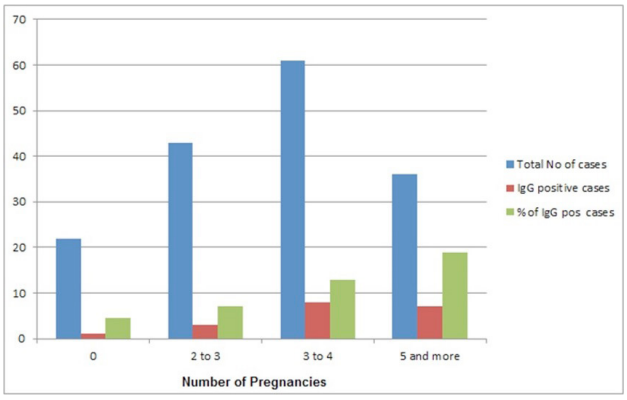

Fig. 4. The percentage of IgG positive cases with increasing number of pregnancies.

No significant association between seroprevalence and other factors was observed; the reason could be smaller number of samples. A future study with larger number of samples may reveal more interesting facts about the topic in this region.

CONCLUSION

Although antibodies against *T. gondii* (IgG and IgM) were seen in some study participants, the overall prevalence of *T. gondii* active infection in young women of Rafha city was very low as compared to other regions of Saudi Arabia and other parts of the world. The ministry of health is providing compulsory active immunization against many infectious diseases including toxoplasmosis. Considering the results of the current study, we hope that the disease will be eradicated from the region very soon.

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Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Alghamdi, J., Elamin, M.H. and Alhabib, S., 2016. Prevalence and genotyping of *Toxoplasma gondii* among Saudi pregnant women in Saudi Arabia. *Saudi pharm. J.*, **24**: 645-651. https://doi.org/10.1016/j.jsps.2015.05.001

Almogren, A., 2011. Antenatal screening for *Toxoplasma gondii* infection at a tertiary care hospital in Riyadh, Saudi Arabia. *Annls. Saudi Med.,* **31**: 569-572. https://doi.org/10.4103/0256-4947.87090

Al-Mohammad, H.I., Amin, T.T., Balaha, M.H. and Al Moghannum, M.S., 2010. Toxoplasmosis among the pregnant women attending a Saudi maternity hospital: seroprevalence and possible risk factors. *Annls. trop. Med. Parasitol.,* **104**: 493-504. https://doi.org/10.1179/136485910X12786389891443

Almushait, M.A., Bin Dajem, S.M., Elsherbiny, N.M., Eskandar, M.A., Al Azraqi, T.A. and Makhlouf, L.M., 2014. Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women in south western, Saudi Arabia. *J. Parasit. Dis.,* **38**: 4-10. https://doi.org/10.1007/s12639-012-0195-z

Aqeely, H., El-Gayar, E.M., Khan, D.P., Najmi, A., Alvi, A., Bani, I., Mahfouz, M.S., Abdalla, S.E. and Elhassan, I.M., 2014. Seroepidemiology of *Toxoplasma gondii* amongst pregnant women in Jazan Province, Saudi Arabia. *J. trop. Med.,* **2014**: Article ID 913950. https://doi.org/10.1155/2014/913950

Bin Dajem, S.M. and Almushait, M.A., 2012. Detection of *Toxoplasma gondii* DNA by PCR in blood samples collected from pregnant Saudi women

from the Aseer region, Saudi Arabia. *Annls. Saudi Med*., **32**: 507-512. https://doi.org/10.5144/0256-4947.2012.14.7.1200

Bouratbine, A., Siala, E., Chahed, M.K., Aoun, K. and Ben Ismail, R., 2001. Sero-epidemiologic profile of toxoplasmosis in northern Tunisia. *Parasite,* **8**: 61-66. https://doi.org/10.1051/parasite/2001081061

Burg, J.L., Grover, C.M., Pouletty, P. and Boothroyd, J.C., 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *J. clin. Microbiol.,* **27**: 1787-1792.

CDC, 2015. *Toxoplasmosis - biology.* Last Updated March 2015, Retrieved on Dec 2016, http://www.cdc.gov/parasites/toxoplasmosis/epi.html

Cook, A.J., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Foulon, W., Semprini, A.E. and Dunn, D.T., 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. *Br. med. J.,* **321**: 142-147. https://doi.org/10.1136/bmj.321.7254.142

Correia, C.C., Melo, H.R. and Costa, V.M., 2010. Influence of neurotoxoplasmosis characteristics on real-time PCR sensitivity among AIDS patients in Brazil. *Trans. R. Soc. trop. Med. Hyg.,* **104**: 24-28. https://doi.org/10.1016/j.trstmh.2009.06.012

Costa, J.M. and Bretagne, S., 2012. Variation of *B1* gene and AF146527 repeat element copy numbers according to *Toxoplasma gondii* strains assessed using real-time quantitative PCR. *J. clin. Microbiol*., **50**: 1452-1454. https://doi.org/10.1128/JCM.06514-11

da Silva, R.C., Langoni, H., Su, C. and da Silva, A.V., 2011. Genotypic characterization of *Toxoplasma gondii* in sheep from Brazilian slaughterhouses: new atypical genotypes and the clonal type II strain identified. *Vet. Parasitol.,* **175**: 173-177. https://doi.org/10.1016/j.vetpar.2010.09.021

Dardé, M.L., Ajzenberg, D. and Smith, J., 2011. Population structure and epidemiology of *Toxoplasma gondii*. In: Toxoplasma Gondii: *The model apicomplexan. Perspectives and methods* (eds. L.M. Weiss and, K. Kim), Academic Press, Elsevier, London, pp. 49-80.

Di Carlo, P., Romano, A., Schimmenti, M.G., Mazzola, A. and Titone, L., 2008. Maternofetal *Toxoplasma gondii* infection: critical review of available diagnostic methods. *Infez. Med*. **16**: 28-32.

Dubey, J.P., Lindsay, D.S. and Speer, C.A., 1998. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin. Microbiol. Rev*., **11**: 267-299.

Dubey, J.P. and Jones, J.L., 2008. *Toxoplasma gondii* infection in humans and animals in the United States. *Int. J. Parasitol.,* **38**:1257-1278. https://doi.org/10.1016/j.ijpara.2008.03.007

Dubey, J.P., 2009. History of the discovery of the life cycle of *Toxoplasma gondii*. *Int. J. Parasitol.,* **39**: 877-882. https://doi.org/10.1016/j.ijpara.2009.01.005

Dubey, J.P., Ferreira, L.R., Martins, J. and Jones, J.L., 2011. Sporulation and survival of *Toxoplasma gondii* oocysts in different types of commercial cat litter. *J. Parasitol*., **97**: 751-754. https://doi.org/10.1645/GE-2774.1

Eida, A.M., 2015. Direct genotyping of *Toxoplasma gondii* in blood samples from pregnant women in Jazan, Saudi Arabia. *J. Egypt. Soc. Parasitol.,* **45**: 603-608. https://doi.org/10.12816/0017921

Elmore, S.A., Jones, J.L., Conrad, P.A., Patton, S., Lindsay, D.S. and Dubey, J.P., 2010. *Toxoplasma gondii:* epidemiology, feline clinical aspects and prevention. *Trends Parasitol.,* **26**: 190-196. https://doi.org/10.1016/j.pt.2010.01.009

El-Shahawy, I.S., Khalil, M.I. and Bahnass, M.M., 2014. Seroprevalence of *Toxoplasma gondii* in women in Najran City, Saudi Arabia. *Saudi med. J*., **35**: 1143-1146.

Flegr, J., Prandota, J., Sovičková, M. and Israili, Z.H., 2014. Toxoplasmosis-a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *PLoS One*, **9**: e90203. https://doi.org/10.1371/journal.pone.0090203

Hill, D. and Dubey, J.P., 2002. *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clin. Microbiol. Infect.*, **8**: 634-640. https://doi.org/10.1046/j.1469-0691.2002.00485.x

Imam, N.F.A., Azzam, E.A.A. and Attia, A.A., 2016. Seroprevalence of *Toxoplasma gondii* among pregnant women in Almadinah Almunawwarah KSA. *J. Taibah. Univ. med. Sci.,* **11**: 255-259.

Jones, C.D., Okhravi, N., Adamson, P., Tasker, S. and Lightman, S., 2000. Comparison of PCR detection methods for B1, P30, and 18S rDNA genes of *T. gondii* in aqueous humor. *Invest. Ophthalmol. Vis. Sci.,* **41**: 634-644.

Jones, J.L., Kruszon-Moran, D., Wilson, M., McQuillan, G., Navin, T. and McAuley, J.B., 2001. *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. *Am. J. Epidemiol.,* **154**: 357-365. https://doi.org/10.1093/aje/154.4.357

Jones, J.L. and Dubey, J.P., 2012. Foodborne toxoplasmosis. *Clin. Infect. Dis.,* **55**: 845-851.

https://doi.org/10.1093/cid/cis508

Kong, Q.M., Lu, S.H., Tong Q.B., Lou, D., Chen, R., Zheng, B., Kumagai, T., Wen, L.Y., Ohta, N. and Zhou, X.N., 2012. Loop-mediated isothermal amplification (LAMP): Early detection of *Toxoplasma gondii* infection in mice. *Parasit. Vectors,* **5**: 2. https://doi.org/10.1186/1756-3305-5-2

Pappas, G., Roussos, N. and Falagas, M.E., 2009. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int. J. Parasitol.,* **39**: 1385-1394. https://doi.org/10.1016/j.ijpara.2009.04.003

Paquet, C. and Yudin, M.H., 2013. Toxoplasmosis in pregnancy: prevention, screening and treatment. *J. Obstet. Gynaecol. Can.,* **35**: 78-81. https://doi.org/10.1016/S1701-2163(15)31053-7

Singh, S., Munawwar, A., Rao, S., Mehta, S. and Hazarika, N.K., 2014. Serologic prevalence of *Toxoplasma gondii* in Indian women of child bearing age and effects of social and environmental factors. *PLoS Negl. trop. Dis*., **8**: e2737. https://doi.org/10.1371/journal.pntd.0002737

Skariah, S., McIntyre, M.K. and Mordue, D.G., 2010. *Toxoplasma gondii:* determinants of tachyzoite to bradyzoite conversion. *Parasitol. Res.,* **107**: 253-260. https://doi.org/10.1007/s00436-010-1899-6

Torgerson, P.R. and Mastroiacovo, P., 2013. The global burden of congenital toxoplasmosis: a systematic review. *Bull. World Hlth. Organ.,* **91**: 501-508. https://doi.org/10.2471/BLT.12.111732

Wahab, T., Edvinsson, B., Palm, D. and Lindh, J.J., 2010. Comparison of the AF146527 and B1 repeated elements, two real-time PCR targets used for detection of *Toxoplasma gondii*. *Clin. Microbiol.,* **48**: 591-592. https://doi.org/10.1128/JCM.01113-09

Weiss, L.M. and Kim, K., 2011. Toxoplasma gondii, 2nd Edition. Academic Press, Elsevier, London, pp. 39, 343, 580.

Yamada, H., Nishikawa, A., Yamamoto, T., Mizue, Y., Yamada, T., Morizane, M., Tairaku, S. and Nishihira, J., 2011. Prospective study of congenital toxoplasmosis screening with use of IgG avidity and multiplex nested PCR methods. *J. clin. Microbiol.,* **49**: 2552-2556. https://doi.org/10.1128/JCM.02092-10