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Detection of *Toxoplasma gondii* infection and associated risk factors among pregnant women in Makkah Al Mukarramah, Saudi Arabia

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ABSTRACT

Objective: To determine the prevalence of *Toxoplasma gondii* (*T. gondii*) in pregnant women in Saudi Arabia and detect the risk factors associated with infection.**Methods:** Data were collected using questionnaires after the participants signed the consent form. ELISA techniques were used to detect both immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against *T. gondii* for all samples from the participants.**Results:** About 326 samples were examined by ELISA to detect IgG and IgM. Seroprevalence of IgG was 21.2% (69) while seroprevalence of IgM was 1.2% (4). The majority of pregnant women [168 (52.0%)] were in the third trimester. The history of abortion occurred in 95 (29.1%) for the first or second time while 31 (9.5%) had abortion three times and more. The study found relationship between previous infection with *T. gondii* and the height of pregnant women. The women with height less than 150 cm were more affected than others ($P < 0.02$). Association was observed between eating undercooked meat and infection with *T. gondii* ($P = 0.008$).**Conclusions:** Infection with *T. gondii* was found among Saudi women. High proportions of women (78.8%) are exposed to primary infection which can lead to abortion. Therefore, it is recommended that educational program to raise awareness to prevent the infection should be commenced without delay.

1. Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite causing toxoplasmosis, which is one of the most predominant chronic infections affecting one third of the human

around the world[1-3]. It is documented as a Category B important pathogen by National Institutes of Health[4]. In pregnancy, particularly primary infection with *T. gondii* has been associated with miscarriage, hydrocephalus, cerebral calcification and chorioretinitis in the newborn[5]. Humans can acquire infection via ingestion of raw or undercooked meat containing cysts of *T. gondii* or by consumption of contaminated food or water with oocysts of *T. gondii* or via contaminated soil. Infection can also occur from infected mother to the fetus via placenta[6-10].

The importance of toxoplasmosis in pregnant women comes from the high prevalence of *T. gondii* infection and its severe consequences to the fetus and infant[10,11], and prevention from infection of the fetus and complications by antibiotic treatment has not been very effective[12].

Diagnosis of *T. gondii* infections during pregnancy is mostly done by detecting immunoglobulin M (IgM) and immunoglobulin

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The study protocol was performed according to the Helsinki declaration and approved by Bioethics Committee in the Faculty of Public Health and Health Informatics, Umm Al Qura University. Informed written consent was obtained from the participants.

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G (IgG) antibodies by using serological methods such as latex agglutination test, ELISA, and indirect immunofluorescence antibodies test[13-16].

T. gondii infection is widespread and the seropositivity varies from less than 10% to over 90%[17,18]. In Saudi Arabia several studies were published recently regarding the prevalence of toxoplasmosis in pregnant women. The overall prevalence of the disease in pregnant women in Jazan Province was 24.1%[19], and in the south western it was 38.8%[20]. In Riyadh, the capital of Saudi Arabia, retrospective study was carried out in pregnant women at King Khalid University Hospital, and the prevalence of 38% was found[21]. In pregnant women in Makkah Al Mukarramah, two studies were conducted using ELISA in 2008 and 2006 with the prevalence of 35.6% and 29.4%, respectively[22,23].

The current study was conducted to determine the prevalence of toxoplasmosis in pregnant women in Makkah Al Mukarramah and to study the risk factors that increase the infection with *T. gondii*.

2. Materials and methods

2.1. Area and study design

Cross-sectional study for toxoplasmosis in pregnant women was performed. The sample size was calculated as 326 samples according to prevalence obtained in 2006 in Makkah Al Mukarramah[23]. The study was conducted during April to August 2014 in Maternal and Child Hospital after approved by scientific committee of the hospital. The target group of this study was Saudi pregnant women who live in Makkah or the surrounding and regularly visited the hospital for follow up.

2.2. Ethical approval

The study protocol was performed according to the Helsinki declaration and approved by Bioethics Committee in the Faculty of Public Health and Health Informatics, Umm Al Qura University. Approval also was given for the study from the administration of the hospital before starting.

2.3. Data collection

Consent form was filled by each individual and signed to agree to participate in the study. Data were collected using a questionnaire. There were questions concerning socio-demographic data including age, education, occupation, residency, and related risk factors including source of drinking water, obstetrical history and type of meat, egg and milk the participants eat also, kitchen hygiene, whether they own cats, history of cleaning cat area or feeding raw meat scraps, eating out, and soil exposure were also explored. Some questions regarding the knowledge of the disease and past history of illness before marriage were also included in the questionnaire.

2.4. Samples collection

The blood samples were collected in the laboratory section

in Maternal and Child Hospital by using 5 mL syringe into heparinized tubes. Plasma was separated by centrifugation of the blood at 5000 r/min for 10 min. Plasma was kept in different labeled cryo tubes at -80°C till used.

2.5. Laboratory work

The ELISA was performed to detect IgG and IgM antibodies against *T. gondii* using plasma samples according to manufacturer instruction (Toxo IgG® and IgM®, Human, Germany). Mean absorbance value of positive and negative controls and cut-off were calculated. Cut-off was calculated using the following formula: Cut-off value = mean of negative control + $0.2 \times$ mean of positive control.

2.6. Data analysis

SPSS version 20.0 (SPSS Inc. Chicago, IL, USA) was used to calculate the descriptive statistics to obtain specified statistics on the categorical (frequency or percentage) and numerical (mean and median) variables. The package was also used to calculate the prevalence of the disease. *Chi*-square test was used for ordinal variables to find the significant association between the infection and risk factors. Statistically *P*-value less than 0.05 was considered as significant.

3. Results

3.1. Seroprevalence of *T. gondii*

Out of 326 samples examined by ELISA, seroprevalence of IgG was 21.2% (69); also IgM was tested in all the samples using ELISA IgM and seroprevalence was 1.2% (4). Combined IgG and IgM positivity was found in one case (0.3%) (Table 1).

Table 1

Seroprevalence of IgG and IgM in pregnant women in Makkah Al Mukarramah.

Sero reaction	n	%
IgG positive	69	21.2
IgG positive and IgM positive	1	0.3
IgG positive and IgM negative	68	20.9
IgG negative and IgM positive	3	0.9
IgG negative and IgM negative	254	77.9

3.2. Socio-demographic profile

A total of 326 pregnant women were enrolled in this study with mean age of (30.19 ± 6.02) years; the age range of pregnant women participated in the current study was 16–40 years. Most of pregnant women participated in this study reside in urban area (86%). Approximately 206 (86.9%) belong to Arab and more than half of them [180 (55.6%)] have been married for over five years, and 117 (36.1%) attended higher education and 263 (80.7%) were housewives. Majority of pregnant women in this study have blood grouping type O positive [123 (48.4%)] while 287 (88.0%) of them has height more than 150 cm, and more than 226 (69.5%) of them has weight more than 60 kg as shown in Table 2.

3.3. Obstetrical features

In the duration of the study most pregnant women participated were grand multigravid [169 (62.6%)]; moreover, the majority of pregnant women were in the third trimester [168 (52.0%)]. The history of abortion occurred in 95 (29.1%) for the first or second time while 31 (9.5%) had aborted three times and more. Out of 325 participants, 12 (3.7%) had stillbirth (Table 2).

Table 2
Socio-demographic profile and obstetric history of participants.

Socio-demographic profile and history	N	%	
Age groups (years) (n = 311)	16–20	17	5.5
	21–25	64	20.5
	26–30	100	32.2
	31–35	72	23.2
	36–40	58	18.6
Residency (n = 307)	Urban	264	86.0
	Rural	43	14.0
Race (n = 237)	Arab	206	86.9
	Asian	19	8.0
	African	12	5.1
Years of marriage (years) (n = 324)	< 1	48	14.8
	1–5	96	29.6
	> 5	180	55.6
Education (n = 324)	Illiterate	18	5.6
	Primary and intermediate	95	29.3
	Secondary	94	29.0
	University	117	36.1
Occupation (n = 326)	Housewife	263	80.7
	Employee	45	13.8
	Student	18	5.5
Blood grouping (n = 254)	O+	123	48.4
	O-	18	7.1
	A+	62	24.4
	A-	8	3.1
	B+	38	15.0
	B-	4	1.6
Height (cm) (n = 326)	121–150	39	12.0
	> 150	287	88.0
	Weight (kg) (n = 325)	< 50	28
Weight (kg) (n = 325)	51–60	71	21.9
	> 60	226	69.5
	Gravidity (n = 270)	Primigravid	40
Multigravid		61	22.6
Grand multigravid		169	62.6
Trimester (n = 323)	1st Trimester	70	21.7
	2nd Trimester	85	26.3
	3rd Trimester	168	52.0
Abortion (n = 326)	0	200	61.4
	1–2	95	29.1
	> 3	31	9.5
Past occurrence of stillbirth (n = 325)	Yes	12	3.7
	No	313	96.3

3.4. Behavioral characteristics

To determinate the exposure of participants to risk factors associated with infection with *T. gondii*, pregnant women were asked about the source of drinking water that was usually used,

and most of them [136 (43.9%)] used health water for drinking. For drinking milk, majority of pregnant women used processed milk [260 (85.0%)]. Approximately 313 (97.2%) of target group in this study were consuming chicken meat and 293 (91.0%) were consuming sheep meat, while 62 (19.3%) consuming goat meat and only 11 (3.4%) consuming beef, but 130 (40.4%) consuming camel meat. On the other hand, the majority of women who participated in the current study handled meat [300 (93.5%)] and about 297 (92.5%) washed hands after handling meat, while 302 (94.1%) reported washing kitchen utensils (Table 3).

Table 3

Behavioral characteristics of pregnant women in Makkah Al Mukarramah.

Variable	N	%	
Source of drinking water (n = 310)	Desalination water	87	28.1
	Health water	136	43.9
	Zamzam	10	3.2
	Desalination water & health water	34	11.0
	Desalination water & zamzam	18	5.8
	Health water & zamzam	19	6.1
	All water type	6	1.9
Type of milk consuming (n = 306)	Processed milk	260	85.0
	Natural milk	3	0.9
	Processed milk & natural milk	43	14.1
Consuming chicken meat (n = 322)	Yes	313	97.2
	No	9	2.8
Consuming sheep meat (n = 322)	Yes	293	91.0
	No	29	9.0
Consuming goat meat (n = 322)	Yes	62	19.3
	No	260	80.7
Consuming beef meat (n = 322)	Yes	11	3.4
	No	311	96.6
Consuming camel meat (n = 322)	Yes	130	40.4
	No	192	59.6
Handling meat (n = 321)	Yes	300	93.5
	No	21	6.5
Washing hands after handling meat (n = 321)	Yes	297	92.5
	No	24	7.5
Washing kitchen utensils (n = 321)	Yes	302	94.1
	No	19	5.9
Frequency of eating meat (n = 320)	Daily	242	75.6
	Weekly	78	24.4
Eating meat in restaurant (n = 318)	Yes	311	97.8
	No	7	2.2
Frequency of eating meat in restaurant (n = 313)	Regularly	89	28.4
	Often	171	54.6
	Rarely	53	17.0
	Partially cooked	57	17.8
Meat prefer (n = 321)	Thoroughly cooked	264	82.2
	Raw	1	0.3
Eating egg (n = 320)	Partially cooked	11	3.4
	Thoroughly cooked	308	96.3
	Contact with cat (n = 326)	Yes	53
Keep indoor cat (n = 326)	No	273	83.7
	Yes	65	19.9
Cleaning cat area (n = 326)	No	261	80.1
	Yes	24	7.4
Feeding cat raw meat scraps (n = 326)	No	302	92.6
	Yes	15	4.6
Contact with soil (n = 326)	No	311	95.4
	Yes	31	9.5
Eating soil (n = 326)	No	295	90.5
	Yes	14	4.3
Cleaning house and dust (n = 326)	No	312	95.7
	Yes	166	50.9
Working in the garden (n = 326)	No	160	49.1
	Yes	29	8.9
	No	297	91.1

3.5. Knowledge of *T. gondii* infection and detection before marriage

According to Table 4, about 40 (12.4%) of infected women have previous knowledge of *T. gondii* while only 14 (4.3%) checked infection with *T. gondii* before getting married.

Table 4

Knowledge of *T. gondii* infection and detection before getting marriage.

Variable	No. of infected	%
Know of <i>T. gondii</i> (n = 323)	Yes	40
	No	283
Check before getting married (n = 323)	Yes	14
	No	309

3.6. Risk factors associated with seropositivity

The current study showed no significant difference between previous infection and age ($P > 0.11$). Pregnant women in rural or urban area in Makkah can get the infection ($P > 0.4$). No relationship between race and previous infection with *T. gondii* ($P > 0.4$) was found. Pregnant women who married recently or long time ago can either get or not the infection with *T. gondii*; no significant different was observed ($P > 0.15$). Relationship between education and previous infection was not observed in this study ($P > 0.2$). Also, there was no association between the infection and the occupation of the pregnant women participated in this study ($P > 0.07$). Also, no association was found between blood group of pregnant women who participated in the current study and the previous infection ($P > 0.15$). The study found relationship between previous infection with *T. gondii* and the height of pregnant women. The women with height less than 150 cm were more affected than others ($P < 0.02$). In the same time no relationship was found between weight and the previous infection ($P > 0.6$).

Association between gravidity and infection was not seen in this study ($P > 0.2$). Also, the relationship between the stage of pregnancy and the infection was not recorded ($P > 0.5$). Association between the history of abortion and infection with *T. gondii* was not detected in the present study ($P > 0.2$). Also, no association between infection and stillbirth was observed in this study ($P > 0.5$) (Table 5).

Table 5

Analyses of factors associated with IgG/ IgM seroprevalence of *T. gondii* among pregnant women.

Variable	IgG seropositivity		P-value	IgM seropositivity		P-value
	Yes	No		Yes	No	
Age groups (years)	16-20	1	16	0.113	1	16
	21-25	13	51		0	64
	26-30	16	84		2	98
	31-35	15	57		0	72
	36-40	18	40		1	57

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Table 5 (continued)

Variable	IgG seropositivity		P-value	IgM seropositivity		P-value
	Yes	No		Yes	No	
Resident	Urban	54	210	0.404	4	260
	Rural	10	33		0	43
Race	Arab	49	157	0.442	1	205
	Asian	5	14		0	19
	African	1	11		0	12
Years of marriage (years)	< 1	6	42	0.159	2	46
	1-5	18	78		1	95
	> 5	44	136		1	179
Education	Illiterate	3	15	0.208	0	18
	Primary & intermediate	21	74		1	94
	Secondary	23	71		0	94
	University	22	95		3	114
Occupation	Housewife	59	204	0.077	3	260
	Employee	10	35		1	44
	Student	0	18		0	18
Blood grouping	O+	17	107	0.154	2	121
	O-	5	13		1	17
	A+	13	49		0	62
	A-	1	7		0	8
	B+	9	29		0	38
	B-	0	4		0	4
	AB+	1	0		0	1
	Height (cm)	< 120	14	25	0.018	0
> 150	55	232		4	283	
Weight (kg)	< 50	5	23	0.672	0	28
	51-60	13	58		1	70
	> 60	51	157		3	223
Gravidity	Primigravid	5	35	0.289	1	39
	Multigravid	14	47		0	61
	Grand multigravid	40	129		2	268
Trimester	1st trimester	17	53	0.568	1	69
	2nd Trimester	20	65		0	85
	3rd Trimester	32	136		3	165
Abortion	0	46	154	0.294	4	196
	1-2	15	80		0	95
	> 3	8	23		0	31
Past occurrence of stillbirth	Yes	2	10	0.649	1	12
	No	67	246		3	309
Source of drinking water	Desalination water	21	66	0.672	1	86
	Health water	26	110		2	134
	Zamzam	4	6		0	10
	Desalination water & health water	6	28		0	34
	Desalination water & Zamzam	5	13		0	18
	Health water & Zamzam	3	16		0	19
	All water type	1	5		0	6
Type of milk consuming	Processed milk	54	204	0.638	2	258
	Natural milk	0	3		0	3
	Processed milk & natural milk	10	33		1	42
Consuming chicken meat	Yes	66	247	0.377	4	309
	No	3	6		0	9
Consuming sheep meat	Yes	62	231	0.709	4	289
	No	7	22		0	29
Consuming goat meat	Yes	9	53	0.14	0	62
	No	60	200		4	256
Consuming beef meat	Yes	3	8	0.631	0	11
	No	66	245		4	307
Consuming camel meat	Yes	23	107	0.179	3	127
	No	46	146		1	191
Handling meat	Yes	61	239	0.055	3	297
	No	8	13		1	20

(continued on next page)

Table 5 (continued)

Variable		IgG seropositivity		P-value	IgM seropositivity		P-value
		Yes	No		Yes	No	
		Washing hands after handling meat	Yes		62	235	
	No	7	17		1	23	
Washing kitchen utensils	Yes	64	238	0.598	3	299	0.104
	No	5	14		1	18	
Frequency of eating meat	Daily	53	189	0.795	3	239	0.977
	Weekly	16	62		1	77	
Eating meat in restaurant	Yes	66	245	0.170	3	308	0.086
	No	3	4		1	6	
Frequency of eating meat in restaurant	Regularly	15	74	0.270	2	87	0.313
	Often	36	135		1	170	
	Rarely	15	38		0	53	
Meat prefer	Partially cooked	5	52	0.008	1	56	0.544
	Thoroughly	64	200		3	261	
Eating egg	Raw	0	1	0.837	0	1	0.924
	Partially cooked	2	9		0	11	
	Thoroughly	67	241		4	304	
Contact with cat	Yes	11	42	0.87	0	53	0.490
	No	58	215		4	269	
Keep indoor cat	Yes	14	51	0.527	0	65	0.409
	No	55	206		4	257	
Cleaning cat area	Yes	6	18	0.608	0	24	0.571
	No	63	239		4	298	
Feeding cat raw meat scraps	Yes	4	11	0.531	0	15	0.828
	No	65	246		4	307	
Contact with soil	Yes	8	23	0.506	1	30	0.331
	No	61	246		3	292	
Eating soil	Yes	4	10	0.505	0	14	0.670
	No	65	247		4	308	
Cleaning house and dust	Yes	32	134	0.395	1	165	0.589
	No	37	123		3	157	
Working in the garden	Yes	9	20	0.231	1	28	0.312
	No	60	237		3	294	

Regarding to the uses of drinking water and the infection with *T. gondii* in the present study, no significant association were detected ($P > 0.6$), and the same results were found between types of milk used and infection ($P > 0.6$). Types of meat consumed including beef, chicken, sheep, goat and camel meat had no association in causing the infection. Handling meat had no association with infection by *T. gondii* in the current study ($P > 0.09$). Washing hands after handling meat and washing kitchen utensils were not significantly associated with infection ($P > 0.3$; $P > 0.5$).

Relationship between eating meat or eating meat in the restaurant or frequency of eating meat in the restaurant and infection was not found in this study ($P > 0.7$; $P > 0.1$; $P > 0.2$). Association was observed between eating undercooked meat and infection with *T. gondii* ($P = 0.008$). The relation between eating raw or undercooked egg and infection was not detected in this study ($P > 0.8$).

The association between contacting cat, keeping indoor cat, cleaning the area of cat or feeding cat raw meat scraps and infection was not observed in this study ($P > 0.8$; $P > 0.5$; $P > 0.6$; $P > 0.5$, respectively) (Table 5).

In addition, no relationship between direct contact with soil, cleaning dust, working in the garden or eating soil and the infection with *T. gondii* was detected ($P > 0.5$; $P > 0.5$; $P > 0.3$; $P > 0.5$, respectively). (Table 5).

4. Discussion

The results obtained from this study were based on serological methods, ELISA for IgG and IgM. Detection of IgG in blood samples was for previous infection and IgM for recent infection in pregnant women. Prevalence of previous *T. gondii* infection among pregnant women in Makkah City in the current study was 21.2% which is similar to previous studies done in the same area and same target group. In 2002, Ghazi *et al.* recorded 35.6% IgG seropositivity by using ELISA[22], and in 2006 in the same target group the prevalence was 29.4% by using ELISA for IgG[23]. According to the prevalence obtained in the three studies, we observed that the prevalence of *T. gondii* among pregnant women in Makkah City was reduced. This reduction may be attributed to climate changes, enhancement in hygienic conditions, changes in feeding habits or good knowledge about avoiding the infection with *T. gondii*. The observation confirmed by seropositivity of IgM for detection of recent infection was done in the same group and area in 2006 (5.6%)[23], but in the current study, the prevalence of recent infection was 1.2%.

Comparing the results obtained in the current study with previous studies done in the different areas in Saudi Arabia in the same target group, we found that the prevalence was contrasting. In the pregnant women in Al Ahsa, prevalence was 51.4% using ELISA for IgG and 8.8% using ELISA for IgM[24]. While prevalence was 41% in pregnant Saudi women in Aseer region by using PCR[25]. Another study done in Saudi pregnant women in Hail using ELISA showed prevalence of 28.9% and 2.8% for IgG and IgM, respectively[26]. Also, study done in Hail in pregnant women in 2014 revealed prevalence of 8.57% for IgG[27]. Recently, a study was done in women in Najran using ELISA, the results showed 29.2% for IgG and 3.1% for IgM[28]. In South Western Saudi Arabia, another study done in pregnant women using ELISA showed prevalence of 38.8% for IgG and 6.2% for IgM[20]. Similar study done in Jazan using same techniques displayed prevalence of 20% for IgG and 6.2% for IgM[19]. The results obtained in the current study and previous ones done in Saudi Arabia were similar and the variation may be due to the difference of climate and geographical area, as the infection with *T. gondii* spread in hotter and wetter area. The results were also similar to that obtained in other countries such as Qatar[29] and Iraq[30], but different from results recorded in pregnant women in Colombia[31], France[32], and Ethiopia[33]; the variation may be due to the difference in climate or life style.

In previous study done in pregnant women in Makkah in 2006, about 10% of participants had the knowledge of toxoplasmosis and none of participants was previously tested for *Toxoplasma* infection. In current study the proportion was increased to more than 12% for women who have the knowledge of toxoplasmosis and more than 4% were tested for *Toxoplasma* infection before getting married[23].

Although the seroconversion was increased among 36–40 age

group, this finding was not significant in the current study but matching with the theory which says that people living longer has chance to expose to more causing agent. This result was different from that obtained by other researchers in the same target groups with different locations in Saudi Arabia[20,23,26,27], but similar to study done in Ethiopia[34], Cameroon[35], and Thailand[36].

Location, race and marital status of pregnant women had no significant association with infection in this study, but the study suggested that people can get the infection in any places and the infection varies even in one location in same area. Women from any race acquire the infection equally. Women with long-time marriage are prone to acquire infection more times than single women because they may be more responsible for cooking and cleaning the house.

In the current study, the significant relationship between seropositivity and education was not found; the infection actually increased with high level of education and uneducated women were less infected. This finding may be due to the lifestyle which depends on hygienic practice regardless of level of education. This finding contrasts with finding in Hail region[27], but matches with finding in the same area of this study[23].

The majority of women who participated in the current study were housewives; few of them were working or students. The relationship between infection and occupation was not observed. This observation confirmed the hygienic lifestyle of participants.

The association between pregnant women and height was found significant in this study. Women higher in heights were more suspected to acquire the infection, as found in Iran[37]. This finding needs to be confirmed in separate study.

The relationship between obstetric history and seropositivity was not observed in this study. These results are in agreement with previous study done in Makkah[23] and in south western region[20].

Part of risk factors surveyed such as drinking water and type of milk or meat consumed, has no role in transmission of toxoplasmosis in this study; this finding matches with other previous study done in the same target group and same area. This may be attributed to religious beliefs and culture habits[23].

Handling meat, washing hands after handling meat, and washing kitchen utensils have no effect on transmission of the disease to the target group because there was no statistical significance found.

Other risk factors regarding eating meat in restaurant or frequency of eating meat in restaurant did not show any significant relationship with seroprevalence in the current study. According to the result obtained in this study, eating partially cooked meat or undercooked meat was highly associated with seroprevalence of toxoplasmosis in the current study, and may be the only source of infection to pregnant women participated in the study. Raw meat was not consumed in the target area of study due to their culture, but partially cooked meat was consumed. This result was well documented in different parts of the world[6,38-41].

Some of the risk factors such as the ownership of cat, feeding or contacting with cat, contacting with soil, cleaning the dust, eating

soil, and working in the garden have shown no significant association with toxoplasmosis. The absence of this significant association may be due to the weather conditions in Makkah.

According to our finding, the infection can be eradicated by using educational programme with emphasis on the role of undercooked meat in transmission of the disease particularly in pregnant women.

In conclusion, the study tried to explore the situation of the disease in Makkah, the source of infection and the risk factors which contributed to increase in the infection. The situation has been compared with previous study done in the same area and similar target group ten years ago. The current study found that the spread of disease reduced and the main source of infection has been determined. The authors believe that educational programme should target this source of infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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