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PREVALENCE OF TOXOPLASMOSIS AND ASSOCIATED RISK FACTORS IN WOMEN IN NORTHERN BORDER AREA, SAUDI ARABIA

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ABSTRACT

Background: Toxoplasmosis is caused by infection with the protozoan parasite *Toxoplasma gondii*, which has the capability to infect all warm-blooded animals. The main aim was to obtain data that contribute to a better understanding of the prevalence of toxoplasmosis in Northern Border area in the Kingdom of Saudi Arabia, and assess the risk factors related to the infection.

Methods: In this cross-sectional study, 508 women were responded to participate; epidemiological data were collected and analysed, IgM, IgG anti-toxoplasma antibodies were screened in the plasma using ELISA method.

Results: Among 508 samples 74 women (14.6%) were reactive to IgG anti-*T. gondii* antibodies; no samples were reactive to IgM. The larger part of women participated in this study were in the

age group (26-30 years), the age group (36-40) years were more infected with toxoplasmosis. The study showed that living in urban areas was related to the infection (p<0.0001). Risk factor analysis showed that contact with cats, keeping indoor cats, and contact with the soil was associated with contagion with *T.gondii* (p<0.001).

Conclusion: Prevalence of toxoplasmosis in women of the Northern Boarders area was low in comparison to other parts of Saudi Arabia, exposure to risk factors was detected, increasing awareness and screening are needed to eliminate and lower the risk of consequences of toxoplasmosis infection.

Keywords: *Toxoplasma gondii*, Prevalence, ELISA, Risk factors, Northern Border Area

INTRODUCTION

Toxoplasmosis is an ailment caused by infection with the commit intracellularly protozoan parasite *Toxoplasma gondii* (*T. gondii*) [1, 2]. Toxoplasmosis is a standout amongst the most pervasive parasitic irresistible infections of medical and veterinary significance because of its association abortion and congenital disease in its intermediate hosts. Toxoplasmosis causes an assortment of infectious disorders in humans, extending from influenza-like side effects in immunocompetent adults, to extreme dispersed illness in immunosuppressed people, and to birth deformity in new-born children when women are infected during pregnancy [3-6]. Toxoplasmosis is among the worldwide main zoonotic infections [7, 8]. Transmission to humans is through direct contact with dirty material infected with *T. gondii* oocysts, consumption of undercooked meat or vegetables contaminated with *T. gondii* oocysts and transmission from an infected mother to the unborn fetus (congenital toxoplasmosis) [9].

Risk factors for infection with *T. gondii* are related to cleanliness, diet, other social habits, and weather [10]. As per infection routes, predominant risk factors for toxoplasmosis include: lacks in ecological sanitization (untreated water), the nearness of household felines and rodents, utilization of polluted feed (rough or havecooked meat and efficiently washed-outveggie) and contactwith defiled soil [11].

Approximately around 25 to 30% of human population around the world is infected with *Toxoplasma gondii* [12]. It was reported that the prevalence shift broadly between humans of different nations (from 10 to 80%) and regularly in a given country or between various groups in a similar area [13].

In Saudi Arabia prevalence of toxoplasmosis varies widely between different areas in the country. Low seroprevalence have generally been reported in different regions of the northern area cities including Arar (14.1%), Hail (10.1%), Almadinah (21.5%) and Tabuk (9.4%) [14-17]. In the eastern region, published data showed that the prevalence was (39.97%) in Alkhobar, and (31.5%) in Dharan[18,19]. Another published articles in central and southern regions of Saudi Arabia documented several rates, which mostly characterized by high prevalence of toxoplasmosis as seen in Ad-Dawadimi (40.9%), and Abha (45%) [20, 21] In the western region, moderate prevalence were founded in Makkah (22.4%) [22].

We aimed to evaluate the pervasiveness and risk factors associated with *Toxoplasma gondii* infection among women of the northern border area in the kingdom of Saudi Arabia.

MATERIALS AND METHODS

Study Area

This study was carried out in the Northern Border Area of Kingdom of Saudi Arabia, in Rafha Province, located 788 km from Riyadh, between the longitude 42.30˚-45˚ east and latitude 28˚-30.27˚ north, the province consists of many cities and villages surrounding it. The climate is desert; it is hot in summer, cold in winter. However, the temperature modifies in the spring, and rain often falls in winter and spring on average.

Study Design

The study was a cross-sectional study for detection of toxoplasmosis in women, the sample estimate was ascertained as (508) according to the equation:

Max Sample Size = (Z-score)² X StdDev X (1-StdDev) / (margin of error)²

Correction for total Pop size = Max Size X Npop / (Max Size + Npop-1)

The study was conducted during the period from October 2016- to October 2017. The present study was performed in Rafha central hospital and health care centers in some villages. The population group of this study was women who expressed an interest in participating in the study and live in Rafha or the surrounding.

Questionnaire

A designed questionnaire was prepared in English and interpreted into Arabic then used to collect data from participants after they agreed to participate in the study by filling and signing the informed consent. The questions were about essential statistic information including age, education, residency, and possibly associated risk factors including drinking water sources, obstetric data, kind of milk, meat favored,

and egg. Also, hygienic behaviors inside the kitchen, the presence of cats inside homes, cleaning cat area, eating in restaurants, gardening, and dust cleaning. Other questions regarding the knowledge and past infection with the parasite and check before marriage was included in the questionnaire.

Collection of the specimen and laboratory work

2ml of venous blood was taken from each participant using a 5 ml syringe aseptically into a heparinized tube, then transported to microbiology laboratory, department of basic health sciences in the college of pharmacy, Northern Border University, KSA. The blood was centrifuged to separate plasma at 5000 RPM, the separated plasma was kept after labeling in -20˚C till use.

Plasma was screened for anti-*T.gondii* IgM and IgG antibodies using commercial enzyme-linked immunosorbent assay (ELISA) Kits (UDI EM127 Toxoplasma IgM, Dammam, KSA**)** and (UDI D91040 Toxoplasma IgG, Dammam, KSA**)** from the United Diagnostic Industries company. The test protocol was carried as indicated by the producer's directions. Plasma samples were diluted to a ratio of 1:101 with the sample diluent. Calibrators and Blank were run with our samples. For IgG and IgM assays, the wavelength used to measure the optical density was 450 nm, the ratios between the OD value of unknown samples and that of the calibrators were calculated. Sample with a ratio more than or equal to 1.1 was considered as positive, and when the proportion was less than 0.9it was approved to be negative.

Ethical clearance

The study was conducted as directed by the Helsinki declaration, and approved by the local bioethics committee, in the Northern Border University Arar, kingdom of Saudi Arabia, (Approval No.4/37/A), approval number (1/38) was also obtained from center of medical education and research, general directorate of health affairs in the northern border region, Ministry of Health, KSA. Approval was also given by the administration of Rafha central hospital.

Data Statistical Analysis

Data obtained were checked for fulfillment and consistency, entered into the computer, and analyzed using SPSS (SPSS Inc. Chicago, IL. USA) version 20.0. Descriptive data were presented as frequencies and percentages. Differences and risk factors associated with the infection and were tested using the Pearson’s chi-square test. P-value less than 0.05 were considered statistically significant.

RESULTS

Seroprevalence of *T.gondii*

From 508 samples investigated using ELISA method, IgG anti-*T.gondii* was detected in 74 women with prevalence rate (14.6%), which indicates either past infection or acquired immunity. All participants presented negative anti-*T.gondii* IgM.

Sociodemographic profile

508 women were participating in this survey, 388 (76.4%) of the studied women were from urban areas, while 120 (23.6%) from rural areas. Most women take a part in this study were in the age group (26-30 years) approximately 154 (30.3%), 390 Saudi women (76.8%) participated in the study, while 118 (23.2%) were non-Saudi. All women in this study were educated, most of them (40.9%) completed or studying at the secondary schools, (37.6%) are studying in university, (17.7%) were studying at intermediate schools, and merely (3.7%) completed only elementary schools. The married status of women in this study showed that 87 (17.1%) were single, and 421 (82.9%) were married. 291 (57.3%) of the study population were pregnant, 217 (42.7%) were not (Table 1).

The obstetrical situation showed that most married women in this study were grand multigravida 193 (45.8%), vast majority of pregnant women participated in the study were in the second trimester 191 (65.3%). About 101 (19.9%) of women aborted in the first or second time, while 17 (3.3%) had aborted three times or more. Stillbirth occurred in 32 (6.1%) of the study population (Table 1).

To determine the vulnerability of participants to risk factors related to infection with *T. gondii*, the study population wasinquired about drinking water source, food habits, behavior in houses, and contacting cats. In Rafha province water sources are either health or home filtered water, in this study 257 (50.6%) of women drink water from home filter, while 251 (49.4%) got health water from the shops. Women in these areas mainly drink bottled milk 234 (46.1%), others drink unpasteurized fresh milk 205(40.4%), and few of them use powdered milk 67(13.23%) (Table 2).In this work, consuming sheep meat by women was found to be 206 (40.6%), followed by camel meat 174 (34.3%), then beef meat 117 (23%), 4 (8%) consume goat meat, and 7 (1.4%) wasn't eating meat at all. Most women in the study area were handling raw meat by themselves 426 (83.9%) (Table 2).

As mentioned in Table 3, Most women in this study had previous knowledge about *T.gondii* 320 (63%), on the other hand, only

14 (4.3%) check the infection before marriage.

Risk factors related to seropositive Participants

The current study showed that Prevalence rate increased with age (p<0.010) women who were in the age group 36-40 years were more infected than women in the age group 31-35, youthful women were less infected than oldest. The study revealed that the prevalence rate in urban areas was highly significant (p<0.0001) and women, who live in there, were under risk of infection by *T.gondii*. The relation between education and infection with *T.gondii* was not significant (p<0.088). The study observed an association between infection and gravidity (p<0.002). A relation between gestational period and mother infected with *T.gondii* was not detected (p<0.061). The relationship between abortion and infection with *T.gondii* was found (p<0.0001). The association between mother infection by *T. gondii* and stillbirth occurrence were observed and significance (p<0.001) (Table 4).

Concerning nutrition risk factors related to *T.gondii* infection, no association between type of milk and disease was found (p<0.5). Also, no relation between water sources and infection (p<0.506). In this study, there was no significant relationship between eating partially or thoroughly cooked eggs and infection by *T.gondii* (p<0.58). The association between eating in restaurants and infection with *T.gondii* was not observed (p>0.15). No relation is found between eating partially or thoroughly cooked meat (p>0.15). The connection between toxoplasmosis and frequency of eating meat was not found (p>0.63). Eating beef, sheep, goat, and camel meat did not associate with the infection with *T.gondii* (p>0.13). No relationship marked between washing hands after meat handling (p>0.62), and washing kitchen utensils (p>0.37) (Table 4).

There was an association between women contacted with cats and *T.gondii* infection (p<0.007). Keeping indoor cats and infection in women was highly significant (p<0.0001). Women who participated in cleaning cat area, significantly affected by the infection with toxoplasmosis (p<0.001) (Table 4).

The association between contact with the soil and infection by *T.gondii* was seen in this study (p<0.001). A relation between infection and eating soil (mud) was observed (p<0.01). Moreover, the association between infection and working in the garden in the house was not reported in this study (p>0.06) (Table 4).

.Table 1: Socio-demographic Profile and Obstetric History of Participants

Socio-demographic Profile and Obstetric History		Number	Percent %
Age groups (years)	16-20	73	14.4
	21-25	105	20.7
	26-30	154	30.3
	31-35	83	16.3
	36-40	83	16.3
	>40	10	2
Race	Arab	414	81.5
	Africa	52	10.2
	Asia	42	8.3
Residence	Urban	388	76.4
	Rural	120	23.6
Education	Elementary	19	3.7
	Intermediate	90	17.7
	Secondary	208	40.9
	University	191	37.6
Marital Status	Single	87	17.1
	Married	421	82.9
Gravidity	Primigravida	49	11.6
	Multigravida	179	42.5
	Grand multigravida	193	45.8
Pregnancy (n=508)	Non Pregnant	217	42.7
	Pregnant	291	57.3
Gestational Period	First Trimester	4	1.3
	Second Trimester	191	65.3
	Third Trimester	96	33
Abortion History	0	390	76.8
	1-2	101	19.9
	>3	17	3.3
Still Birth (n=508)	No	476	93.7
	Yes	32	6.1

Table 2: Behavioral Characteristics of Women Participated in the Study

Variable		Number	Percent %
Source of Drinking Water (n=508)	Home Filter	257	50.6
	Health Water	251	49-4
Type of Drinking Milk (n=508)	Bottled	234	46.1
	Fresh	205	40.4
	Powder	67	13.2
	No	2	0.4
Type of Meat Consuming (n=508)	No	7	1.4
	Goat	4	8
	Sheep	206	40.6
	Camel	174	34.3
	Cow	117	23
Handling Meat (n=508)	No	82	16.1
	Yes	426	83.9
Washing Hands After Meat Handling (n=508)	No	65	12.8
	Yes	443	87.2
Washing Kitchen Utensils (n=508)	No	92	18.1
	Yes	416	81.9
Frequency of Eating Meat (n=508)	No	7	1.4
	Daily	405	79.7
	Weekly	96	18.9
Eating Meat in Restaurant (n=508)	No	126	24.8
	Yes	382	75.2
Meat Prefer (n=508)	No	7	1.4
	Partially Cooked	41	8.1
	Thoroughly Cooked	460	90.5

Eating Egg (n=508)	**No**	**3**	**0.6**
	Partially Cooked	**249**	**49**
	Thoroughly Cooked	**256**	**50.4**
Direct Contact with Cat (n=508)	**No**	**441**	**86.8**
	Yes	**67**	**13.2**
Keeping Indoor Cat (n=508)	**No**	**372**	**73.2**
	Yes	**136**	**26.8**
Cleaning Cat Area (n=508)	**No**	**248**	**48.8**
	Yes	**260**	**51.2**
Contact Soil (n=508)	**No**	**128**	**25.2**
	Yes	**380**	**47.8**
Cleaning House Dust (n=508)	**No**	**85**	**16.7**
	Yes	**423**	**83.3**
Working In Garden (n=508)	**No**	**111**	**21.9**
	Yes	**397**	**78.1**
Eating Soil (Mud) (n=508)	**No**	**394**	**77.6**
	Yes	**114**	**22.4**

Table 3: Knowledge of *Toxoplasma gondii* and screening before marriage

Variable		Number	Percent %
Know T.gondii	**No**	**188**	**37**
	Yes	**320**	**63**
Check Before Marriage	**No**	**494**	**97.2**
	Yes	**14**	**2.8**

Table 4: Analysis of Factors Associated with IgG Seroprevalence of *T.gondii* Among Respondent Women

Variable		Ig G seropositivity		*P*-Value
		Yes	No	
Age groups	**16-20**	**7**	**66**	
	21-25	**11**	**94**	
	26-30	**19**	**135**	**0.019**
	31-35	**17**	**66**	
	36-40	**20**	**63**	
	>40	**0**	**10**	
Race	**Arab**	**63**	**351**	
	Africa	**6**	**46**	**0.683**
	Asia	**5**	**37**	
Residence	**Urban**	**51**	**337**	
	Rural	**23**	**97**	**0.0001**
Education	**Elementary**	**5**	**14**	
	Intermediate	**15**	**75**	**0.088**
	Secondary	**35**	**173**	
	University	**19**	**172**	
Marital Status	**Single**	**6**	**81**	**0.062**
	Married	**68**	**353**	
Gravidity	**Primigravida**	**3**	**46**	
	Multigravida	**21**	**158**	**0.002**
	Grand multigravida	**44**	**149**	
Pregnancy	**Non Pregnant**	**26**	**191**	**0.06**
	Pregnant	**48**	**243**	
Gestational Period	**First Trimester**	**0**	**4**	
	Second Trimester	**26**	**165**	**0.061**
	Third Trimester	**22**	**74**	
Abortion History	**0**	**23**	**367**	
	1-2	**44**	**57**	**0.0001**
	>3	**7**	**10**	
Still Birth	**No**	**62**	**414**	
	Yes	**12**	**20**	**0.001**

Table 4: Continued

Variable		Ig G seropositivity		*P*-Value
		Yes	No	
Source of Drinking Water	Home Filter	37	220	
	Health Water	37	214	0.506
Type of Drinking Milk	Bottled	36	198	
	Fresh	32	173	0.505
	Powder	6	61	
	No	0	2	
Type of Meat Consuming	No	1	6	
	Goat	0	4	
	Sheep	37	169	0.128
	Camel	27	147	
	Cow	9	108	
Handling Meat	No	7	75	
	Yes	67	359	0.091
Washing Hands After Meat	No	7	57	0.619
Handling	Yes	67	376	
Washing Kitchen Utensils	No	7	85	0.37
	Yes	67	349	
Frequency of Eating Meat	No	1	6	
	Daily	62	343	0.630
	Weekly	11	85	
Eating Meat in Restaurant	No	10	116	0.15
	Yes	64	318	
Meat Prefer	No	1	7	
	Partially Cooked	2	39	0.181
	Thoroughly Cooked	71	388	
Eating Egg	No	1	2	
	Partially Cooked	34	215	0.575
	Thoroughly Cooked	39	217	
Direct Contact with Cat	No	57	384	0.007
	Yes	17	50	
Keeping Indoor Cat	No	34	338	0.0001
	Yes	40	96	
Cleaning Cat Area	No	23	225	0.001
	Yes	51	209	
Contact Soil	No	7	121	0.001
	Yes	67	313	
Cleaning House Dust	No	3	82	0.002
	Yes	71	352	
Working In Garden	No	10	101	0.06
	Yes	64	333	
Eating Soil (Mud)	No	49	345	0.01
	Yes	25	89	

DISCUSSION

This study was done to investigate prevalence percentage of *T.gondii* of IgG and IgM antibodies in women from Rafha Province, KSA, by serological based method ELISA. The samples were taken from women, regardless of their pregnancy and obstetric history. The prevalence was found 14.6% IgG, and no samples were reactive to IgM anti-*T.gondii.* This result is similar to the previous one reported as (12%) in Rafha city [23]. Another survey conducted in Arar city was consistent with our findings [20]. In spite of our big sample size, but percentages remain similar which ensure the lower prevalence of the infection with *T. gondii*

found in the northern border region. Recent surveys carried out in the Kingdom of Saudi Arabia showed that the prevalence in Ad-Dawadimi (40.9%), Al Khobar (39.4%), and Alriyadh (32.5%) with rates higher than our study [14, 19, 24].

In the current study, the seroprevalence increases with age, a significantly higher infection rate of *T.gondii* among women in the age group 36 to 40 years was found which proposes that numerous women become infected at a more youthful age and toxoplasma IgG can be detectable for a long time. Our outcomes are similar to investigations performed in Pakistan, Brazil and Ethiopia [25-27].

The work discovered that living in urban areas is a potential risk factor associated with *T.gondii* infection, that maybe is relevant to respondents habits, this determination was also stated by [28, 29]. However, recent study in Makkah showed no connection between the location and infection [22].

Race, education, and marital status of women participated in this study had no relation to the infection, women can get the infection regardless of their race and education.

In this research, relation of the gestational period of pregnant women and infection was not significant. The number of pregnancies was reported as a risk factor for gaining toxoplasmosis [30]. In this study abortion history, gravidity, and occurrence of stillbirth had a strong relationship with the infection, these outcomes were demonstrated by [21]. As reported by many studies, unimproved water sources can be affected from contaminated soil by cat faeces contains oocyst [31, 32]. In this study, there was no association between water sources and infection, and this is due to the pure water consumption by the participant either home filtered using the fixed purification system or bottled water. Astudy conducted in Zaria, Nigeria found a higher seroprevalence in pregnant women drinking ground water in contrast to others utilizing health water [33].

Type of drinking milk and meat consumed in the present study was not associated with the transmission of infection with toxoplasmosis. Milk is known as a possible transporter for diffusion of toxoplasmosis in humans, as referred to in numerous trials, milk from infected animals contains tachyzoites and this will spread the infection [34]. As risk factors we found that handling meat, washing hands after meat handling, and washing kitchen utensils, have no role in the transmission of toxoplasmosis in participants.

Consuming raw or undercooked meat has been perceived as an essential method of transmission [11, 35]. However, eating

partially or thoroughly cooked meat did not show any significant relationship with the seropositivity of toxoplasmosis in the current study. These outcomes are in concurrence with the former investigation done in Dhahran [18].

Contact with home cats was orderly correlated with *T.gondii* transmission, because cats are the only definitive host for *T.gondii*. There is a contraindicated report about contacting cats as a threat of infection. Our findings stated a statistically significant association between seropositivity of toxoplasmosis and contact with cats. This finding was listed in studies reported from Ethiopia and Taiwan [25-27, 36]. In contrast, some investigations in some areas of Saudi Arabia like Makkah, Dhahran, and southwestern region did not show any association between the infection and cat contact [18, 21, 22].

Contact with soil in public parks or gardens at home have been widely indicated as a risk factor for toxoplasmosis [5, 35, 37]. Our study found that contact with soil, cleaning house dust, and eating soil or mud, were highly significant factors for the transmission of *T.gondii.* However, some studies did not agree with our results [38].

CONCLUSION

The study demonstrates that the prevalence of *T. gondii* was generally lower amongst women in Rafha province, in addition to a significant exposure to most of the risk factors, this may lead to infection of susceptible women. There is a need for increasing the awareness level, health education about toxoplasmosis way of transmission, and risk factors avoidance. Prenatal screening of toxoplasmosis is recommended to minimize the risk of congenital toxoplasmosis.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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