

## TOXOPLASMOSIS IN PREGNANT WOMEN: THE IMMUNE STATUS AND POTENTIAL RISK FACTORS

### ABSTRACT

**Background and aims:** Toxoplasmosis is caused by a protozoan intracellular organism, *Toxoplasma gondii*, and is a widespread disease. Toxoplasmosis can progress to a severe systemic disease (congenital form); when a mother becomes infected for the first time during pregnancy, a temporary haematopoietic parasite can appear with focal lesions generated within the placenta, thus infecting the fetus. Therefore, this cross-sectional study aimed at assessing the immune status of the pregnant woman towards toxoplasmosis, determining the prevalence of *T. gondii* in Amran city - Yemen, and identifying the potential risk factors that affect the pregnant woman towards toxoplasmosis. **Material and methods:** This study included 280 pregnant women who were admitted to maternity clinics at Amran General Hospital and Family Health Center in Amran City - Yemen during the period from December 2016 to June 2017. The questionnaire was used to obtain relevant demographic data and potential risk factors. Then, blood samples were collected from pregnant women and tested for identification of IgM and IgG antibodies against *T. gondii* using the ELISA technique. **Results:** The total positive rate for IgM antibodies was 3.6%, while for IgG was 27.9%. A high IgM rate equal to 5.3% was observed in the 31-35 age group, and a higher IgG rate (36.8%) was observed in the same age group. There were 69.3% of pregnant women susceptible to infection with toxoplasmosis, 2.9% recently infected, 27.1% protected, and 0.7% had secondary infection. The high rate of IgM positivity was observed in the second trimester of pregnancy (4.7%), while the lowest rate was in the third trimester of pregnancy (2%). A slight increase in the rate of IgG positivity was observed in the third trimester of pregnancy (32%). There was a significant association between contact with animals such as cats and infection with toxoplasmosis, where OR = 5.4, CI = 1.01-30.13,  $\chi^2 = 4.59$ ,  $p = 0.04$ . While there was no significant association with the other studied factors. **Conclusion:** Yemen has less seropositivity than other Arab and African countries due to differences in risk factors. Data on *T. gondii* infection during pregnancy is scarce in many countries, especially where there is a lack of political stability such as Yemen. The risk factors identified included proximity to the cats and domestic animals, and increased maternal age. Toxoplasmosis in pregnancy in Yemen may be a really underestimated health problem. More research is needed.

**Key words:** Immunological Status, Pregnant Women, risk factors, Toxoplasmosis, Yemen

### INTRODUCTION

*Toxoplasma gondii* (*T. gondii*), a single-celled parasite that is widespread obligate intracellular parasite, is one of the most successful eukaryotic pathogens in infecting warm-blooded vertebrates including humans, and is capable of causing toxoplasmosis<sup>1,2</sup>. Distribution and its ability to maintain a benign symbiosis with its host are features that allow it to be widely viewed as one of the most successful parasites on Earth<sup>2</sup>. It is estimated that up to a third of the world's population is carriers of toxoplasma infection, and the life cycle of this parasite plays a large role in its ease of transmission<sup>3,4</sup>. Toxoplasmosis is found in every country and rates of seropositivity range from 10% to 90%<sup>5,6</sup>. Globally, more than 6 billion people are infected with *toxoplasma gondii*<sup>7</sup>. Humans are commonly infected by oral ingestion of water, food, or soil contaminated with oocysts, or by consumption of raw vegetables and undercooked meat contaminated with cysts of *T. gondii* [8]. Though *T. gondii* infection is very common, the clinical implication in the immunocompetent host is benign and asymptomatic and only in about 10% of cases, cervical or occipital lymphadenopathy and ocular disease may occur<sup>9</sup>. The significance is mainly attributable to primary infection during pregnancy that may result in congenital Toxoplasmosis, abortions, perinatal deaths and still births<sup>5</sup>. This includes a wide range of manifestations, extending from mild chorioretinitis, which can present many years after birth, to miscarriage, mental retardation, microcephaly, hydrocephalus and seizures<sup>10,11</sup>. Moreover, reactivation of latent infection in immunocompromised conditions may occur resulting in *T. gondii* encephalitis or disseminated infections<sup>9,12</sup>. Its infection induces several immunological changes in the body which are characterized by the production of the immunoglobulins IgM, IgG and IgA<sup>13</sup>. Detection of Toxoplasmosis is currently dependent on serological techniques including screening for IgM and IgG antibodies, the former indicates recent infection while the latter indicates a past exposure with the existence of protective immunity<sup>2,14</sup>.

In Yemen, data on infection among females of childbearing age or during pregnancy are limited. Moreover, few studies have been conducted recently on vulvovaginal candidiasis during pregnancy, vaccination against tetanus among pregnant women, its prevalence, potential risk factors, awareness of congenital CMV, rubella virus infection in pregnant women and markers of hepatitis B virus serum among pregnant women<sup>16-20</sup>. In Yemen, pregnant women are not routinely screened for *T. gondii* during pregnancy, and there is no follow-up. Thus, the current study was carried out to reveal the state of sero-epidemics and potential risk factors for *T. gondii* in pregnant women in Amran area in Yemen.

## SUBJECTS AND LABORATORY METHODS

A cross-sectional study was carried out during the period from December 2016 to June 2017. Two hundred and eighty pregnant women, who were attended to the obstetrical clinics in General Amran Hospital, and Family health Centers in Amran city, Yemen, were included in this study.

**Data collection:** A full history was taken from each studied pregnant woman, and the findings were written down in predesigned questionnaire. The collected data included: Demographic characteristics of participant, and information about the risk factors.

**Sample Collection:** Four to five ml of venous blood samples were obtained from each pregnant into a plain tube. Then sera were separated from clotted blood and kept in freezer at (- 20 °C) until tested. The samples were tested for the presence of anti-toxoplasma (IgM and IgG) antibodies by using an Enzyme Linked Immunosorbent Assay (ELISA) commercial available (Ratio Diagnostics, RD, Germany).

**Ethical considerations:** During data collection, pregnant women who gave their written consent for screening of their blood to detect *T. gondii* antibodies were selected. Brief explanation of the objective and the significance of the study were given to each participant in order to obtain verbal consent and to have the signature to avoid misunderstanding. The study proposal was evaluated and approved by the Ethics Committee of Faculty of Medicine and Health Sciences, Sana'a University.

## RESULTS

The study results illustrated on 5 tables: Table 1 shows the serum level of *Toxoplasma* IgM and IgG antibodies for different age groups of pregnant women. The total positive rate for IgM antibodies was 3.6%, while for IgG was 27.9%. A high IgM rate equal to 5.3% was observed in the 31-35 age group, and a higher IgG rate (36.8%) was observed in the same age group. Table 2 shows the serological patterns of *Toxoplasma gondii* pregnant women. There were 69.3% of pregnant women susceptible to infection with toxoplasmosis (negative for IgG and IgM), 2.9% recently infected (positive for IgM only), 27.1% protected (IgG positive only), and 0.7% were secondary infected (IgM and IgG positive). Table 3 shows the association of *Toxoplasma* IgM antibodies with stages of pregnancy. The high rate of IgM positivity was observed in the second trimester of pregnancy (4.7%), while the lowest rate was in the third trimester of pregnancy (2%). Table 4 shows the association of *Toxoplasma* IgG antibodies with stages of pregnancy. A slight increase in the rate of IgG positivity was observed in the third trimester of pregnancy (32%). Table 5 shows the association of *toxoplasma* IgG antibodies with potential risk factors. There was a significant association between contact with animals such as cats and infection with toxoplasmosis among pregnant women, where OR = 5.4, CI = 1.01-30.13,  $\chi^2 = 4.59$ ,  $p = 0.04$ . While there was no significant association with the other studied factors.

## DISCUSSION

This study showed an overall 27.9% sero-prevalence of anti-*T. gondii* IgG antibody among pregnant women in Amran City (Table 1). This result is within the range of seroprevalence of anti-*T. gondii* IgG antibody among pregnant women reported in previous studies in which they have shown a seropositivity rate in Arab region ranging from 22.9 to 58.2%<sup>21-23</sup>. This result was lower than that reported from Saudi Arabia, which it range from 38-61%<sup>24-26</sup>. While our 27.9% seroprevalence of anti-*T. gondii* antibody was slightly higher than that reported from UAE (22.9%)<sup>21</sup>.

When we considered age as a factor effects the prevalence of anti-*T. gondii* IgG antibody among pregnant women there was slight evaluating in the prevalence of anti-*T. gondii* IgG antibody with increasing age of pregnant women, e.g. rate in age group 20-24 years was 23.8%, this rate rise to 36.8% in age group 30-34 years (Table 1). This is similar to the results in the North Africa region, where the seroprevalence of *Toxoplasma gondii* is higher in older age groups compared to younger age groups<sup>27, 28</sup>, indicating that *T. gondii* may increase with age. This high rate of seropositivity at older ages may be the result of prolonged exposure to high environmental contamination of toxoplasma oocytes from animal sources, inadequate hygiene, and climatic factors appropriate for oocyst survival<sup>29</sup>.

There was a significant association between *T.gondii* infection and contact with domestic animals or farm

animals (OR = 5.4, CI = 1.01-30.13,  $\chi^2 = 4.59$ ,  $p = 0.04$ ). Our results are similar to what Fakhfakh *et al.*<sup>28</sup> and Zemene *et al.*<sup>30</sup> findings showing that contact with animals, especially cats, is significantly associated with *toxoplasmosis* seropositivity. There was no association between eating uncooked or raw meat (OR rate 1.08, 95% CI = 0.13-8.99;  $p = 0.939$ ) and *toxoplasma gondii* seropositivity in the current study. This finding is contradicted by Walle *et al.* study<sup>31</sup> in Africa where there is a strong association between eating undercooked or raw meat (OR= 5.73, 95% CI= 1.35-24.39;  $p = 0.02$ ) and *toxoplasmosis*<sup>31-34</sup>. These results can be explained by the fact that daily consumption of raw meat is very common in many parts of Africa<sup>35-37</sup>.

There was no significant association between residence in rural areas (OR= 1.05, 95% CI= 0.41 -1.6;  $P = 0.47$ ) and *T.gondii*-positive antibody (Table 5). These results differ from studies conducted in Egypt and Saudi Arabia, where they found that living in a rural area was an independent predictor of *toxoplasmosis*[<sup>24,36</sup>]. The high prevalence in rural areas is an acceptable result in places where there are poor sanitation facilities, contact with soil or animals, and drinking unpasteurized or unboiled water and milk, but these conditions are similar in rural and urban areas in Yemen, so there was a similar prevalence in rural areas and urban areas in our study.

Consumption of unwashed raw vegetables or fruits has been reported as a non-significant factor for *T. gondii* infection in the current study ( OR=0.86, 95% CI= 0.46 -1.6;  $p = 0.38$ ). This result differs from studies conducted in the Arab countries and China where the seropositivity of *toxoplasmosis* was significantly associated with eating vegetables that had not been washed<sup>32,37</sup>. It was found that the seropositivity of *Toxoplasma gondii* is not significantly correlated with soil contact in the current study ( OR=0.75, 95% CI= 0.35-1.0;  $p = 0.29$ ). This is separate from the results reported by several studies elsewhere which found that soil contact was associated with increased *toxoplasmosis* infection<sup>24,29, 38-39</sup>.

## CONCLUSION

Yemen has the lowest seropositivity than other Arab and African countries due to differences in risk factors. Data on *T. gondii* infection during pregnancy is scarce in many countries, especially where there is a lack of political stability such as Yemen. Risk factors identified included proximity to cats and pets, and increased maternal life. *Toxoplasmosis* during pregnancy in Yemen may be a health problem that has not really been underrated. More research is needed.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

"No conflict of interest associated with this work".

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**Table 1:** Seroprevalence of *Toxoplasma* IgM and IgG antibodies for different age groups of pregnant women in Amran city – Yemen 2017.

Age groups(years)	Toxoplasma antibodies positive			
	IgM		IgG	
	No.	%	No.	%
16-20 (n=30)	1	3.3	8	26.7
21-25 (n=101)	3	3.0	24	23.8
26-30 (n=63)	2	3.2	16	25.4
31-35 (n=57)	3	5.3	21	36.8
≥36 (n=29)	1	3.4	9	31.0
<b>Total (n=280)</b>	<b>10</b>	<b>3.6</b>	<b>78</b>	<b>27.9</b>

Table 2: Serological patterns in pregnant women for *Toxoplasma gondii* in Amran city –Yemen 2017.

Serological pattern	Number	Percentage%	Status
Negative Toxo-IgM with Negative Toxo-IgG	194	69.3	Susceptible
Positive Toxo-IgM only	8	2.9	Infected (recently)
positive Toxo-IgG only	76	27.1	Protected
Positive Toxo-IgM with positive Toxo-IgG	2	0.7	Infected (secondary)
<b>Total</b>	<b>280</b>	<b>100.0</b>	

**Table 3:** The association of *Toxoplasma* IgM antibodies with gestational stages of pregnant women in Amran city -Yemen 2017.

Trimesters	<i>Toxoplasma</i> IgM antibodies				$\chi^2$	P
	Positive		Negative			
	No.	%	No.	%		
First trimester (n=74)	2	2.7	72	97.3	1.06	0.58
Second trimester (n=148)	7	4.7	141	95.3		
Third trimester (n=50)	1	2.0	49	98.0		
<b>Total N=272*</b>	<b>10</b>	<b>3.6</b>	<b>262</b>	<b>96.3</b>		

**Table 4:** The association of *Toxoplasma* IgG antibodies with stages of pregnancy in Amran city-Yemen 2017.

Trimesters	<i>Toxoplasma</i> IgG antibodies				$\chi^2$	<i>P</i>
	Positive		Negative			
	No.	%	No.	%		
First trimester (n=74)	22	29.7	52	70.3	0.90	0.63
Second trimester (n=148)	38	25.7	110	74.3		
Third trimester (n=50)	16	32.0	34	68.0		
<b>Total (n=272*)</b>	<b>76</b>	<b>27.9</b>	<b>196</b>	<b>72.1</b>		

\*8 missing cases.  $\chi^2$  Chi square  $\geq 3.84$  (significant), *P* Probability value  $\leq 0.05$  (significant)

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Table 5: The association of *Toxoplasma* IgG antibodies with potential risk factors among pregnant women in Amran city –Yemen 2017.

Risk factors		<i>Toxoplasma</i> IgG antibodies				OR	CI	$\chi^2$	P
		Positive		Negative					
		NO	%	No	%				
Contact with animals (cat)	Yes (n=6)	4	<b>66.7</b>	2	33.6	5.40	1.01-30.13	4.59	<b>0.04*</b>
	No (n=274)	74	27.0	200	73.0				
Contact with soil	Yes (n=43)	10	23.3	33	76.7	0.75	0.35-1.60	0.54	0.29
	No (n=233)	67	28.8	166	71.2				
Consumption of uncooked meat	Yes (n=29)	5	17.2	24	82.8	0.49	0.18-1.36	1.90	0.12
	No (n=248)	73	29.4	175	70.6				
Consumption of raw vegetables	Yes (n=209)	57	27.3	152	72.7	0.86	0.46-1.61	0.20	0.38
	No (n=63)	19	30.2	44	69.8				
Residence	Urban (n=150)	42	28.0	108	72.0	1.05	0.62-1.78	0.03	0.47
	Rural (n=124)	36	29.0	88	71.0				

OR Odd ratio > 1 at risk

CI Confidence intervals

$\chi^2$  Chi square  $\geq 3.84$  (significant)

P Probability value  $\leq 0.05$  (significant)