



## SEROEPIDEMIOLOGY OF *TOXOPLASMA GONDII* AMONGST PREGNANT WOMEN WITH BAD OBSTETRIC HISTORY AT A TERTIARY CARE HOSPITAL IN CENTRAL INDIA

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### ABSTRACT

**Background:** Toxoplasmosis is a zoonosis caused by infection with *Toxoplasma gondii*. It is usually asymptomatic, but infection in pregnant women can pose serious health problems for the fetus like chorioretinitis, brain calcification, and microcephaly. It is therefore important to prevent transplacental transmission of *T.gondii* in pregnant females. **Aims & Objectives:** Considering that the seroepidemiology of *Toxoplasma* infection in the pregnant women is poorly studied, the main objective of this study was to estimate the seroprevalence and potential risk factors for *T. gondii* infection in women with BOH seeking antenatal care. **Materials & Methods:** Total 172 women with Bad obstetric history attending antenatal clinic were tested for *Toxoplasma* IgM and IgG antibodies by ELISA and a detailed history was taken according to case proforma to assess for potential risk factors. **Results:** The overall seroprevalence of anti-*T. gondii* antibodies was 33.7% (58/172). Among the seropositive women, 20.9% and 12.8% were positive for IgG antibody and IgM antibody respectively. Among the risk factors evaluated, residence in an urban area, contact with soil, consumption of raw or undercooked meat and unwashed vegetables, drinking unpasteurised milk were observed to be risk factors for Toxoplasmosis. No significant relationship was found between the seroprevalence of *T. gondii* infection and older maternal age, gestational age, gravida, level of education, contact with cats and drinking untreated water. **Conclusion:** Health education and awareness is important in pregnant women and women in childbearing age group regarding the risk factors associated with Toxoplasmosis to avoid deleterious effects of the infection on mother and fetus.

**KEYWORDS :** *Toxoplasma gondii*, Seroprevalence, Antibodies, Pregnancy

### INTRODUCTION

Human toxoplasmosis is a re-emerging, cosmopolitan zoonotic disease caused by parasite *Toxoplasma gondii*. It is an obligate intracellular protozoan parasite belonging to Apicomplexa phylum and its carrier and definite host are domestic cats and other felines<sup>1</sup>. *T. gondii* infects up to one third of the world's population. High prevalence has been reported among pregnant women and women of childbearing age across the globe. The infection occurs widely, and a lot of variations are seen depending on social and cultural habits, geographic factors, climate, and route of transmission. Higher prevalence has been reported in warm and humid areas<sup>2</sup>.

*Toxoplasma gondii* infection may occur vertically by cross-placental transmission and infect the fetus (congenital transmission), or horizontally by oral ingestion of infectious oocysts from the environment, oral ingestion of tissue cysts contained in raw or undercooked meat or primary offal (viscera) of intermediate hosts, unpasteurized milk and tissue transplants<sup>3,4</sup>.

The maternal-fetal transmission rate of *T.gondii* and its severity of causing congenital infection varies in different trimesters, with severity of infection being highest and the transmission rate being lowest during the first trimester whereas the opposite is true for the third trimester<sup>5</sup>.

Women are usually asymptomatic when they acquire *T. gondii* infection in pregnancy. Vertical transmission from a recently infected pregnant woman to her fetus may lead to congenital toxoplasmosis that causes chorioretinitis, intracranial calcification, hydrocephalus, and mental retardation of the infant<sup>6</sup>. If the pregnant woman is detected with *Toxoplasma* infection, the most commonly used drug is spiramycin because it can be absorbed efficiently and has little side effects to the fetus<sup>7</sup>.

It is important to understand the socio-demographic profile of the pregnant women for formulating primary and secondary health-care policy to treat toxoplasmosis<sup>1</sup>.

### AIMS AND OBJECTIVES:

This study aims to determine the seroprevalence of IgM and IgG anti-*Toxoplasma* antibodies and the associated risk factors among pregnant women with BOH using enzyme linked immunosorbent assay (ELISA) method.

### MATERIALS & METHODS:

We performed an observational study enrolling 172 women with BOH

attending Antenatal clinic at a tertiary care hospital in Central India, from November 2016 to March 2019.

Study group consisted of women in the age group 19-36 years. Approximately 5 mL of venous blood samples were collected, and their sera was separated and kept at -20°C until further testing.

After informed consent, detailed history was obtained from each woman according to the case proforma. These included obstetric history, socio-demographic background like age, education, residence, contact with cat, contact with soil and dietary habits (like eating undercooked or raw meats, such as pork, mutton, beef or consuming unwashed vegetables and fruits, drinking unpasteurised milk or untreated water)

**Inclusion criteria:** Antenatal women with BOH depending on previous unfavorable fetal outcome in terms of

- Two or more consecutive spontaneous abortion
- History of intrauterine fetal death
- Intrauterine growth retardation
- Preterm labor
- Still births
- Early neonatal death
- Congenital anomalies

**Exclusion criteria:** No specific exclusion criteria

### Serological methods

Testing for *Toxoplasma* infection was done by Indirect Enzyme linked Immunosorbent Assay (ELISA) for specific IgM and IgG antibodies using commercial kit according to manufacturer's instructions. Ratio Diagnostics E-TXM-K19 *Toxoplasma* IgM ELISA kit and Ratio Diagnostics E-TXG-K18 *Toxoplasma* IgG ELISA kit provided by RD-Ratio Diagnostics Westerbachstraße 47, 60489 Frankfurt, Germany were used.

### Statistical assessment

The SPSS 27.0 software for Windows was used to record data and for analyses. The Chi-Square test was used for the analytic assessment. The differences were considered to be statistically significant when the *p* value obtained was less than 0.05.

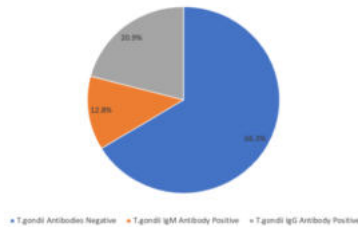
### Ethics

Ethical clearance was obtained from the Ethics committee of the institute.

**RESULTS**

*Toxoplasma gondii* seroprevalence varies widely in different parts of the world. Overall, the seroprevalence of *Toxoplasma* infection in our study was 33.7% (58/172) of which 12.8% (22/172) were positive for anti-*Toxoplasma* IgM antibodies and 20.9% (36/172) were positive for anti-*Toxoplasma* IgG antibodies.

Figure 1. Seroprevalence of T.gondii Antibodies in Pregnant Women with BOH (n = 172)



**Table 1: Demographic & Obstetric Factors Associated with *Toxoplasma gondii* Infection in Pregnant women**

Demographic and Obstetric factors	Total No of Cases (n=172)	<i>T.gondii</i> Positive Cases(n=58)	<i>T.gondii</i> Negative Cases(n=114)	p value
<b>Age (in years)</b>				
19-24	60	18(30%)	42(70%)	p = 0.7013
25-30	78	27(34.6%)	51(65.4%)	
31-36	34	13(38.2%)	21(61.8%)	
<b>Gestational Age</b>				
1 <sup>st</sup> Trimester	88	36(41%)	52(59%)	p = 0.1051
2 <sup>nd</sup> Trimester	43	10(23.2%)	33(76.8%)	
3 <sup>rd</sup> Trimester	41	12(29.2%)	29(70.8%)	
<b>Gravida</b>				
≤3	99	31(31.3%)	68(68.7%)	p = 0.55
4	42	14(33.3%)	28(66.7%)	
≥5	31	13(41.9%)	18(58.1%)	
<b>Place of Residence</b>				
Urban	106	44(41.5%)	62(58.5%)	p = 0.00617
Rural	66	14(21.2%)	52(78.8%)	
<b>Education Level</b>				
Uneducated	70	29(41.4%)	41(58.6%)	p = 0.2071
Primary	80	25(31.2%)	55(68.8%)	
Secondary	18	3(16.7%)	15(83.3%)	
Higher	4	1(25%)	3(75%)	

*p* value was calculated using Chi-Square test  
 In the present study, highest positivity rate for *Toxoplasma* antibodies of 38.2% (13/34) was seen in age group of 31-36 years followed by 34.6% (27/78) among 25-30 years and 30% (18/60) among 19-24 years as shown in table 1. Thus, showing an increasing trend with age. However, this difference in seropositivity in different age groups was not significant ( $p>0.05$ ).

Seroprevalence of *Toxoplasma* was 41%, 23.2%, and 29.2% for first, second, and third trimester, respectively.

There was gradual increase of seropositivity for *Toxoplasma* infection with increasing gravida. However, the *p* value was more than 0.05 and no significant association was found.

Regarding the participants residential area, 41.5% (44/106) of the *Toxoplasma* positive women lived in an urban area, while 21.2% (14/66) lived in rural areas. There was a significant association between seropositivity among participant coming from urban areas and testing positive for *Toxoplasma* infection. ( $p=0.00617$ )

**Table 2: Risk Factors Associated With *Toxoplasma Gondii* Infection In Pregnant Women**

Risk factors	Total No of Cases (n=172)	<i>T.gondii</i> Positive Cases(n=58)	<i>T.gondii</i> Negative Cases(n=114)	p value
<b>Contact with Cats</b>				
Yes	41	12(29.3%)	29(70.7%)	p = 0.4895
No	131	46(35.1%)	85(64.9%)	
<b>Contact with Soil</b>				
Yes	54	32(59.2%)	22(40.8%)	p = 0.00001
No	118	26(22.1%)	92(77.9%)	

<b>Consumption of Raw or Undercooked Meat</b>				
Yes	61	27(44.3%)	34(55.7%)	p = 0.030171
No	111	31(27.9%)	80(72.1%)	
<b>Consumption of Raw or Unwashed Vegetables &amp; fruits</b>				
Yes	103	43(41.7%)	60(58.3%)	p = 0.006518
No	69	15(21.7%)	54(78.3%)	
<b>Drinking Unpasteurized Milk</b>				
Yes	90	37(41.1%)	53(58.9%)	p = 0.031729
No	82	21(25.6%)	61(74.4%)	
<b>Drinking Untreated Water</b>				
Yes	105	41(39.1%)	64(60.9%)	p = 0.06433
No	67	17(25.4%)	50(74.6%)	

*p* value was calculated using Chi-Square test  
*Toxoplasma* positive women who reported having contact with cats were found to be 29.3% (12/41) compared to 35.1% (46/131) who did not have contact with cats.

A strong association was present between seropositive women and their contact with soil ( $p=0.00001$ ). 59.2% (32/54) women who tested positive for *T.gondii* antibodies had history of contact with soil.

44.3% (27/61) women who consumed raw or undercooked meat tested positive for *Toxoplasma* antibodies. 41.7% (43/103), 41.1% (37/90) and 39.1% (41/105) women who consumed raw or unwashed vegetable & fruits, unpasteurised milk and untreated drinking water respectively also showed presence of *Toxoplasma* antibodies.

In terms of dietary characteristics, those who ate undercooked or raw meat ( $p=0.03$ ), consumed raw vegetables and fruits ( $p=0.006$ ), those drinking unpasteurised milk ( $p=0.03$ ) were more likely to be seropositive.

**DISCUSSION**

Among the 172 women tested, 58 showed presence of anti-*Toxoplasma* antibodies i.e., 33.7%. The seroprevalence of *T. gondii* ranges from 6.1% to 74.5% among different regions of the world. The overall anti-*Toxoplasma* antibody prevalence was only 10.3% by a study done by Sakikawa *et al*<sup>8</sup>. Our results were similar to Mustafa *et al* (2018)<sup>9</sup> who observed a seroprevalence of 10% for *Toxoplasma* IgM Antibody. The study was also in concordance with a report by Sadik *et al* (2012)<sup>10</sup> who showed a prevalence of 20.93% for *Toxoplasma* IgG antibodies in BOH cases. While higher seropositivity for *Toxoplasma* IgM antibodies and IgG antibodies i.e., 21.54% and 50% were reported by Agrawal *et al* (2016)<sup>11</sup> and Kumari *et al* (2011)<sup>12</sup> respectively. It is observed that active *Toxoplasma* infection can occasionally persist from one pregnancy to the other and may be one of the cause for repeated abortions or still births. In such cases, *T. gondii* may encyst in the uterine endometrium and is stirred into activity by the process of placentation. This may lead to low grade local endometritis which may persist. Hence, it is essential to test for both IgG and IgM antibodies, as chronic infection can also lead to fetal wastage<sup>13</sup>.

Although seroprevalence of Toxoplasmosis increased with age in our study but there was no significant association ( $p=0.7013$ ). This suggests that the probability of coming into contact with sources of infection increases with age. As the number of high-risk pregnancies, including late childbearing, has continued to increase in recent years, the probability of contacting potential sources of infection may also increase. Older pregnant women should be followed carefully through antenatal care<sup>8</sup>. Increased seroprevalence with age can be because of increasing time of exposure to various risk factors<sup>14</sup>. In contrast to our study, Chintapalli S *et al* (2013)<sup>15</sup> in Visakhapatnam observed that seropositivity of *Toxoplasma* IgM antibodies decreased with age.

The first trimester showed 41% seroprevalence while it was lower 29.2% and 23.2% in the third and second trimester respectively. But this difference was not statistically significant. However, studies done in Sri Lanka by Iddawela *et al*<sup>3</sup> and Aqeely *et al*<sup>15</sup> in Saudi Arabia all showed IgG seropositivity to be highest in the 2nd trimester. Pregnant women at their first, second and third trimesters showed different rates of the *Toxoplasma* infection in reports by Mahmoudvand *et al*<sup>7</sup> and Hajssoleimani *et al*<sup>16</sup>. Thus, showing inconsistent results.

The current study reported a higher seroprevalence among participants from urban areas than among those from rural areas. Urbanization in developing countries may have been associated with poor socio-economic conditions caused by overpopulation and poverty. This

suggests that factors affecting the risk of contracting infection are very complex than a simple urban-rural division and may be confounded by other factors<sup>1</sup>. This association was also found in other studies conducted in various parts of the world like Peru and Sri Lanka<sup>1,5</sup>.

Despite some studies have stated that low level of education was associated with higher rate of Toxoplasmosis and vice versa, we did not find a significant relationship between the seroprevalence of *T. gondii* infection and the level of education. High level of education may reduce risk exposure and increase awareness to follow hygienic measures regarding food and cooking behavior such using different chopping board for meats and vegetables, washing chopping boards with soap, frequent washing of knives and hands while cooking and avoiding contamination of food by protecting it from dust and flies<sup>3,17</sup>.

There was no significant difference ( $p = 0.4895$ ) in *Toxoplasma* seropositivity among women reported to have contact with cats compared to those having no contact with cats. The association of cats and human toxoplasmosis is difficult to assess by only epidemiological surveys because soil, not the cats, is the main culprit. Oocysts are absent on cat fur and are often buried in soil along with cat faeces, and soil contact is universal and unavoidable<sup>14</sup>. A study conducted in Sri Lanka by Iddawela *et al*<sup>5</sup> showed presence of pet cats in household was not statistically meaningful with the seropositivity of pregnant women similar to our study. However, pregnant females and women in childbearing age who own cats should avoid coming in contact with cat litter boxes<sup>5</sup>. On the other hand, study from Ethiopia<sup>18</sup> showed a significant association between contact with cats and seroprevalence of *Toxoplasma* infection.

The current study identified contact with soil as a significant risk factor associated with *Toxoplasma* seropositivity. Under favourable environmental conditions oocysts can survive in soil and remain infective for many years. Contact with soil was found to be highly significant in other parts of the world as well for seropositivity of *T. gondii*<sup>5</sup>.

In our study consumption of undercooked or raw meat and unwashed raw vegetables and fruits were strong predictors of *T. gondii* infection. Similar results have been observed in studies done in Cameroon, Sudan and London<sup>2,19,20</sup>. Pregnant women should refrain from eating raw or undercooked meat. Toxoplasmosis can largely be prevented by cooking meat at a safe temperature<sup>8</sup>.

No significant association was observed between drinking untreated water and *Toxoplasma* infection similar to a study by Wam *et al*<sup>5</sup>. In contrast, other study in Thailand<sup>21</sup> showed statistically significant association between the two. Sporulated oocysts can survive in moist soil for months to years. Though no significant association was found between *T. gondii* infection and untreated water, may be oocysts were absent in untreated water in some of our study areas.

## CONCLUSION

In a developing country like India majority of the population is unaware of this parasitic disease. Lack of hygienic practices, Illiteracy, epidemiological factors like unsafe drinking water and climate can contribute to increased risk of acquiring the disease. Our findings indicate a strong correlation between Toxoplasmosis in pregnant women and their contact with soil, consumption of raw or undercooked meat and raw or unwashed vegetables, drinking unpasteurised milk. Therefore, an awareness campaign should be conducted to educate pregnant women during their Antenatal visits regarding transmission routes and preventive measures to minimize exposure to *T. gondii*. Thus, avoiding unfavourable fetal outcome.

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