SEROPREVALENCE OF TOXOPLASMA GONDII INFECTION AND ASSOCIATED RISK FACTORS AMONG ASYMPTOMATIC PREGNANT FEMALES IN EGYPT

By
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Abstract
Protozoan parasite Toxoplasma gondii is considered as one of the most critical risk factor for recurrent abortion in pregnant females and resulted in multi congenital malformation in fetus world-wide. The present study was carried out on 693 pregnant females from Alexandria, Beheira, Gharbia, Menoufia, Qalyoubia and Fayoum provinces. The study determined the prevalence of toxoplasmosis in pregnant females during pregnancy trimesters and shed the lights on the main risk factors and possible contamination routes. Detection for the presence of Toxoplasma IgG antibodies were done by enzyme linked fluorescence assay (ELFA). It was found that the overall seroprevalence of T. gondii was 30.16%. The seroprevalence increased with age. Significant relations were observed between Toxoplasma IgG antibodies and abortion history, maternity trimester and consumption of under-cooked meat. No significant differences were reported due to parity, occupation, abortion trimester, contact with cats and/or other animals and exposure to soil.

Keywords: Toxoplasma gondii, Pregnancy, Prevalence, Egypt, ELFA

Introduction
Infection with the intracellular parasite Toxoplasma gondii causes a foodborne, zoonotic disease known as toxoplasmosis with a worldwide distribution. In both developing and developed countries, the parasite infects approximately 30-50% of the human population (Flegr et al, 2014). In USA, toxoplasmosis was the fourth cause of hospitalization after salmonellosis, campylobacteriosis and norovirus infections (Scallan et al, 2011). For humans, sources of T. gondii infection were reported such as food and/or drinking water contaminated with sporulated oocysts, by ingestion of tissue cysts from undercooked meat, and congenital vertical transmission (Pomares et al, 2011).

T. gondii infection causes encephalitis, brain abscesses and death when it is reactivated in immuno-compromised or immunodeficient patients (Walker and Zunt, 2005; Robert-Gangneux and Dardé 2012; Rostami et al, 2014). Moreover, several recent studies have reported that latent toxoplasmosis was related to male and female infertility (Dalimi and Abdoli, 2013; Shiadeh et al, 2016) and associated with neuropsychiatric disorders such as schizophrenia, suicide, bipolar disorder, parkinson disease, obsessive compulsive disorder and anxiety (McConkey et al, 2013; Sutterland et al, 2015). During pregnancy T. gondii can cause abortion in pregnant females, ocular and neurological impairment like mental retardation, blindness, seizures, epilepsy, microcephaly and hydrocephalus in fetus (Pappas et al, 2009; Robert-Gangneux and Dardé 2012). Therefore, screening of antibodies against T. gondii in different groups of patients to determine those who are at risk of getting a primary Toxoplasma infection help in increasing of epidemiological data on toxoplasmosis in the general population, aid in the establishing control measures and prohibitions of toxoplasmosis complications (Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012). Enzyme linked fluorescence assay (ELFA) showed higher sensitivity and specificity than enzyme linked immunosorbent assay (ELISA) and immunosorbent agglutination assay (ISAGA) methods and therefore had more trustful results (Gharavi et al, 2011).
Meat products was proved as critical source of human toxoplasmosis, *T. gondii* as foodborne disease, was prevalent and detected in cattle (Ibrahim *et al.*, 2009), camels (Hilali *et al.*, 1998), water buffaloes (Dubey *et al.*, 1998), sheep and goats (Ibrahim *et al.*, 1997; Shaapan *et al.*, 2008; Mahboub *et al.*, 2013; Younis *et al.*, 2015), ducks, turkeys, rabbits free-range and caged chickens (El-Massey *et al.*, 2000; Dubey *et al.*, 2003; Ibrahim *et al.*, 2009; Harfoush and Tahoon, 2010; Ibrahim *et al.*, 2016), ostrich (El-Madawy and Metawea, 2013), and pigs (Hassanain *et al.*, 2013). Regardless meat consumption the parasite was detected in draught horses (Haridy *et al.*, 2009) and working donkeys and donkey’s milk (Haridy *et al.*, 2010). Moreover, *T. gondii* antibodies were detected in Egyptian pregnant women using different serological assays but not ELFA (Azab *et al.*, 1993; Ibrahim *et al.*, 2009; Abd El-Ghany and Amin, 2012; Mohamed and Ibrahim, 2012; Kamal *et al.*, 2015). In Arabian countries, *T. gondii* antibodies were detected in pregnant women in Jordan (Morsy and Michael, 1980), Libya (Kassem and Morsy, 1991) and Saudi Arabia (Eidi, 2015).

The present study aimed to assess the seroprevalence and risk factors of *T. gondii* in asymptomatic pregnant females in different regions of Egypt using ELFA.

**Subjects, Materials and Methods**

Sample collection and detection of anti-*Toxoplasma* IgG by ELFA: A total of 693 asymptomatic pregnant female samples were collected during 2014 from different provinces, Gharbiya, Qalyoubiya, Menoufia, Beheira, Alexandria and Fayoum, in Egypt. The samples were obtained from the brachial vein in private clinical laboratories. Blood samples were collected and centrifuged at 1000×g for 10 min, and the separated serum was stored at -80 °C till examination. The serological tests were determined using Mini-VIDAS machine (BioMérieux S.A., France) which can measure antibodies by using an ELFA. Mini-VIDAS kits (TOXO-IgG) were obtained from BioMérieux Company, France and utilized in accordance with manufacturer’s procedure.

Ethical approval: The current study was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice and approved by the scientific ethical committee of the National Liver Institute, Menoufia University, Egypt. A written informed consent was obtained from each participant.

![Map of sampling area. Sera collected from 693 pregnant females at private clinical laboratories in Menoufia, Gharbia, Qalyoubia, Beheira, Alexandria and Fayoum.](image)

Fig. 1: Map of sampling area. Sera collected from 693 pregnant females at private clinical laboratories in Menoufia, Gharbia, Qalyoubia, Beheira, Alexandria and Fayoum.

Questionnaire: All participants were interviewed using a standardized questionnaire, which included data about location, age, socio-demographic characteristics (parity, occupation, maternity trimester, abortion and abortion trimester) and lifestyle habits concerning to parasite transmission risk factors. These contain consumption of undercooked meat and meat products exposure to soil (farming, and gardening in home), exposure to cats (pet cat ownership or have a contact with street cats) and contact with other animals (pet dog ownership or domestic field animals). The answers were recorded in dichotomous form (yes or no); patients were considered exposed to a factor if they gave a positive response to any of its manner.

Statistical analysis: Chi square test was utilized to assess significant differences (*P* < 0.05) of infection rate in pregnant females of
different location, age, socio-demographic characteristics and infection risk factors. Means in same columns assigned with different letter showed significant differences between these values \((P < 0.05)\).

**Results**

Table 1: Seroprevalence of *T. gondii* in pregnant females in six provinces using ELFA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Examined</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexandria</td>
<td>101</td>
<td>13</td>
<td>12.87**</td>
</tr>
<tr>
<td>Beheira</td>
<td>34</td>
<td>10</td>
<td>29.41**</td>
</tr>
<tr>
<td>Gharbia</td>
<td>78</td>
<td>21</td>
<td>26.92**</td>
</tr>
<tr>
<td>Menoufia</td>
<td>376</td>
<td>124</td>
<td>32.98**</td>
</tr>
<tr>
<td>Qalyoubiya</td>
<td>78</td>
<td>21</td>
<td>26.92**</td>
</tr>
<tr>
<td>Fayoum</td>
<td>26</td>
<td>20</td>
<td>76.92***</td>
</tr>
<tr>
<td>Total</td>
<td>693</td>
<td>209</td>
<td>30.16</td>
</tr>
</tbody>
</table>

Means in same columns assigned with same letter showed insignificant differences between these values \(P > 0.05\).

* Indicate that seroprevalence of *T. gondii* is significantly different \((P < 0.05)\).

Overall prevalence of *T. gondii* was 30.16% in asymptomatic pregnant women, using ELFA (Tab. 1). The higher seropositivity was detected in Fayoum 76.92\% (20/26) then Menoufia 32.98\% (124/376), Beheira 29.41\% (102/347), Gharbia 26.92\% (21/78), Qalyoubia 26.92\% (21/78) and Alexandria with values of 12.87\% (13/101). Alexandria showed the lowest seropositivity. Statistically significant increase was detected in Fayoum when compared to Alexandria. No significant difference was detected among other areas when compared to each other or compared to Fayoum or Alexandria.

Table 2: Socio-demographic characteristics and seroprevalence of toxoplasmosis among pregnant females.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (year)&lt; = 25</td>
<td>347</td>
<td>102</td>
<td>29.39</td>
</tr>
<tr>
<td>&gt; 25</td>
<td>346</td>
<td>107</td>
<td>30.92</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One time</td>
<td>536</td>
<td>167</td>
<td>31.16</td>
</tr>
<tr>
<td>Two times or more</td>
<td>157</td>
<td>42</td>
<td>26.75</td>
</tr>
<tr>
<td>Maternity trimester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>639</td>
<td>191</td>
<td>29.89</td>
</tr>
<tr>
<td>2nd</td>
<td>54</td>
<td>18</td>
<td>33.33*</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>11</td>
<td>36.67</td>
</tr>
<tr>
<td>No</td>
<td>663</td>
<td>198</td>
<td>29.86</td>
</tr>
<tr>
<td>Abortion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>530</td>
<td>116</td>
<td>21.89</td>
</tr>
<tr>
<td>Present</td>
<td>163</td>
<td>93</td>
<td>57.06*</td>
</tr>
<tr>
<td>Abortion trimester 1st</td>
<td>110</td>
<td>59</td>
<td>53.64</td>
</tr>
<tr>
<td>2nd</td>
<td>43</td>
<td>28</td>
<td>65.12</td>
</tr>
<tr>
<td>3rd</td>
<td>10</td>
<td>6</td>
<td>60.00</td>
</tr>
</tbody>
</table>

* Indicate that seroprevalence of *T. gondii* significantly different \((P < 0.05)\, chi-square test).  

Regarding age, toxoplasmosis seropositivity was higher in asymptomatic pregnant females aged more than 25 years old, 30.92\% (107/346) compared to those 25 years old or less 29.39\% (102/347) without significant difference (Tab. 2). According to parity, seropositivity was higher in pregnant females with less than two times pregnancy 31.16\% (167/536) than those had two times or more pregnancy 26.75\% (42/157). Seroprevalence was significantly \((P < 0.05)\) higher in pregnant females in second maternity trimester 33.33\% (18/54) than those in first maternity trimester 29.89\% (191/639). Regarding occupation, working females showed higher seropositivity 36.67\% (11/30) than housewives 29.86\% (198/663). Aborted females showed significant increase in positivity 57.06\% (93/163) than those without history of abortion 21.89\% (116/530). On the basis of abortion trimester, females who aborted in the second or third trimesters rec-
recorded high seropositivity 65.12% (28/43), 60.0% (6/10) respectively, compared to females aborted in the first trimester 53.64% (59/110). No significant changes were recorded in age, parity, occupation, or abortion trimester in relation to the parasite seropositivity.

### Table 3: Risk factors associated with Toxoplasma positivity in pregnant females.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with cats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>19</td>
<td>54.29</td>
</tr>
<tr>
<td>No</td>
<td>658</td>
<td>190</td>
<td>28.88</td>
</tr>
<tr>
<td>Contact with other animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>668</td>
<td>196</td>
<td>29.34</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>13</td>
<td>52.00</td>
</tr>
<tr>
<td>Exposure to soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>8</td>
<td>66.67</td>
</tr>
<tr>
<td>No</td>
<td>681</td>
<td>201</td>
<td>29.52</td>
</tr>
<tr>
<td>Consumption of undercooked meat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>193</td>
<td>100</td>
<td>51.81*</td>
</tr>
<tr>
<td>No</td>
<td>500</td>
<td>109</td>
<td>21.80</td>
</tr>
</tbody>
</table>

*Indicate that seroprevalence of *T. gondii* significantly different (*P < 0.05, chi-square test).

Pregnant females who had a contact with cats showed higher seropositivity 54.29% (19/35) than those who did not contact with cats 28.88% (190/658) (Tab. 3). On the other hand, pregnant females who didn’t have a contact with other animals had high seropositivity 52.0% (13/25) when compared to those had contacted with other animals 29.34% (196/668). Pregnant females exposed to soil displayed higher seropositivity compared to those didn’t exposed 29.52% (201/681). No significant differences were recorded in *T. gondii* positivity on basis of contact with cats, other animals or exposure to soil. Parasite prevalence was significantly increased in pregnant females eat undercooked meat, especially ovine meat, 51.81% (100/193) when compared to those didn’t eat undercooked meat 21.8% (109/500).

### Discussion

In Egypt, many pregnant females lack knowledge of the risk factors related to toxoplasmosis (Mohamed and Ibrahim, 2012). Pregnant females who acquired *Toxoplasma* infection might remain asymptomatic, although they could transmit the infection vertically to their fetuses with many congenital complications (Kravetz and Federman, 2002). During pregnancy, females who lived with oocysts carriers were highly at risk of infection (Avelino et al, 2004). The present results showed that 30.16% of the pregnant females were *T. gondii* chronically infected patients. Based on location, seroprevalence was significantly high in Fayoum as compared to Alexandria, and insignificant in Gharbia, Qalyoubia, Menoufia and Beheira as compared to either Alexandria and/or Fayoum. The present high prevalence agreed with Egyptian authors who found seroprevalence (>50%) in Egyptian pregnant females (Rifaat et al, 1973; El-Nawawy et al, 1996; Ibrahim et al, 2009; Saleh et al, 2014; Kamal et al, 2015). The serologic evidence showed that toxoplasmosis prevalence and incidence among women varied worldwide (Zuber and Jacquier, 1995). High seropositivity of *T. gondii* during pregnancy and after abortion was 54% & 58.2% in Jordan (Abdel-Hafez et al, 1986; Nimri et al, 2004), 48% in India (Borkakoty et al, 2016), 42.47% & 30.70% in Malaysia and Myanmar (Andiappan et al, 2014) and 44.9% in Mexico (Galvan Ramirez et al, 1995).

In the current study, no significant differences were detected between seropositivity of *T. gondii*, and age, parity and occupation. Regarding age, infection percentage increased with increasing age, the present study recorded 30.92% in pregnant females with age more than 25 years old and...
The present study agreed with previous reports (Mohamed and Ibrahim, 2012; Sarkar et al., 2012; Zemene et al., 2012; Majid et al., 2016). Regarding parity, the current study showed increasing levels of *T. gondii*-IgG in women who were pregnant for one time than those pregnant for two times or more, which agreed with Ayi et al. (2009) in Ghana. Others detected higher prevalence of *T. gondii* in housewives compared to employees (Nijem and Al-Amleh, 2009; Al Se’adawy, 2010; Kamal et al., 2015), adversely with others the present prevalence of *Toxoplasma* was higher in working women than housewives, who might consume fast food.

In the present study, the seroprevalence showed significant increase in pregnant females during the second trimester compared to those in the first trimester. Previous reports displayed significant or insignificant elevation in the positivity of *Toxoplasma* with increasing pregnancy trimester in India and Yemen (Saif et al., 2014; Borkakoty et al., 2016). Moreover, similar results were detected in Myanmar (Andiappan et al., 2014). One of the most critical risk factor of miscarriage is a *Toxoplasma* infection in human pregnant females. The present study demonstrated high statistically significant *T. gondii* seroprevalence in females with abortion history, than those with no history of abortion. Females who were aborted in the second trimester or the third trimester showed high parasite seropositivity compared to those aborted in the first trimester. Several previous studies come in concordance with the current results (Saif et al., 2014; Desta, 2015; Nassef et al., 2015).

Toxoplasmosis is a zoonotic disease and human in close contact with cats had a high possibility to be infected (Kravetz and Federman, 2002; Roman et al., 2006). Cats are the definitive hosts of *Toxoplasma* in which the sexual cycle takes place (Frenkel et al., 1970) therefore; cats play a main role in the epidemiology of this parasite through shedding infective parasite oocysts with their feces. For this reason, contact with cats was considered as a main risk factor for human community’s infection with *T. gondii* (McAllister, 2005). This study demonstrated that seropositivity of *T. gondii* in pregnant woman contacted with cats was higher than those did not contact with cats. In Nigeria (Ishaku et al., 2009) and Ethiopia (Zemene et al., 2012) reported association between contact with cats and *T. gondii* seropositivity. In the present study, high prevalence was detected among owners of cats but without significant difference between *T. gondii* and cat’s contact. These pet cats might be treated by safety standard measures (Dabritz and Conrad, 2010). These results were consistent with those done in Tanzania (Doehring et al., 1995) and Palestine (Nijem and Al-Amleh, 2009).

The present study showed prevalence of *T. gondii* in pregnant females in contact with other animals was lower than females who didn’t contact them. Saif et al. (2014) found the similar results in Yemen. The current study reported that seropositivity of pregnant females exposed to soil was higher than females who didn’t deal with soil, but without significant differences, which agreed with Andiappan et al. (2014) and Cong et al. (2015). The association between toxoplasmosis and soil contact was not surprising. Yaghoub and Yagoob (2014) reported that the availability of appropriate climate for oocyst sporulation in infected soil was a one of the major routes of zoonosis. In the current study, pregnant females who ate under-cooked meat showed significant increase compared to those who didn’t eat under-cooked meat. Studies recorded that consumption of under-cooked infected meat elevated the incidence of zoonosis (Moazeni Jula et al., 2013, Abd El-Razik et al., 2014; Andiappan et al., 2014).

**Conclusion**

The study reported frequent occurrence of *T. gondii* among asymptomatic Egyptian pregnant females and consequently increase the risk of abortion. This finding suggests
the need for rapid involvement in public campaign to increase awareness of toxoplasmosis risk factors. Despite the lack of statistical correlation between infection and many risk factors, preventive measures must be followed to prevent toxoplasmosis.

Conflict of interest: The authors declare no conflict of interest related to this work.

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Authors’ contributions: Ibrahim conceived the study, Ibrahim, and El-Shqanqery designed and performed the experiments, Ibrahim, Mohamed and El-Shqanqery analyzed the results, and all authors wrote and revised the manuscript.

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