Journal of the Egyptian Society of Parasitology, Vol. 47, No. 1, April 2017
J. Egypt. Soc. Parasitol. (JESP), 47(1), 2017: 93- 100

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 ºC 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.

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.

Variable	Total	Examined	Percentage
Alexandria	101	13	12.87 [a]*
Beheira	34	10	29.41 [ab]
Gharbiya	78	21	26.92 [ab]
Menoufia	376	124	32.98 [ab]
Qalyoubiya	78	21	26.92 [ab]
Fayoum	26	20	76.92 [b]*
Total	693	209	30.16

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 seropositivty was detected in Fayoum 76.92% (20/26) then Menoufia 32.98% (124/376), Beheira 29.41% (10/34), 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.

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

* 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-

orded 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.

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

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

Pregnant females who had a contact with cats showed higher seropositivty 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

29.39 % in those 25 years old or less. 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 undercooked meat showed significant increase compared to those who didn't eat undercooked 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.

Acknowledgements

The authors would like to thank the Laboratory Medical Staff for collecting blood samples.

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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