



Serological Survey on *Toxoplasma gondii* in some Dairy Animals and Pregnant Women in Qena, Egypt

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ABSTRACT

Toxoplasmosis is an important reason of reproductive failure in human and farm animals causing significant socioeconomic losses worldwide. Additionally, infection in pregnant women can cause severe health problems in the child as mental retardation and blindness. In this work the seroprevalence of *T. gondii* infection was estimated in dairy goats, sheep, and cows as well as in pregnant women in Qena Province, Egypt using the Enzyme Linked Immunosorbent Assay (ELISA). The study included 150 raw milk samples which were collected from the previously mentioned animals in different localities (50 from each) as well as 100 pregnant women serum samples with a history of abortion. Our results revealed that *T. gondii* IgM and both IgM & IgG antibodies were detected in 20, 40 and 28, 6% of goat and sheep milk samples, respectively. While *T. gondii* IgM antibodies could be detected in 64% of cow milk samples. A total of 28 (28%) women were seropositive for toxoplasmosis, divided into 2 (2%) were seropositive for *T. gondii* IgM antibodies and 26 (26%) for IgG. There was an association between IgG seroprevalence and age and the times of abortion in pregnant women.

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Introduction

Toxoplasmosis is a common parasitic zoonosis worldwide, caused by an obligate intracellular cyst-forming Apicomplexans protozoan parasite called *Toxoplasma gondii* that can infect almost all warm-blooded animals, including humans and domestic animals which act as intermediate hosts (Witkowski *et al.*, 2015). Several studies estimated that about one-third of the world's population has been infected with *T. gondii* (Montoya and Liesenfeld, 2004; Weiss and Dubey, 2009) and about 20.7% of deaths due to foodborne infectious agents is caused by toxoplasmosis (Ortega, 2006). Members of family Felidae play a significant role in the epidemiology of the disease since they are the definitive hosts of the parasite excreting thousands of environmentally resistant oocysts (Dubey *et al.*, 2014).

Goats, sheep, and cattle are highly susceptible to *T. gondii* infection leading to great economic losses through causing embryonic death, fetal death, abortion, stillbirth, and reduced

milk production (Liu *et al.*, 2011; Wang *et al.*, 2011). Goats, sheep, and cows' raw milk plays an important role in the transmission of *T. gondii* to humans (Al-Khatib, 2011; Mancianti *et al.*, 2013; Ding *et al.*, 2017; Tonouhewa *et al.*, 2017).

Regarding public health, toxoplasmosis has been constituted a zoonotic disease with the highest human incidence by the European Food Safety Authority (EFSA, 2007). Human infection usually occurs horizontally through ingestion of undercooked meat containing tissue cysts, drinking milk contaminated with the parasite, accidental ingestion of sporulated oocysts from the environment or vertically by passing tachyzoites to the fetus through the placenta. However sub-clinical infections occur in immunocompetent individuals, *T. gondii* infection can cause severe health problems in those with immune suppression (Weiss and Dubey, 2009; Wang *et al.*, 2017). In pregnant women, *T. gondii* infection is highly serious as it leads to abortion, stillbirth, and other ocular congenital infections in acute cases, while in chronic infection the fetal encephalitis is the most common sign (Kamani *et al.*, 2010) with different severity, depending on the trimester at which the pregnant woman infected; during the first trimester the infection is more severe than in the second and third trimester (Remington *et al.*, 2001).

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So this study was designed to assess the presence of *T. gondii* IgM and IgG antibodies in goats, sheep, and cows' raw milk samples as well as in pregnant women serum samples in Qena Province, Egypt using Enzyme Linked ImmunoSorbent Assay (ELISA).

Materials and methods

Samples

A total of 150 raw milk samples were collected randomly from different milking in-housed reared animals (ewes, does and cows, 50 samples for each) from different localities in Qena Province, Egypt. 10 mL of raw milk were collected from each animal manually after disinfection of the teats with alcohol in a sterile glass-stopper bottle. Samples were transported to the Molecular Biology Research Unit in Assiut University (Certified ISO/IEC: 17025-2017) in an insulated box surrounded by ice (Sadek et al., 2015). The samples were kept at -20 °C until examination by ELISA in accordance with the manufacturer's instructions (DRG *Toxoplasma* IgM, IgG (TORCH), EIA-1799, USA).

In addition, pregnant women (n.=100) that admitted to antenatal clinics (Obstetrics and Gynecology Department, Qena General Hospital, Egypt) with a case history of previous abortion were included in the study after obtaining an oral consent from each participant woman. A questionnaire was applied including age, history and the number of abortion, duration of pregnancy for each abortion, duration of current pregnancy, history of *Toxoplasma* infection, drinking of raw milk, and contact with pets. Then, blood samples were collected in clean, dry, sterile screw-capped labeled tubes and were left to clot at room temperature for 30 min, and then separated by centrifugation at 336 g for 4 min to obtain sera. Sera were stored at -20 °C till analysis (Shao et al., 2014).

Preparation of milk samples

The frozen milk samples were thawed at room temperature and centrifuged at 224 g for 20 min and the interface between the lipid layer and the pelleted cellular debris (lacto-sera) was examined for *T. gondii* antibodies (Tavassoli et al., 2013).

ELISA assay

Toxoplasma antibodies were detected in milk and serum samples as described in the manual kits of *Toxoplasma* IgG and IgM Enzyme Immunoassay Test Kit (DRG *Toxoplasma* IgM, IgG (TORCH), EIA-1799, USA). The lab work was done in the Molecular Biology Research Unit in Assiut University (Certified ISO/IEC: 17025-2017).

Results

Data postulated in Table 1 showed that 60% of does' raw milk samples were seropositive for *T. gondii*; 20% for IgM and 40% for both IgM & IgG antibodies. The overall seroprevalence of *T. gondii* in ewes' milk was 34% represented by 28% and 6% for IgM and both IgM & IgG, respectively. While a higher level of *T. gondii* IgM antibodies could be recovered from cows' raw milk (64%) at the time both IgM & IgG antibodies couldn't be detected. Raw milk samples in our study were collected from different localities in Qena Province (Qena, Qeft, and Dshna) as shown in Table 2. Among examined does: the lowest seroprevalence rate (10, 30%) was reported in samples collected from Dshna locality followed by Qena (15, 25%) however, Qeft reported the highest range of infection (30, 60%) for IgM and both IgM & IgG, respectively. Ewes' milk samples collected from Qeft showed the highest seroprevalence rate (40, 10%) followed by Dshna (20, 5%)

Table 1. Seroprevalence of *T. gondii* antibodies in animals' raw milk samples and pregnant women serum samples

Animal species	No.	Positive <i>T. gondii</i> antibodies			Seropositive cases No. (%)
		IgM No. (%)	IgG No. (%)	IgM & IgG No. (%)	
Does	50	10 (20)	0	20 (40)	30 (60)
Ewes	50	14 (28)	0	3 (6)	17 (34)
Cows	50	32 (64)	0	0	32 (64)
Total	150	56 (37.3)	0	23 (15.3)	79 (52.7)
Pregnant women	100	2 (2)	26 (26)	0	28 (28)

Table 2. Distribution of *T. gondii* antibodies in animals' milk samples according to location

Animal species	Locality	No. of examined samples	<i>T. gondii</i> antibodies	
			IgM No. (%)	IgM & IgG No. (%)
Does	Qena	20	3 (15)	5 (25)
	Qeft	20	6 (30)	12 (60)
	Dshna	10	1 (10)	3 (30)
Ewes	Qena	10	2 (20)	0
	Qeft	20	8 (40)	2 (10)
	Dshna	20	4 (20)	1 (5)
Cows	Qena	20	10 (50)	0
	Qeft	10	10 (100)	0
	Dshna	20	12 (60)	0
Total		150	56 (37.3)	23 (15.3)

and Qena (20, 0%) for IgM and both IgM & IgG, respectively. All examined cows' milk samples collected from Qeft were positive for IgM, followed by Dëshna (60%) and Qena in the third rank (50%).

The overall seroprevalence of *T. gondii* was 28% in pregnant women; 2% recorded as recent infection (IgM) and 26% as chronic infection (IgG) (Table 1). Regarding age, Table 3 showed that toxoplasmosis seropositivity was the highest in pregnant females aged >30-35 years old (58.8%) followed by those aged more than 35 years (57.1%) then group >23-30 (21.7%) while pregnant women aged 17-23 years showed the lowest incidence of infection (13.3%). *T. gondii* seropositivity was higher in pregnant females with the case history of one time of abortion and this seropositive occurrence decreased by increasing the number of abortions till reaching the lowest incidence in those with history of four times of abortion (Table 4).

Discussion

Dairy farms industry suffers from major economic losses as results of reproductive failure and reduced milk production. *T. gondii* is one of the most important causes of infectious abortion in livestock worldwide (Ortega-Mora et al., 2007). One of the strongest risk factors of acquiring *T. gondii* infection is drinking raw contaminated milk (Ghoneim et al., 2009). Milk contamination with *T. gondii* originated from the infected stray cats' feces which found within milk production area as well as animals acquired *T. gondii* infection from stray dogs and cats during grazing. *Toxoplasma* tachyzoites are resistant to milk media and preserve their infectivity for up to 30 min due to its oral transmission. In goat milk, tachyzoites may survive for 3-7 days at 4 °C (Walsh et al., 1999; Asgari et al., 2011). Although tachyzoites are vulnerable to proteolytic enzymes and are destroyed by gastric digestion, they can be survived for up to two hours in acid pepsin solutions resulting in infection (Dubey, 1998).

In the present study, higher rate of *Toxoplasma* acute infection (40%) than subacute (20%) were detected in the examined goats with an overall seroprevalence of 60%. This high incidence may be attributed to the presence of cats, which were observed during samples collection. Similar seropositive

result (60.6%) was obtained by Mancianti et al. (2013) and closely related result (58.9%) by Abdel-Rahman et al. (2012). Higher result (65%) was obtained by Sroka et al. (2017), while lower seroprevalence was reported as 8.9% by Dehkordi et al. (2013); 38.3% by Sadek et al. (2015); 50.6% by Younis et al. (2015) and 37.1% by Attia et al. (2017). These variations may be owing to differences in study locality, breeding conditions, and management. Furthermore, higher incidence of *Toxoplasma* acute infection among examined goats was recorded by Hassanain et al. (2005), who recovered *T. gondii* IgM in 72.6 % of examined samples as clarified in our study. In contrast, Saad et al. (2018) detected *T. gondii* IgG in 90% of the examined goat milk samples.

Results from this study revealed that *T. gondii* antibodies were detected in 34% of sheep raw milk samples, which may be due to the widespread of a large number of infected stray cats as well as the widespread grazing area for these animals. Closely related results were recorded as 31.1% by Ghazi et al. (2006); 39.7% by Sadek et al. (2015) and 30.95% by Ossani et al. (2017), while lower percent to those were observed as 25% by Camossi et al. (2011); 10.5% by Moura et al. (2011) and 6.5% by Santana Rocha et al. (2015). In our study, acute infection (*T. gondii* IgM antibodies) was observed in most cases (28%) this is in contrary to Robert et al. (2001), who reported that most infected sheep showed chronic infection. These variations can be attributed to differences in the animal breed, the climate and environmental conditions.

In this study, high level of recent infection was detected in the examined cows (64%). This result may have a relation to the presence of cats in the area adjacent to the dairy farms from which samples were collected. In concordance with our results, a high level of recent infection was clarified as 44.8% by El-Fahal et al. (2013) and as 89.3% by Ibrahim et al. (2014). Lower incidences of *T. gondii* IgM antibodies were obtained by Robert-Gangneux and Darde, (2012) and Bartova and Sedlak (2015); 16.8 and 9.7%, respectively. El Deeb et al. (2012) and Ahmed et al. (2014) couldn't detect *T. gondii* antibodies in cows' raw milk samples explaining this result by the ability of cattle to eliminate toxoplasma cysts from their tissues or even reduce their number.

In the obtained results, *T. gondii* antibodies were detected in goat milk at higher percentage than sheep milk, this finding supports the result obtained by Ramzan et al. (2009) who

Table 3. Distribution of *T. gondii* antibodies in relation to the age of examined pregnant women

Age groups (Years)	No.	<i>T. gondii</i> antibodies		Seropositive cases No. (%)
		IgM No. (%)	IgG No. (%)	
17 to 23	30	1(3.3)	3 (10)	4 (13.3)
>23 to 30	46	1(2.2)	9 (19.6)	10 (21.7)
>30 to 35	17	0	10 (58.8)	10 (58.8)
> 35	7	0	4 (57.1)	4 (57.1)
Total	100	2 (2)	26 (26)	28 (28)

Table 4. Distribution of *T. gondii* antibodies in relation to abortion number in pregnant women

No. of abortion	No.	<i>T. gondii</i> antibodies		Seropositive cases No. (%)
		IgM No. (%)	IgG No. (%)	
1 time	40	1 (2.5)	15 (37.5)	16 (40)
2 times	20	1 (5)	5 (25)	6 (30)
3 times	25	0	4 (16)	4 (16)
4 times	15	0	2 (13.3)	2 (13.3)
Total	100	2 (2)	26 (26)	28 (28)

mentioned that the seroprevalence of *T. gondii* in goat is higher than in sheep attributing this to their greater susceptibility to *T. gondii* infection, the animals feeding habits, and their higher activity and movement compared with sheep, which increases the probability of contact with contaminated sources.

Regarding location, higher prevalence of infection in animals was recorded in Qeft, which may be due to the warm and moist environmental conditions in that area, which are suitable conditions for the survival of *T. gondii* oocysts (Dubey et al., 2011; Bezerra et al., 2015), also it may be attributed to the massive distribution of infected stray cats in this location.

The presence of recent *Toxoplasma* infection in animals as recorded in the current study could be a significant source of infection transmission to human. A significant correlation between *T. gondii* seroprevalence in pregnant women and the consumption of contaminated raw milk in Egypt was reported by Ahmed et al. (2014) and Sadek et al. (2015).

The overall *T. gondii* seroprevalence in pregnant women was 28%, closely related results (30.2%) were obtained by El-Shqanqery et al. (2017), while higher percentages were observed as 46.1% by Tammam et al. (2013); 82% by Ahmed et al. (2014); 57.9% by Bassiony et al. (2016) in Egypt. *T. gondii* IgG antibodies were detected in most cases diagnosed in this study, similar results were reported by Abd El-Ghany and Merwad (2012); Abdel-Kareem and Eltayeb (2015) and Khademi et al. (2019). Lower percentage of acute infection was also obtained by Njunda et al. (2011); El Deeb et al. (2012) and Ahmed et al. (2014).

These results highlighted a serious problem especially in Qena governorate, Egypt that many pregnant females lack knowledge of the risk factors related to toxoplasmosis. It was reported that pregnant females infected with *T. gondii* might stay asymptomatic, although they could transmit the infection vertically to their fetuses with many congenital complications (Kravetz and Federman, 2002).

The obtained results clarified that the incidence of *T. gondii* chronic infection increased by age; pregnant women aged >30 to 35 years old showed the highest percentage of IgG antibodies while the lowest was in women aged 17-23 years. This result not only means that older age is a risk factor predisposing to *T. gondii* infection but also may be illustrated by increasing the time being exposed to the causing agent and may retain a steady level of *Toxoplasma* in serum for years. Similar results detected by Al-Harathi et al. (2006); Majid et al. (2016) and El-Shqanqery et al. (2017).

The current study also showed increased levels of *T. gondii* IgG in women with single abortion than those abort for two times or more, which agreed with Ayi et al. (2009); Jafer (2011); El-Shqanqery et al. (2017) and Darweesh et al. (2018), and disagreed with Birgisdottir et al. (2006), who mentioned that *T. gondii* IgG seroprevalence was higher in multigravida than in primigravida and attributed that to suppression of the immune response or hormonal imbalance.

Conclusion

Dairy animals in Qena Province, Egypt play an important role in the epidemiology of human toxoplasmosis, which has been proved by this study through detecting high rate of *T. gondii* IgM and IgG antibodies in raw milk, representing the strongest source of *Toxoplasma* infection for humans. The study clarified that high incidence of toxoplasmosis occurs in warm and moist areas which are favorable for oocysts survival. From this study, it can suggest that boiling or pasteurization of milk before consumption can help in decreasing the incidence of human toxoplasmosis beside increasing awareness about toxoplasmosis risk factors.

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Conflict of interest

The authors declare that they have no conflict of interest.

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