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Detection of Genomic *Toxoplasma gondii* DNA and Anti-*Toxoplasma* Antibodies in High Risk Women and Contact Animals

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Abstract: Toxoplasmosis is a disease of zoonotic nature; being reported to be widespread in animals and humans. Serological diagnosis represents the first and the most widely used approach to define the stage of toxoplasmosis and diagnosis of primary and late infection in pregnancy can be improved by determination of *Toxoplasma* DNA. Eighty-eight and 88 coagulated and non coagulated blood samples were collected from high risk women {68 pregnant that had bad obstetric history and 20 non pregnant that aborted in different times (1st or 2nd trimester)} with an average age (17 -45 years)} and their contact animals (62 sheep and 24 goats) in three centers at El-Fayoum in Egypt. Results showed that the prevalence of anti -*Toxoplasma* IgM and IgG among pregnant women (30.5 and 20.45%, respectively) was higher than non pregnant women (13.6 and 7.95%, respectively). The positive percents of PCR in the examined positive ELISA (IgG and IgM) pregnant and non pregnant women were (21.5 and 9.0, respectively) suggesting a recent or late infection. The high risk pregnant and non pregnant women aged 35-45 years old showed the highest percent of IgG (66.7 and 62.5), IgM (50.0 and 50.0) and positive PCR (50.0 and 37.5), respectively. Sheep and goats showed high seroprevalence of *Toxoplasma* IgG (98.4 and 41.7 %) and positive PCR (67.7 and 25%), respectively and those animals may constitute a potential source of infection to the investigated women at El-Fayoum. The relationship between positivity and some risk factors was assessed by ELISA and data collected by questionnaire. The strongest risk factors associated with acquiring toxoplasmosis were eating undercooked sheep or goat meat, drinking unpasteurized sheep or goat milk and handling raw sheep or goat meat.

Key words: Toxoplasmosis · High risk women · Sheep and goat · Seroprevalence · ELISA IgG · ELISA IgM · PCR

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by a protozoan parasite called *Toxoplasma gondii* which can infect all mammals and birds species throughout the world. Approximately one-third of humanity has been exposed to the parasite world wide [1,2]. All animal species act as intermediate hosts, except feline species which acts as a definitive host [3,4].

T. gondii infection in humans may occur vertically by tachyzoites that are passed to the fetus via the placenta, or horizontal transmission which may involve three life-cycle stages i.e. ingesting sporulated oocysts from cats or ingesting tissue cysts in raw or under cooked meat or tachyzoites in blood products or primary offal (viscera) of many different animals, tissue transplants and unpasteurized milk [5].

While, infection of healthy adult humans is usually mild, serious disease can result in utero or when the host is immunocompromised. The fetus is only at risk of congenital disease when acute infection occurs during pregnancy. Congenital infection has also been reported from a chronically infected immunocompromised mother with a reactivation of toxoplasmosis [6,7].

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Economical losses of toxoplasmosis are of medical and veterinary importances, in humans are due to abortion and fetal abnormalities [8] as well as morbidity and mortality in congenitally infected and immunocompromised individuals [9, 10]. In small ruminants (sheep and goat), economical losses occur due to prenatal death and abortion [8].

Alvelino *et al*. [11] mentioned that pregnant women living under unfavorable environmental conditions had an approximately two times increased risk of being infected for each risk factor (contact with host animals and presence of vehicles of oocysts transmission). Previous pregnancy was the risk factor that had the strongest influence on acquiring toxoplasmosis. Han *et al*. [12] stated that *T. gondii* infection in Korea is positively correlated with eating raw meat, but is not associated with the consumption of unwashed vegetables, drinking untreated water, history of raising a cat, or blood transfusion. Fallah *et al*. [13] reported that age, consumption of fresh undercooked meat and frequent consumption of raw vegetables were associated with higher infection rates.

The present work aimed to detect *Toxoplasma* infection among high risk women {pregnant women that had bad obstetric history and non pregnant women that aborted in different times (1^{st} or 2^{nd} trimester)} in relation to some risk factors e.g. age, contact animals, eating raw meat) in El-Fayoum, Tamyia and Senoris centers at El-Fayoum (Egypt) using ELISA and PCR.

MATERIALS AND METHODS

Blood samples were collected from 88 women (68 pregnant and 20 non pregnant subjects) with an average age (17 -45 years) in three centers (El-Fayoum, Tamyia and Senoris centers) at El-Fayoum during the period from October 2005 to December 2006. Blood samples were also collected from their contact animals (62 sheep and 24 goats). The separated serum and whole blood samples with EDTA were stored at -20°C until used. A questionnaire was carried out with the investigated women to detect the relationship between positivity and some risk factors (eating undercooked meat, drinking raw sheep or goat milk, preparation of raw sheep or goat meat, own or exposures to cats or Feline species).

ELISA Assay

ELISA in Women: The collected serum samples from pregnant and non pregnant women were tested for the presence of the specific IgM and IgG antibodies by using Clinotech Toxo ELISA IgM and IgG kits (Clinotech Diagnostics and Pharmaceuticals, Canada). Clinotech Toxo IgM and IgG ELISA kits are microwell ELISA test designed for the qualitative detection of IgM or IgG antibodies to *T. gondii* in human serum.

ELISA in Small Ruminants: ELISA was carried out according to Voller *et al*. [14]. Whole soluble tachyzoite antigens were prepared as described by Waltman *et al*. [15]. The optimal antigens (soluble tachyzoites antigen preparation) concentration, antibody and conjugate dilutions were chosen after preliminary checker board titration. In the present study, the optimum conditions were 10 µg/ml coating buffer antigen concentration, 1:100 sheep and goat serum dilutions. 1:1000 Horse radish peroxidase-labeled anti-sheep-IgG and anti-goat-IgG (Sigma Co.) as conjugate and 1 mg *p*-nitrophenyl phosphatase dissolved in one ml substrate buffer as substrate. The absorbance of the colored reaction was read within 30 min at 405 nm using a titertek multiskan ELISA reader. All incubation steps were carried out at 37°C in a moist chamber. The positive threshold value was determined to be two-fold the mean cut-off value of negative sera.

PCR

DNA Extraction:

Extraction of Genomic DNA from the RH *T. Gondii* strain: It was carried out according to Sambrook *et al*. [16]. The DNA pellet was dissolved in 50µl of TE (pH 8) and stored at -20°C till used as positive control.

Extraction of Genomic DNA of *T. Gondii* from the Collected Blood Samples: The genomic DNA from blood samples collected from women (88) and animal (86) was extracted with the Biospin Blood Genomic DNA Mini-Prep Kit (BioFlux Cat # BSJ040100001S80) as manufacture instructions. DNA concentration and purity was measured according to Sambrook *et al*. [17].

PCR Amplification of B1 Gene: B1 gene was amplified [16] using primers 1 (5′-*TCG GAG AGA GAA GTT CGT CGC AT* -3′ and 2 (5′-*AGC CTC TCT CTT CAA GCA GCG TA*-3′) [18]. The following reaction mixture was added in a 0.2 ml PCR tubes: DNA tamplet (100 ng/µl), 10 µl; Taq polymerase (5u/µl), 1 µl; 10x enzyme buffer, 2 µl; dNTPs, 0.8 µl; each Primer, 1 µl and Bidist. water to 20 µl. The mixture was briefly spined and placed in the thermal cycler (T gradiant, Biometra, Germany), which was programmed as follow: initial denaturing (95°C/2 minute) and 40 cycles consisting of denaturing (95°C/1 minute), annealing (55°C/30 seconds), extension (72°C/45 seconds) and

final extension (72°C/10 minutes). PCR product was electrophoresed at 80 v/15 minutes [16] and finally examined using UV transilluminator. 100 bp DNA ladder (Finzyme) was used as a marker.

RESULTS

Table 1 and 3 show that high risk women of the age group 35-45 years gave the highest total percent of anti-*Toxoplasma* IgG (66.0 and 62.5) and positive PCR (50.0 and 37.5) for pregnant and non pregnant subjects, respectively. Also, they showed the highest and equal total percents of anti-*Toxoplasma* IgM (50.0) (Table 2).

Sheep showed higher percent of anti-*Toxoplasma* IgG and positive PCR (98.4 and 67.7, respectively) than goats (41.7 and 25.0, respectively) as shown in Table 4. Table 5 shows that consumption of raw or undercooked sheep or goat meat, drinking raw sheep or goat milk and handling raw sheep or goat meat are the strongest risk factors of acquiring *T. gondii* infection by high risk women at El-Fayoum. The PCR product (300 bp) was detected in positive blood samples in women (Fig. 1) and small ruminants (Fig. 2).

Table 1: Percentage of anti-Toxoplasma IgG antibodies by ELISA in high risk women with different ages at El-Fayoum

	17-25y		25-35y		35-45y		Total positive	
Locality	Pr	Non pr	Pr	Non pr	Pr	Non pr	Pr	Non pr
El-Fayoum Center	40.0	33.3	37.5	33.3	75.0	50.0	47.0	37.5
SenorisCenter	44.4	25.0	42.8	33.3	33.3	66.6	42.5	40.0
Tamyia Center	25.0	33.3	42.8	40.0	80.0	66.6	47.8	45.5
*Total	38.8	30.0	41.3	36.3	66.6	62.5	45.8	41.4
Total samples in pr and non pr women at El-Fayoum							30.7	13.6

* = Total samples in pregnant or non pregnant women at El-Fayoum - Pr = pregnant women - Non pr = non pregnant women

Table 2: Percentaceof anti-Toxoplasma IgM antibodies by ELISA in high risk women with different ages at El-Fayoum

	17-25y		25-35y		35-45y		Total positive	
Locality	Pr	Non pr	Pr	Non pr	Pr	Non pr	Pr	Non pr
El-Fayoum Center	20.0	0	25.0	0	50.0	100	29.4	25.0
Senoris Center	22.2	0	14.2	0	33.3	33.3	21.0	10.0
Tamyia Center	50.0	33.3	28.5	40.0	60.0	33.3	39.1	36.4
*Total	27.7	10	24.13	18.1	50.0	50.0	30.5	24.2
Total samples pr and non pr women at El-Fayoum							20.45	7.95

* = Total samples pregnant or non pregnant women at El-Fayoum - Pr = pregnant women - Non pr = non pregnant women

Table 3: Detection of Toxoplasma gondii DNA by PCR in high risk women with different ages at El-Fayoum

	17-25y		25-35y		35-45y		Total positive	
Locality	Pr	Non pr	Pr	Non pr	Pr	Non pr	Pr	Non pr
El-Fayoum Center	20%	33.3%	37.5%	33.3%	50%	50%	35.3%	37.5%
Senoris Center	11.1%	0%	28.5%	33.3%	66.6%	33.3%	26.3%	20%
Tamyia Center	50%	33.3%	28.5%	20%	40%	33.3%	34.7%	27.3%
*Total	22.2%	20%	31.3%	27.3%	50%	37.5%	32.2%	27.5%
Total samples pr and non pr women at El-Fayoum Governorate							21.5%	9%

* = Total samples pregnant or non pregnant women at El-Fayoum Governorate - Pr = pregnant women - Non pr = non pregnant women

Table 4: Detection of Toxoplasma gondii IgG and DNA in small ruminants in different localities at El-Fayoum

	ELISA IgG		PCR	
Locality	Sheep	Goat	Sheep	Goat
EL-Fayoum Center	95%	37.5%	90%	37.5%
Senoris Center	100%	33.33%	60%	16.7%
Tamyia Center	100%%	50%	54.5%	20%
Total	98.4	41.7%	67.7%	25%

Table 5: Risk factors for the investigated women at El-Fayoum

Risk factors	Pregnant women	Non pregnant women
-Eating undercooked sheep or goat meat	+++	+++
-Drinking raw sheep or goat milk	+++	+++
-Preparation of raw sheep or goat meat	+++	+++
-Any raw meat exposure or drinking any raw milk of different animals (Cow's milk or buffaloes)	++	++
-Own or exposures to Cats or Feline species	+	+

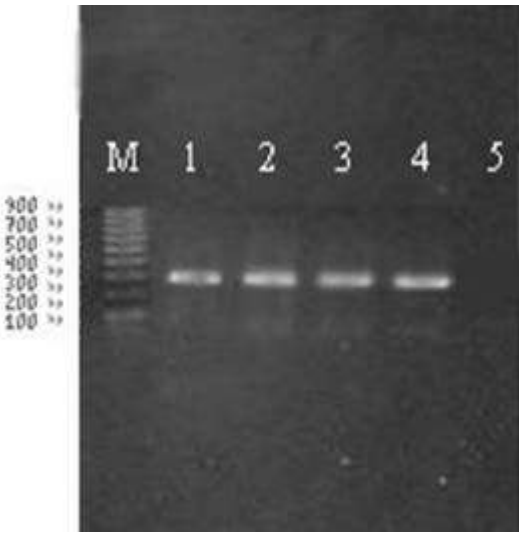

Fig. 1: Electrophoretic pattern of the PCR products (300 bp) from human samples. Lane 1: positive control, lane 2, 3 and 4: positive women samples and lane 5: negative blood samples M: DNA marker (100 bp).

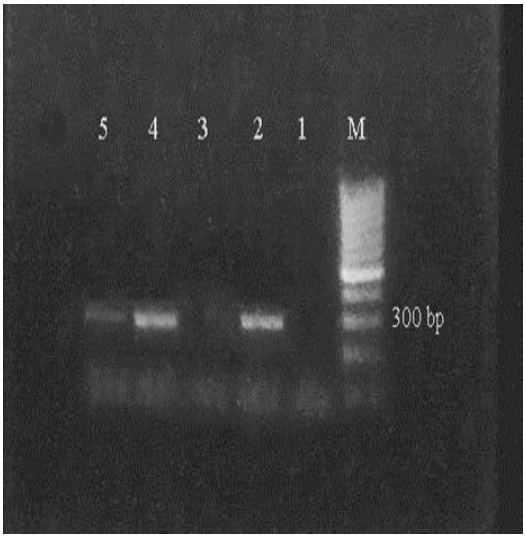

Fig. 2: Electrophoretic pattern of the PCR products (300 bp) from small ruminants. Lane 1: negative control, lane 2: positive control, lane 3: negative sheep blood samples, lane 4 and 5: positive sheep and goat blood samples. M: DNA marker (100 bp).

DISCUSSION

Routine serologic diagnosis of toxoplasmosis provides high sensitivity, but specificity varies depending on the test used [19]. Pelloux *et al*. [20] stated that diagnosis of primary and late infection with *T.gondii* in pregnancy can be improved by determination of *Toxoplasma* DNA.

In the present study, the seroprevalence of *T .gondii* IgG among pregnant (47, 42.5 and 47.8 %) was higher than non pregnant women (37.5, 40 and 45.5 %) at El-Fayoum (El Fayoum, Senoris and Tamyia centers, respectively). This higher seroprevalence may be due to alterations in the immune mechanisms during pregnancy leading to increase of the invasion of this parasite [21, 22]. Hussein *et al*. [23] determined the seroprevalence of *Toxoplasma* IgG by ELISA in 31 full term parturient (57.9%), 38 aborted (58.1%) and prematurely delivered women (44.7%). El-Fakahany *et al*. [24] reported that seropositivity to specific IgG antibodies was 36.4, 59.2 and 57.9 % in complicated gestation, uncomplicated gestation and randomly population, respectively. On the other hand, Kurnatowska and Tomczewska [25] found that the incidence of *T. gondii* specific IgG was significantly higher in non pregnant women than pregnant women.

The total seroprevalence of *T .gondii* IgG and IgM among pregnant (66.6 and 50%, respectively) and non pregnant women (62.5 and 50.0%, respectively) of the age group 35-45 years at El-Fayoum were the highest. Also, These high risk group women showed the highest positive PCR results (62.55 for pregnant and 37.5% for non pregnant). Valcavi *et al*. [26] determined the prevalence of IgG antibodies to *T. gondii* in Italy by ELISA; being 48.5% with correlation of infection with age, showed a significant increase of positivity until 30-40 approximately years. Remington *et al*. [27] mentioned that the prevalence of the infection with *T. gondii* increases with age and there are considerable geographic differences in prevalence rates. Hung *et al*. [28] mentioned that older age group of ≥ 35 years had a significantly higher seroprevalence than that of the younger age group of 15-25 years. Also, Fallah *et al*. [13] reported that age was statistically significantly associated with higher infection rates.

Sheep and goats showed high positive percent of *Toxoplasma* IgG and genomic DNA (98.4 and 67.7 and 41.7and 25.0, respectively) at El-Fayoum. This finding is in agreement with Tenter *et al*. [5] who reported that sheep showed high seroprevalences in many areas of the world up to 92%. On the other hand, Dodriguez-Ponce *et al*. [29] detected higher seroprevalence rate of *Toxoplasma* IgG in goats (63.3 %) in the island of Grand Canary. Clementino *et al*. [30] reported lower seroprevalence rate of anti-*T. gondii* specific IgG in sheep (29.41%) in Brazil.

The high prevalence of *T. gondii* infection in sheep and goats may be due to sheep free range livestock associated with *T. gondii* infection. They are kept on pastures with an increased pressure of infection due to contamination of environment with oocysts. The frequency of stray cats in a humid rainy climate favoring the survival of oocysts has contributed to the high *Toxoplasma* prevalence in Central America [27]. In Egypt, stray cats are widely spread as in El-Fayoum which is in favor of a higher prevalence of oocysts in humid environment and farming animal rearing are also common. Alvelino *et al*. [11] mentioned that pregnant women living with host animals or vehicles of oocysts transmission had an approximately two times increased risk of being infected for each risk factor.

The data collected by questionnaire and ELISA positivity showed that eating undercooked sheep or goat meat, drinking raw sheep or goat milk and preparation of raw sheep or goat meat were the risk factor that had the strongest influence on acquiring toxoplasmosis by the investigated women at El-Fayoum. While, any raw meat exposure or drinking any raw milk of different animals (cow or buffalo milk) had less influence followed by own or exposure to cats. In this respect, it was found that *T. gondii* infection is positively correlated with eating raw meat [12,13]. In Jordan, it was found that the increase of infection with *Toxoplasma*, is due to consumption of lamb greater than that of beef and these animals are reared outdoors which put them at greater risk of environmental exposure than animals reared indoors [31]. However, Han *et al*. [12] reported that *T. gondii* infection is not associated with a history of raising a cat.

It was concluded that the high prevalence of toxoplasmosis among the investigated high risk women at El-Fayoum is due to many risk factors including age, contact with host animals (small ruminants), eating undercooked meat, drinking raw sheep or goat milk, preparation of raw sheep or goat meat and own or exposure to cats. It is recommended to consider routine serological testing in pregnancy due to high prevalence of toxoplasmosis in the investigated pregnant women. Women are advised to avoid the numerous risk factors, making compliance difficult.

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