Detection of genomic *Toxoplasma gondii* DNA and anti-*Toxoplasma* antibodies

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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 Governorate 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 %, 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 and 50%) and positive PCR (50% 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 Governorate. 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. [Life Science Journal. 2009; 6(3): 54 – 60] (ISSN: 1097 – 8135)

Key words: Toxoplasmosis; ELISA IgG; ELISA IgM

1 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]. Except feline species which acts as a definitive host, all animal species act as intermediate hosts[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[6,7]. The fetus is only at risk of congenital disease when acute infection occurs in pregnancy. Congenital infection has also been reported from a chronically infected immunocompromised mother with a reactivation of toxoplasmosis.

Economical losses of toxoplasmosis are of medical and veterinary importance, in humans are due to abortion, fetal abnormalities[8], 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].

Avelino 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, a 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 statistically significantly associated with higher infection rates.

Therefore, 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 (1st or 2nd 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 Governorate (Egypt) using ELISA and PCR.

2 Materials and Methods

Blood samples were collected from 88 women (68 pregnant and 20 non pregnant women with an average age (17 -45 years) in three centers (El fayoum, Tamyia and Senoris centers) at El fayoum Governorate during the period from October 2005 to December 2006. Blood samples were also collected from their contact animals (62 sheep and 24 goats). Serum was separated and blood samples with EDTA were stored at -20oC 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 & 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 antigen (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 amplified16 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^{o}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 (72oC/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. 3 Results

Table (1 & 3) show that high risk women of the age group 35-45 years gave the highest total percent of anti-Toxoplasma IgG (66 and 62.5%) and positive PCR (50 and 37.5%), respectively. Also, they showed the highest and equal total percents of anti-Toxoplasma IgM (50 %) (Table 2). Sheep showed higher percent of anti-Toxoplasma IgG and positive PCR (98.4 and 67.7% %) than goats (41.7 and 25%), respectively (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 Governorate. The PCR product (300 bp) was detected in positive blood samples in women (Figure 1) and small ruminants (Figure 2).

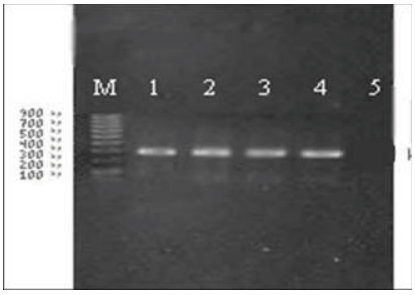

Figure 1. Electrophoretic pattern of the PCR products (300 bp) from human samples. Lane 1: positive control; lane 2, 3 and 4: positive women samples; lane 5: negative blood samples; M: DNA marker (100 bp).

4 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 Governorate (El Fayoum, Senoris and Tamyia centers, respectively). This higher seroprevalence may be due to alterations in the immune mechanisms in 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 seroprevalences of *T .gondii* IgG and IgM among pregnant (66.6 and 50%, respectively) and non pregnant women (62.5 and 50%, respectively) of the age group 35-45 years at El-Fayoum Governorate were the highest. These high risk group women showed also the highest positive PCR results (62.550 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. This may be due to decrease the immunity with advanced in age. 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%, respectively) at El-Fayoum Governorate. 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 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 governorate which is in favor of a higher prevalence of oocysts in humid environment and farming animal rearing are also common. Avelino 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 Governorate. 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 or Feline species. Han et al[12]and Fallah et al[13] stated that *T. gondii* infection is positively correlated with eating raw meat. Laila Nimri et al[31] found that the increase of infection with *Toxoplasma*, in Jordan, 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. Han et al[12] reported that *T. gondii* infection is not associated with a history of raising a cat.

We can conclude that the high prevalence of toxoplasmosis among the investigated high risk women at El-Fayoum Governorate 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 or Feline species). 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.

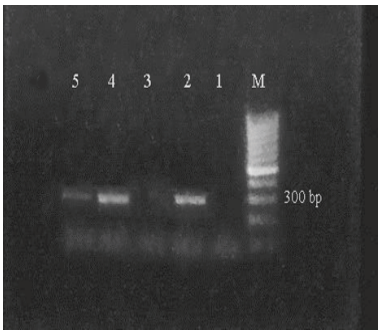

Figure 2. Electrophoretic pattern of the PCR products (300 bp) from small ruminants. Lane 1: negative control; lane 2: positive control; lane 3: negative sheep blood samples; lane 4 and 5: positive sheep and goat blood samples. M: DNA marker (100 bp).

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

locality	17-25y		25-35y		35-45y		Total positive	
	Pr	Non pr	Pr	Non pr	Pr	Non pr	Pr	Non pr
El-Fayoum	40	33.3	37.5	33.3	75	50	47	37.5
Senoris	44.4	25	42.8	33.3	33.3	66.6	42.5	40
Tamyia	25	33.3	42.8	40	80	66.6	47.8	45.5
Total*	38.8	30	41.3	36 .3	66.6	62.5	45.8	41.4
Total samples in pr and non pr women at El-Fayoum Governorate							30.7	13.6

* = Total samples in prgnant or non pregnant women at El-Fayoum Governorate;
-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 Governorate.

locality	17-25y		25-35y		35-45y		Total positive	
	Pr	Non pr	Pr	Non pr	Pr	Non pr	Pr	Non pr
El-Fayoum	20	0	25	0	50	100	29.4	25
Senoris	22.2	0	14.2	0	33.3	33.3	21	10
Tamyia	50	33.3	28.5	40	60	33.3	39.1	36.4
Total*	27.7	10	24.13	18.1	50	50	30.5	24.2
Total samples in pr and non pr women at El-Fayoum Governorate							20.45	7.95

***** = Total samples in prgnant or non pregnant women at El-Fayoum Governorate;
-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 Governorate.

locality	17-25y		25-35y		35-45y		Total positive	
	Pr	Non pr	Pr	Non pr	Pr	Non pr	Pr	Non pr
El-Fayoum	20%	33.3%	37.5%	33.3%	50%	50%	35.3%	37.5%
Senoris	11%	0%	28.5%	33.3%	66.6%	33.3%	26.3%	20%
Tamyia	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 in pr and non pr women at El-Fayoum Governorate							21.5%	9%

* = Total samples in prgnant 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 Governorate.

locality	ELISA IgG		PCR	
	sheep	goat	sheep	goat
EL-Fayoumcenter	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 Governorate

Non pregnant women	Pregnant women	Risk factors
-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	+	+