PUBLIC HEALTH IMPLICATIONS OF TOXOPLASMOSIS IN ANIMALS AND WOMEN IN SELECTED LOCALITIES OF MENOUFIA GOVERNORATE, EGYPT

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ABSTRACT

Toxoplasma gondii is an important protozoan with serious obstetric impact in animals and women. The work aimed to relate public health significance of toxoplasmosis among some animals and women in Menoufia Governorate. A total 50 blood samples were collected from 50 adult sheep (30 from Kafr Tanbidi and 20 from Shebein El-Kom) and a total of 31 cattle milk samples were collected from different localities in Shebein El-Kom. Sheep sera were examined by ELISA IgG and recorded seropositivity of 60% (30/50) with 66.67% (20/30) were positive in Kafr Tanbidi and 50% (10/20) in Shebein El-Kom. PCR on sheep blood recorded 22% (11/50), of them 20% (6/30) were positive in Kafr Tanbidi and 25% (5/20) in Shebein El-Kom while PCR on cattle milk revealed 19.35% (6/31) positive for *Toxoplasma* DNA. On the other hand, *Toxoplasma* seroprevalence among 250 women was 54.4% (136/250) by ELISA IgG and 8.4% (21/250) by ELISA IgM. There was a significant association between IgG seroprevalence and age, increased gravidity, abortion, climate, cat contact, consumption of raw cow milk, undercooked meat and contaminated water from wells and between IgM seroprevalence and age, abortion and climatic conditions prevailed during sampling.

Key words: Toxoplasma gondii, ELISA IgG, ELISA IgM, PCR.

INTRODUCTION

Toxoplasmosis is one of the global major anthropozoonotic parasitic diseases infecting humans directly from animals (Torgerson and Macpherson, 2011). *Toxoplasma* can be transmitted by variety of ways either horizontally or vertically, it was known by its capacity to invade wide range of cell types this is attributed to its invasive forms which called tachyzoites and bradyzoites that are related to the intermediate hosts and merozoites and sporozoites that are related to the definitive hosts (Ferguson and Dubremetz, 2013).

In animals, The disease may be ranged from asymptomatic infection as in cattle, horses and poultry species that are immunologically resistant (Jones and Dubey, 2012) to the serious disease which can be expressed as storms of abortions and prenatal deaths as in sheep (Dubey, 2008). Toxoplasmosis may also be fatal as in sea otters (Miller *et al.,* 2008).

In human, *Toxoplasma* was known to infect 30-50 % of the world's human population with great epidemiological variations among populations within different countries, different geographical areas in the same country and even among the different ethnic groups living in the same area (Deji-Agboola *et al.,* 2011). Although most *Toxoplasma* infections are asymptomatic in immunocompetent adults, the disease expresses itself violently among immunocompromized individuals as causing acute life-threatening toxoplasmic encephalitis in the people who have HIV/AIDS and a T-cell count less than 100 cells/μL (Weiss and Dubey, 2009). Sever infection also can occur in vertical transmission when the mother becomes infected for the first time during pregnancy causing sever congenital defects and also eye diseases that may end with permanent loss of vision (Olariu *et al.,* 2011). Also, foodborne toxoplasmosis is considered a leading cause of death in the United States (Jones *et al.,* 2014).

The diagnosis of the disease in animals and human is investigated by direct methods as polymerase chain reaction (PCR) since a single *T. gondii* parasite could be directly detected by PCR using the 35- fold repetitive B1 gene as a target for amplification (Wahab *et al.,* 2010) and indirect methods as enzyme linked immunosorbent assay (ELISA) that is simple and rapid test that testing serum antibodies for large number of samples with the chosen anti-species conjugate (Naot and Remington, 2008).

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The aim of the current work was to perform a surveillance of toxoplasmosis among sheep to get an indication of their possible role in human toxoplasmosis, examination of some cattle raw milk samples by PCR technique to highlight their potential role in foodborne toxoplasmosis. We also aimed to determine the prevalence and the relevant risk factors of toxoplasmosis among women in Menoufia Governorate.

MATERIALS AND METHODS

Specimen collection:
A total of 50 adult sheep of Baladi breed (in contact with cats, soil and stream water) from 2 different localities in Menoufia Governorate (30 samples were collected from a sheep flock reared in Kafr Tanbidi while the other 20 samples were collected from a sheep flock in Shebein El Kom) in the period from June 2014 to April 2015. 50 sheep serum samples were examined by ELISA IgG as well as 50 sheep whole blood samples were examined by PCR technique for detecting Toxoplasma DNA. Moreover, a total of 31 cattle milk samples were collected from different dairy cows that were reared by different dairy farmers in different localities in Shebein El Kom, Menoufia Governorate in the period from March 2015 to May 2015 for advanced DNA diagnosis of *Toxoplasma* tachyzoites in milk.

Furthermore, a total of 250 serum samples were collected from women in the child bearing age (18 – 40 years) attended various clinic laboratories in Shebein El-Kom, Menoufia Governorate in the period from June 2014 to August 2015 for examination by ELISA IgG and ELISA IgM. Some of the potential risk factors were investigated by using a short questionnaire including age, occupation, residence, history of previous gestations, eating undercooked meat, ingestion of raw milk or milk products and contact with cat or with soil and the type of consumed water.

Detection of anti-*T. gondii* IgG and IgM antibodies by ELISA assay:
All samples of blood were collected and centrifuged at 3000 rpm for 10 min and the separated serum was stored in labeled aliquots at -20°C and examined for IgG and IgM antibodies According to El Gamal *et al.* (2013) by using commercial ELISA kits (Pishtaz Teb Zaman Diagnostics, Tehran, Iran). Further modification was carried during examination of sheep sera by Substitution of the Pishtaz IgG conjugate with another conjugate solution contains peroxidase-labeled affinity purified antibody to sheep IgG (γ) poly clonal antibody which was produced in rabbit (cat. no. 04-23-02, KPL, Inc., USA) that was used in dilution of 1:100 in a dilution buffer solution of 0.5% bovine serum albumin (BSA) in phosphate buffered saline solution (PBS) containing 0.05% tween. The simplest way to prepare a PBS solution is to use PBS buffer tablets of PH 7.4 (Medicago AB, Sweden) to give a ready-to-use PBS solution when dissoluted in a specified quantity of distilled water.

Extraction of *T. gondii* DNA:
The blood samples of sheep and cattle milk samples were frozen at -20°C till examination by PCR. DNA extraction from blood samples and milk samples was performed in accordance with the manufacturer's instructions of Biospin Whole Blood Genomic DNA Extraction Kit (catalog. no. BSC06S1, Bioflux Corporation, Arrow Plaza, Tokyo, Japan) and G-spinTM Total DNA Extraction Kit (cat. no. IBT-QMS-GT1704_R01-2012-01, intron biotechnology, Seoul, Korea), respectively.

Amplification and detection of *T. gondii* DNA:
The primers were used according to Rahdar *et al.* (2012) as follows: forward 5'-AAAAATGTGGGAATGAAAGAG-3' and reverse 5'-ACGAATCAACGGAACTGTAAT-3'. The final volume of PCR reaction was set up in a 25 μl. Each PCR tube contained 10 μl of master mix from (cat. no. 25027, Intron Biotechnology, Korea), 0.5 μl of each primer, 10 μl of distilled water, and 4 μl of extracted DNA. In a thermal cycler model (GS1 Base Unit GS00001, USA), the cycling conditions were performed as denaturation at 95°C for 10 min followed by 35 cycles at 94°C for 1 min, at 52°C for 45 sec and at 72°C for 1 min for each cycle. The final cycle was followed by an extension step at 72°C for 10 min. A 10 μl of each PCR product was subjected to gel electrophoresis in 2% agarose gel electrophoresis (cat. no. 35-1010, peQ Lab GmbH, Erlangen, Germany) with using GeneRuler™100 bp plus DNA ladder (cat. no. SM0323, Thermo Scientific Inc., USA) as a marker. The expected PCR product size was 469 bp. RH strain of *T. gondii* which was used as positive control, was obtained kindly together with the primers from Animal Medicine and Infectious Diseases Department, Fac. of Vet. Med., Univ. of Sadat City.

Statistical analysis:
Data were analyzed using Chi- Square analysis test (X2) by using SPSS version 17 as was performed by Deji-Agboola *et al.* (2011). Sensitivity = true positives (TP) / [true positive (TP) + false negative (FN)] x100. Specificity =true negatives (TN) / [true negative (TN) + false positives (FP)] x100. Accuracy =number of correct results divided by the total number of results x100. Positive Predictive Value (PPV) = true positive (TP) / [true positive (TP) + false positive (FP)] x100. Negative Predictive Value (NPV) = true negatives (TN) / [true negatives (TN) +false negatives (FN)] x100.

RESULTS

Table 1: Detection of anti-*T. gondii* IgG antibodies and *Toxoplasma* DNA in sheep in relation to areas of collection by using ELISA IgG and PCR:

Area of collection	Positive ELISA IgG		Positive PCR	
	No.	%	No.	%
Kafr Tanbidi (No.= 30)	20	66.67	6	20
Shebein El Kom (No.=20)	10	50	5	25
Total (No.=50)	**30**	**60**	**11**	**22**
Chi- square	1.39^{NS}		0.18^{NS}	
P-value	0.24		0.68	

^{NS} Non Significant

Table 2: Results and accuracy of ELISA IgG in comparison with PCR among the examined sheep:

Item	PCR		Total
	Positive	Negative	
ELISA IgG	No.	No.	
Positive	8	22	**30**
Negative	3	17	**20**
Total	**11**	**39**	**50**
Chi-square	0.952^{NS}		
P value	0.33		

Sensitivity = 72.73%

Specificity = 43.59%

Accuracy = 50%

PPV = 26.67%

NPV = 85%

^{NS} Non Significant

Table 3: Molecular detection of *T. gondii* DNA among the examined cattle milk samples by using PCR.

Test	No. tested	Positive		Negative	
		No.	%	No.	%
PCR	**31**	6	19.35	25	80.65
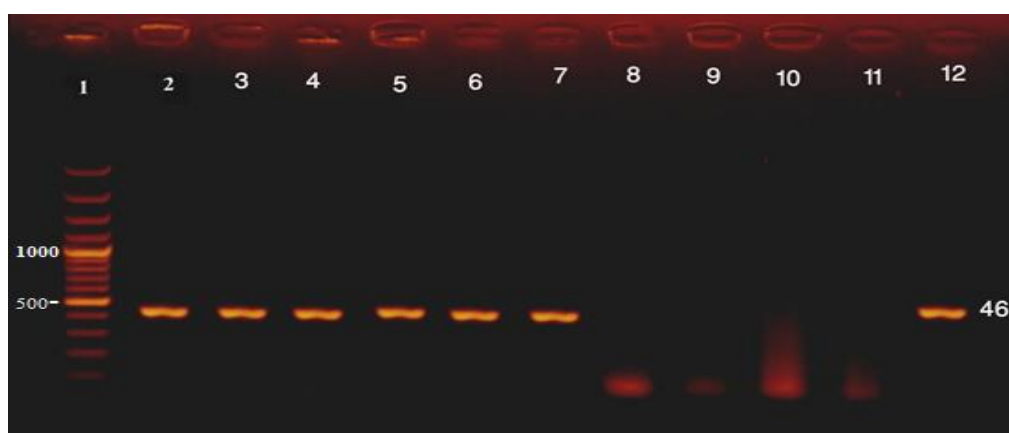
Figure (1): Ethidium bromide-stained agarose gel electrophoresis showing results of PCR amplification of *B1* gene of *T. gondii* in whole sheep blood and cattle milk. Lane 1: DNA ladder, lane 2, 3 and 4: milk positive results, lane 5, 6 and 7: blood positive results, lane 8, 9, 10: negative results, lane 11: negative control and lane 12: positive control.

Table 4: Seroprevalence of anti-*T. gondii* IgG and IgM antibodies in the tested women by ELISA test.

No. tested	Positive IgG		Positive IgM	
	No.	%	No.	%
250	136	54.4	21	8.4

Table 5: Seroprevalence of anti-*T. gondii* IgG antibodies in relation to demographic data, relevant obstetric history and risk factors:

Variables	Positive IgG		Negative IgG		Chi-square	P-value
	No.	%	No.	%		
Age groups:						
- ≥ 20 (No.=25)	15	60	10	40	12.63*	0.027
- 21-24 (No.=103)	59	57.28	44	42.72		
- 25-28 (No.=61)	26	42.6	35	57.38		
- 29-32 (No.=33)	14	42.42	19	57.38		
- 33-36 (No.=20)	16	80	4	20		
- 37-40 (No.=8)	6	75	2	25		
Occupation:						
- House wives (No. =186)	105	56.45	81	43.55	1.23 NS	0.27
- Others (No. =64)	31	48.44	33	51.56		
Residence:						
- Rural (No. =137)	75	54.74	62	45.26	0.015 NS	0.90
- Urban (No. =113)	61	53.98	52	46.02		
Prevailing climatic conditions:						
- Warm conditions (No. =111)	72	64.86	39	35.14	8.81**	0.003
- Cold conditions (No. =139)	64	46.04	75	53.96		
Gravidity:						
- primi (No.=200)	102	51	98	49	4.66*	0.03
- multi (No.=50)	34	68	16	32		
Abortion:						
- Recently aborted (No.=111)	70	63.06	41	36.94	6.04*	0.014
- Healthy pregnant (No.=139)	66	47.48	73	52.52		
Complicated gestational history:						
- Complicated (previous abortions or still births) (No.=43)	26	60.47	17	39.53	0.77 NS	0.38
- Normal gestations (No.=207)	110	53.14	97	46.86		
Eating undercooked meat:						
- Consumers (No.=132)	81	61.36	51	38.64	5.47**	0.019
- Non consumers (No.=118)	55	46.61	63	53.39		
Drinking cow raw milk:						
- Consumers (No.=176)	105	59.66	71	40.34	6.63**	0.01
- Non consumers (No.=74)	31	41.9	43	58.11		
Cat contact:						
- Cat contactors (No.=78)	51	65.4	27	34.62	5.52*	0.019
- Non contactors (No.=172)	85	49.42	87	50.58		
Contact with soil:						
- Contactors (No.=61)	35	57.38	26	42.62	0.288 NS	0.59
- Non contactors (No.=189)	101	53.44	88	46.56		
water consumed:						
- Filtered/treated tape water (No.=222)	115	51.80	107	48.20	5.39*	0.02
- wells water (No.=28)	21	75	7	25		

** Highly significant   * Statistically Significant   NS Non Significant

Table 6: Seroprevalence of anti-*T. gondii* IgM antibodies in relation to demographic data, relevant obstetric history and risk factors.

Variables	Positive IgM		Negative IgM		Chi-square	P-value
	No.	%	No.	%		
Age groups:						
- ≥ 20 (No.=25)	0	0	25	100		
- 21-24 (No.=103)	13	12.62	90	87.38	12.66*	
- 25-28 (No.=61)	3	4.92	58	95.08		0.027
- 29-32 (No.=33)	0	0	33	100		
- 33-36 (No.=20)	3	15	17	85		
- 37-40 (No.=8)	2	25	6	75		
Occupation:						
- House wives (No. =186)	18	9.68	168	90.32	1.54 NS	0.21
- Others (No. =64)	3	4.69	61	9.31		
Residence:						
- Rural (No. =137)	13	9.49	124	90.51	0.467 NS	0.49
- Urban (No. =113)	8	7.08	105	92.92		
Prevailing climatic conditions:						
- Warm conditions (No. =111)	14	12.61	97	87.39	4.60*	
- Cold conditions (No. =139)	7	5.04	132	94.96		0.032
Gravidity:						
- primi (No.=200)	17	8.5	183	91.5	0.013 NS	0.91
- multi (No.=50)	4	8	46	92		
Abortion:						
- Recently aborted (No.=111)	15	13.51	96	86.49	6.78*	0.009
- Healthy pregnant (No.=139)	6	4.32	133	95.68		
Complicated gestational history:						
- Complicated (previous abortions or still births) (No.=43)	6	13.95	37	86.05	2.08 NS	0.15
- Normal gestations (No.=207)	15	7.25	192	92.75		
Eating undercooked meat:						
- Consumers (No.=132)	14	10.61	118	89.39	1.769 NS	0.18
- Non consumers (No.=118)	7	5.93	111	94.07		
Drinking cow raw milk:						
- Consumers (No.=176)	18	10.23	158	89.77	2.58 NS	0.11
- Non consumers (No.=74)	3	4.05	71	95.95		
Cat contact:						
- Cat contactors (No.=78)	10	12.82	68	87.18	2.88 NS	0.09
- Non contactors (No.=172)	11	6.4	161	93.60		
Contact with soil:						
- Contactors (No.=61)	6	9.84	55	90.16	0.216 NS	0.64
- Non contactors (No.=189)	15	7.94	174	92.06		
water consumed:						
- Filtered/treated tape water (No.=222)	17	7.66	205	92.34	1.42 NS	0.23
- wells water (No.=28)	4	14.29	24	85.71		

* Statistically Significant   NS Non Significant

Table 7: Number of women with positive and negative ELISA IgG in relation to ELISA IgM results.

Item	Positive IgM	Negative IgM	Total
	No.	No.	
Positive IgG	15	121	**136**
Negative IgG	6	108	**114**
Total	**21**	**229**	**250**
Chi- square		2.68 NS	
P- value		0.102	

NS Non-Significant.

DISCUSSION

The results presented in table (1) revealed that 60% of tested sheep was positive for IgG antibodies with 66.67% were positive in Kafr Tanbidi and 50% in Shebein El-Kom. The obtained result was in agreement with Bártová *et al*. (2009) who declared prevalence of 59% in Czech Republic in Central Europe and Hassanain *et al*. (2013) who showed a prevalence of 61.4% in a previous study performed at National Research Center (NRC) in Cairo Governorate, Egypt. On the other hand, higher IgG seropositivity was recorded 98.4% in El-Fayoum Governorate, Egypt and 71% in Grenada, West Indies by Ghoneim *et al*. (2009) and Sharma *et al*. (2015), respectively.

On the contrary, lower seropositivity rates were detected: 28.86 % in the Delta region of Egypt (Mahboub *et al*., 2013) and 20% in Central Ethiopia (Gebremedhin *et al*., 2014).

The high seroprevalence in the current study could be referred to the grazing pattern where the tested sheep flocks were grazed daily with a free access to contaminated feed and water, the high *Toxoplasma* seroprevalence in sheep confirms the environmental contamination of pastures and food during grazing with infective oocysts (Prelezov *et al*., 2008). In Egypt, Al-Kappany *et al*. (2010) reported that the Egyptian stray cats are very abundant and widely spread with 97.4 % of them are *Toxoplasma* infected indicating the high risk of environmental contamination with sporulated oocysts that remain infective in soil and water for a long time. Younis *et al*. (2015) showed that toxoplasmosis increases with age of sheep and Mahboub *et al.* (2013) illustrated presence of a significant association between toxoplasmosis and the sheep breed, the highest seropositivity 35.42% was found among Baladi breeds in the Delta region of Egypt in comparison with 13.33% among Ossemi breeds, 21.43% among Rahmani breeds while not detected among Barki breeds.

It was no surprise that molecular detection of *Toxoplasma* DNA showed only 22% of sheep were PCR positive as PCR used to detect the parasite antigen while ELISA IgG used to detect the parasite antibody. From them 20% was PCR positive in Kafr Tanbidi and 25% was PCR positive in Shebein El-Kom.

Concerning the comparison of ELISA IgG in relation to PCR (PCR technique was used as a standard test) that was illustrated in table (2), ELISA IgG detected (8 out of 11) positive PCR samples (true positive result) indicating chronic infection with the *Toxoplasma* antigen still in blood, while ELISA IgG could not detect (3 out of 11) positive PCR samples (false negative), which could be corresponding to a recent infection at the time of examination leading to insufficient production of IgG. On the other side, ELISA IgG was able to detect (22 out of 39) negative PCR samples (false positive results) indicating chronic infection with no antigen in blood but only IgG specific antibodies are present. About 17 samples were negative by both ELISA IgG and PCR (true negative) indicating their susceptibility for acquiring recent infection.

Furthermore, the accuracy of ELISA IgG compared to PCR for diagnosis of toxoplasmosis in the tested sheep was evaluated. The calculated sensitivity (i.e. the probability that the assay will be positive when the infection is present) for ELISA IgG was 72.7%, specificity (i.e. the probability that the assay will be negative when the infection is absent) was 43.59%, PPV was 26.67%, NPV was 85% and so the accuracy of ELISA IgG was 50% as compared with PCR. The obtained results were somewhat in agreement with the findings of El Gamal *et al*. (2013).

More attention should be given to raw milk and milk products as potential sources for human toxoplasmosis. Camossi *et al*. (2011) illustrated that the tachyzoites present in the milk of affected animals can be horizontally transmitted to offspring via consumption. Moreover, Spišák *et al*. (2010) confirmed experimentally that the survival of tachyzoites in milk occurs for 3-7 days at 4 °C, proving the role of raw milk as a source of *T. gondii* infection.

The results tabulated in table (3) represented 19.35% of the examined milk samples were PCR positive. A previous molecular study was done on 889 raw milk samples from different species in Iran resulted in a positivity rate of 5.17% among the whole examined milk samples with a positivity rate of 3.5% among examined cattle milk itself (Dehkordi *et al*., 2013).

In Egypt, Ahmed *et al*. (2014) failed to detect any *T. gondii* DNA by PCR in raw cow milk in Sharkia Governorate. However, they concluded a significant association between toxoplasmosis in pregnant women and the consumption of raw cow milk and homemade cheese.

The considerable high positivity of *T. gondii* in cow's milk showed in the present study may be referred to many factors such as management and hygienic standards in breeding, abundance of stray cats which are in contact with herds and the humid climate and agricultural nature of collecting areas in Menoufia Governorate that favor survival of sporulated oocysts in soil and water, some farmers depend on wells and stream water for watering cows (infective oocysts can maintain their infectivity in water for long time) (Jones and Dubey, 2010). Moreover, the considerable high seropositivity rate of *Toxoplasma* specific antibodies detected among sheep in the current study

are in accordance with the increasing positivity rate among tested cattle selected from the same areas (Boughattas et al., 2014).

Available investigations on the epidemiology of *Toxoplasma* in cattle milk are not enough in Egypt, although previous studies were done to detect *Toxoplasma* antibodies in cows in Egypt and other parts of the world but the current study is considered the first Egyptian study detected *T. gondii* DNA in cattle raw milk so, we recommend intensifying the studies on this part.

The seroprevalence of anti-*T. gondii* IgG antibodies in the tested women by ELISA was shown 54.4% in table (4). The obtained result was in agreement with the Egyptian researchers Nassef et al. (2015) and Shehata et al. (2016) who reported seropositivity of IgG antibodies 52.2% and 50% in a previous study in Menoufia Governorate and Alexandria Governorate, respectively. Moreover it agrees with results of other parts of the world as: 50.6% in Morocco (El Mansouri et al., 2007), 51.4% in Saudi Arabia (Al Mohammad et al., 2010) and 56.2% in Iran (Sharif et al., 2016).

On the other hand, relatively higher rates were documented in Egypt as: 67.5% in Menoufia Governorate by El Deeb et al. (2012), 62% in Sharkia Governorate by El Gamal et al. (2013). Another high rate 81.4% was also detected in Central Ethiopia (Gebremedhin et al., 2013).

On the contrary in Egypt, lower seroprevalence rates were illustrated in different Governorates as: 35.2% in Beni-Suef (Aboelhadid et al., 2013), 46.1% in Qena Governorate (Tammam et al., 2013) and 44% in Dakhalia Governorate (El-Tantawy et al., 2014). Moreover, lower rates were shown in other parts of the world as: 9.35% in Italy (Tomasoni et al., 2010), 44.8% among pregnant women in Libya (Mousa et al., 2011) and 6.4% among pregnant women in South Africa (Kistiah et al., 2012). The high IgG antibodies prevalence reflects the chronic *Toxoplasma* infection (Remington et al., 2004).

Moreover, the seroprevalence of IgM specific antibodies was 8.4% that was in agreement with those of some Egyptian investigators who reported IgM rates of 9.78% and 10% at Sharkia and Menoufia Governorates by Abd El-Ghany and Amin (2012) and Nassef et al. (2015), respectively. Moreover in Ethiopia a seroprevalence rate 8.98% was noticed by Tilahun et al. (2016).

On the contrary, relatively higher rates were detected in some Egyptian Governorates as: 60% in Dakahlia Governorate (Soliman et al., 2001), 47% in Sharkia Governorate (El Gamal et al., 2013) and 18% in Qena Governorate (Tammam et al., 2013). Moreover,

higher prevalence rates were declared in other parts of the world as: 45.89% in Italy (De Paschale et al., 2008) and 46% in Tanzania (Swai and Schoonman, 2009).

On the other hand, a low IgM seroprevalence rate 2.8% was recorded in Menoufia Governorate by El Deeb et al. (2012) that still greatly different from the very low rate 0.8% which had been reported in Nigeria by Ishaku et al. (2009). The importance of IgM positivity is that it indicates presence of a recently acquired infection (Goldstein et al., 2008).

The worldwide variation in seroprevalence of anti-*T. gondii* IgG and IgM specific antibodies from one geographic area to another occurs relating to the environmental and socioeconomic conditions, including eating habits, general level of hygiene, host susceptibility, geographical location and humidity of the soil (Furtado et al., 2011).

Tables (5) and (6) revealed seroprevalence of anti-*T. gondii* IgG and IgM antibodies respectively, in relation to demographic data, relevant obestitric history and risk factors, regarding to age, it was noticed that the majority of positive IgG percentage was 80% at age group (33-36 years) followed by 75% at age group (37-40 years) while the majority of positive IgM percentage was 25% at age group (37-40 years) followed by 15% at age group (33-36 years). Ayi et al. (2009) and Deji-Agboola et al. (2011) failed to find any association between women age and *Toxoplasma* prevalence. The prevalence of *Toxoplasma* specific antibodies increases with age in accordance with increasing exposure to sources of infection during life.

The present study failed to find any significant association between toxoplasmosis and neither occupation nor residence in accordance with Flatt and Shetty (2012); Kamal et al. (2015) and Nassef et al. (2015). Concerning to women occupation, seroprevalence was 56.45% by ELISA IgG and 9.68% by ELISA IgM for housewives while was 48.44% and 4.69% for women with other occupations by ELISA IgG and ELISA IgM, respectively.

The high seroprevalence rate among house wives can be explained as they spend more time cooking at home handling raw meat, handling pet animals including cats at home and manipulating with dust or dirt during frequent home cleaning. Furthermore, working women with higher socioeconomic standers, higher education levels are adopted for the appropriate hygienic measures regarding food and cooking habits which tend to eradicate the sources of disease transmission. Ayi et al. (2009) reported that *Toxoplasma* infection through handling of raw meat cannot be ruled out since penetration of tachyzoites through broken skin has been reported in some countries.

As regards to residence, 54.74% of women residing rural areas were IgG positive and 9.49% were IgM positive while seroprevalence was 53.98% and 7.08% for women residing urban areas by ELISA IgG and ELISA IgM, respectively. The insignificant relationship between residing and acquiring *Toxoplasma* antibodies in the current study might be referred to the fact of urbanization with absence of rural life style within Egypt as a whole. As well, individuals residing urban areas in Menoufia Governorate had more opportunities of acquiring toxoplasmosis by several routes rather than contact with soil and farm animals or watering from wells (the main sources of infection among rural residing women), they could acquire infection by frequent cleaning litter boxes of indoor infected cats, eating contaminated undercooked meat outside in restaurants, the wide preference of Menoufia individuals to consume organic raw milk and milk products (as: Karish cheese).

A significant association was shown between prevailing climatic conditions and increasing *Toxoplasma* prevalence in agreement with Dehkordi *et al*. (2013). The seropositivity in women was 64.86% and 12.61% in warm climate while was 46.04% and 5% in cold climate by using ELISA IgG and ELISA IgM, respectively. *Toxoplasma* sporulated oocysts are able to remain infective in warm, humid soil (that distinguishes the climate of Menoufia Governorate) and do not survive in dried, cold climates (Robert-Gangneux and Dardé, 2012).

According to obstetric history of the examined women, a significant association was found between increasing IgG seroprevalence and increasing gravidity and presence of abortion in accordance with Flatt and Shetty (2012) and Nassef *et al*. (2015), our obtained result was referred to suppressed immune response or hormonal imbalance among multigravida women as well as increasing in pregnancies is mainly associated with age increase. Moreover, persistence of *Toxoplasma* cysts in chronically infected uteri with their rupture during placentation infects the fetus in the first trimester of pregnancy causing recurrent abortions (Malik *et al*., 2014) while it was failed to find any significant statistical association with presence of complicated gestational history in agreement with Babaie *et al*. (2013).

On the other side, the current work documented no significant relationship between IgM seroprevalenc and neither gravididity nor presence of complicated gestational history in accordance with Robert-Gangneux and Dardé (2012). However, a statistical significant relationship was detected between IgM prevalence and abortion that was referred to the fact that recent *Toxoplasma* infection is accompanied with presence of tachyzoites circulating in blood that can distribute in all body fluids and pass the placental barrier infecting the fetus in the first trimester of pregnancy causing miscarriage or other congenital defects (Reis *et al*., 2006) the obtained result was on the contrary with findings of Deji-Agboola *et al*. (2011).

Concerning the behavioral risk factors, a significant association was found between IgG seroprevalence and contact with cats in accordance with Nassef *et al*. (2015) while Abd El-Ghany and Amin (2012) and Kamal *et al*. (2015) failed to find any association between toxoplasmosis and cat contact. Exposure to cats' faeces via owning cat(s) or having cats in the immediate surrounding with handling cats' sandboxes containing their faeces was found to be associated with *Toxoplasma* infection (Al-Kappany *et al.,* 2010).

Moreover, a significant association was found between IgG seroprevalence and consumption of raw cow milk in agreement with Ahmed *et al.* (2014) in accordance with the fact that tachyzoites in the milk can survive for a week at refrigerator (Spišák *et al*., 2010). A significant association was also found between IgG seroprevalence and eating undercooked meat in accordance of Abd El-Ghany and Amin (2012) and Kamal *et al.* (2015) because of the habit of eating some Egyptian food containing undercooked meat as: kabbab, shawarma, Hawawshy, Basterma and luncheon) that contain *Toxoplasma* encysted form which mainly present in skeletal muscles, brain, heart and visceral organs as: lungs, liver and kidneys (Skariah *et al.,* 2010). On the other hand, Moura *et al.* (2013) failed to find any association between toxoplasmosis and consumption of neither raw cow milk nor undercooked meat.

Furthermore, a significant association was also found between IgG seroprevalence and drinking water from wells in agreement with findings of Moura *et al.* (2013). Contaminated water was previously recorded as the main cause of *Toxoplasma* outbreaks among humans in Canada and USA (Jones and Dubey, 2010).

The current study did not found any significant statistical association between increasing prevalence of IgG antibodies and soil contact unlike the findings of Deji-Agboola *et al.* (2011) and Nassef *et al.* (2015). Soil contact has many forms as farming, gardening, removing cat feces or even ingestion of crops directly from soil without washing.

On the other side, no significant association was able to be demonstrated between IgM seroprevalence and any of the studied behavioral risk factors in agreement with Deji-Agboola *et al.* (2011) and Nassef *et al.* (2015).

Table (7) declared the number of women with positive and negative ELISA IgG in relation to ELISA IgM results, 108 of the examined women were negative for both IgG and IgM antibodies

indicating their susceptibility for primary infection through exposure to risk factors with the risk of vertical transmission of infection to fetus if they became infected for the first time during pregnancy and causing serious sequelae. On the other hand, 15 of women were positive for both IgG and IgM antibodies indicating sub-acute *Toxoplasma* infection or as acutely infected <1 year since IgM antibodies could be detected early in acute infection and might remain for short periods. 121 women were immune to *Toxoplasma* infection (IgG+/IgM-) as prevalence of chronic infection for >1 year while 6 of tested women were possibly considered in early acute infection (IgG-/IgM+) (Robert-Gangneux and Dardé, 2012).

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الآثار الصحية العامة لمرض التوكسوبلازما فى الحيوانات والسيدات فى مناطق مختارة بمحافظة المنوفية ، مصر

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يعتبر طفيل التوكسوبلازما من الأوليات الهامة المتسببة فى حدوث تاريخ سيئ من الولادات فى الحيوانات والنساء، وقد أجريت هذه الدراسه لتلقى الضوء على الأهمية الصحية و المشتركة لمرض التوكسوبلازما فى النساء وبعض الحيوانات بمحافظة المنوفية. تم تجميع ٥٠ عينة سيروم و٥٠ عينة دم من عدد ٥٠ من الأغنام البالغة ذوات السلالة البلدى (٣٠ عينة من كفر طنبدى و ٢٠ من شبين الكوم) كما تم تجميع ٣١ عينة من لبن الأبقار من مناطق مختلفة بشبين الكوم. تم فحص السيروم الخاص بالأغنام بواسطة اختبار الاليزا الخاص بالأجسام المناعية نوع IgG لتحديد نسبة انتشار التوكسوبلازما بين الأغنام وقد سجلت نسبة ٦٠ % (٣٠ من أصل ٥٠) كانوا موجبين للأجسام المناعية IgG ومنهم نسبة ٦٦.٦٧ % (٢٠ من أصل ٣٠) كانت موجبة بكفر طنبدى ونسبة ٥٠ % (١٠ من أصل ٢٠) كانت موجبة بشبين الكوم. بتطبيق تقنية تفاعل البلمرة المتسلسل على ٥٠ عينة من بلازما الأغنام سجلت نسبة ٢٢ % (١١ من أصل ٥٠) كانوا موجبين لتفاعل البلمرة المتسلسل ومنهم نسبة ٢٠ % (٦ من أصل٣٠) كانت موجبة بكفر طنبدى ونسبة ٢٥ % (٥ من أصل ٢٠) كانت موجبة بشبين الكوم. كما تم تطبيق اختبار تفاعل البلمرة المتسلسل على عينات لبن الأبقار لتسليط الضوء على دور اللبن فى نقل داء المقوسات وكانت النتيجة ١٩.٣٥ % (٦ من أصل ٣١) كانوا يحتوون على الحمض النووى للتوكسوبلازما. كما أشتملت الدراسة على قياس معدل انتشار داء المقوسات بين ٢٥٠ سيدة فى المرحلة العمرية (من ١٨ الى ٤٠ سنة) تم اختيارهم فى الفترة من (يونيو ٢٠١٤ الى أغسطس ٢٠١٥) من معامل تحاليل مختلفة بشبين الكوم بمحافظة المنوفية مع تقييم عوامل الخطر المحتملة لاكتساب التوكسوبلازما. وقد كانت نسبة ٥٤.٤ % (١٣٦ من أصل ٢٥٠) موجبة للأجسام المناعية نوع IgG بينما كانت نسبة ٨.٤ % (٢١ من أصل ٢٥٠) هى الموجبة للأجسام المناعية نوع IgM. وقد تبين وجود علاقة احصائية مابين نسبة الأجسام المضادة نوع IgG وعمر المريضة وعدد مرات الحمل ووجود الاجهاض وكذلك الاتصال بالقطط وتناول الألبان واللحوم الغير معاملة حراريا جيدا وكذلك مياه الابار الملوثة والمناخ ، كما وجدت علاقة احصائية مابين نسبة الأجسام المضادة نوع IgM وعمر المريضة ووجود حالات اجهاض وكذلك الأحوال المناخية السائدة وقت تجميع العينات.