Epidemiology and Molecular Detection of Zoonotic *Toxoplasma gondii* in Cat Feces and Seroprevalence of Anti-*Toxoplasma gondii* Antibodies in Pregnant Women and Sheep

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Abstract: *Toxoplasma gondii* is one of the most prevalent zoonotic parasites being responsible for major economic losses in sheep and abortion in pregnant women. One hundred samples of cat feces were examined for *T. gondii* oocysts using sheather's sugar flotation. The prevalence of *T. gondii* oocysts was 2% at Sharkia Province, Egypt. For experimental infection, twelve kittens were randomly fed on the diaphragm meat (100 gm/ each kitten), collected from freshly slaughtered sheep at EL-Bassatein abattoir, Cairo, while other two kittens were left as a control group. Eleven out of twelve kittens shed unsporulated oocyst with an infection rate of 91.7%. The prepatent period was ranged from 4-7 days, while the patent period was within the range of 7- 11 days. After DNA extraction of *T. gondii* oocyst from feces of four experimentally infected kittens and two naturally infected cats, the B1 gene was amplified in all samples with a PCR product of 115bp. Also, one hundred blood samples of pregnant sheep, with a history of previous abortions, were collected from three flocks in Sharkia Province. While, one hundred sera of pregnant women in their first trimester were collected from private labs after obtaining a comprehensive questionnaire that investigates the risk factors associated with prevalence of toxoplasmosis.The collected sera of sheep and pregnant women were serologically investigated for *T. gondii* antibodies by indirect hemagglutination test using Toxo-HAI Fumouze Kits. The seroprevalence rate of anti-*T. gondii* IgG antibodies was 85% in pregnant sheep, while IgM antibodies of Toxoplasma infection were negative. The anti-*T.gondii* IgG antibodies in seropositive sheep were evaluated with titers ranging from 1:160 to 1:2560 Moreover, the seroprevalence rates of anti-*T.gonii* IgG and IgM antibodies in pregnant women were 30 and 10%, respectively, but only 10% revealed a mixed seroprevalence for *Toxoplasma* IgG and IgM antibodies. The titers of anti-*T.gondii* IgG and IgM antibodies in seropositive women were ranged from1:160 to1: 2560 and 1:160 to 1:320, respectively. There were significant correlations between the seropositivity of *T. gondii* specific IgG antibodies in pregnant women and the most investigated risk factors including; knowledge about transmission modes, contact with cats, luncheon and sausage consumption, gardening or contact with soil, washing hands before meals and unwashed raw vegetables or fruits consumption. Mean while, no significant association between *T. gondii* seropositivity and ingestion of undercooked meat and viscera. This study emphasized that cats and sheep play a great role in epidemiology of *T. gondii* that having a public health hazard in pregnant women. Thereby it is recommended a further genotyping for *T. gondii* strains from different hosts to predict a recent strategy for prevention and control of such zoonotic parasite.

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1. Introduction

Toxoplasmosis is a cosmopolitan parasitic zoonosis, infecting human and other warm-blooded animals [1,2,3]. Up to one-third of the human population is chronically infected with *Toxoplasma gondii* [1,4].It is a common infection of sheep worldwide [5,6]. It is caused by an obligate intracellular protozoan parasite; *Toxoplasma gondii* [7]. Felids are the only known definitive hosts of *T. gondii* in which the sexual cycle can take place, and hence domestic cats play a central role in the epidemiology of *T. gondii*, constituting the only known source of environmental contamination with the infective oocyst stage [8].

In general, human can become infected horizontally with *T. gondii* by ingesting raw or undercooked meat or insufficiently treated meat containing viable Tissue cyst [9-12], or by ingesting food or water contaminated with sporulated oocysts [13,14], or by accidentally ingesting oocysts from the contaminated environment with infected cat feces [15] or vertically via transplacental transmission from pregnant non-immune mother to the fetus via tachyzoites in the circulation [8,13,16,17]. Also, in some animal species; mice, rats, and sheep, serial transplacental infection of subsequent generations has been proposed as another route of vertical transmission [18].

Acute primary maternal toxoplasmosis if acquired during the first trimester of pregnancy can cause significant morbidity and mortality in

developing fetuses [11,19,20], and can induce abortion and loss of vision [3,21]. Therefore, World Health Organization has repeatedly encouraged the collection of accurate data about *T. gondii* due to its medical importance as a major source of parasitic zoonosis. However, only few countries regularly monitor toxoplasmosis in human and animals.

Infection of sheep with *T. gondii* has important veterinary implications and heavy economic losses due to early embryonic death and resorption, fetal death and mummification, abortion, stillbirth and neonatal death [17, 22-26]. Also, infection of these herbivores with *T. gondii* has implications for public health since consumption of undercooked meat infected with the parasite could facilitate zoonotic transmission [17,27,28].

Little is known about the prevalence and molecular detection of *T. gondii* oocysts in feline feces in Egypt. The prevalence of *T. gondii* in cat feces collected from different localities in Sharkia province was 50% [29]. Also, previous studies investigated the prevalence of *T. gondii* oocysts from feces of naturally-exposed cats from different countries: Australia [30]; Germany [31,32]; Netherlands [33]; Chile [34].; Brazil [35].; USA [36]; Qatar [37] and Europe [38]. The development of molecular diagnostic methods particularly the polymerase chain reaction (PCR) for detection of *T. gondii* were potentially very useful especially when the serological tests and clinical symptoms are not evident. The locus most often routinely used for PCR identification was the tandemly analysed 35-fold repetitive B1 gene. As this gene is the most conserved gene sequence among various strains of *T. gondii* has shown to be a potential candidate to assure better diagnosis of toxoplasmosis in cats, sheep and pregnant women [39].

The diagnosis of *Toxoplasma gondii* infection is most commonly made by detecting IgG and IgM antibodies in the human and herbivores animals' serum using indirect hemagglutination test (IHAT). In Egypt, there is limited data on the seroprevalence of *T. gondii* or the proportion of women at risk of acquiring *Toxoplasma* infection during pregnancy [40]. However, some studies were carried out to investigate the prevalences of Toxoplasma-specific IgG in pregnant women in different geographic areas in Egypt: Sharkia Province [29,41].; Alexandria [42] and Menoufia [43].

Concerning the seroprevalence of anti-*Toxoplasma gondii* antibodies in sheep, different studies were carried out in Egypt [29,44]. Recent epidemiologic studies have identified different risk factors for *T. gondii* infection: contact with cats, eating raw or undercooked mutton, beef or minced meat products, gardening or contact with soil, eating raw or unwashed vegetables or fruits [43,45-47].Thereby, in this study, it is of great importance to investigate the prevalence and molecular detection of *T. gondii* oocyst in cat feces using amplification of B1 gene, as well as to determine the seroprevalence of anti-*Toxoplasma gondii* antibodies (IgG and IgM) in pregnant women and sheep to assess the infection risk for pregnant women, and consequently to predict update prevention and control strategies for such zoonosis.

2. Material and Methods:

Examination of cat feces for *T. gondii* oocysts:-

One hundred samples of cat feces were collected from different localities in Sharkia Province (Minia Elkamah, Hehia and Abo-kabeer cities) in the period from January to July, 2012. Cats were hunted and placed in a separate cage. The cat feces were examined for the presence of *T. gondii* oocysts using sheather's sugar flotation as was previously described by [4,8,17,31,48]. Briefly, feces (2-10 gm) of each cat were floated in sucrose solution (454 gm sugar, 355 ml water and 6 ml formalin; specific gravity, 1.203), filtered through gauze, and centrifuged in a 15 ml tube at 1500 rpm for 10 min. A drop of the float from the meniscus was examined microscopically between cover slip and glass slide at 400X magnification. Measurements of oocysts were taken with the aid of an ocular micrometer. If oocyst size was ranged from 9-12 µm, fecal floats were sedimented in water and aerated in 2% sulfuric acid, for sporulation, on a shaker at 22 ºC for 1 week and stored in a refrigerator at 4 ºC [4,49].

Experimental infection of kittens with sheep diaphragm containing tissue cyst of *T. gondii*:

Fourteen kittens, of 2-2.5 month's age, were kept separately in cages and feed only boiled milk and bread. The feces of each kitten was examined daily by sheather's sugar floatation for one week before starting the experiment to ensure that kittens were free from *T. gondii* oocyst. The meat samples were collected from 120 freshly slaughtered sheep at EL-Bassatein abattoir, Cairo, Egypt. About 30 gm of meat were obtained from diaphragm of each slaughtered sheep. The collected meat samples were pooled and minced using electric blinder before being fed to kittens. Twelve kittens were randomly fed on the diaphragm meat (100 gm/ each kitten), while the remaining two kittens were left as a control group.

Each individual kitten feces was examined daily starting from the 2nd day after feeding on sheep diaghragm for detection of non sporulated *T. gondi*i oocysts [50].The average range of prepatent and patent periods was recorded. Sporulation of the detected oocysts was also carried out according to **Dubey and Beattie[4] and Lindsay *et al.*** [49].

Also, the faecal floats of experimentally fed kittens or examined cats were placed in Eppendorf tubes and washed 3 times using distilled water and the

centrifuged pellets, that containing *T. gondii* oocysts, were preserved in phosphate buffer saline at -80◦C for molecular studies.

Molecular detection of *Toxoplasama gondii* in feces of both surveyed cats and experimentally infected kittens:-

A-Oocyst DNA extraction procedure:-

The infected centrifuged pellets obtained from experimentally fed kittens and examined cats were submitted to three cycles of freezing and thawing for at least 4 h at +20 ◦C and −80 ◦C. The oocyst DNA was extracted from feces of four experimentally infected kittens and two naturally infected cats, as previously described by **Sambrook *et al.*** [51]. Briefly, to each prepared sample, 1% and 0.3mg/ml final concentration of SDS and Proteinase K, respectively were added and the mixture incubated at 37◦C overnight. DNA was extracted by phenol-chloroform-isoamyl alcohol followed by ethanol precipitation and resuspended in TE buffer. The purity of extracted DNA was spectrophotometery determined by reading optical density (OD) at 260 and 280 nm. An infected sheep blood containing tachyzoite of *T. gondii* (muton isolate, genotype I) was obtained from Department of Zoonotic Diseases, Veterinary Research, National Research Center, Dokki, Giza, Egypt; and considered as a positive control. The tachyzoite DNA was extracted using the same protocol.

B. PCR amplification of B1 gene:

The coding regions of the B1 gene in the positive control strain and also in *T. gondii* oocysts, isolated from feces of naturally infected cats and also experimentally fed kittens, were amplified using 25µl PCR reaction volume by a conventional PCR assay according to **Contini *et al.*** [52]. Two oligonucleotides were designed from the B1 gene sequences (**Bretagne *et al.*** [53]; forward primer, B1-B22; 5'-AACGGGCGAGTAGCACCTGAGGAGA -3' and reverse primer, B1-B23; 5'-TGGGTCTACGTCGATGGCATGACAAC -3' (positions 1793-818 and 1907-1882, respectively). Amplification was performed using Primus thermal Cycler, M. W. G Biotech, Germany. The following PCR components were added in each PCR tube: 5X PCR master mixes (Jena Bioscience), 25 pmol from primer, 5 µl of DNA template and volume completed by nuclease free water. The amplification program include, initial denaturation of 95 ◦C for 5 minutes. Followed by thirty five cycles, each cycle included a denaturation step at 93◦C for 1 minute, a primer annealing step at 60◦C for 1 minute and an extension step at 72◦C for 3 minutes. The final elongation step was prolonged for 10 minutes to ensure a complete extension of amplified DNA. Aliquots (10µl) of PCR products were electrophoresied on 1.5% agarose gels and staining with ethidium bromide followed by visualisation under UV.

Prevalence of anti- *Toxoplasma gondii* IgG and IgM antibodies in pregnant women and sheep:

Sera collection:

One hundred blood samples of pregnant sheep were collected from three flocks in Sharkia Province with a history of previous abortions. Ten milliliters of blood samples were collected and allowed to clot for 0.5-1 hour. The sera were separated by centrifugation at 3000 rpm for 10 minutes. While, one hundred sera of pregnant women were collected in their first trimester, from private labs, after obtaining a comprehensive questionnaire that investigates the risk factors associated with toxoplasmosis such as knowledge about transmission modes, contact with cats, luncheon and sausage consumption, undercooked meat and viscera consumption, gardening or contact with soil, washing hands before meals and unwashed raw vegetables or fruit consumption.

Determination of IgG and IgM antibodies to *T. gondii* infection in the collected sera:

Serological investigation of the collected sera for the presence of anti- *T. gondii* IgG and IgM antibodies was done using Toxo-HAI Fumouze Kits (Indirect hemagglutination test), according to the manufacturer's instructions [54]. Also, anti- *T. gondii* IgG and IgM antibodies can be differentiated by treating serum with 2-mercaptoethanol which inhibits the agglutinating power of IgM. Briefly for IHA assay, sera were added into U-microplates, and diluted serially starting from 1:80 to 1:2560. Then, one drop of sensitized red blood cells (composed of sheep red blood cells coated with a *Toxoplasma* antigen) was distributed in diluted sera, and the plates were shaken gently for 2 minutes before incubation at 37 ºC for 2 hrs without shaking. The test was considered positive (titer ≥ 1:80) when a reddish brown film can be observed in the well, while the negative reaction of the test (titer < 1:80) when these red blood cells deposit and form a ring in well bottom. Positive and negative control sera were included in each test.

Statistical analysis:-

Statistical analyses were done using computerized statistical software program IBM SPSS 19.0. The Pearson Chi-square test was used to examine the relationship between two qualitative variables. Statistical significance was defined as *p*-values < 0.05.

3. Results:

In this study, the prevalence of *T. gondii* oocysts in cat feces was 2% (2 out of 100). Concerning the experimentally fed kittens for sheep diaphragm possibly containing tissue cyst of *T. gondii*, eleven out of twelve kittens shed unsporulated oocyst with an infection rate of 91.7%. The prepatent period was

ranged from 4-7 days, while the patent period was within the range of 7- 11 days.

Oocysts in freshly passed feces are unsporulated (non-infective), subspherical to spherical in shape and measures 9-13 µm. The sporont or zygote almost fills the entire oocyst and appears to be in close contact with the oocyst wall (**Figure 1; A&B**). Sporulated oocysts are subspherical to ellipsoidal in shape and measure 10-14 µm. The sporulated oocyst contains two ellipsoidal sporocyst measures 6-8 µm. Each sporocyst contains four sporozoites (**Figure 1; C**). The result of amplification of *T. gondii* DNA using primer set (B1-22& B1-23) for B1 gene are shown in **Figure (2)**. The PCR product of B1 gene in all samples was 115bp.

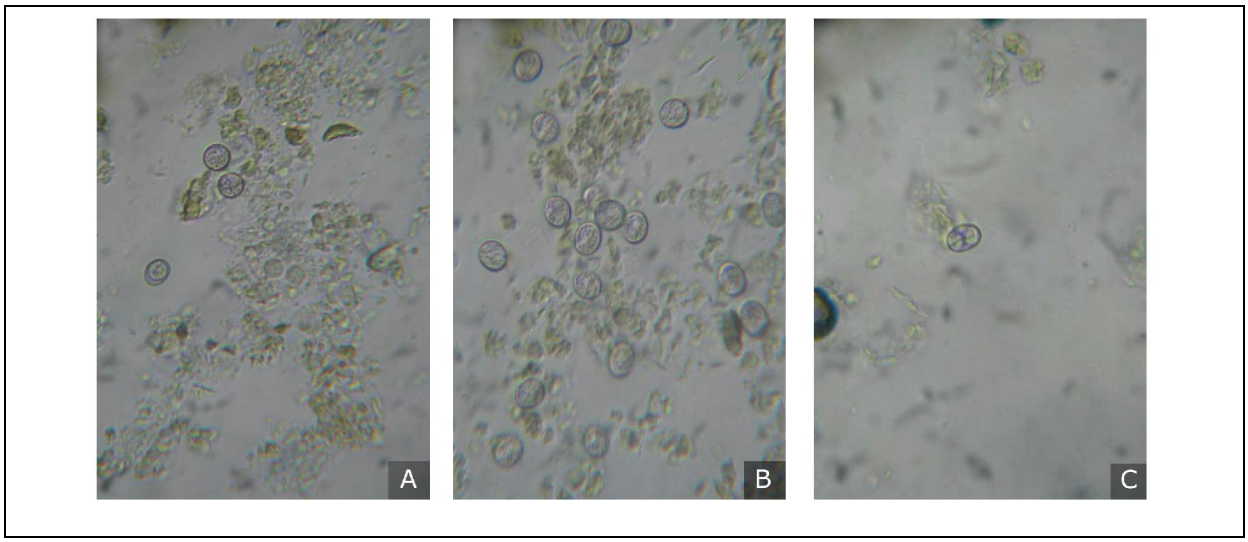

Figure (1): A- Unsporulated Oocysts of *T. gondii* in naturally infected cat feces, **B-** Unsporulated Oocysts of *T. gondii* in sucrose solution**, C-** Sporulated Oocyst of *T. gondii*.

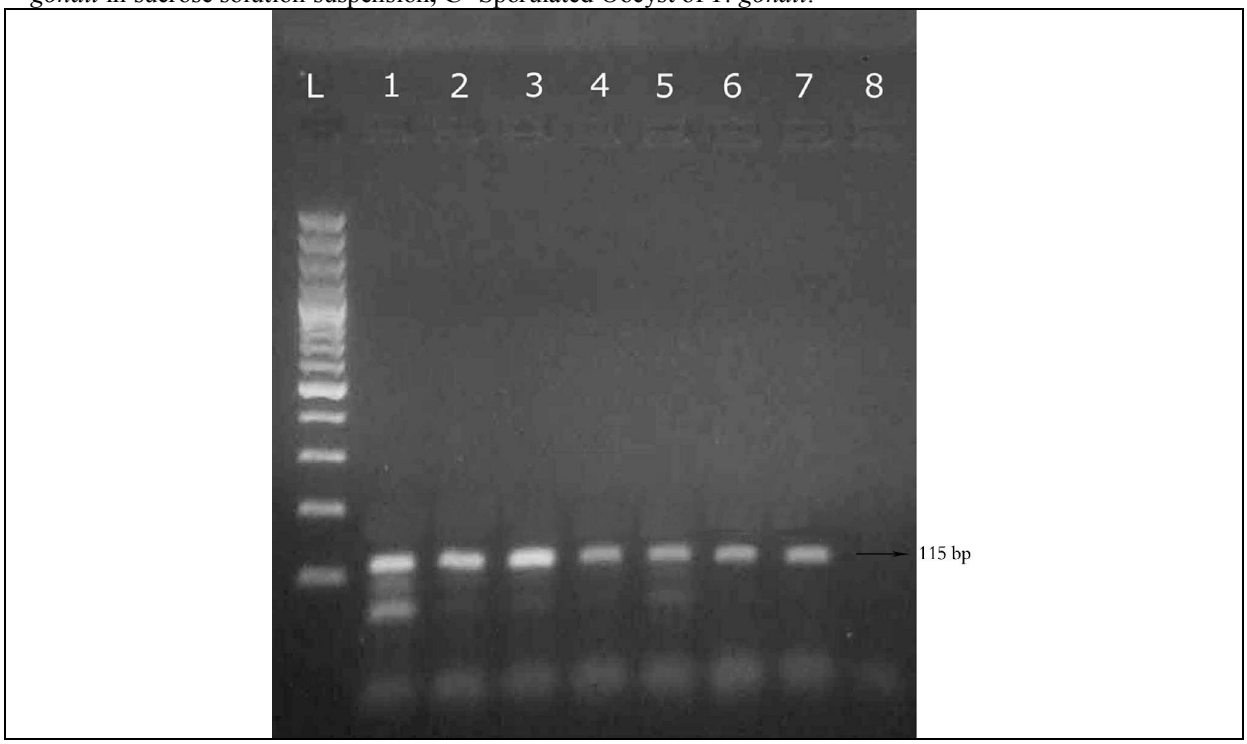

Figure (2): PCR products of B1 gene of *T. gondii* oocysts. Lane 1: positive control; Lanes 2, 3, 4 and 5: *T. gondii* oocysts in feces of experimentally infected kittens; Lanes 6 and 7, *T. gondii* oocysts in feces of naturally infected cats; Lane 8, negative control and L, 100 bp ladder.

Concerning the seroprevalence of anti-*Toxoplasma gondii* antibodies, thirty out of 100 examined serum samples of pregnant women (30%) showed the presence of anti-*T. gondii* IgG antibodies with titers ranging from 1:160 to 1: 2560 by IHA test (**Tables 1 & 2**).While, only ten (10%) showed the presence of anti-*T. gondii* IgM antibodies with titers ranging from 1:160 to 1:320 (**Tables 1& 2**). On the other hand, 85 out of 100 examined serum samples of pregnant sheep (85%) were positive for anti- *T. gondii* IgG antibodies with titers ranging from 1:160 to 1:2560 by IHA test (**Tables 1&2**). While, anti-*Toxoplasma* IgM antibodies were negative (**Table 2**). As shown in **Table 3**, there were significant correlations between the seropositivity of *T. gondii* IgG antibodies in pregnant women and the studied risk factors that included knowledge about transmission modes, contact with cats , luncheon and sausage consumption, gardening or contact with soil, washing hands before meals and unwashed raw vegetables or fruits consumption (P<0.05). Mean while, no significant association between *T. gondii* seropositivity and ingestion of undercooked meat and viscera (P>0.45).

Table (1): Seroprevalence of anti-*T.gondii* antibodies in pregnant women and sheep in relation to type of antibodies.

Host species	Total examined	IgG		IgM		IgG + IgM	
		Positive	%	Positive	%	Positive	%
Pregnant women	100	30	30	10	10	10	10
Pregnant sheep	100	85	85	0	0	0	0

Chi-square value in comparison of IgG and IgM in pregnant women = 13.49 (P<0.01)
Chi-square value in comparison of IgG and IgG +IgM in pregnant women = 13.49 (P <0.01)
Chi-square value in comparison of IgG and IgM in pregnant sheep= 9.30 (P <0.05)
Chi-square value in comparison of IgG and IgG +IgM in pregnant sheep = 9.30 (P <0.05)

Table (2): Seroprevalence of anti-*T. gondii* IgG and IgM antibodies in pregnant women and sheep with respect to titer of antibodies.

Host species	IgG No. positive	IgM No. positive	IgG titer					IgM titer	
			1/160 No. (%)	1/320 No. (%)	1/640 No. (%)	1/1280 No. (%)	1/2560 No. (%)	1/160 No. (%)	1/320 No. (%)
Pregnant women	30	10	5(16.7)	5(16.7)	15(50)	0(0)	5(16.7)	5(50)	5(50)
Pregnant sheep	85	0	10(11.7)	0(0)	25(29.4)	5(5.9)	45(52.9)	0(0)	0(0)

Chi-square value in comparison of IgG and IgM titre 1/160 in pregnant women = 6.11 (P >0.05)
Chi-square value in comparison of IgG and IgM titre 1/320 in pregnant women = 6.11 (P >0.05)
Chi-square value in comparison of IgG and IgM titre 1/160 in pregnant sheep = 13.45 (P <0.05)
Chi-square value in comparison of IgG and IgM titre 1/320 in pregnant sheep = 2.17(P >0.05)

Table (3): Risk factors associated to seropositivity for *T. gondii*-specific IgG antibody in pregnant women.

Risk factor	Total (100) No.	No. (%) positive for *T. gondii* specific IgG antibody	*P*- value
Knowledge about transmission modes			
Yes	16	1 (6.25)	Chi-square=23.25
No	84	29 (34.5)	P<0.001
Contact with cats			
Yes	21	4 (19.05)	Chi-square=20.53
No	79	26 (32.9)	P<0.001
Luncheon and sausage consumption			
Yes	67	28 (41.8)	Chi-square=16.57
No	33	2 (6.1)	P<0.01
Undercooked meat and viscera consumption			
Yes	26	13 (50)	Chi-square=9.56
No	74	17 (22.9)	P>0.45
Gardening or contact with soil			
Yes	13	4 (30.8)	Chi-square=30.17
No	87	26 (29.9)	P<0.001
Washing hands before meals			
Yes	11	2 (18.2)	Chi-square=25.23
No	89	28 (31.5)	P<0.05
Unwashed raw vegetables or fruits consumption			
Yes	5	1 (20)	Chi-square=21.56
No	95	29 (30.5)	P<0.01

4. Discussion

Toxoplasmosis is a zoonosis arising from close contact of human with felids [55]. As far as the domestic cats are concerned, they play a crucial role in the epidemiology of this disease as they are the only definitive hosts and are the only ones to shed oocysts in their faeces. It is generally assumed that cats play a major role in transmitting *T. gondii* through the faecal contamination of soil, food or water since they may excrete millions of oocysts over a period of 1–2 weeks [56]. However, the presence of those infected pets indicates a contaminated environment, posing a risk to the human population and small ruminants [47]. Proximity of cats to human homes and smaller space for deposition of cat feces in urban areas could increase the possibility of oocyst contamination [10]. So, cats have also been used extensively for the isolation of *T. gondii* strains by feeding tissue samples to cats and then examining the feces for shedding of oocysts from day 3 to 14 post-infection [57]. As a matter of fact, in Egypt, stray cats are very abundant, and toxoplasmosis was reported in 97.4% of feral cats, indicating high environmental contamination with oocysts [58].

In the current study, the prevalence of *T. gondii* in feces of examined cats was 2%. This result was in accordance with finding of **Edelhofer and Aspöck**[59] in Austria and **Venturini *et al.*** [60] in Argentina. Nearly similar results were recorded in previous studies: **Svobodova and Svobodá**[61](1.3%) in Czech Republic; **Dubey *et al.*** [62] (1.8%) in U.S.A.; **Barutzki and Schaper**[31] (1.1%) in Germany and **Pena *et al.*** [35] (1.3%) in Brazil. However, lower findings were cited in various studies: **Epe *et al.*** [63] (0.6%, north Germany); **Robben *et al.*** [33] (0.3%, Netherlands); **Dabritz *et al.*** [36] (0.9%, USA); **Schares *et al.*** [38] (0.11% in Germany, 0.1% in Australia and 0.23% in France) and **Berger--Schoch *et al.*** [64] (0.4%, Switherland). The prevalence of *T. gondii* in cat feces in the present study and other epidemiologic reports showed lower percentages because oocyst shedding varies with the life style of the cat (indoor versus outdoor), age of the cat, and the prevalence of *T. gondii* in intermediate hosts (rodents, birds) in the vicinity of cats [4]**.** Cats less than 1 year of age produce the largest numbers of *T. gondii* oocysts, and generally become infected shortly after they are weaned and begin to hunt. Most cats that have excreted oocysts once generally develop immunity and do not repeatedly excrete oocysts after challenge with *T. gondii* [65]. Otherwise, no *T. gondii* oocyst was detected in cat feces by many authors: **Hill *et al.*** [66] in USA; **Miro *et al.*** [67] in Spain; **Afonso *et al.*** [68] in France; **Dubey *et al.*** [48] in Colombia and **Qian *et al.*** [69] in China.

The current prevalence of *T. gondii* in cat feces was in marked contrast to higher prevalence rates of 23.2% in Cost Rica [70]; 12% in South Germany [71]; 15 % in Australia [30]; 4.3% in Chile [34]; 10% in Qatar [37] and 50% in Egypt [29]. These higher infection rates may be attributed to younger age of cats and a very wide pool of potential sources that may be a source of infection for cats [8] as cats may be infected from eating infected sheep meat or from eating wild birds and rodents infected with *T. gondii*.

Specific and sensitive methods are developed for other protozoa but are not yet available for *T. gondii* oocysts detection. Microscopy as well as bioassay may be not sufficient to perform sensitive and simple detection. So, it may support rather than replace molecular investigation. The B1 gene of 35 repetitive fold has been routinely used for PCR detection of *T. gondii* in clinical specimens since the early 1990s [53,72]. In the present study**,** molecular detection of *T. gondii* DNA from feces of two naturally infected cats and four experimentally infected kittens were obtained via amplification of B1 gene with a molecular weight of 115 bp **(Figure 2)**. In accordance to our finding, **Lass *et al.*** [73] cited a molecular identification for *T. gondii* oocysts from contaminated vegetables and fruits with cat feces via B1 gene amplification. However, other studies in Egypt reported molecular detection of *T. gondii* using B1 gene from blood of pregnant women and Sheep [74]; from peritoneal fluid of experimentally infected mic [75] and from Blood of sport horses [76].

Sheep represent an important source of meat, milk and wool for humans in many countries, and toxoplasmosis causes abortion and great economic losses to sheep industry worldwide [26,28]. In addition, these small ruminants have a significant role in the epidemiology of toxoplasmosis, since Meat from these animals is regarded as an important source of human *T. gondii* infection especially in countries and regions where mutton and goat meat is regularly eaten [77].

In **Table 1**, the seroprevalence of anti-*T. gondii* IgG antibody in pregnant sheep using IHAT was 85%, however the seroprevalence of anti-*T. gondii* IgM and mixed seroprevalence of IgG and IgM antibodies were negative. There was a significant difference between the seroprevalence of anti-*T. gondii* IgG and IgM or between anti-*T. gondii* IgG and mixed serum IgG and IgM antibodies ($P<0.05$). In Egypt, elevated seroprevalence rates for anti-*T. gondii* IgG antibodies in sheep were reported by **El-Ghaysh and Mansour**[78] (50 %); **Shaapan *et al.*** [44] (43.7%) ; **Ghoneim *et al.*** [74] (98.4%) and **Hassanain *et al.*** [79] (61.4%). Also, several studies reported different anti-*T. gondii* IgG seroprevalences in various geographic areas: **Dubey *et al.*** [80]

recorded 41% in Maryland; **Dubey and Welcome**[81] cited 73.8 % in New York; **Dubey and Kirkbride**[82] (65.8%, South Dakota); **Malik *et al.*** [83] (58.5%, USA); **Cabannes *et al.*** [84] (92%, France); **Aktas *et al.*** [85] (47%, Turkey); **van der Puije *et al.*** [86] (40.9%); **Dumètre *et al.*** [87] (65%, France); **Mainar-Jaime and Barberán**[88] (40.4%); **Vesco *et al.*** [89] (49.9%, Sicily); **Bártová *et al.*** [26] (59%, the Czech Republic); **Lopes *et al.*** [90] (52.0% , Brazil); **Rossi *et al.*** [91] (61%) and **Tzanidakis *et al.*** [92] (48.6%, Greece). As shown in **Table 1,** the present study showed higher seropositivity of sheep with *T. gondii*; this may be attributed to easily acess of cats to sheep flocks, and consequently contamination of sheep pasture with feces of infected cats containing *T. gondii* oocysts as was previously supported by **Shaapan *et al.*** [44] and also may be explained to increase sheep age[93]**.** The presence of cats on farms are the putative risk factor for higher seroprevalence in sheep in European and South American studies [89,90].

On the other hand, there were marked decreases in the incidence rate of anti-*T.gondii* IgG antibodies in sheep using different serological tests in several reports: in Egypt (**Maronpot and Botros**[94], 12.1%, indirect immunofluorescent antibody test**; Rifaat *et al.*** [95], 26.4%, Sabin Feldman test**; Esmat**[96], 24.3%, IHAT**; Mohamed and Eisa**[97], 18.2%**; Saleh *et al.*** [41], 21.98% and **Maysa**[29], 18%, IHAT); in Iran (**Hoghooghi-rad &Afraa**[98], 13.1%, **Hashemi-Fesharki**[99], 24.50%, IHAT and **Bonyadian *et al.*** [100], 29.1%); in Chile (**Gorman *et al.*** [101]; 12%, IHAT); in Uruguay (**Freyre *et al.*** [102]; 28.7%); in Brazil (**da Silva and Langoni**[103]; 8.3%, IFAT); in Italy (**Masala *et al.*** [104]; 28.4% and **Fusco *et al.*** [105]**;** 28.5%, IFAT); in Kerman (**Bahrieni et al**[106]; 24.7%, modified agglutination test); in Pakistan (**Ramzan *et al.*** [107]; 11.2%); in Portugal (**Sousa *et al.*** [108]; 17.1%), and in Nigeria (**Kamani *et al.*** [109]; 6.7%). The great variations for seroprevalence of toxoplasmosis amongst sheep from one study to another may be due to differential environment contamination with *T. gondii* oocysts, frequency of felines on the farms, age of the animals and the climatic variations from one region to another [13]**;** and also could be due to difference in the sensitivity and specifity of the used serological tests as well as the size of the animal sample [1,77].

Moreover from **Table 2**, anti-*T.gondii* IgG antibodies in seropositive sheep using IHAT were evaluated with the following titers: 1:160 in 10 (11.7%) animals; 1:640 in 25(29.4%) animals; 1:1280 in 5(5.9%) animals and 1:2560 in 45 (52.9%) animals. In one study, 20.8% of ewes were seropositive at a titer of 1:640 using IHAT [110]. In another study in Brazil; antibodies against *T. gondii* infection in sheep were detected with the following titers: 16 in 19 (47.5%) animals; 64 in 15 (37.5%) animals; 256 in 5 (12.5%) animals, and 1024 in 1 (2.5%) animal using IFAT [103]**.** Besides, **Fusco *et al.*** [105] in Italy recovered antibody titres of seropositive sheep were as follows: 142(42.6%) animals with titer 1:200; 120(36%) animals with titer 1:400; 43(12.9%) animals with titer 1:800 and 28(8.4%) animals with titer >1:800 using IFAT. According to finding of **Rossi *et al.*** [91] in Brazil, 72 sheep (46.5%) were reagent for *T. gondii* (titer≥ 64), with 80% of samples presenting titers between 512 and 2048 and the most frequent titer was 512 (30.5%). Higher seroprevalence of toxoplasmosis in sheep in this study emphasizing the need of regular monitoring of this infection due to its zoonotic potential and its economic losses.

The diagnosis of *T. gondii* infection is most commonly made by detecting IgG and IgM antibodies in the sera of pregnant women; however, these tests can not estimate the time of infection precisely enough to properly manage the risk to the fetus of a maternal infection [111]**.** There were significant differences between anti-*T.gondii* IgG and IgM antibodies; and also between anti-*T.gondii* IgG and mixed IgG &IgM antibodies ($P< 0.01$). The overall prevalence of anti-*T. gondii* IgG antibodies in pregnant women (30%), as in **Table 1,** was similar to finding of **Carmen *et al.*** [112] in Slovakia. Nearly relevant seroprevalece rates of *T. gondii* IgG antibodies in pregnant women were found to be 27.3% using IHAT and IFAT in Egypt [113]**;** 30.1% by ELISA in Turkey [46]; 29% in Saudi Arabia [114]; 23.4% in Iran [115]; 37% in Turkey [116] and 32.6% in Nigeria [117].

On the contrary, higher seroprevalence rates of anti-*T. gondii* IgG antibodies were cited in different countries: in Cuba (**Gonzalez-Morales *et al.*** [118]; 71%, ELISA); in Argentina (**Fuentes et al**[119]; 59%, IFAT); in India (**Singh and Pandit**[120]; 45%, direct agglutination test); in Grenada (**Asthana *et al.*** [111]; 57 % ELISA); in Egypt (**Awadalla *et al.*** [42], 46.2% in Alexandria; **Ibrahim *et al.*** [40], 51.49% in Dakahlia and **El Deep et al**[43]**,** 67.5% in Menoufia governorate); in Brazil (**Spalding *et al.*** [121], 74.5%, IFAT; **Heukelbach *et al.*** [122], 70%, ELISA and **Vaz *et al.*** [123], 53.03%); in Turkey (**Tekay and Özbek**[124]; 69.5%); in Morocco (**El Mansouri *et al.*** [125]; 50.6%); in Kuwait (**Iqbal and Khalid**[126]; 53.1%, Vitek Immuno Diagnostic Assay System); in Colombia (**Gomez-Marin et al**[127]; 61%, IFAT and **Rosso et al**[128]; 45.8%); in France (**Berger et al**[129]; 43.8%); in Nigeria (**Akinbami**[130]; 40.8%, ELISA); in Gabon (**Mickoto *et al.*** [131]; 56%); in Lebanon (**Bouhamdan *et al.*** [132], 62.2%) and in Cameroon (**Njunda *et al.*** [133]**,** 70%).

In comparison to the present result of anti-*T. gondii* IgG antibodies in pregnant women, previous studies recorded lower incidence rates to be 0.8% in Korea [134]; 11.2% in Vietnam [135]; 12% in Jordan [136]; 7.9% in China [137]; 9.1% in U.K [138]; 0.8 % in Korea [139]; 11% in U.S. [140]; 8.2% in Mexico [141]; 17.9% in Palestin [142]; 20.45 % in Egypt [74]; 10.6% in China [143];16% in Egypt [29] and 12.0% in Spain [144]. The difference in *T. gondii* IgG seropositivity between current study and other studies may be accounted for the sensitivity of different serological tests; the wide range of exposure to *T. gondii* [43], and also may be explained to climatic conditions as well as the factors associated with lifestyle and diet [1].

It is widely agreed that anti-*T. gondii* IgM is an excellent marker for the initial evaluation of expectant mothers at public health services [145] where individuals can remain positive for months or even years after infection [146,147]. As regards to anti-*T. gondii* IgM prevalence of 10% in pregnant women (**Table 1**), higher prevalence rates of 13.8, 12.8, 30.5 and 17.8% were cited by **Iqbal and Khalid** [126]; **Al-Hindi and Lubbad** [142]; **Ghoneim *et al.*** [74] and **Higa *et al.*** [148], respectively. Otherwise, lower incidence rates for *T. gondii* IgM antibodies were found to be 3.3% [120]; 6.6% [37]; 2.22% [149]; 2.8% [128]; 2.3% [141]; 8% [29]; 2.73% [133] and 2.8% [43]. However, anti-*T. gondii* IgM antibodies in women were negative in many reports [46,112,136,143].

From **Table 2**, there were no significant differences in comparison of IgG and IgM titers of 1:160 and 1:320 in serpositive pregnant women (P>0.05). Each titer of 1:160, 1:320 and 1:2560 for *T.gondii* IgG antibodies was recorded in seropositive women with the same percentage of 16.7%; while 50% of seropositive IgG cases had a titer of 1:640. Also, each titer of 1:160 and 1:320 for *T.gondii* IgM antibodies was recorded with the same percentage of 50% in seropositive cases. In brief, this study revealed that the overall seropositivity for IgG antibodies was 30% in pregnant women. A large proportion of pregnant women (70%) were susceptible to primary infection (IgG −/IgM−). Also, the rate of probable acute *Toxoplasma* infection (IgG+/IgM+) in this study was 10%. Therefore, 20% of pregnant women were immune to *Toxoplasma* infection (IgG+/IgM−), as prevalence of chronic infection. This was in agreement with finding of **Lindsay *et al.*** [150], who explained that increased levels of IgG and IgM antibodies in acute *Toxoplasma* infections usually appear within the first or second week of infection. High levels of specific IgG antibodies indicate that the individual has been previously infected. However, these antibodies do not distinguish a recent infection from one acquired a long time before. Detection of specific IgM antibodies can help to determine if infection was recent [151]; although, these antibodies can persist for months or even years after acute infection [152].

In the current study, a significant relationship was detected between the seropositivity of *T. gondii* IgG antibodies in pregnant women and the most investigated risk factors ((**Table 3,** P<0.05). In support to our findings, similar significant relations between relevant risk factors and toxoplasmosis in pregnant women were reported by many authors. These included knowledge about modes of transmission [43], contact with cats [9,46,115,117,121,141,143,153], gardening or contact with soil and washing hands before meals [43,154]and consumption of unwashed raw vegetables or fruits [43,112,143]. In this study, luncheon and sausage consumption was significantly related with *Toxoplasma* infection as similarly recorded by **Nimri *et al.*** [136]. Indeed, other relevant studies cited no significant association between consumption of undercooked meat and seroprevalence [43,46,155].

In contrast with our data, some authors reported no association between *Toxoplasma* infection and contact with cats [43,46,155,156]. In this study, ingestion of undercooked meat and viscera showed no relation with seroprevalence of *T. gondii* IgG antibodies. However, other reports ruled out a significant correlation between consumption of undercooked meat and prevalence of infection [74,112,143,157]. In conclusion, cat and sheep play a great role in the epidemiology of *T. gondii* that posing a zoonotic risk for pregnant women. Thereby, this study recommended further molecular diagnosis of acute and chronic toxoplasmosis in the future, as well as, genoptyping of *T. gondii* strains from different hosts in order to predict a recent strategy for prevention and control of such zoonotic parasite.

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