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Prevalence and associated risk factors of *Toxoplasma gondii* infection in sheep and aborted women in Egypt

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Abstract

Toxoplasmosis has been associated with economic and public health concerns due to abortion in humans and animals. Detection for the presence of anti-Toxoplasma gondii antibodies in sera of 174 healthy sheep (IgG) and sera of 89 aborted women (IgG, IgM, and IgG avidity) using enzyme-linked immunosorbent assay and T. gondii in 59 sheep milk samples identified by PCR. The primary risk factors for sheep and women seropositive samples were also analyzed. Relatively higher seroprevalence of T. gondii, 62.6%, was recorded in sheep than 57.3% in aborted women. The IgM/IgG antibody responses in aborted women were 42.7%, 41.6%, 15.7%, and 0% for IgM -ve/IgG -ve (negative toxoplasmosis), IgM -ve/IgG +ve (chronic toxoplasmosis), IgM +ve/IgG +ve (maternal infection) and IgM +ve/IgG -ve (acute infection), respectively. IgM and IgG-positive samples showed a low avidity rate 14.3%, denoting acute maternal infection, while a much higher rate 78.6%, denoting chronic maternal infection. Molecular investigation of T. gondii in sheep milk samples revealed that 28.8% were positive by PCR at 470-bp. The respectable significant association between many risk factors and the seroprevalence of T. gondii in humans and sheep was recorded. The current study indicates elevated toxoplasmosis antibodies in sheep and aborted women from El-Beheira and Alexandria governorates, Egypt Additionally, it demonstrates a correlation between age, mutton intake, miscarriage, cat exposure, drinking water source, and breeding practices with seropositivity to T. gondii. These results largely confirm the need for greater in-depth toxoplasmosis epidemiological research and public health education initiatives.

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Introduction

Toxoplasma gondii (*T. gondii*), an obligate intracellular protozoan parasite capable of infecting all warm-blooded species, including humans, is the cause of toxoplasmosis. This opportunistic zoonotic infection is widespread throughout the world (1). Cats are the only definitive host of this parasite in which sexual reproduction occurs and capable of excreting the environmentally resistant oocysts stage, while all mammals, including man and birds, serve as

intermediate hosts (2). Around the world, up to one-third of the population is seropositive for *T. gondii* and at risk of infection (3). Humans can become infected by consuming raw or undercooked meat with tissue cysts (bradyzoites), drinking water, or eating food contaminated with cat excrement containing sporulated oocysts (4). Handling raw meat and consuming unpasteurized milk containing the rapidly growing stage (tachyzoites) may also provide risks for human *T. gondii* infection (5).

Human toxoplasmosis is frequently asymptomatic in immunocompetent, healthy people, the primary toxoplasmosis infection in pregnant women has only mild symptoms, and approximately 70-90% of infants born with congenital toxoplasmosis are asymptomatic at birth. However, an infection may result in a spontaneous abortion, a stillbirth, a premature birth, or severe fetal harm (6,7). Serious clinical signs such as stillbirth, microcephaly, hydrocephalus, and retinochoroiditis in newborns and encephalitis, myocarditis, and pneumonia in immunocompromised individuals are present in these situations (8). Additionally, severe latent congenital or ocular toxoplasmosis may develop, with the latency most likely caused by treatment with anti-inflammatory corticosteroids (9). The prenatal period's health follow-up does not always include a requirement for serological screening for T. gondii. Therefore, the number of pregnant women at risk of contracting toxoplasma infection is unknown (10). The possibility of toxoplasmosis transmission by blood transfusion to seronegative recipients draws attention to the importance of Toxoplasma screening as a preliminary test before blood donation (11).

Toxoplasmosis in sheep is one of the most prevalent infectious agents that causes reproductive failure. It results in placentitis, abortion, and stillbirth, generating big economic losses over time (12,13). During grazing in a polluted environment, sheep are mostly infected by ingesting T. gondii oocysts; 61.4% of seropositive Egyptian sheep were accepted with mutton-containing cysts (14). Moreover, it was determined that 47.5% of seropositive lambs fed on waste material harbored virulent T. gondii strains (15). There are only three clonally types of T. gondii isolates found in sheep meat: type I, II, and III. These types are defined by the pathogenicity of the isolates in mice as measured by LD₅₀ and LD_{100} , as well as genetic diversity (16). It was feasible to have a mixed infection in the same meat sample. However, the more virulent ones masked the biological characteristics of the less virulent strains (17). Because sheep are more frequently infected than other farm animals, several studies have found that T. gondii seroprevalences in sheep are high worldwide (18). Various infection rates of T. gondii in sheep from different localities in Egypt have been reported, which range from 34 to 100%. This is compared to 11.8% to 67.6% seroprevalence rates reported in pregnant and aborted women from the same country (10,19).

Serological assays that identify anti-*T.gondii* specific immunoglobulins IgM and IgG antibodies in sera samples are the primary methods used to diagnose toxoplasmosis in sheep and humans (7,20). *T.gondii* IgM levels fall after 1-6 months. However, they can still be found for a year or more, indicating that an older infection cannot be ruled out despite the high levels indicative of an acute or recent infection (11). Evaluation of the IgG avidity index in pregnant women is also a very helpful diagnostic technique for establishing the time of Toxoplasma infection (7). However, the presence of anti-*T.gondii* IgG without IgM indicates that the infection was remote and had occurred at least six months earlier (21). After identifying both IgM and IgG antibodies in the same serum sample, the anti-*Toxoplasma* IgG avidity test can distinguish between recent and prior infections. A higher than 4-month-old toxoplasma infection is indicated by high IgG avidity (22).

Mutton is a prominent source of meat, and due to its nutritional advantages over other red meats, its consumption has expanded globally, especially in less developed countries. In addition to giving people a sufficient amount of dietary protein (23). The primary cause of human toxoplasmosis is T. gondii tissue cysts found in edible animal flesh, particularly sheep mutton, because they may survive in undercooked tissues (24). Besides mutton, T. gondii tachyzoites have been identified in the milk of several food animal species, including cows, sheep, and goats. The infective tachyzoites are excreted in the milk and can survive in fresh cheese (25). In Egypt, there is a large overlap between the use of milk and mutton from sheep and the population at risk of contracting toxoplasmosis, particularly pregnant women. However, limited information is known regarding the frequency of toxoplasmosis in sheep and humans in these overlapped areas (26).

Therefore, the present study was initiated to investigate the incidence of toxoplasmosis in apparently healthy sheep in El-Beheira and Alexandria governorates, Egypt, and determines the infection prevalence in aborted women from the same governorates using the indirect ELISA (serological) and PCR (molecular) techniques. Moreover, risk factors associated with seropositive samples of both sheep and women were also assessed.

Materials and methods

Ethical approval

The experiment was carried out according to the institutional guidelines of the National Research Centre's Animal Research Committee, number 20/139. Project number 12010139 NRC, Egypt.

Study area description

An epidemiological survey was conducted between September 2021 and July 2022 to determine the prevalence of toxoplasmosis in sheep and aborted women and to identify the associated risk factors. The study was carried out in 2 governorates: El-Beheira (X/Y coordinates; 30.343551/ 30.848099) and Alexandria (X/Ycoordinates; 29.924526/ 31.205753). Both localities are located on the West North Coast of Egypt. Sheep were randomly selected from different herds from the two localities. Human samples were obtained from El Shatby Hospital for Obstetricians and Gynecologists, Alexandria, which provides obstetrics and gynecology services to all sick and pregnant women from the governorates of El-Beheira and Alexandria.

Sample collection

One hundred seventy-four blood samples from apparently healthy sheep were collected for serological examination. Additionally, 59 milk samples were obtained from some of these animals in the active milking period. Samples were collected after obtaining verbal consent from the herd's owners. Collected data including age, pregnancy status, history of abortion, source of drinking water, presence of cats, and herd size. For a human, a total of 89 blood samples were collected from aborted women. Written and verbal consent was also obtained before sample collection. Relevant data such as age, residence, mutton consumption, abortion history, and miscarriage trimester were obtained.

Sample preparation and processing

Sera from sheep and human blood samples were separated and put into 1.5 ml Eppendorf tubes, which were then stored at -20 C until additional serological investigation. Before collecting 10 ml of milk samples from each teat of a sheep, the teats were cleaned, sanitized, and the first few jets of milk from each teat were thrown away. After correctly recognized and kept in a chilling ice box, milk samples were submitted to the lab for molecular analysis.

Serological examination

Sheep serum samples were tested for anti-T. gondii IgGspecific antibodies using a commercially available ELISA kit from the ID vet (ID Screen® Toxoplasmosis Indirect Multispecies. Product code: TOXOS-MS-2P. Innovative Diagnostic vet Laboratories, France). On the other hand, human serum samples were tested for anti-T detection. gondii IgM and IgG-specific antibodies using SERION ELISA classic Toxoplasma gondii IgM (Germany, order Nr ESR110M) and SERION ELISA classic Toxoplasma gondii IgG (Germany, order Nr ESR110G) respectively. The Seropositive samples with IgM and IgG antibodies were retested using IgG avidity assay by the SERION ELISA classic Toxoplasma gondii IgG test in combination with the corresponding SERION ELISA classic Avidity Reagent (Germany, order Nr B110AVID) to differentiate between recent and past infection. The indirect ELISA assays were carried out under methods described by Shaapan et al. (27).

Molecular examination

Sheep milk samples kept at -20 °C were thawed and then processed for the total genomic DNA extraction. Pelleted samples were diluted in 200 µL of PBS and processed for total genomic DNA extraction. PCR targeted a 470-bp fragment of the repetitive B1 gene (35-fold repeats/genome). The used primer pair Tg1-5was AAAAATGTGGGAATGAAAGAG-3 Tg2-5and ACGAATCAACGGAACTGTAAT-3. For each PCR run, positive and negative controls were included. The positive control consisted of 3 µl genomic DNA extracted from T.

gondii RH strain tachyzoites. Amplified DNA samples were subjected to gel electrophoresis on 1.2 % agarose gel (28).

Risk factors assessments

The correlation between risk variables and the seroprevalence of *T. gondii* in humans and sheep was determined using logistic regression analysis. In humans, five risk factors were examined: age, place of residency, mutton consumption, previous miscarriages, and trimester of miscarriage. In sheep, Age group, pregnancy status, abortion history, interaction with cats, drinking water source, and breeding system were the six risk factors examined (29).

Statistical analysis

Data were analyzed statistically using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Logistic regression analysis was performed to detect the association between seroprevalence rates and risk factors for *T. gondii* infection, in which adjusted odds ratio (AOR) and the confidence interval at 95% (CI 95%) were calculated to estimate the probability of association. Differences were considered statistically significant when P-values less than 0.05 were recorded (30).

Results

Seroprevalence data of *T. gondii* infection in sheep and human

Seropositive samples were detected from both sheep and aborted women; out of 174 sheep sera samples, 109 were positive, with a prevalence rate of 62.6%. A relatively lower seropositive rate of 57.3% (51/89) was recorded in aborted women's sera samples (Table 1).

When classified according to IgM/IgG antibody responses, four categories of aborted women samples were determined; Out of 38 cases (42.7%) were negative for both IgM and IgG (IgM -ve / IgG -ve) indicating a negative status of toxoplasmosis. On the other hand, IgM -ve / IgG +ve was detected in 37 (41.6%) cases indicating past/chronic toxoplasmosis. IgM +ve / IgG +ve was detected in 14 (15.7%) denoted with the maternal infection that was most likely acquired during pregnancy. Nevertheless, none of the tested sera samples were detected with IgM +ve / IgG -ve for the current acute status (Table 2).

IgM and IgG antibodies were detected simultaneously in 14 cases (maternal infection), so an anti-*Toxoplasma* IgG avidity test was conducted to discriminate between recent and past infections. Out of 14 samples, only two (14.3%) showed low avidity, denoting a most likely acute maternal infection. In contrast, a much high rate of 78.6% (11/14) of cases were recorded with high avidity, denoting a chronic maternal infection. Only a single case (7.1%) showed an undefined maternal infection status, as it had a borderline avidity index.

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	Number Examined	Number Positive (%)	OR* (95% CI**)	<i>p</i> -value ^{***}
Sheep	174	109 (62.6%)	0.69 (0.46-1.04)	0.310
Human	89	51 (57.3%)	1.44 (0.96-2.16)	0.080

* Odd Ratio ** 95% Confidence Interval *** Differential data are significant at P<0.05

Table 2: Differential Immunoglobulin (Ig)-based seroprevalence data of aborted women

Ig-Category	Prevalence (%) Out of 89 Subjects	Interpretation
IgM -ve /IgG -ve	38 (42.7%)	Negative case
IgM -ve /IgG +ve	37 (41.6%)	Past (chronic) infection
IgM +ve /IgG +ve	14 (15.7%)	Most probably a recent infection (Valid to Avidity Test)
IgM +ve /IgG -ve	0	
Total	89	

Molecular examination of sheep milk samples

Sheep milk Molecular detection by PCR successfully amplified a 470-bp DNA fragment from the positive control sample and some of the examined sheep milk samples. After testing this on 59 sheep milk samples, 17 (28.8%) samples were positive by PCR (Figure 1).



Figure 1: Detection of *T. gondii* DNA using PCR. Lane L: 100 bp Ladder; lane 1: negative control, lane 2: positive control and lanes 3-5: positive sheep milk samples (470 bp)

Risk factors associated with *T. gondii* seropositivity in aborted women

The risk factors and relations with the seroprevalence of *T. gondii* in humans revealed that mutton consumption and miscarriage history were the most significantly associated with the seropositive cases of aborted women. 48 out of the 68 aborted cases (70.6%) with seropositive *T. gondii* had reported a history of mutton consumption. This is significantly higher than cases with no consumption of mutton history who reported a seropositivity rate of 14.3%.

On a similar line of evidence, there is a significant association between a history of miscarriage and *T. gondii* seroprevalence rate, as cases with repeated miscarriages (two or more) reported a significantly higher seropositive rate 71.2% than those with a single miscarriage history 37.8%. Other risk factors have affected, albeit with no statistical significance, *T. gondii* seroprevalence as those cases aged > 25 years and women came from rural residency reported higher seropositivity rates 69.8 and 61.8% than younger women and those of urban residency 26.9 and 30.8%, respectively. Finally, cases with reported miscarriages during the second trimester reported a higher susceptibility to infection than cases with the first-trimester miscarriage 62.8% versus 18.2%, respectively, even though in a non-significant difference (Table 3).

Risk factors associated with *T. gondii* seropositivity in sheep

In sheep, the most important determinant risk factors of T. gondii infection included the history of abortion and the presence of cats which were associated with significant differences in animal susceptibility to T. gondii infection. 11 out of 13 sheep with abortion history 84.6% turned out to be seropositive when compared with 60.9% (98/161) of animals with no history of abortion. The presence of cats is another significant determinant of infection susceptibility, as 82.6% of animals with a history of contact with cats were seropositive compared to 40.2% of animals with no cat contact history. Some differences existed for other risk factors and their association with T. gondii susceptibility in sheep, even with no statistical significance, such as age group, pregnancy status, drinking water source, and breeding system. Higher susceptibility and seropositive rates were recorded in sheep 1-2 years old, pregnant, and those with surface water as a drinking source. Sheep are kept in an extensive breeding system (\geq 50 heads), then other animal groups (Table 4).

	Examined No. (%)	Seropositive No. (%)	AOR* (95% CI)	<i>p</i> -value
Age group				0.085
< 25 Y**	26 (29.2%)	7 (26.9%)	0.65 (0.42-1.40)	0.085
> 25 Y	63 (70.8%)	44 (69.8%)	2.33 (0.95-2.10)	
Residence				
Rural	76 (85.4%)	47 (61.8%)	0.98 (0.55-1.86)	0.850
Urban	13 (14.6%)	4 (30.8%)	3.25 (2.40-6.75)	
Mutton consumption				
Yes	68 (76.4%)	48 (70.6%)	9.34 (6.10-17.50)	0.001
No	21 (23.6%)	3 (14.3%)	0.85 (0.08-0.16)	
History of miscarriage				
One	37 (41.6%)	14 (37.8%)	4.55 (2.23-7.86)	0.005
Two or more	52 (58.4%)	37 (71.2%)	0.42 (0.23-0.63)	
Trimester of miscarriage				
First	11 (12.4%)	2 (18.2%)	0.82 (0.34-3.52)	0.000
Second	78 (87.6%)	49 (62.8%)	3.32 (0.65-17.90)	0.900

Table 3: Risk factors associated with T. gondii seropositivity in aborted women

* AOR = adjusted odds ratio ** Y = Years

Table 4: Risk factors associated w	vith T. y	gondii sero	positivity	in sheep
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	Examined No. (%)	Seropositive (%)	AOR (95% CI)	<i>p</i> -value
Age group				
<1Y	16 (9.2%)	1 (6.3%)	1.73 (0.85-3.85)	0.200
1-2 Y	113(64.9%)	81 (71.7%)	1.65 (0.84-2.22)	0.200
> 2 Y	45 (25.9%)	27 (60%)	1.60 (1.90-3.45)	
Pregnancy status				
Pregnant	75 (43.1%)	52 (69.3%)	0.65 (0.65-1.90)	0.330
Non-pregnant	99 (56.9%)	57 (57.6%)	2.30 (0.95-3.22)	
History of abortion				
Yes	13 (7.5%)	11 (84.6%)	3.88 (2.35-4.20)	0.001
No	161(92.5%)	98 (60.9%)	0.65 (0.45-0.85)	
Contact with cats				
Yes	92 (52.9%)	76 (82.6%)	3.50 (2.852-6.950)	0.030
No	82 (47.1%)	33 (40.2%)	2.44 (0.930-3.150)	
Drinking water source				
Surface	112(64.4%)	78 (69.6%)	1.25 (0.80-2.15)	0.141
Underground	62 (35.6%)	31 (50%)	0.90 (0.65-1.30)	
Breeding system				
Non-extensive (≤ 10 heads)	12 (6.9%)	3 (25%)	1.35 (1.10-1.90)	0.260
Semi-extensive (10-50 heads)	93 (53.4%)	58 (62.4%)	0.66 (0.80-1.30)	0.200
Extensive (\geq 50 heads)	69 (39.7%)	48 (69.7%)	0.85 (0.75-1.90)	

Discussion

The current study focused on toxoplasmosis as a significant foodborne infection that might affect pregnant women. Our study's relatively higher seroprevalence rate of T gondii infection was 62.6% in sheep and 57.3% in aborted women from El-Beheira and Alexandria, Governorates using ELISA. In Egypt, nearly similar high seroprevalence, 61.76% in sheep and 63.79% in pregnant women from Menoufia and Gharbiya provinces in the Delta, respectively (31). At the same time, 39.1% and 22.9% were lower

seroprevalences recorded in sheep and pregnant women from El-Minya Governorate, respectively (32). In the Cairo governorate, previous studies found that 46% of sheep and 60.7% of aborted women were positive for Toxo IgG by ELISA (20, 33). These previously noted variations in seroprevalence may be caused by variations in ecological conditions, serological assays employed, sample size, breed of sheep, site, and time of sampling, rather than a lower prevalence in the urbanized Cairo governorate than in the rural governorates (34). Numerous studies on toxoplasmosis in Egyptian pregnant women have been published. However,

they often use small sample sizes and lack information on the population researchers attempted to investigate. Because of the sample size, diagnostic test utilized, and living situations of the women evaluated, results cannot be compared between publications. There are scant statistics on seroconversion before and during pregnancy (35). Anti-T. gondii specific IgG and IgM antibodies identification is a vital diagnostic technique for T. gondii infection through serological testing (36). In this study, the IgM/IgG antibody responses in aborted women sera detected by ELISA were 42.7% of (IgG-/IgM-) as negative toxoplasmosis, 41.6% of (IgG+/IgM-) as chronic toxoplasmosis, 15.7% of (IgG+/IgM+) as maternal infection and 0% of (IgG-/IgM+) for recent acute infection. In a previous parallel study for the detection of T. gondii infection in the antenatal population in Menoufia governorate, Egypt, the serological pattern analysis showed that 32.5% of cases were (IgG-/IgM-) susceptible to harmful infection and 64.7% were (IgG+/IgM-) as the frequency of chronic infection, and 2.8% were (IgG+/IgM+) of possible maternal Toxoplasma infection was with only one instance 0.3% had low avidity (37).

Additionally, a recent study revealed that a high percentage of HIV patients had chronic toxoplasma infections of 45.8% (IgG+/IgM-), and 6.5% had acute infections (IgG-/IgM+), which can be explained by the improved HIV treatment regimen that shortens the duration of immune suppression (38). It is quite obvious that the presence of IgM antibodies alone (IgG-/IgM+) is rarely seen due to the short period between the appearance of IgM and IgG (39), as consequently, in the current investigation, 0% of (IgG-/IgM+) was detected denoting acute or recent infection. This finding was also consistent with Abd El Wahab et al. (11), where IgM was identified alone in 0.7%. IgM with IgG was found in 5.4% of tested blood donor samples. It may be complicated to distinguish between acute and chronic infection since determining IgG avidity has effectively identified recent Toxoplasma infections (40). IgM and IgGpositive aborted women serum samples in this study showed a low avidity rate 14.3%, denoting acute maternal infection, while a much high rate (78.6%), denoting chronic maternal infection. Also, another study (7) advised applying the IgG avidity test in all pregnant women as good diagnostic efficacy for differentiation between the acute and chronic infected cases.

T. gondii DNA was found in the milk samples of 6.5% sheep from South Bahia, Salvador (41), 5.4 % sheep from São Paulo State, Brazil (42), and 28% sheep from the Slovak Republic (43). The variance in results could be attributed to the used diagnostic techniques, environmental factors, farm management, and the presence of cats in the herds (44). In Egypt, a study on raw sheep milk recorded *Toxoplasma* tachyzoites in 8.62% (5/58) by microscopic examination and detection of *T. gondii* DNA in 10.71% (3/28) by PCR assay (45). In our investigation, molecular detection of *T. gondii* in sheep milk samples proved that 28.8% (17/59) were positive

by PCR at 470-bp. The most likely time for *T. gondii* tachyzoites to be excreted in infected animal milk is during the acute infection phase (28). These study's findings also reinforce the theory that consuming raw sheep's milk and its unpasteurized derivatives increases the risk of T. gondii transmission to humans (46).

In this study, the most significant risk factors associated with the T. gondii seropositive cases of aborted women were a history of mutton consumption and repeated miscarriages 70.6 and 71.2%. Other risk factors affected but with no statistical significance were cases aged > 25 years, rural residency, and second-trimester miscarriages 26.9, 30.8, and 62.0 %. These findings follow the assessment of risk factors of toxoplasmosis in Ethiopian pregnant women where a strong significant correlation was also found between the history of abortion, age range, eating raw meat and vegetables with T. gondii infection (47). Our results were also in line with the earlier researchers who found that eating insufficiently cooked mutton (kabab and kofta) was a considerable risk factor for toxoplasmosis seropositivity in pregnant women (14,20,48). Additionally, the custom of consuming sheep meat, the third trimester of pregnancy, and interaction with cats all demonstrated a statistically significant correlation with toxoplasma seropositivity in pregnant women (32). Furthermore, eating mutton has been linked to a significant source of T. gondii infection in people, presenting a threat to the public's health. As a result, eating infected, undercooked ovine meat was seen as a significant danger for human toxoplasmosis (5).

In sheep, the most important determinant risk factors associated with T. gondii infection in the current investigation included abortion history and contact with cats 84.6 and 82.6%. Some other risk factors have existed without statistical significance, such as age group, pregnancy status, drinking water source, and breeding system. Also, similar results of our study on T. gondii seroprevalence in sheep from southeast Mexico were identified, and significant differences in pregnancy, age, and water source were not found. Although, the presence of cats was not identified, and the semi-intensive breeding system presented a high seroprevalence (49). On the other hand, no statistical significance was discovered based on the history of abortion. A considerably increased seroprevalence was connected with growing sheep age and location (32). Likewise, hormonal differences suppress the immune system during breastfeeding and pregnancy, which increases the risk of toxoplasmosis in female sheep. (30). Overall, the high incidence of the parasite in sheep suggests that T. gondii is widely distributed, suggesting that ovine meat may be a significant source of human infection, especially since pregnant women are one of the main target populations (31).

Conclusion

The current study indicates elevated toxoplasmosis antibodies in sheep and aborted women from El-Beheira and Alexandria governorates, Egypt. Based on our study findings, milk samples may only be utilized to directly demonstrate toxoplasmosis by identifying Toxoplasma DNA during the acute phase of infection. Additionally, it demonstrates a correlation between age, mutton intake, miscarriage, cat exposure, drinking water source, and breeding practices with seropositivity to *T. gondii*. These results largely confirm the need for greater in-depth toxoplasmosis epidemiology research, public health education initiatives, and the importance of proper food safety behaviors to avoid the risk of infection.

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Conflict of interest

The authors have declared no conflict of interest.

References

- Shaapan RM. The common zoonotic protozoal diseases causing abortion. J Parasit Dis. 2016;40(4):1116-1129. DOI: <u>10.1007/s12639-</u> <u>015-0661-5</u>
- Kolören Z, Dubey JP. A review of toxoplasmosis in humans and animals in Turkey. Parasitol. 2020;147(1):12-28. DOI: 10.1017/S0031182019001318
- Ammar AI, Moharm IM, Shaapan RM. *Toxoplasma gondii* infection in cattle egret (*Bubulcus ibis*): First report from Shebin El-Kom, Menoufia governorate, Egypt. Pak J Biol Sci. 2020;23(11):1442-1449. DOI: 10.3923/PJBS.2020.1442.1449
- Barakat AM, Salem LM, El-Newishy AM, Shaapan RM, El-Mahllawy EK. Zoonotic chicken toxoplasmosis in some Egyptians governorates. Pak J Biol Sci. 2012;15(17):821-286. DOI: <u>10.3923/pjbs.2012.821.826</u>
- Markon AO, Ryan KA, Wadhawan A, Pavlovich M, Groer MW, Punzalan C, Gensheimer K, Jones JL, Daue ML, Dagdag A, Donnelly P. Risk factors for *Toxoplasma gondii* seropositivity in the old order Amish. Epidemiol Infect. 2021;149:e89. DOI: 10.1017/S0950268820002897
- Hill D, Dubey JP. *Toxoplasma gondii*: Transmission, diagnosis and prevention. Clin Microbiol Infect. 2002;8(10):634-640. DOI: 10.1046/j.1469-0691.2002.00485.x
- Hassanain NA, Shaapan RM, Hassanain H. Associated Antenatal health risk factors with incidence of toxoplasmosis in Egyptian pregnant women. Pak J Biol Sci. 2018;21(9):463-8. DOI: 10.3923/PJBS.2018.463.468

- Khan K, Khan W. Congenital toxoplasmosis: An overview of the neurological and ocular manifestations. Parasitol Int. 2018;67:715-721. DOI: <u>10.1016/j.parint.2018.07.004</u>
- Elfadaly HA, Hassanain MA, Shaapan RM, Hassanain NA, Barakat AM. Corticosteroids opportunist higher *Toxoplasma gondii* brain cysts in latent infected mice. Int J Zool Res. 2015;11(4):169-176. DOI: <u>10.3923/ijzr.2015.169.176</u>
- Ibrahim HM, Huang P, Salem TA, Talaat RM, Nasr MI, Xuan X, Nishikawa Y. Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in northern Egypt. Am J Trop Med Hyg. 2009;80:263-267. DOI: <u>10.4269/ajtmh.2009.80.263</u>
- Abd El Wahab WM, Shaapan RM, Hassanain MA, Elfadaly HA, Hamdy DA. *Toxoplasma gondii* infection and associated sociodemographic and behavioral risk factors among blood donors. Asian J Epidemiol. 2018;11:52-58. DOI: 10.3923/aje.2018.52.58
- Ali S, Zhao Z, Zhen G, Kang JZ, Yi PZ. Reproductive problems in small ruminants (sheep and goats): A substantial economic loss in the world. Large Anim Rev. 2019;25:215-223. [available at]
- Clune T, Beetson S, Besier S, Knowles G, Paskin R, Rawlin G, Suter R, Jacobson C. Ovine abortion and stillbirth investigations in Australia. Aust Vet J. 2021;99:72-78. DOI: <u>10.1111/avj.13040</u>
- Hassanain MA, Elfadaly HA, Shaapan RM, Hassanain NA, Barakat AM. Biological assay of *Toxoplasma gondii* Egyptian mutton isolates. Int J Zool Res. 2011;7(4):330-337. DOI: <u>10.3923/ijzr.2011.330.337</u>
- Elfadaly HA, Hassanain MA, Shaapan RM, Hassanain NA, Barakat AM. Detection of *Toxoplasma gondii* from wastage nourished small ruminant and poultry: Zoonotic significance. Int J Zool Res. 2017;13:6-11. DOI: <u>10.3923/ijzr.2017.6.11</u>
- Elfadaly HA, Hassanain NA, Shaapan RM, Hassanain MA, Barakat AM, Abdelrahman KA. Molecular detection and genotyping of *Toxoplasma gondii* from Egyptian isolates. Asian J Epidemiol. 2017;10(1):37-44. DOI: 10.3923/aje.2017.37.44
- Shaapan RM, Toaleb NI, Abdel-Rahman EH. Significance of a common 65 kDa antigen in the experimental fasciolosis and toxoplasmosis. J Parasit Dis. 2015;39(3):550-556. DOI: 10.1007/s12639-013-0394-2
- Stelzer S, Basso W, Silván JB, Ortega-Mora LM, Maksimov P, Gethmann J, Conraths FJ, Schares G. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. Food Waterborne Parasitol 2019;15:e00037. DOI: 10.1016/j.fawpar.2019.e00037
- El-Shqanqery HE, Ibrahim HM, Mohamed AH, El-Sharaawy AA. Seroprevalence of *Toxoplasma gondii* infection and associated risk factors among asymptomatic pregnant females in Egypt. J Egypt Soc Parasitol. 2017;47:93-100. DOI: 10.21608/jesp.2017.77989
- Shaapan RM, El-Nawawi FA, Tawfik MA. Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. Vet Parasitol. 2008;153(3-4):359-362. DOI: <u>10.1016/j.vetpar.2008.02.016</u>
- Begeman IJ, Lykins J, Zhou Y, Lai BS, Levigne P, El Bissati K, Boyer K, Withers S, Clouser F, Noble AG, Rabiah P. Point-of-care testing for *Toxoplasma gondii* IgG/IgM using Toxoplasma ICT IgG-IgM test with sera from the United States and implications for developing countries. PLOS Negl Trop Dis. 2017;11(6):e0005670. DOI: 10.1371/journal.pntd.0005670
- Garnaud C, Fricker-Hidalgo H, Evengård B, Álvarez-Martinez MJ, Petersen E, Kortbeek LM, Robert-Gangneux F, Villena I, Costache C, Paul M, Meroni V. *Toxoplasma gondii*-specific IgG avidity testing in pregnant women. Clin Microbiol Infect. 2020;26:1155-1160. DOI:10.1016/j.cmi.2020.04.014
- Mahmoud MA, Ghazy AA, Shaapan RM. Review of diagnostic procedures and control of some viral diseases causing abortion and infertility in small ruminants in Egypt. Iraqi J Vet Sci. 2021;35:513-521. DOI: 10.33899/ijvs.2020.127114.1461
- 24. Toaleb NI, Shaapan RM, Hassan SE, El Moghazy E. High diagnostic efficiency of affinity isolated fraction in camel and cattle

toxoplasmosis. World J Med Sci. 2013;8:61-66. DOI: 10.5829/idosi.wjms.2013.8.1.72161

- Almeria S, Dubey JP. Foodborne transmission of *Toxoplasma gondii* infection in the last decade. An overview. Res Vet Sci. 2021;135:371-385. DOI: <u>10.1016/j.rvsc.2020.10.019</u>
- Shaapan RM, Toaleb NI, Abdel-Rahman EH. Detection of *Toxoplasma* gondii-specific immunoglobulin (IgG) antibodies in meat juice of beef. Iraqi J Vet Sci. 2021;35:319-324. DOI: <u>10.33899/ijvs.2020.126829.1390</u>
- Shaapan RM, Hassanain MA, Khalil FA. Modified agglutination test for serologic survey of *Toxoplasma gondii* infection in goats and water buffaloes in Egypt. Res J Parasitol. 2010;5:13-17. DOI: 10.3923/jp.2010.13.17
- Vismarra A, Barilli E, Miceli M, Mangia C, Bacci C, Brindani F, Kramer L. *Toxoplasma gondii* and pre-treatment protocols for polymerase chain reaction analysis of milk samples: A field trial in sheep from southern Italy. Ital J Food Saf. 2017;6:6501. DOI: 10.4081%2Fijfs.2017.6501
- Elfadaly HA, Hassanain NA, Hassanain MA, Barakat AM, Shaapan RM. Evaluation of primitive ground water supplies as a risk factor for the development of major waterborne zoonosis in Egyptian children living in rural areas. J Infect Public Health. 2018;11:203-208. DOI: 10.1016/j.jiph.2017.07.025
- Elfadaly HA, Hassanain MA, Shaapan RM, Barakat AM, Toaleb NI. Serological and hormonal assays of murine materno-fetal *Toxoplasma gondii* infection with emphasis on virulent strains. World J Med Sci. 2012;7:248-254. [available at]
- Ibrahim HM, Mohamed AH, El-Sharaawy AA, El-Shqanqery HE. Molecular and serological prevalence of *Toxoplasma gondii* in pregnant women and sheep in Egypt. Asian Pac J Trop Med. 2017;10:996-1001. DOI: <u>10.1016/j.apjtm.2017.09.012</u>
- 32. Abdelbaset AE, Hamed MI, Abushahba MF, Rawy MS, Sayed AS, Adamovicz JJ. *Toxoplasma gondii* seropositivity and the associated risk factors in sheep and pregnant women in El-Minya governorate, Egypt. Vet World. 2020;13:54-60. DOI: 10.14202%2Fvetworld.2020.54-60
- Hassanain MA, El-Fadaly HA, Hassanain NA, Shaapan RM, Barakat AM, Abd El Razik, KA. Serological and molecular diagnosis of toxoplasmosis in human and animals. World J Med Sci. 2013;9:243-247. DOI: <u>10.5829/idosi.wjms.2013.9.4.8185</u>
- Hassan SE, Toaleb NI, Shaapan RM, Abdel-Rahman EH, Elmahallawy EK. Diagnosis of toxoplasmosis using affinity purified fraction of tachyzoites local isolate. Res J Parasitol. 2016;11:13-19. DOI: 10.3923/jp.2016.13.19
- Abbas IE, Villena I, Dubey JP. A review on toxoplasmosis in humans and animals from Egypt. Parasitol. 2020;147:135-159. DOI: 10.1017/S0031182019001367
- 36. Al-Namroty AO, Shaapan RM, El-Moamly AR, Al-Hamshary EM. Correlation between behavioral alterations and dopamine changes in mice experimentally infected with *Toxoplasma gondii*. Neuropsychol Trends. 2020;28:39-57. DOI: <u>10.7358/neur-2020-028-alna</u>
- El Deeb HK, Salah-Eldin H, Khodeer S, Allah AA. Prevalence of *Toxoplasma gondii* infection in antenatal population in Menoufia governorate, Egypt. Acta Trop. 2012;124:185-191. DOI: <u>10.1016/j.actatropica.2012.08.005</u>
- Fang EE, Nyasa RB, Ndi EM, Zofou D, Kwenti TE, Lepezeu EP, Titanji VP, Ndip R. Investigating the risk factors for seroprevalence and the correlation between CD4+ T-cell count and humoral antibody responses to *Toxoplasma gondii* infection amongst HIV patients in the Bamenda health district, Cameroon. PloS One. 2021;16:e0256947. DOI: 10.1371/journal.pone.0256947
- Fricker-Hidalgo H, Cimon B, Chemla C, Dardé ML, Delhaès L, L'Ollivier C, Godineau N, Houze S, Paris L, Quinio D, Robert-Gangneux F. *Toxoplasma* seroconversion with negative or transient immunoglobulin M in pregnant women: Myth or reality?. A French multicenter retrospective study. J Clin Microbiol. 2013;51:2103-2111. DOI: <u>10.1128/JCM.00169-13</u>
- 40. Teimouri A, Mohtasebi S, Kazemirad E, Keshavarz H. Role of

Toxoplasma gondii IgG avidity testing in discriminating between acute and chronic toxoplasmosis in pregnancy. J Clin Microbiol. 2020;58:e00505-20. DOI: <u>10.1128/JCM.00505-20</u>

- 41. de Santana Rocha D, de Sousa Moura RL, Maciel BM, Guimarães LA, O'dwyer HN, Munhoz AD, Albuquerque GR. Detection of *Toxoplasma* gondii DNA in naturally infected sheep's milk. Genet Mol Res. 2015;14:8658-8662. DOI: <u>10.4238/2015.July.31.14</u>
- Camossi LG, Greca-Júnior H, Corrêa AL, Richini-Pereira VB, Silva RC, Da Silva AV, Langoni H. Detection of *Toxoplasma gondii* DNA in the milk of naturally infected ewes. Vet Parasitol. 2011;177:256-261. DOI: <u>10.1016/j.vetpar.2010.12.007</u>
- Luptakova L, Benova K, Rencko A, Petrovova E. DNA detection of *Toxoplasma gondii* in sheep milk and blood samples in relation to phase of infection. Vet Parasitol. 2015;208:250-253. DOI: 10.1016/j.vetpar.2014.12.002
- 44. Amairia S, Rouatbi M, Rjeibi MR, Nouasri H, Sassi L, Mhadhbi M, Gharbi M. Molecular prevalence of *Toxoplasma gondii* DNA in goats' milk and seroprevalence in northwest Tunisia. Vet Med Sci. 2016;2:154-160. DOI: <u>10.1002/vms3.29</u>
- 45. Sadek OA, Abdel-Hameed ZM, Kuraa HM. Molecular detection of *Toxoplasma gondii* DNA in raw goat and sheep milk with discussion of its public health importance in Assiut governorate. Assiut Vet Med J. 2015;61:166-177. [available at]
- 46. Deljavan N, Moosavy MH, Hajipour N. Molecular detection of *Toxoplasma gondii* DNA in goats (*Capra hircus*), sheep (*Ovis aries*), and donkey (*Equus asinus*) milk using PCR in east Azerbaijan province. Iran J Vet Res. 2022;152:58-60. DOI: <u>10.1016/j.rvsc.2022.07.020</u>
- 47. Negero J, Yohannes M, Woldemichael K, Tegegne D. Seroprevalence and potential risk factors of *T. gondii* infection in pregnant women attending antenatal care at Bonga hospital, southwestern Ethiopia. Int J Infect Dis. 2017;57:44-49. DOI: 10.1016/j.ijjd.2017.01.013
- Elnahas A, Gerais AS, Elbashir MI, Eldien ES, Adam I. Toxoplasmosis in pregnant Sudanese women. Saudi Med J. 2003;24:868-870. [available at]
- Suazo-Cortez R, Martínez-Herrera DI, Pardío-Sedas VT, Cruz-Vázquez CR, Morales-Álvarez JF, Sánchez-Viveros G, Galindo-Tovar ME. Seroprevalence and risk factors associated with *Toxoplasma gondii infection* in sheep of Veracruz state, southeast Mexico. Vet Res Forum. 2020;11:77-81. DOI: <u>10.30466/vrf.2019.96751.2313</u>

الانتشار وعوامل الخطر المصاحبة لعدوى المقوسة الكوندية في الأغنام والنساء المجهضات في مصر

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الخلاصة

ارتبط داء المقوسات بمخاطر اقتصادية وصحية عامة حيث انه يسبب الإجهاض في الإنسان والحيوانات. وقد تم الكشف عن وجود الأجسام المضادة للمقوسة الكوندية في مصل ١٧٤ من الأغنام السليمة ظاهريا وفي مصل ٨٩ امرأة مجهضة وذلك باستخدام اختبار الممتز المناعي المرتبط بالانزيم وتم تحديد طفيلي المقوسة الكوندية في حليب الأغنام

بواسطة اختبار تفاعل البلمرة المتسلسل. كما تم تحليل عوامل الخطر المتعلقة بالعينات الموجبة من الأغنام والنساء. وقد أظهرت النتائج تسجيل معدل انتشار للطفيل بلغ 62.6% أعلى نسبياً في الأغنام مقارنة بـ ٥٧,٣% عند النساء وكانت استجابات الأجسام المضادة من نوعي IgM / IgG في النساء المجهضات ٢,٢٤%، ٢,٦٤%، ٧,٥١% و ٠% بالنسبة لـ IgM في or. IgG - ve / IgG + ve / 10,9 / 90 - 10 (العدوى المرمنة)، ve / IgG + ve (عدوى الأم) و - IgM + ve / IgG الع or (العدوى الحادة)، على التوالي. وأظهرت العينات الموجبة انخفاضًا في معدل التقارب ٢٤,٣٪ مما يدل على عدوى الأمهات الحادة، وقد أظهر الفحص الجزيئي في عينات حليب الأغنام أن ٢٨٨٪ كانت موجبة

بواسطة تفاعل البلمرة عند ٤٧٠ -نقطة أساس. بالإضافة انه تم تسجيل ارتباط معنوي واضح بين العديد من عوامل الخطر والانتشار المصلي لداء المقوسات في الإنسان والأغنام. وتشير الدراسة الحالية إلى ارتفاع الأجسام المضادة للمرض في كل من الأغنام والنساء المجهضات من محافظتي البحيرة والإسكندرية، مصر بالإضافة لوجود علاقة بين الإيجابية المصلية وبين العمر للمصابين وتناول لحم الضأن وحدوث الإجهاض والتعرض للقطط ومصدر مياه الشرب. كما تؤكد هذه النتائج إلى حد كبير الحاجة إلى مزيد من البحوث الوبائية المتعمقة لداء المقوسات.