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SEROPREVALENCE OF TOXOPLASMOSIS IN REPRODUCTIVE-AGE WOMEN AND ANIMALS WITH FIRST MOLECULAR CHARACTERIZATION OF TOXOPLASMOSIS GONDII ISOLATED FROM CATS IN ASWAN GOVERNORATE, EGYPT

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ABSTRACT

Toxoplasmosis is a widespread parasitic zoonosis caused by *Toxoplasma gondii*. Toxoplasmosis can cause congenital abnormalities during pregnancy, abortions, and encephalitis in both people and animals. The present study aimed to investigate the seroprevalence of toxoplasmosis in reproductive-age women and ruminants. Moreover, molecular identification was assessed for *T. gondii* isolated from cats, for the first time, in Aswan Governorate, Egypt. Blood samples were obtained, and serum was isolated from reproductive-age women (n=178) and ruminant animals (n=150) from the same locality. Seroprevalence of toxoplasmosis was determined using a rapid test (RT) and ELISA kits. Additionally, fecal samples from 100 cats were collected for the detection of *T. gondii* oocysts, and then processed by PCR for molecular and phylogenetic analysis. The phylogenetic tree was designed using the sequences collected and uploaded to the GenBank database. For human toxoplasmosis, IgM (ELISA) was more diagnostic twice than IgM (RT), and the percentages were 24.2% and 10.1%, respectively. However, IgG (ELISA) prevalence rate was 66.3 %, and the IgG (RT) was 62.9 %. Moreover, the seroprevalence rate of toxoplasmosis in ruminants was 23.3% and 25.3% for IgM (ELISA) and IgM (RT), respectively. The results of toxoplasmosis prevalence in humans and ruminants were statistically significant ($P \leq 0.001$). Toxoplasmosis was 30% of the examined cats. The present study has identified two distinct gene sequences for *T. gondii* that are associated with the gender of cats. Our findings detected that women of reproductive age and companion animals are more likely to be exposed to *T. gondii*.

Keywords: Toxoplasmosis, Seroprevalence, Human, Animals, Molecular, Phylogenetic, Zoonotic, Aswan

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INTRODUCTION

Toxoplasma gondii is an apicomplexan and obligatory intracellular parasite that causes toxoplasmosis in both humans and animals globally. Approximately 30% of people worldwide possess the toxoplasmosis infection (Aguirre *et al*., 2019; Omonijo *et al*., 2022; Qosimov *et al*., 2025). The Felidae family, which includes cats, is the final host for *T. gondii*, while humans and other warm-blooded animals serve as intermediate hosts. (Abdelbaset *et al*., 2022; González-Barrio *et al*., 2024). Oocyst, tachyzoite, and bradyzoite are the three main morphologies that *T. gondii* displays. Humans contract the parasite by eating undercooked meat that has bradyzoites or by ingesting water or food tainted with sporulated oocysts from cat excrement. Additionally, organ donations, blood transfusions, and congenital transmission to fetuses from infected mothers can also transmit infection (Ducrocq *et al*., 2021; Schwenk *et al*., 2021; Araujo Coelho *et al*., 2024). Recently, some studies indicated that *T. gondii* can spread in people through sexual contact (Tong *et al*., 2023).

Immunocompromised patients may develop life-threatening consequences such as myocarditis, encephalitis, or disseminated toxoplasmosis, but immunocompetent individuals often show no symptoms from human toxoplasmosis infections (Dalimi *et al*., 2012; Abdoli *et al*., 2016). Furthermore, congenital toxoplasmosis is a potentially lethal condition that can cause early delivery, stillbirth, and abortion (Tűzkő *et al*., 2024). Toxoplasmosis infections have been connected to several behavioral and neuropsychiatric conditions (Milne *et al*., 2020). Some patients may have ocular problems, seizures, and impaired coordination are possible indications of a weakened immune system (Flegr *et al*., 2013). There are no particular clinical indicators of human toxoplasmosis, so it is crucial to use *T. gondii*-specific diagnostic techniques to get an accurate diagnosis (Chorawala *et al*., 2024).

Toxoplasmosis is a major source of communal infection among animals worldwide. It causes reproductive problems, that lead to significant financial losses in the food animal sector. Given the high incidence of toxoplasmosis in ruminant animals, eating undercooked meat or milk contaminated with *T. gondii* can be a major way for human toxoplasmosis infection (Shariatzadeh *et al*., 2021; Thebault *et al*., 2021; Omonijo *et al*., 2022). Epidemiological investigations of toxoplasmosis are spreading beyond humans to cover other animal hosts in the pursuit of preventative measures. To date, several surveys have been carried out to ascertain toxoplasmosis prevalence rates in humans and animals in different countries, using both serological and molecular techniques (Alzaheb *et al*., 2018; Bigna *et al*., 2020; Khan & Noordin, 2020; Sameeh *et al*., 2021). Serological screening for antibodies to *T. gondii* is recommended for reproductive-age women to identify those at risk of contracting toxoplasmosis (Fanigliulo *et al*., 2020). Therefore, the current study was conducted to assess the seroprevalence of toxoplasmosis in reproductive-age women and the accompanying animals in Aswan Governorate, Egypt. Additionally, determining the molecular characterization of *T. gondii*, which was isolated from domestic cats for the first time in this study area.

MATERIALS AND METHODS

Ethical considerations

The study was performed in compliance with the instructions of Aswan University Ethical Committee (No. Asw.U./446 /3/20). All patients were counselled, and informed consent was collected from every patient. Ethical approval for the study on animals was conducted in compliance with all relevant Egyptian laws pertaining to research and publication.

Sampling and processing

Blood samples were obtained from 178 reproductive-age women attending the gynecology and obstetrics clinic of Aswan University, Aswan, Egypt, during the period from December 2022 to January 2024. The patients were between 18 to 50 years old with various gynecological and obstetric complaints. Additionally, blood samples were obtained from 150 ruminant animals (cattle, sheep, and camels). All samples were centrifuged at 3000xg for 10 minutes for serum separation. The separated sera were stored at -20 °C for serological examination. Moreover, one hundred fecal samples from cats were collected separately for fecal examination and molecular analysis. All animals appeared healthy and were in contact with humans in the same study area.

Seroprevalence assays

Antibodies of IgM and IgG against *T. gondii* were detected in both human and animal sera using rapid test (RT) kits. IgM and IgG ELISA kits (GMBH, Wiesbaden, Germany) were utilized following the guidelines provided by the manufacturer. The ELISA plate reader (Humareader, Germany) was used to measure the optical densities at two different wavelengths (450 nm and 630 nm). The ELISA plate reader's built-in software was used in point-to-point mode to ascertain the serum's level of anti-Toxoplasma IgM and IgG, based on a standard curve (Hassanain *et al*., 2013).

Fecal examination

The fecal samples of cats were examined using the direct smear method to detect *T. gondii* oocysts. Moreover, oocysts were identified using flotation/sedimentation procedures. After being collected and placed in 2.5% potassium dichromate (1:5), the unsporulated oocysts were inspected under a microscope for the presence of sporulated oocysts after five days.

DNA extraction and molecular analysis

As directed by the manufacturer, DNA was extracted from cat fecal samples using the QIAamp Fast Genomic DNA micro-Kit (Qiagen, Germany). The 529-RE mRNA gene primers were amplified using PCR (Table 1). DreamTaq Green PCR Master Mix (2X) (K1081, Thermo Fisher, USA) was used to amplify specified genes according to the manufacturer's specifications using a Creacon (Holland, Inc) Polymerase Chain Reaction (PCR) system cycler. The data were analyzed using a gel documentation system (Geldoc-it, UVP, England) and Totallab analysis software (www.totallab.com, Ver.1.0.1). Positive amplicons (513 bp) were recovered from the agarose gel. The resultant PCR products were purified using microspin filters and quantified spectrophotometrically. The phylogenetic tree of *T. gondii* was constructed and compared to previously registered sequences in GenBank.

Statistical Analysis

Statistical analysis was done by using SPSS version 25. Data was presented, and a suitable analysis was done using the chi-square test. The *p*-values ($P \leq 0.05$) were statistically significant.

RESULTS

Seroprevalence rate of toxoplasmosis in women & ruminants

The results of anti-*Toxoplasma* specific antibodies (IgM & IgG) using rapid test (RT) and ELISA assay are presented in Table (2). For human toxoplasmosis, IgM (ELISA) was more diagnostic twice than IgM (RT), and the percentages were 24.2% and 10.1%, respectively. On the other hand, regarding the old infection, the two techniques were nearly equal, with IgG (ELISA) prevalence rate being 66.3 % and the IgG (RT) being 62.9 %. Moreover, the seroprevalence rate of toxoplasmosis in ruminant animals was 23.3% and 25.3% for IgM (ELISA) and IgM (RT), respectively. The results of toxoplasmosis prevalence in humans and ruminant animals were statistically significant (*P*≤ 0.001).

Toxoplasmosis in cats and molecular characterization

Out of 100 cat fecal samples, 30% had a prevalence rate with *T. gondii* by microscopic examination of the oocysts. *T. gondii* oocyst appears approximately spherical, measuring 10 to 12 μm in diameter by micrometry and has a smooth, thin shell, each containing two sporocysts, each with four sporozoites (Figure 1). The present study has identified two distinct gene sequences for *T. gondii* that are associated with gender. Specifically, the gene sequence related to male cat fecal matter is catalogued under accession number PQ558151, while the sequence for female cats is registered under accession number PQ558152. Utilizing the MAGA program, a total of 24 gene sequences of *T. gondii* were analyzed across various hosts and countries, including one out-group gene, *Entamoeba nuttalli*, which is listed under accession number AB282671. The resulting phylogenetic tree revealed three clusters based on the Euclidean distance among the 24 gene sequences. Notably, the two gene sequences from male and female cats were positioned closely together within the first cluster, which also included six other genes exhibiting a high similarity percentage exceeding 99%. The base pair (bp) length of these related genes was consistently 546, except for one gene, registered under accession number M33572.1, found in Egypt, which had a bp length of 1404. Furthermore, the two gene sequences found in this investigation shared a tight relationship with the *T. gondii* gene found in Northwestern Italy (Table 3 & Figure 2).

Table 1: The primer sequences for PCR.

Primer name	Sequence (5'-3')	Gene	bp	Reference
Tox-4	CGCTGCAGGGAGGAAGACGAAA GTTG	529-RE mRNA	**532**	**(Homan et al.,2000)**
Tox-5	CGCTGCAGACACAGTGCATCTG GATT			

Table 2. The seroprevalence rate of toxoplasmosis in humans and ruminant animals**.**

Species	Test	Total examined	Infected (+)	Not infected (-)	Prevalence (%)	*P* value
Human	IgM (RT)	178	18	160	10.1	*P* value 0.0000 X2= 173.67
	IgM (ELISA)		43	135	24.2	
	IgG (RT)		112	66	62.9	
	IgG (ELISA)		118	60	66.3	
Ruminant animals	IgM (RT)	150	38	112	25.3	*P* value 0.0000 X2= 61.13
	IgM (ELISA)		35	115	23.3	
	IgG (RT)		91	59	60.6	
	IgG (ELISA)		71	79	47.3	

Table 3. The gene sequences of *T. gondii* according to different hosts and countries.

#	Detected pathogens	Accession number	Country	bp	Host	Identity (%)	Query Cover
1	*T. gondii*	PQ558151	Upper Egypt	513	Males Cat	Current gene sequence	
2		PQ558152	Upper Egypt	513	Female Cat	Current gene sequence	
3		KT881317	Australia	507	Domestic cats	99.21%	98%
4		MG587997.1	Northwestern Italy	546	Cattle	99.60%	98%
5		MG587998.1	Northwestern Italy	546	Cat	99.60%	98%
6		MG588009.1	Northwestern Italy	546	Swine	99.60%	98%
7		M33572.1	Egypt	1404	Cat	99.60%	98%
8		MG588006.1	Northwestern Italy	546	Sheep	99.60%	98%
9		MG588008.1	Northwestern Italy	546	Wild boar	99.60%	98%
10		MG587992.1	Northwestern Italy	546	Fox	99.60%	98%
11		MG588002.1	Northwestern Italy	546	Goat	99.60%	98%
12		MG588005.1	Northwestern Italy	546	Deer	99.60%	98%
13		MG587996.1	Northwestern Italy	546	Bovine	99.60%	98%
14		MG588011.1	Northwestern Italy	546	Bovine	99.41%	98%
15		MG587988.1	Northwestern Italy	546	Wild boar	99.41%	98%
16		MG587995.1	Northwestern Italy	546	Bovine	99.41%	98%
17		AF249698.1	Iraq	1307	Cat	99.41%	98%
18		MG588010.1	Northwestern Italy	546	Wild boar	99.41%	98%
19		LN714498.1	North Africa	6937759	Dog	99.41%	98%
20		MG588000.1	Northwestern Italy	546	Swine	99.41%	98%
21		MG587999.1	Northwestern Italy	546	Wild boar	99.41%	98%
22		MG588004.1	Northwestern Italy	546	Fox	99.41%	98%
23		MG588003.1	Northwestern Italy	546	Fox	99.41%	98%
24		MG588001.1	Northwestern Italy	546	Bovine	99.21%	98%

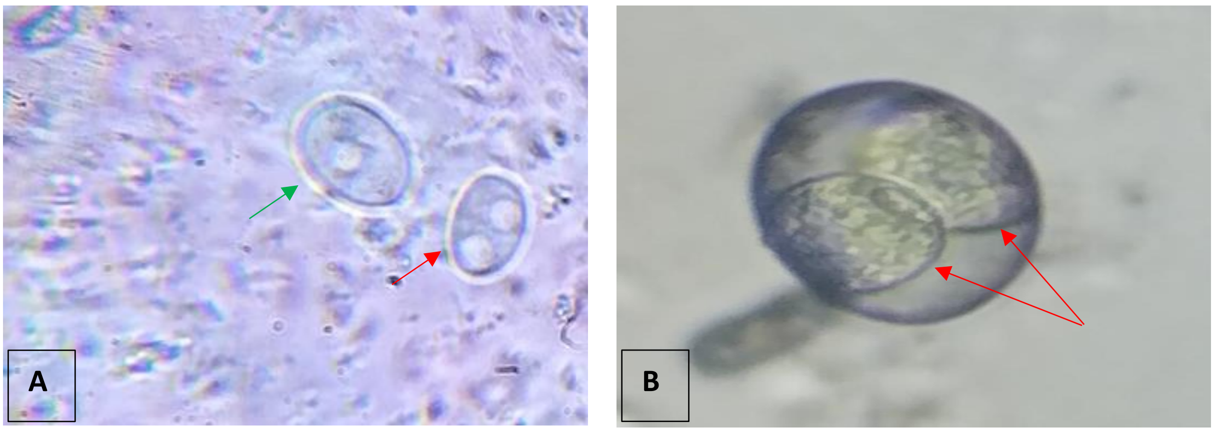

Fig. 1. *Toxoplasma gondii* oocyst isolated from feces of cats showing: A). Unsporulated oocyst (green arrow) and sporulated oocyst (red arrow), B). Sporulated oocyst has a smooth, thin shell, and each contains two sporocysts (red arrows).

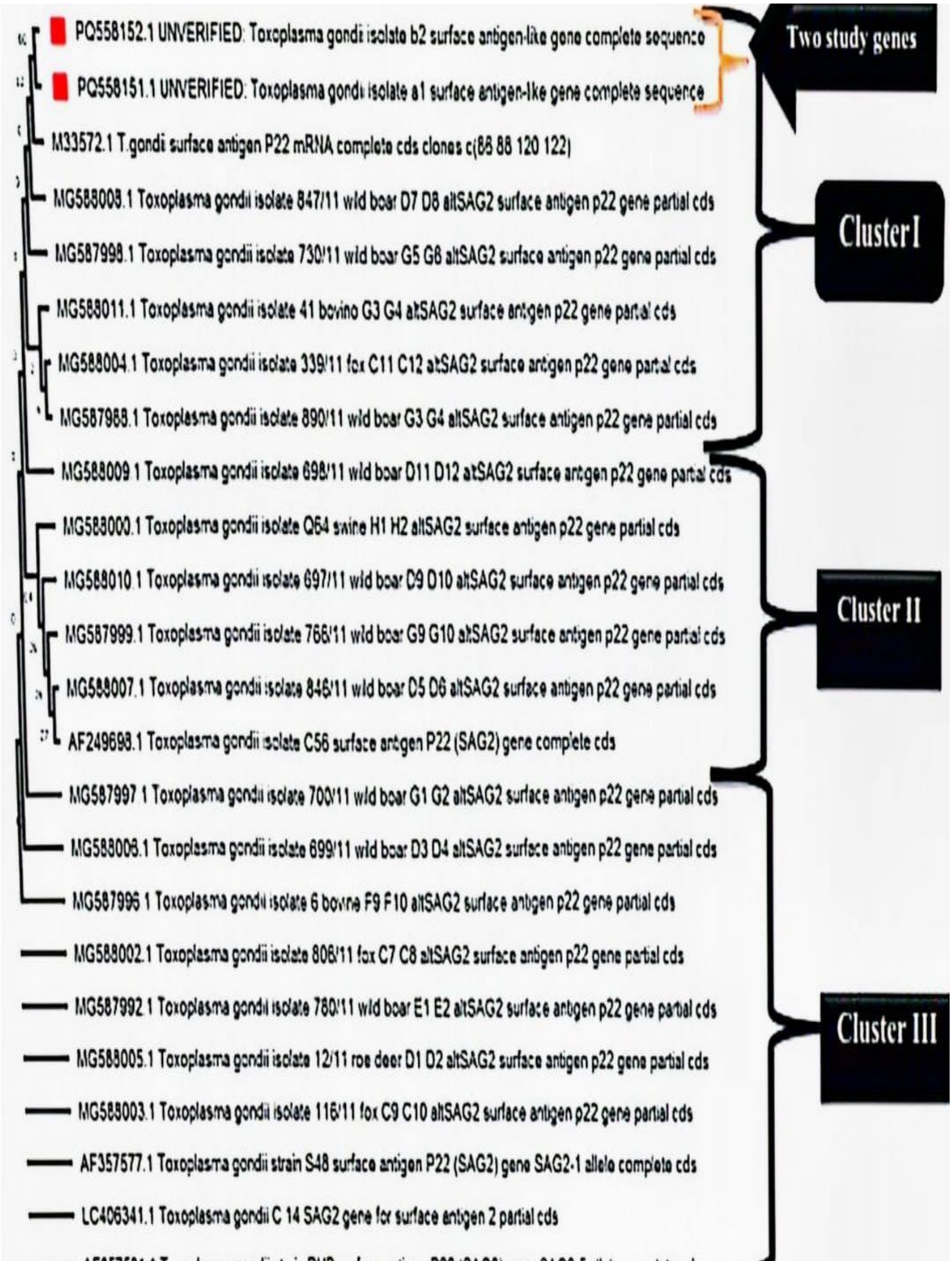

Fig. 2. Phylogenetic tree of *T. gondii* isolates from cats.

DISCUSSION

The seroprevalence of *T. gondii* in ruminant animals and reproductive-age- in the current investigation provided evidence of toxoplasmosis in these hosts. Human toxoplasmosis serological IgM results were 24.2% (ELISA) and 10.1% (RT), while IgG (ELISA) prevalence rate was 66.3 % and IgG (RT) was 62.9 %. Toxoplasmosis in ruminants was (23.3%) and (25.3%) of IgM (ELISA) and IgM (RT), respectively. The results of toxoplasmosis infection were statistically significant in both human & ruminant animals. These findings align with those reported by Ibrahim *et al.* (2017), indicating ELISA seroprevalence of toxoplasmosis in pregnant women (33.79 %) and sheep (17.65 %) in Gharbiya and Menoufia, Egypt. *T. gondii* high frequency in sheep was matched by considerable levels of human positivity. (Shaapan *et al*., 2008) used a range of serological tests to detect elevated levels of antibodies of *T. gondii* in sheep, Cairo, Egypt, and prevalence rates ranging from 37.0% to 43.7%. Additionally, the presence of antibodies of *T. gondii* was identified in 52.4% and 44.1% of the workers and the animals, respectively, at Tanta abattoir, utilizing the indirect hemagglutination test. The crude *T. gondii* tachyzoite antigen is the starting material for making fractions that are utilized in various serologic assays to diagnose toxoplasmosis, which include IgM and & IgG antibodies. The actual antigenic molecules of *T. gondii* are intricate and varied. It is thought that interactions between the ligands of *T. gondii* and receptors on the target cells are responsible for the remarkably broad variety of hosts that are vulnerable to the parasite (Mohammadpour *et al*., 2016).

The higher human and animal toxoplasmosis infections in Aswan Governorate, in the present results, may be due to its location in upper Egypt with a humid and warm environment, which is conducive to *T. gondii* oocysts survival. Various seroprevalence rates were noted at various locations, which may be due to changes in the environment, hygienic conditions, and management techniques (Tavalla *et al*., 2017; Zhu *et al*., 2023). It is necessary to update recent ecological and etiological research on risk variables and new sources of human toxoplasmosis to address the mystery of its high prevalence in both humans and animals used for meat and milk production (Tonouhewa *et al*., 2017; Nayeri *et al*., 2022). The prevalence rate of toxoplasmosis in final & intermediate hosts varies worldwide depending on the diagnostic techniques, the socioeconomic structure of housing cats, and the eating habits of humans and animals. Whereas environmental variables in many communities show the importance of food and cultural habits in contributing to the socio-epidemiological features assumed to be crucial in the spread of this zoonosis (Al-Malki *et al*., 2021; Karakavuk *et al*., 2021; Adel *et al*., 2024; Amouei *et al*., 2025).

Our findings revealed a 30% prevalence rate of toxoplasmosis in cats accompanying women owners. This was consistent with previous studies that identified an interaction with cats, which are a key source of human toxoplasmosis infection (Ding *et al*., 2017; Montazeri *et al*., 2020). Cats are the final hosts of *T. gondii* and the main source of human & animal toxoplasmosis by releasing *T. gondii* oocysts into the environment. Therefore, consuming contaminated unwashed fruits and vegetables or drinking polluted water might spread the infection (Eslahi *et al*., 2024). Hence, consuming undercooked contaminated meat of animals is a significant source of human toxoplasmosis (Belluco *et al*., 2016).

Recent investigations have revealed gender-based distinctions in *T. gondii*, with two cat fecal matter gene sequences catalogued under accession numbers PQ558151 and PQ558152. The phylogenetic analysis, based on Euclidean distances among 24 distinct genes, delineates three clusters. Notably, the genes are situated in close proximity within the first cluster, suggesting a potential overlap or prior identification in other studies, leading to their recent inclusion in Genebank. Furthermore, research conducted by (Zamora-Vélez *et al*., 2020), which focused on genotyping of *T. gondii* DNA in cat feces in Colombia, indicated that the phylogenetic analysis resulted in the isolates forming a singular cluster. The analysis incorporating Brazilian and Chinese strains revealed robust support (PP < 0.99) for three principal clades, despite overall backing for the majority of the nodes. In the present study, a cluster of six genes exhibiting a high similarity percentage exceeding 99% was identified, along with the nearest base pair (bp) genes, all of which shared a base pair count of 546, with the exception of one gene under accession number M33572.1, which was located in Egypt and had a base pair count of 1404. Additionally, the two genes closely linked to *T. gondii* were discovered in Northwestern Italy. This aligns with the findings of (Prince *et al*., 1990) and (Holec-Gąsior *et al*., 2014), which indicated that the gene sequences of two new monoclonal antibodies produced against a membrane-enriched fraction of *T. gondii* tachyzoites were grouped with the present two genes. These antibodies successfully detected protein P22 on the surface of *T. gondii* through immunoblotting, complement-mediated cytolytic assays, and immunofluorescence.

CONCLUSION

T. gondii seroprevalence was detected for reproductive-age women & animals living in the same area. This study identified *T. gondii* genotypes, that are currently circulating in cats in Aswan governorate, Egypt. Additional research is required to evaluate *T. gondii* genotypes in various intermediate hosts in Egypt.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Data Availability Statement

The datasets generated during and/or analyzed during the current study can be found in the main text.

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التقييم المصلي لانتشار داء المقوسات لدى السيدات في سن الإنجاب والحيوانات مع التوصيف الجزيئي الأول لطفيل المقوسات المعزول من القطط في محافظة أسوان، مصر

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داء المقوسات مرض طفيلي حيواني المنشأ ومنتشر على نطاق واسع ويسببه طفيل التوكسوبلازما غوندي. يمكن أن يسبب داء المقوسات تشوهات خلقية أثناء الحمل والإجهاض والتهاب الدماغ لدى كل من البشر والحيوانات. هدفت الدراسة الحالية إلى دراسة معدل انتشار داء المقوسات المصلي لدى النساء في سن الإنجاب والحيوانات المجترة، مع التوصيف الجزيئي لطفيل التوكسوبلازما غوندي المعزول من القطط في محافظة أسوان، مصر. جُمعت عينات دم وفُصلت مصليات من السيدات في سن الإنجاب بعدد (١٧٨) والحيوانات المجترة بعدد (١٥٠) من نفس المنطقة. حُدد معدل انتشار داء المقوسات المصلي باستخدام الاختبار السريع (RT) ومجموعات اختبارات المقايسة الامتصاصية المناعية للانزيم المرتبط (ELISA) . جُمعت عينات براز عددها (١٠٠) من القطط للكشف عن أكياس المقوسة الغوندية، ثم تم تحليلها بواسطة تفاعل البوليميراز المتسلسل للتحليل الجزيئي والتطوري. أُرسلت التسلسلات التي تم الحصول عليها إلى قاعدة بيانات الجينات وصُممت شجرة التطور. وكانت النتائج بالنسبة لمعدلات الاصابة بداء المقوسات في السيدات IgM (ELISA) أكثر تشخيصًا بحوالي مرتين من IgM (RT) وكانت النسبة ٢٤,٢٪ و ١٠,١٪ على التوالي. ومع ذلك، كان معدل انتشار IgG (ELISA) 66.3٪ و 62.9 IgG (RT)٪. علاوة على ذلك، كان معدل الانتشار المصلي لداء المقوسات في المجترات (٢٣,٣٪) و (٢٥,٣٪) من IgM (ELISA) و IgM (RT), على التوالي. كانت نتائج انتشار داء المقوسات في البشر والمجترات ذات دلالة إحصائية ($P \leq 0.001$). كان داء المقوسات مُصيبًا بنسبة ٣٠٪ من القطط التي خضعت للفحص. وقد حددت هذه الدراسة لأول مرة تسلسلين جينيين مُميزين للمقوسة الغوندية يرتبطان بجنس القطط. وتُشير نتائجنا إلى أن التعرض للمقوسة الغوندية مرتفع لدى السيدات في سن الإنجاب لما له من اثار علي الأم والجنين. ينصح باجراء اختبارات الاليزا للسيدات الحوامل لتشخيص المرض مبكراً.