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Development and Evaluation of Glycoprotein-Based ELISA for Diagnosing *Toxoplasma gondii* Infection in Sheep

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ABSTRACT

Practical, sensitive, and specific techniques are required for the detection and management of Toxoplasma disease. One of the most commonly used techniques for this purpose is the Enzyme-Linked Immunosorbent Assay (ELISA). The current study was carried out to create a protocol for the isolation and characterization of glycoprotein antigen (TGA) from *Toxoplasma gondii* (*T. gondii*). The diagnostic performance of an isolated glycoprotein antigen was evaluated in comparison with the crude antigen using an indirect ELISA. The TGA-based ELISA consistently yielded higher optical density (OD) values than the crude antigen using twofold serially diluted sheep serum, with a cut-off value of 0.35. Furthermore, the TGA-ELISA demonstrated a remarkable sensitivity of 100.00% and a specificity of 96.67%, along with a positive predictive value (PPV) of 92.3% and a negative predictive value (NPV) of 100.0%. The Receiver Operating Characteristic (ROC) analysis highlighted the TGA-ELISA's outstanding diagnostic accuracy, with an Area Under the Curve (AUC) of 0.963, compared with 0.867 for the crude antigen-based ELISA. Additionally, the TGA-ELISA showed no significant cross-reactivity with other parasitic diseases, including cryptosporidiosis, Blastocystis, giardiasis, and sarcocystosis, confirming its specificity for *T. gondii* detection. This study underscores the potential of TGA-based ELISA as a more reliable and accurate diagnostic tool for *T. gondii* infection in sheep, offering enhanced sensitivity and reduced cross-reactivity. When employed in a seroprevalence study, the TGA-ELISA detected anti-*Toxoplasma* IgG antibodies in 83.6% of the tested sheep sera**.** This in-house TGA-based ELISA presents a cost-effective alternative to expensive commercial products, enabling broader application in serodiagnosis in animals and humans.

Keywords: Enzyme linked immunosorbent assay, Glycoprotein, *Toxoplasma gondii,* IgG, ROC curve.

INTRODUCTION

Toxoplasmosis, caused by the apicomplexan intracellular protozoan parasite *T. gondii*, is a globally prevalent zoonotic infection affecting a wide range of warm-blooded vertebrates, including humans and animals (Nayeri et al. 2021; El-Kady et al. 2024; El Shanawany et al. 2025a). It threatens approximately one-third of the world's population, with prevalence rates varying significantly across different regions (Robert-Gangneux and Dardé 2012; Robert-Gangneux et al. 2022). Toxoplasmosis is typically asymptomatic in immunocompetent individuals but can lead to severe complications in immunocompromised patients, such as those with HIV/AIDS, and congenitally infected infants, resulting in conditions like encephalitis, retinochoroiditis, and even death (De-La-Torre and Gómez-Marín 2020; Tawfeek et al. 2023). *T. gondii* is one of the recognized foodborne zoonotic parasites that can be fatal to humans, according to the Centers for Disease Control and Prevention (CDC) (Hasan and Nishikawa 2022). *T. gondii* has a host-specific sexual cycle in the definitive host and a two-stage asexual life cycle in the intermediate host. Cats are the definitive hosts of *T. gondii*, whose infectious stage can infect almost any warm-blooded animal including humans (Dessì et al. 2022). Although sporulated oocysts from water or plants can infect humans as well as animals, tissue cysts from raw or undercooked animal meat are

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significant way for human illnesses to spread (Paștiu et al. 2023). Sheep are a crucial component of Egypt's agricultural sector, with an estimated population of over 5 million sheep (FAO, 2015). These animals are vital for the country's economy, providing essential products such as meat, wool, and milk (Egyptian Ministry of Agriculture and Land Reclamation. Agricultural Statistics Bulletin 2022). Most reports used sera from sheep at abattoirs, while few studies were conducted on sheep in farms (Abbas et al. 2020). Many epidemiological investigations have demonstrated that sheep in Egypt face significant challenges with *T. gondii* infections (Fereig et al. 2022; Barghash et al. 2025).

The diagnostic approach to toxoplasmosis has seen significant advancements, integrating both traditional methods and emerging molecular techniques. Traditional diagnostics often involve immunological tests and imaging tools. The use of imaging tools can be time-consuming and require a high level of expertise to yield reliable results. Etiological diagnosis involves isolating various disease materials, which is impractical for large-scale testing in intensive farming environments. Imaging techniques, such as computed tomography, magnetic resonance imaging, nuclear imaging, and ultrasonography, are typically used to diagnose cerebral and ocular toxoplasmosis. However, these imaging results may lack reliability and necessitate expert interpretation (Rostami et al. 2018). Molecular methods, known for their accuracy and sensitivity, are increasingly used in both epidemiological studies and clinical diagnosis (Switaj et al. 2005). These techniques include PCR, and real-time PCR. Despite their effectiveness, molecular methods are often costly.

On the other hand, immunological methods assess the host's immune response by analyzing specific antibody changes (IgA, IgM, IgG, and IgE) following *T. gondii* infection (Ghazy et al. 2007; Robert-Gangneux and Dardé 2012; Zhang et al. 2016). Common immunological tests include enzyme-linked immunosorbent assays (ELISA) and the modified agglutination test (MAT) (Garcia et al. 2006; Shaapan et al. 2008; Weiss and Dubey 2009). ELISA is an easily performed serological method that can be used on a large scale (Minic and Zivkovic 2020). After an acute *Toxoplasma* infection IgM antibodies can remain detectable in the body for years and determining accurate cut-off values for the IgG avidity test is challenging, making both methods less effective for accurate early diagnosis. (Kotresha and Noordin 2010). The ELISA sensitivity and specificity are based mainly on immobilized antigens used; these antigens include antigens of crude tachyzoites, chimeric peptide and recombinant *Escherichia coli* however they showed low specificity and sensitivity results in diagnosis of toxoplasmosis (Ferra et al. 2015; Pishkari et al. 2017). Moreover, commercial tests for antibody detection primarily use preparations of crude parasite antigens, which can lead to false-positive and false-negative results in serodiagnostic assays (Araujo and Ferreira 2010).

Glycans and glycan-binding proteins are known to play a role of paramount importance in host-pathogen interactions (El Shanawany 2021). Zhang et al. (2001) proved the reactivity of lectins *Dolichos biflorus* and succinyl Wheat Germ Agglutinin with *T. gondii* tissue cyst wall, also, *T. gondii* react with *Concanavalin ensiformis* A (Cona) (Luo et al. 2011). Genomic analysis of *T. gondii* (www.toxodB.org) identify enzymes responsible for N and O-linked modifications synthesis (Stwora-Wojczyk et al. 2004a; Samuelson et al. 2005). Different studies has shown that cell-free extracts of *T. gondii* exhibit both N-glycosylation and O-glycosylation activity when synthetic peptide substrates are used (Stwora-Wojczyk et al. 2004a & b). Additionally, mass spectrometry studies have identified the presence of N-glycans, including Man6 (GlcNAc)2, Man7(GlcNAc)2, and Man8(GlcNAc)2, in *T. gondii* (Fauquenoy et al. 2008). Glycans have roles in promoting parasitic infections and helping the parasite evade host immune responses. The understanding of that role could contribute to the development of new therapeutic agents, the identification of potential vaccine candidates, and the creation of novel diagnostic tools with improved sensitivity and specificity (Guha-Niyogi et al. 2001; Rodrigues et al. 2015; Goddard-Borger and Boddey 2018; Veríssimo et al. 2019).

Previous studies and trials highlight that glycoprotein antigens have been shown to improve the sensitivity and specificity of diagnostic assays for the detection of different parasitic diseases such as fasciolosis (Abdel-Rahman et al. 2016), sarcocystosis (El Shanawany et al. 2025b), echinococcosis (El Shanawany et al. 2019a), toxocariasis (El Shanawany et al. 2019b), and chagas disease (Marcipar et al. 2003). However, little work has been done to use glycoprotein antigens as diagnostic markers for toxoplasmosis.

This lack of emphasis on effective, sensitive, and specific diagnosis and monitoring can lead to undetected infections, which in turn can result in severe health issues for the sheep, including abortions and stillbirths, as well as potential zoonotic risks to humans. Addressing these challenges is crucial for improving flock health and reducing the risk of toxoplasmosis transmission. Therefore, study aimed to isolate glycoprotein antigens from a local strain of *T. gondii* and evaluate their efficacy, sensitivity, and specificity in the serological diagnosis of toxoplasmosis in sheep.

MATERIALS AND METHODS

Experimental animals

Two-month-old *T. gondii*-free cats were used. Swiss albino male mice weighing 20-25g were purchased from the animal house, National Research Centre. Six male ewes for experimental infection were purchased from a farm of the Agriculture Research Centre, Cairo University, Egypt.

Ethical approval

All animals were conditioned for fifteen days before the experiment began. All animal experimentation was performed in accordance with ethical animal guidelines and regulations set by the Alexandria University Ethics Committee, Egypt. This is in accordance with the internationally accepted principles for laboratory animal use and care. (Approval number 0306846).

T. gondii local strain isolation

Liver and heart tissues were collected from slaughtered sheep in Giza Governorate, Egypt. Tissue samples were cut into small cubes and subjected to pepsin-

HCl digestion at 4°C for several days until complete tissue breakdown, as confirmed by light microscopy (El-Nawawi et al. 2008). Positive tissue samples were containing *T.gondii* infective stage (Bradyzoites) subsequently inoculated intra-peritoneal into the Swiss albino mice (Elfadaly et al. 2023). The molecular detection and genotyping of the *T. gondii* isolate we have previously published, confirms the virulent nature of the isolated strain (Elfadaly et al. 2017). After two months, mouse brains were tested microscopically for the presence of tissue cysts by brain print examination. Cyst-positive brain tissue was homogenized in 0.85% NaCl solution. An aliquot of each homogenate was bioassayed in mice according to Ghazy et al. (2007) to ensure the presence of at least 10,000 infective *T. gondii* bradyzoites. Four cats orally infected with tissue cysts and fecal samples from each cat were collected daily for 21 days post-infection. Fecal samples from cats subjected to sucrose flotation and sedimentation, for isolation of *T. gondii* oocysts and the total oocyst count was determined using a hemocytometer using the method described by Dubey and Lindsay (2004).

Experimental infection of sheep with *T. gondii* local strain

Six experimental ewes were orally inoculated with 5 x 10^3 sporulated oocytes of a locally isolated *T. gondii* strain according to methods described by Shaapan et al. (2008). After four months of infection, the sheep were euthanized, Blood samples were collected from these infected sheep, and sera were prepared and their meat was examined for *T. gondii* tissue cysts. The prepared sera, considered gold standard positive controls, were stored at -20°C until use. There were another six positive control sera supplied by Prof. Raafat M. Shaapan, Zoonotic Department, Veterinary Research Institute, National Research Centre

Samples collection

Negative control samples

Thirty young sheep bred on a special farm were examined for free toxoplasmosis infection using a modified agglutination test according to Dubey (1997). Also, examined for free from other parasitic infections by fecal microscopic examination using techniques of flotation, sedimentation, and modified Zeil-Neelsen (Henriksen and Pohlenz 1981; Brandelli et al. 2012). Sera from these parasite-free sheep were collected, aliquoted, and stored at -20°C as negative controls.

Cross-Reactivity controls samples

Preparation of antisera

Twenty buffalo sera naturally infected with *Sarcocystis* were obtained from locally slaughtered animals. These buffaloes were also screened for other parasites and *T. gondii*. Ten Swiss albino male mice aged 3–4 weeks old was used for induction of cryptosporidiosis. Oocysts 3×10^3 were used to infect mice individually using esophageal tube. Oocysts obtained from Theodor Bilharz Research Institute (TBRI). To ensure successful experimental infection, the fecal pellets were individually collected and examined after 8 day post infection (El Shanawany et al. 2024a). Nine Swiss albino male mice aged 5–6-week-old was used for induction of blastocystis disease with 2×10^6 live *Blastocystis* that obtained from TBRI. Serum samples were collected following infection (Abdel-Hafeez et al. 2015).

Giardiasis infection was induced in 11 Swiss albino mice which were orally infected with 10^3 *Giardia duodenalis* cyst which obtained from TBRI. Following the infection confirmation serum samples were collected (Dreesen et al. 2014).

Study population samples

A total of 250 serum samples from slaughtered sheep were collected from the main Giza abattoir, labeled, and stored at -20°C for further analysis.

Antigen preparation

T. gondii tachyzoites crude antigen was prepared according to the method described by Hughes et al. (1982) and El Shanawany et al. 2024b. Briefly, peritoneal fluid was collected from infected mice at 6 $^{th}_{dpi}$ (Eid et al. 2023). The collected peritoneal fluid was then centrifuged and sediment was then resuspended in 0.9% sodium chloride then incubated in a shaking water bath to destroy red blood cells. The disposable syringe (25 ml, needle size 27) was used to filter the suspension. The tachyzoites were then washed by centrifugation three times in phosphate-buffered saline (PBS, PH 7.2), the tachyzoites were sonicated several times in an ice bath for 20s each time at 100mAmp and the soluble antigen was collected after centrifugation at 12,000rpm for 30minutes in a cooling centrifuge. The protein content was determined according to Lowry et al. (1951). The antigen was aliquoted and stored at −20°C until it was used.

Lectin affinity chromatography

The glycoprotein of *T. gondii* local isolate strain was isolated according to Abdel-Rahman et al. (2016). Briefly, columns of Agarose bound to *Concanavalin ensiformis* A (Cona) (Sigma Chem Co. St. Louis) were equilibrated with buffer (10mM Tris HCl, pH 7.5, 150mM NaCl, 1mM $CaCl_2$). *T. gondii* crude extract was initially loaded onto the Con A column, incubated overnight at 4°C and then the Con A column was washed extensively with PBS PH 7.2 until no proteins could be detected in washes. Bound glycoproteins were eluted with eluting buffer (50mM D-(+) glucose, 50mM Tris HCl, and 300mM NaCl) (Sigma Chem Co. St. Louis). The eluted *Toxoplasma* glycoprotein antigen (TGA) was then measured for protein content by Lowry et al. (1951).

Evaluation of TGA-based ELISA

The sensitivity and specificity of TGA-ELISA were compared to those of crude *T. gondii*-based ELISA using sheep sera positive and negative for anti-*T. gondii* IgG antibodies. The diagnostic performance of two antigens was evaluated using two folded serially diluted sheep-positive sera. The cross-reactivity of TGA based ELISA was evaluated by diagnosing four closely related protozoal parasitic diseases (sarcosystosis, cryptosporidiosis, blastocystis, and giardiasis) along with a control group comprising sheep sera positive and negative for anti-*T. gondii* antibodies. Also, 250 sheep serum samples were tested by TAG-ELISA to assess the diagnostic potential of an isolated glycoprotein fraction.

Checkerboard titration was used to assess the ideal antigen concentration, serum, and conjugate dilution. The indirect ELISA method was performed according to El Shanawany et al. (2025c); Connick et al. (2023). Briefly, The ELISA micro titer plate's 96-well flat-bottom were coated with 100µl microliter of both purified TGA and crude *T.gondii* antigen in coating buffer (pH 9.6) and incubated overnight at 4°C. The plates were washed three times using washing buffer PBS with 0.05% Tween 20. Bovine serum albumin in carbonate/bicarbonate buffer was added to block the unbound sites and incubated for 1 hour at room temperature. The plate was washed three times before adding serum samples diluted at 1:100. The plate was incubated at 37°C for 90 minutes. Following another wash, 100µl of anti-host IgG horseradish peroxidase conjugate (1:1000 dilution) (Sigma Chem. Co., St. Louis) was added to the designated wells and incubated for one hour at 37°C. Subsequently, the substrate ortho-phenylenediamine (OPD) and H_2O_2 (Sigma-Aldrich, USA) were added to each well. ELISA reader (Bio Tek, Germany) was used to measure the absorbance at 450nm. Our lab Parasitology and Immunology Lab at the Parasitology and Animal Diseases Department was guaranteed ISO 17025 accreditation with calibration for all instruments and validation of the ELISA method for the diagnosis of different parasitic diseases. All the tests were performed in triplicate wells. The cutoff value was determined using the mean value of *T. gondii* negative sheep sera (cutoff = mean + 3SD) (Almazán et al. 2001; Hegazi et al. 2023).

Statistical Analysis

Results were presented as mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$. To assess the diagnostic accuracy of both crude and isolated *T. gondii* glycoprotein antigens, ROC curves were generated. Area under the curve (AUC), sensitivity, and specificity were calculated (Schisterman et al. 2008). Data analysis and graph generation were performed using MedCalc® Statistical Software version 20.215 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023).

RESULTS

Diagnostic performance of TGA-ELISA

This study compared the diagnostic performance of crude and purified *T. gondii* glycoprotein antigen (TGA) using indirect ELISA. IgG antibody detection was assessed across a range of serum dilutions. Compared to using crude antigen, the TGA-based ELISA maintained higher OD values at most serum dilutions. Additionally, a cut-off value of 0.324 was created by the analysis, below which OD results are not considered significant (Fig. 1). Interestingly, the TGA-ELISA showed the greatest sensitivity and detected anti-*T.gondii* IgG antibodies at much higher serum dilutions (up to 1:2048). These results demonstrate the TGA-based ELISA's potential as a more effective diagnostic method for toxoplasmosis.

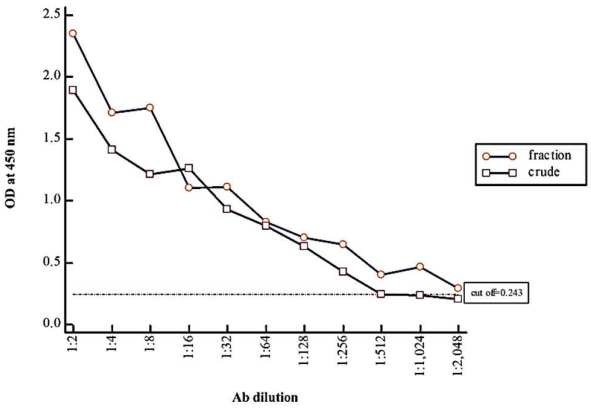

Fig. 1: Diagnostic performance of *T.gondii* crude and isolated glycoprotein antigen using two-fold serial diluted sheep positive serum.

TGA -ELISA sensitivity and specificity

To assess the sensitivity and specificity of TGA-ELISA in comparison with crude *T. gondii* antigen, ROC curves, and AUC were assessed (Fig. 2). When considering the serological results, the ELISA using glycoprotein isolated antigen is the best discrimination, showing a sensitivity of 100% and specificity of 96.67% with 92.3% positive predictive value and 100% negative predictive value. However, using crude *T.gondii* antigen in ELISA showed 83.33% sensitivity and 86.67% specificity, positive and negative predictive values were 71.4% and 92.9% respectively. The AUC values can range from 0.7 (indicating moderate diagnostic ability) to 0.9–1.0 (representing perfect diagnostic ability. The highest value was revealed by TGA-ELISA (AUC=0.963) while the AUC revealed by ELISA based on the use of crude *T. gondii* is equal to 0.867 (Table 1). These findings unequivocally establish the TGA ELISA as a more accurate and reliable diagnostic tool for toxoplasmosis, capable of effectively differentiating infected from non-infected animals.

Cross-Reactivity

The cross-reactivity of the isolated glycoprotein fraction was evaluated against various parasitic diseases, including cryptosporidiosis, blastocystis, giardiasis, and sarcocystosis. The analysis revealed that TGA-ELISA did not exhibit obvious cross-reactivity with non-toxoplasmosis infections, with reactivity values consistently below the calculated cut-off of 0.35 (Fig. 3). For instance, the mean reactivity values for cryptosporidiosis, blastocystis, giardiasis and sarcocystosis were all below 0.3, with standard deviations indicating minimal variability (SD≤0.1). The results indicate that isolated glycoprotein fraction is a highly specific marker for toxoplasmosis, with negligible cross-reactivity to other closely related protozoan parasitic infections.

Table 1: Efficacy parameters measured to assess the diagnostic potency of TGA- ELISA in comparison with crude *T. gondii* -ELISA in the diagnosis of sheep toxoplasmosis

ELISA	Sensitivity %	Specificity %	PPV %	NPV %	AUC
TGA ELISA	100.00	96.67	92.3	100.0	0.969
Crude *T. gondii* ELISA	83.33	86.67	71.4	92.9	0.867

PPV= positive predictive value, NPV = negative predictive value, AUC = area under the curve.

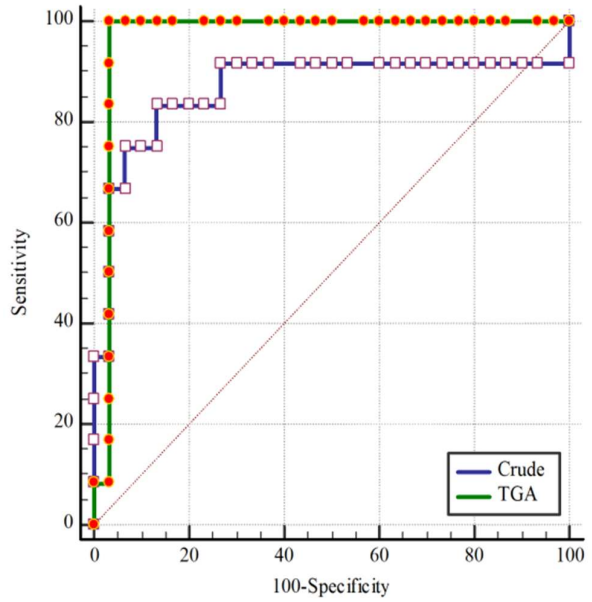

Fig. 2: ROC curve graphical illustration. The curve was conducted to calculate and compare the sensitivity and specificity of both TGA and crude *T. gondii* antigens used for the diagnosis of sheep toxoplasmosis.

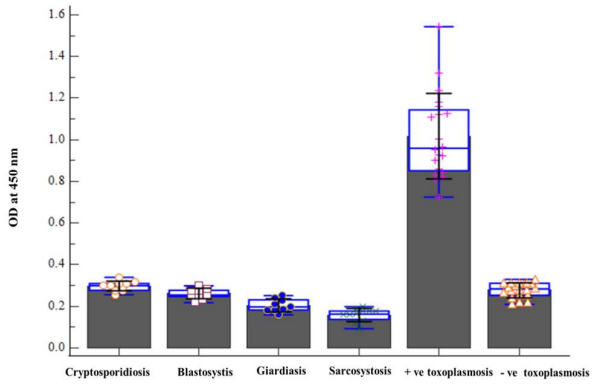

Fig. 3: TGA-ELISA cross-reactivity was evaluated by the diagnosis of five closely related protozoan diseases (cryptosporidiosis, blastosystis, giardiasis, sarcocystosis). Cut-off value 0.35.

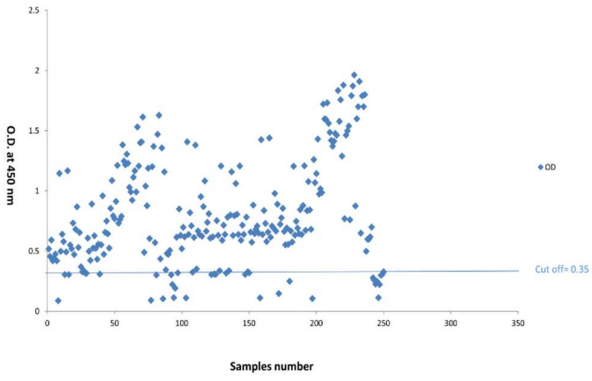

Fig. 4: Seroprevalence of toxoplasmosis in sheep as determined by Indirect TGA-ELISA. A cut-off absorbance value of 0.35 was established to differentiate between seropositive and seronegative animals.

Evaluation of TGA- ELISA for detection of *Toxoplasmosis* antibodies in sheep's sera

The scatter plot displays the optical density (OD) values at 450nm for approximately 250 samples of sheep sera tested using the TGA-ELISA (Fig. 4). The results showed that indirect ELISA based on glycoprotein antigen indicated that 209 (83.6%) out of 250 examined serum samples were positive for IgG *Toxoplasma* infection in sheep.

DISCUSSION

Toxoplasmosis is a significant zoonotic disease. *T. gondii* infects a wide range of hosts, including humans and livestock (Almallah et al. 2023). In sheep, *T. gondii* is considered a major cause of abortion, stillbirth, and weak lambs (Innes et al. 2009), resulting in considerable economic losses in the livestock industry (Stelzer et al. 2019). Detecting *T. gondii* infection in sheep is crucial for controlling the disease and preventing transmission to humans, particularly through contaminated meat (Abbas et al. 2020). Given the widespread nature of *T. gondii* and its potential impact on public health, there is a crucial need for sensitive, specific, and cost-effective diagnostic tools to facilitate screening and management of toxoplasmosis in livestock. The value of this study lies in the use of a locally isolated strain of *T. gondii*, which has been genetically identified in our lab (Elfadaly et al. 2017). By using this isolate, we aimed to ensure that our diagnostic approach would be more relevant and effective for detecting *T. gondii* infections within our specific population. This is important because local strain variations may differ from standard laboratory strains, such as RH and Me 49 and strain-specific antigenic diversity can influence the sensitivity and specificity of diagnostic tests.

One of the most widely used diagnostic methods for detecting antibodies against *T. gondii* is ELISA due to its simplicity and cost-effectiveness. However, the sensitivity and specificity of an ELISA largely depend on the used antigen (García-Maceira et al. 2020; Lagousi et al. 2021). In our study, the ELISA based on purified *T. gondii* glycoprotein antigen (TGA-ELISA) demonstrated effective diagnostic performance. Compared to the crude antigen-based ELISA. The TGA-ELISA was found to have achieved a sensitivity of 100% and a specificity of 96.67%, significantly higher than the 83.33% sensitivity and 86.67% specificity observed with the crude antigen ELISA. The AUC from the ROC analysis further supports the superior diagnostic ability of the TGA-ELISA. The TGA-ELISA achieved an AUC of 0.969, which is within the range indicative of excellent diagnostic accuracy (0.9–1.0). In contrast, the crude antigen ELISA had an AUC of 0.867, suggesting only moderate diagnostic ability. This significant difference in AUC values reinforces the conclusion that the TGA-ELISA is a more reliable method for diagnosing toxoplasmosis in sheep. Moreover, the current results showed higher sensitivity of TGA-ELISA which indicates its potential to detect lower concentrations of antibodies, as evidenced by its ability to detect anti-*T. gondii* antibodies at serum dilutions as high as 1:2048. This is a marked improvement over the crude antigen-based ELISA, demonstrating the effectiveness of the purified glycoprotein antigen in enhancing the test's diagnostic

capacity. The inconsistency between the two tests' results was because of the antigens used in ELISA. The enhanced performance of the TGA-ELISA in our study can be attributed to the specific selection of glycoprotein antigens, which are known to elicit a strong immune response and are less likely to cross-react with antibodies against non-*T. gondii* pathogens. The detection of antibodies to *T. gondii* is particularly useful for diagnosing the disease. In the search for antigens of diagnostic interest, Sharma et al. (1983) identified a carbohydrate-containing "low molecular weight antigen" that elicits an antibody immune response in humans. This antigen was later discovered to be a family of glycoprotein structures (Striepen et al. 1997). Azzouz et al. (2006) reported that sera from individuals infected with *T. gondii* reacted with the glucose–N-acetylgalactosamine-containing structure, which is highly immunogenic and can induce a strong immune response, including TNF-α production through NF-κB activation. This evidence underscores the significance of glycan structures in the immune response to *T. gondii*, highlighting their potential as diagnostic markers in serological assays to improve diagnostic accuracy. The presented results are consistent with those reported by Abdel-Rahman et al. (2016) who found that the use of *Fasciola* glycoprotein antigens in ELISA is regarded as a good immunodiagnostic target for fasciolosis in buffaloes. Moreover, El Shanawany et al. (2019b) discussed that the use of *Toxocara vitulorum* glycoprotein antigen in ELISA significantly improved the accuracy of serological tests for toxocariasis in comparison with crude *T. vitulorum* antigen.

A key concern in serological testing is cross-reactivity, which can lead to false positives and undermine the reliability of the test. Our study showed that the TGA-ELISA did not exhibit cross-reactivity with other protozoan infections, including cryptosporidiosis, blastocystis, giardiasis, and sarcocystosis. This result is consistent with the findings of El Shanawany et al. (2025b), who reported that serological assays using more refined antigens, like glycoproteins, result to have higher specificity and lower cross-reactivity than those using crude antigens. The lack of significant cross-reactivity observed in the TGA-ELISA suggests that this assay could be particularly useful in endemic regions where multiple parasitic infections are prevalent. In contrast, the crude antigen ELISA, which contains many antigenic *T. gondii* components, results in cross-reactivity, as demonstrated by previous studies by Dubey and Lindsay (2004).

The results of this study demonstrated that the application of TGA-ELISA in seroprevalence studies detected anti-*T. gondii* antibodies in 83.6% of the tested samples, indicating the high sensitivity of this antigen. This finding aligns with the results of Ibrahim et al. (2017), who reported an infection rate of 88% in sheep from Menoufiya and Gharbia Governorates using ELISA for diagnosis. Similarly, Abd El-Ghany and Amin (2012) recorded an infection rate of 85% among sheep in Sharkia Governorate, and Kuraa and Malek (2016) found an 86% infection rate among sheep in rural areas of Assiut when using ELISA for diagnosis. In Fayoum Governorate, Ghoneim et al. (2010) reported a 90% positivity rate for toxoplasmosis in sheep using ELISA for serodiagnosis.

However, the results of the current study differ from those of Abd El-Razik et al. (2018), who found a lower seroprevalence of toxoplasmosis in live female sheep and goats using ELISA and On-Site Toxo IgG/IgM Rapid test cassettes. Their study reported seroprevalence rates of 58.3% in Sharkia, 43.8% in Giza, and 28.1% in Cairo. Similarly, Barakat et al. (2009) recorded a prevalence among sheep in Giza Governorate of 44%. Moreover, Farag et al. (2023) showed that the total seroprevalence of toxoplasmosis in Upper Egypt was 47.9% with an individual seropositivity of 59.4% (63/106), 58.6% (17/29), 38.8% (54/139) and 46% (63/137) in cattle, buffalo, sheep and goats, respectively. These differences between the present study and those studies could be attributed to several factors, including variations in geographical location, sample size, and environmental conditions. The presence of intermediate hosts in different regions may also influence seroprevalence rates. The studies by Abd El-Razik et al. (2018) and Barakat et al. (2009) were conducted on sheep farms with special care and controlled environments, which likely reduced the chances of exposure to oocysts shed by cats. In contrast, the present study was conducted on sheep slaughtered at an abattoir, where animals might have been exposed to a wider range of environmental risk factors, increasing the likelihood of *T. gondii* infection. Moreover, in future studies, it will be recommended to apply this study to a large number of animals for more accurate results about the seropositivity of disease in Egypt.

Moreover, to enhance public health and disease control, future studies should include a larger and more diverse population of animals to obtain more accurate data on the seroprevalence of *T. gondii*. Additionally, integrating parallel serological surveys in humans, alongside improved diagnostic tools such as glycoprotein-based ELISA, would strengthen early detection, monitoring, and prevention strategies, benefiting both animal and human health.

Conclusion

In conclusion, the TGA-ELISA demonstrated superior diagnostic performance compared with crude antigen-based ELISA, showing higher sensitivity and specificity and absence of cross-reactivity. The assay is recommended for use in routine diagnostic screening of sheep at farm and slaughterhouse levels, as well as in veterinary diagnostic laboratories. Future validation should include multi-region field trials in different climatic and husbandry systems. In addition, its applicability in other livestock species, such as goats and cattle, should be investigated, and its utility for large-scale epidemiological surveillance should be further evaluated.

DECLARATIONS

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