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The Accuracy of Developed Peroxidase *Toxoplasma gondii* IgG ELISA Plates for Evaluating Toxoplasmosis in Sheep

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ABSTRACT

One of the main sources of human toxoplasmosis is mutton from chronically infected sheep. The accuracy of the developed *T. gondii* IgG ELISA plates (IgG/POD) were evaluated. A total number of 311, both blood and the matching tissue sheep samples were collected. Serological detection of toxoplasmosis in all samples with IgG/POD plate compared with the Latex agglutination test (LAT), Sabin Feldman dye test (SFDT), and conventional ELISA. The identical tissue samples to seropositive sera were bio-assayed through a mouse viability test to define the LD50 and LD100, and the histopathological assay was also done. The designed IgG/POD ELISA plate confirms higher accuracy against the total positive percentage of IgG compared to a conventional ELISA (44 and 38%). It also recognized the highest rates of IgG (48 and 26%). LAT-positive serum markers were (53, 33, and 49%) versus SFDT (68, 41, and 63%), while (12.5, 22 and 14%) were the result percentages of positive microscopic exam of ewes, rams, and total sheep. The mice viability test successfully isolated tachyzoites from 11(9 ewes and two rams) isolates. However, bio-typing detected (27%) of isolates of Type II and (73 %) of isolates of Type III. Finally, tachyzoites and tissue cyst stages were histo-pathological demonstrated within the experimentally infected mice and mutton tissues, respectively. In this study, the IgG/POD developed plate confirmed greater accuracy against IgG compared to the reference ELISA test. This test, along with mouse viability and the histopathological confirmation, are excellent bio-indicators reflecting the zoonotic hazard via the spotted mutton harboring *T. gondii* virulent strains.

Key words: IgG ELISA plates, Toxoplasmosis, Sheep, Mice bioassay, Histo-pathological assay.

INTRODUCTION

Toxoplasmosis is a common latent opportunistic zoonosis that can infect both humans and animals (Shaapan 2016). The infection was caused by one of the most common intracellular tissue cysts-stimulating protozoa, *T. gondii*, one-third of the human population worldwide is *T. gondii* sero-positive (Ammar et al. 2020). Cats are the only final hosts capable of excreting the environmentally resistant oocyst stage, in their feces that causes oral infection in all vertebrates (Barakat et al. 2012). Therefore, postnatal humans' toxoplasmosis is regularly via consuming undercooked meat containing tissue cysts or possibly due to oocysts contaminated food or water (Belluco et al. 2016). Sheep *T. gondii* tissue cysts from chronic carter lambs are an impossible source for human toxoplasmosis. Therefore, the regular update of the sero-prevalence of sheep is required with unconventional sensitive tests (Aghwan et al. 2021).

Serological detection of IgM and IgG, respectively, distinguishes acute and chronic toxoplasmosis in human (Hassanain et al. 2018). In animal species, the latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA) are utilized as reference tests (Shaapan et al. 2008). Matching results of the conventional ELISA with Anti-Sheep IgG Peroxidase Conjugates to Antigen Coated-ELISA (IgG/POD), will provide an accurate view of peroxidase's capacity to improve the test's accuracy and sensitivity. It also assesses the quality of antigen generated from local *T. gondii* isolates rather than the RH strain utilized in earlier studies (Hassan et al. 2012). Also, comparing the LAT with SFDT results, consider the evaluation of the difference in accuracy between the RH tachyzoites strain used in the conventional LAT and tachyzoites collected from local *T. gondii* isolates for SFDT (Elfadaly et al. 2012). Enzymatic sero-diagnostic kits contain peroxidase enzyme (POD). It's a commercial product made from horseradish roots, and the development of a functionally dynamic site between the peroxidase enzyme (HRPO) and an antigen-specific IgG antibody is required for conjugation of peroxidase to antibodies (Krainer et al. 2016). Therefore, labelled (POD)

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with a tracer has been commonly used as secondary antibody to quantify the connection of antibody-antigen complexes (Puchades and Maquieira 2013).

Sheep meat (Mutton) consumption has increased worldwide, primarily in less developed nations, because of its nutritional advantages over other red meats. In addition to providing human beings with a good supply of dietary protein (Mahmoud et al. 2021). *T. gondii* tissue cysts in food animal's meat are the main source of human toxoplasmosis, attributed to their viable persistence in undercooked tissues (Toaleb et al. 2013; Shaapan et al. 2021). In Egypt, 61.4% of seropositive Egyptian sheep were accepted with mutton containing cyst (Hassanain et al. 2011). Also, 47.5% of seropositive from wastage nourished Egyptian lambs were confirmed harboring virulent strains (Elfadaly et al. 2017a). *T. gondii* mutton isolates are classified into only three clonally types: I, II, and III, based on their pathogenicity in mice as determined by LD_{50} and $LD1_{00}$ as well as genetic diversity (Elfadaly et al. 2017b). Because the Type I strain is acute and non-cyst producing, it kills 100% of mice in less than 3 days following inoculation with one tachyzoite (LD100). As a result, it is responsible for severe congenital toxoplasmosis and ocular illness in humans (Belluco et al. 2016). Type-II, on the other hand, is a less virulent cyst-forming strain with neurological symptoms that kills 50% of mice within 15 days of inoculation with 10^2 tachyzoites (LD50 10^2), it is the most common (75%) in human toxoplasmosis (Hassan et al. 2016). The mixed infection in the same meat sample was possible, however more virulent strains obscured the biological features of less virulent strains (Shaapan et al. 2015).

Human mainly suffer severe latent congenital or ocular toxoplasmosis and the latency probably due to anti-inflammatory corticosteroid therapy (Elfadaly et al. 2015). Toxoplasmosis techniques have laid the groundwork for more successful and precise detection of human and animal *T. gondii* infection in the long term. (Mikaeel and Al-Saeed 2020). So, this study aimed to evaluate *T. gondii* anti-Sheep IgG-Peroxidase conjugate with antigen coated- ELISA compared with other sero-diagnostic tests in Egyptian sheep to improve the accuracy of the test Furthermore, mice viability tests along with histopathological confirmation were used as good bio-indicators of mutton-borne zoonotic bio-hazard.

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 19/139. Project number 12010135 National Research Centre (NRC), Egypt.

Samples and Study Design

From December 2018 to November 2020, practical trials were conducted. Blood samples were taken from 311 Egyptian sheep (257 ewes and 54 rams) at the Giza governorate slaughterhouse, with tissue samples. Sera were separated by centrifugation at 4000 x g for 10min, then divided into four copies and stored at -20C until serological examination using LAT and SFDT to identify total immunoglobulin. Plus for characteristic IgG by the conventional ELISA. Then, compared with the Lab-made Anti-Sheep IgG Peroxidase Conjugate with *T. gondii* Antigen Coated-ELISA (IgG/POD).

The corresponding tissue samples to positive sera using the LAT were exposed to pepsin digestion and microscopic examination, where the probable T. gondii positive mutton samples were intra-peritoneal injected in 2 mice for each sample and bio-typing of the succeeded isolates through viability test (El-Nawawi et al. 2008).

Latex Agglutination Test (LAT)

According to the manufacturer's instructions, conduct a total immunoglobulin screening test (Toxocheck-MT; Eiken Chemical, Tokyo, Japan), using RH strain tachyzoites as antigen. The procedures were performed as instructed by Shaapan et al. (2008), positive result when agglutination was observed at dilutions ≥1:164. The mutton samples of animals that were seropositive were subjected to digestion and microscopic examination.

Sabin Feldman Dye Test (SFDT)

Although SFDT is the gold standard for T. gondii antibodies, it carries a personal risk due to the use of live tachyzoites, which requires a high level of technical expertise. Positive results: around half of the extracellular tachyzoites appear unstainable. Dilutions of 1/16 and higher were considered positive (Ghazy et al. 2007).

Detection of IgG ELISA Titre

Antibodies of IgG was detected using ELISA diagnostic kits (VIRO, Germany), the procedure was described by Toaleb et al. (2014). ELISA was performed out by using an automatic ELISA reader to record the optical density of produced color at 450 nm (BIOTEK, INC. ELx, 800UV USA). Checkerboard titration was used to estimate the concentrations of antigen, serum, and conjugate dilutions. Negative mean OD values +2SD were used to determine the cut off value (Abdel-Shafy et al. 2015).

T. gondii IgG Peroxidase Coated-ELISA. (IgG/POD)

T. gondii antigen coated ELISA as a solid phase was prepared from fresh tachyzoites of local isolate, harvested in mice exudate for 72 h after injection, then, sonication disruption of the tachyzoite cells in Tris-HCl buffer, pH 7.2 (Shaapan et al. 2012). Production of rabbits-anti-sheep antibodies (IgG) was obtained in relation to the next, purification of the rabbit's anti- Toxoplasma serum by 50% ammonium sulfate precipitation method. The IgG coupling was conjugated through glutaraldehyde technique with peroxidase (POD) (Saraiva et al. 2007). The ELISA procedures and evaluation were performed as described by Shaapan et al et al. (2010).

Digestion and Microscopic Examination of Mutton Samples

About 20g of sero-positive mutton sample was homogenized separately in 100mL of 2.5% pepsin in PBS and incubated at 37°C for 3-6 hours with continuous shaking. Each homogenate digest tissue was microscopically examined at low and high powers, and the suspected positive microscopic samples were subcutaneously injected into three mice at a dose of about 1 mL/mouse for viability bioassay (Elfadaly et al. 2018).

Mice Viability and Bio-typing of Isolates

Mice viability tests were performed on the microscopically suspected bradyzoites containing mutton samples. The infected mice were checked daily for symptoms of ascites, mortality, or neurological manifestations, usually within 72-84h with highly virulent strains or partial off food with moderately virulent strains (Shaapan et al. 2021). Bio-typing of *T. gondii* isolates was also detected by determining the lethal dose (LD) for each isolate. Where LD_{100} is a lethal dose that kills 100% of mice after 84h of inoculation. The term LD_{50} refers to a deadly dose that kills 50% of mice in 15 days (Abdalhamed et al. 2019).

Histo-pathological Assay

Portion of the matching preserved copy of mutton specimens that microscopy verified to include T. gondii bradyzoites, as well as the tissues of the experimental symptomatic mice were subjected to a histo-pathological examination. Tissue specimens measuring around 0.5cm were fixed in a 10% neutral buffered formal saline solution, sectioned, and further sliced at 5µm before being stained with hematoxylin and eosin (H and E). The acute or chronic T. gondii related lesions with pathological abnormalities were next evaluated under an optical microscope (Hassanain et al. 2013).

RESULTS

The SFDT scored the highest total immunoglobulin overall positivity rate above the LAT test. Also, the percentage differences were 15, 8 and 14% corresponded to ewes, rams and total lamb (Table 1).

Regarding serological tests that identified IgG immunoglobulin, the IgG/POD test detected a higher overall sheep-positive IgG immunoglobulin percentage (44%) versus to ELISA Kit (38%). Also, IgG/POD recorded the highest percentage values, while the ELISA Kit recorded lower values within ewes and rams, respectively. Besides, the IgG/POD test resulted in a high percentage difference (6%) versus ewes, rams and total sheep (Table 2).

Even though the positive serological markers were 136(53%) ewes and 18 (33%) rams by the LAT, but only 17 (12.5%) ewes and 4 (22%) rams mutton samples were microscopically explained bradyzoites and were exposed to mouse viability test. While mice viability test succeeded in isolating tachyzoites percentages from only (7, 11 and 8%) corresponding to ewes, rams and total lamb samples, where only11 (9 ewes and 2 rams) mutton samples were harboring bradyzoites, and were subjected to histopathological assay (Table 3).

Bio-typing of the eleven T. *gondii* isolates based on the detection of fatal doses, LD_{50} and LD_{100}, with varied virulence, capable of killing 50 or 100% of inoculated mice, respectively. The results indicated that only 2 types; (27%) of moderately virulent type-II, where 50% of the dead mice occurred within 15 days through massive tissue cyst formation. While the A virulent type-III recording 8 isolates; (73%) with live mice persistence through moderate tissue cysts. However, the highly virulent type-I was not isolated or recorded (Table 4).

Table 1: Comparative positive results of SFDT and LAT for ewes, rams and total animals

Sheep Groups	No. of samples	Total immunoglobulin		
		SFDT Positive (%)	LAT Positive (%)	Difference (%)
Ewes	257	174 (68)	136 (53)	(15)
Rams	54	22 (41)	18 (33)	(8)
Total	311	196 (63)	154 (49)	(14)

Values in parenthesis are percentage.

Table 2: Comparative positive results and IgG Titer of IgG/POD and ELISA Kit

Sheep Groups	No. of samples	Total immunoglobulin		
		IgG/POD Positive (%)	ELISA Kit Positive (%)	Difference (%)
Ewes	257	123 (48)	108 (42)	≥ (6)
Rams	54	14 (26)	11 (20)	≥ (6)
Total	311	137 (44)	119 (38)	≥ (6)

Values in parenthesis are percentage.

Table 3: Comparison of results among LAT, microscopic exam and mice viability

Sheep Groups	No. of blood and mutton samples	Total immunoglobulin		
		LAT Positive (%)	Microscopic exam Positive (%)	Viability test (%)
Ewes	257	136 (53)	17 (12.5)	9 (6)
Rams	54	18 (33)	4 (22)	2 (6)
Total	311	154 (49)	21 (14)	11 (6)

Values in parenthesis are percentage.

Table 4: Bio-typing of *T. gondii* isolates from mutton

	Type I	Type II	Type III
11 isolates	0	3/11 (27%)	8/ 11 (73%)
virulent	Highly	Moderately	Mildly
Death time	5 DPI	45 DPI	A virulent
Tissue cysts	0	Huge ≥30DP I	Moderate ≥45 DPI
LD	LD_{100} 1	LD_{50} 10^2	

Where, mutton cells that infected by Type-II strain bradyzoites showed diffuse vacuolar degeneration and mononuclear inflammatory cell infiltration with cystic dilatation (Fig. 1). Additionally, the symptomatic mice were injected with the type-II isolates. Their cells showing inflammatory tachyzoites infiltration (Fig. 2).

DISCUSSION

In this study, SFDT had a greater overall positive of total immunoglobulin (63%) than the LAT in this investigation (49%). It also revealed that ewes, rams, and total sheep had greater percentage differences (15, 8, and 14%) than the LAT (Table 1). So, the efficient diagnosis using SFDT is related to the use of freshly aspirated peritoneal harvest of local strain tachyzoites as antigen, which was employed fresh within 72 h and no more, with the possibility of increased sensitivity and specificity to the same native strain antibodies (Elfadaly et al. 2015; Al-Namroty et al. 2020). The IgG/POD test was verified with the highest positive IgG percentage values (48 and 26%) compared to ELISA (42 and 20%) within ewes and rams, respectively (Table 2). The IgG/POD test is more accurate and sensitive than the traditional ELISA test, according to these findings. As a result, the current study's greater percentage values could be attributed to the employment of specialized antibodies to sheep as a replacement to non-specific antibodies in

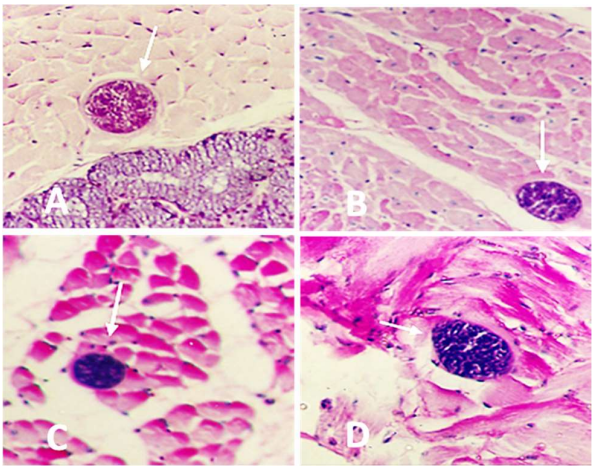

Fig. 1: *T. gondii* tissue cysts (arrows) in sheep muscles, Tongue (A), (B) Heart (B) Thigh (C) and Diaphragm (D). (H&E stained, x400).

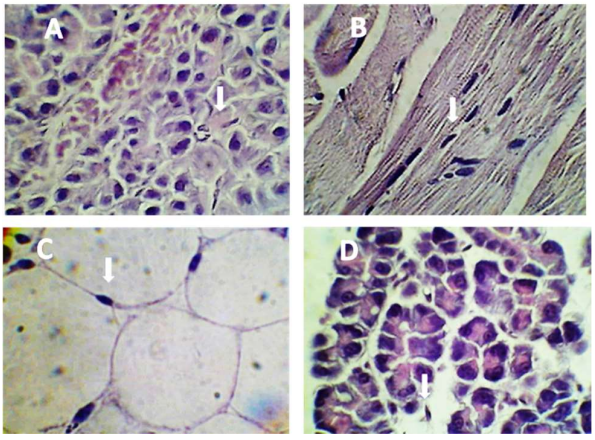

Fig. 2: *T. gondii* acute stage tachyzoites (arrows) in Liver (A), Heart (B), Lung (C) and (D) Kidney (D) tissues of inoculated mice. (H&E stained, x400).

traditional ELISA (27%). (Saraiva et al. 2007). Given that we employed a highly specific antigen product from a *T. gondii* local strain, we also used a highly specific antigen product from a *T. gondii* local strain. Furthermore, the use of the horse radish peroxidase enzyme improves coupling ability and increased antigen-antibody binding processes (Hassanain et al. 2011; Shaapan et al. 2018). As a result, the current study is concerned with elevated both IgG (49%) reflecting chronic conditions without the need for IgM investigation with the possible formation of tissue cysts, as the presence of IgG with or without IgM immediately confirms a previous infection without the need for serological follow-up (Fricker-Hidalgo et al. 2007).

Many previous studies agreed with the current study in proving that sheep are one of the most *T. gondii* susceptible species (Shaapan et al. 2008; Yan et al. 2016; Zeedan et al. 2022). Where, 49% of overall sheep were LAT positive. However, only (14%) mutton samples were microscopically harboring bradyzoites (Table 3). This finding demonstrates that tissue cysts are not present in all positive animals, and that cross-reactivity with non-cyst producing parasites is possible. It's also conceivable that the distribution of mutton cysts and the tissue samples taken are incompatible (Elfadaly et al. 2017b). *T. gondii* tissue cysts are generally difficult to isolate. However, in the current investigation, when tachyzoites percentages were isolated from only 11(8%) isolates from total mutton samples, this finding confirms that most isolates are virulent strains that could not cause mouse morbidity or were misdiagnosed with others. protozoa that create cysts (Fricker-Hidalgo et al. 2007). Few studies concerning bio-typing of *T. gondii* are available (Shaapan and Ghazy 2007; Boothroyd 2009). The current study confirmed only 3 isolates (27%) of moderately virulent type-II and mice dead within 15 days due to massive tissue and brain cysts (Table 4). However, type II was definite the most prevalent strain in mutton and was the most prevalent strain in human toxoplasmosis (Hassanain et al. 2011), as a result, the present study's findings demonstrate that mutton is linked to human biohazards via the pathogenic type-II. Although type-I is the most highly virulent human strain (Hassanain et al. 2013), it was not isolated or recorded in this study. This is because this strain is susceptible to mutation within the host, particularly in immunocompromised individuals, or as a result of corticosteroid medication (Elfadaly et al. 2015). With live mice persistence, the virulent type-III recorded 8 isolates (73%). These findings support the theory that virulent strains comprise up the great majority of animal and human stock isolates (Elfadaly et al. 2017b).

Sheep, unlike humans, are herbivores who eat on the ground. As a result, *T. gondii* oocysts are the most common infective stage in sheep. As a result, the high sero-positive percentage in sheep is used as a bio-indicator to assess the degree of oocyst pollution in the environment (Elfadaly et al. 2018). Meat tissue cysts, on the other hand, are the most common source of human toxoplasmosis, owing to their ability to survive in undercooked meat (Sakban and A'aiz, 2020). The findings could be linked to the vast new restaurants that serve undercooked fast beef dishes, as well as the Egyptians' changing eating habits. Differences in sero-prevalence could be attributable to climatic conditions, serological assays utilized, sample size, sheep strain, cat occurrence, seasonal fluctuations, as well as feeding culture and meat processing processes (Zhang et al. 2016; Elfadaly et al. 2017b). As they were IgG seropositive, over 40% of Egyptians consider themselves chronic carriers of latent opportunistic toxoplasmosis from at-risk groups and Mutton containing virulent strains of both sheep and human toxoplasmosis due to human-sheep interaction. (Barakat et al. 2012; Elfadaly et al. 2017a).

Conclusion

Our customized laboratory manufacturing *T. gondii* peroxidase-IgG coated-ELISA test set higher accuracy compared to a reference conventional ELISA kit. This test, coupled with the mouse viability test and histo-pathological confirmation, were strong bio-indicators for determining mutton's zoonotic role in human toxoplasmosis. Because of the recently local strain tachyzoites antigen employed, SFDT was more accurate than the LAT. The data support the impact of private awareness programs on risk factors, specifically against undercooked mutton or raw goat milk consumption, as well as the removal of cats from farms. In addition to the intensive routine screening of pregnant women regularly.

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Conflict of interest

The authors of the current work declare that they have no conflicts of interest in this work.

Author's contribution

Hassan Elfadaly and Raafat Shaapn: planned and designed the study, serological, molecular investigation, and drafting the paper. Ashraf Barakat: sharing in the idea and study design, and participated in drafting the manuscript. Nawal Hassanain and Ahmed Maher: sharing study conception, helped in manuscript preparation and involved in samples collection and preparation, sharing serological tests. All the authors have read and approved the final manuscript.

REFERENCES

Abdalhamed AM, Hassanain MA, Zeedan GS and Shaapan RM, 2019. Evaluation of Toxoplasma gondii propagated in specific pathogen free embryonated chicken egg, for diagnosis of toxoplasmosis in equids and human. Journal of Parasitic Diseases 43(3): 498-505. https://doi.org/10.1007/s12639-019-01117-3

Abdel-Shafy S, Shaapan RM, Abdelrahman KA, El-Namaky AH, Abo-Aziza FA and Zeina HA, 2015. Detection of *Toxoplasma gondii* Apicomplexa: Sarcocystidae in the brown dog tick *Rhipicephalus sanguineus* Acari: Ixodidae fed on infected rabbits. Research Journal of Parasitology 10: 142-150. https://doi.org/10.3923/jp.2015.142.150

Aghwan SS, Al-Bakri HS and Albaqqal SM, 2021. Comparison the efficiency of different techniques for the diagnosis of Toxoplasma gondii infection in slaughtered ewes, Iraq. Iraqi Journal of Veterinary Science 35: 19-23. http://dx.doi.org/10.33899/ijvs.2021.127058.1452

Al-Namroty AO, Shaapan RM, El-MoamLy AAR and Al-Hamshary EM, 2020. Correlation between behavioral alterations and dopamine changes in mice experimentally infected with *Toxoplasma gondii*. Neuropsychogical Trends 28: 39-57. http://dx.doi.org/10.7358/neur-2020-028-alna

Ammar AI, Moharm IM and Shaapan RM, 2020. *Toxoplasma gondii* Infection in Cattle Egret (*Bubulcus ibis*): First report from Shebin El-Kom, Menoufia Governorate, Egypt. Pakistan Journal of Biological Sciences 23(11): 1442-1449. https://dx.doi.org/10.3923/pjbs.2020.1442.1449

Barakat AM, Salem LM, El-Newishy AM, Shaapan RM and El-Mahllawy EK, 2012. Zoonotic chicken toxoplasmosis in some Egyptians governorates. Pakistan Journal of Biological Sciences 15(17): 821-286. https://dx.doi.org/10.3923/pjbs.2012.821.826

Belluco S, Mancin M, Conficoni D, Simonato G, Pietrobelli M and Ricci A, 2016. Investigating the determinants of *Toxoplasma gondii* prevalence in meat: a systematic review and meta-regression. PloS One 11(4): e0153856. https://doi.org/10.1371/journal.pone.0153856

Boothroyd JC, 2009. *Toxoplasma gondii*: 25 years and 25 major advances for the field. International Journal for Parasitology 39(8): 935-946. https://doi.org/10.1016/j.ijpara.2009.02.003

Elfadaly HA, Hassanain MA, Shaapan RM, Barakat AM and Toaleb NI, 2012. Serological and hormonal assays of murine materno-fetal *Toxoplasma gondii* infection with emphasis on virulent strains. World Journal of Medical Science 7: 248-254.

Elfadaly HA, Hassanain MA, Shaapan RM, Hassanain NA and Barakat AM, 2015. Corticosteroids opportunist higher *Toxoplasma gondii brain* cysts in latent infected mice. International Journal of Zoological Research 11(4): 169-176. http://dx.doi.org/10.3923/ijzr.2015.169.176

Elfadaly HA, Hassanain MA, Shaapan RM, Hassanain NA and Barakat AM, 2017a. Detection of *Toxoplasma gondii* from wastage nourished small ruminant and poultry: zoonotic significance. International Journal of Zoological Research 13: 6-11. https://dx.doi.org/10.3923/ijzr.2017.6.11

Elfadaly HA, Hassanain NA, Shaapan RM, Hassanain MA, Barakat AM and Abdelrahman KA, 2017b. Molecular detection and genotyping of *Toxoplasma gondii* from Egyptian isolates. Asian Journal of Epidemiology 10(1): 37-44. https://doi.org/10.3923/aje.2017.37.44

Elfadaly HA, Hassanain NA, Hassanain MA, Barakat AM and Shaapan RM, 2018. Evaluation of primitive ground water supplies as a risk factor for the development of major waterborne zoonosis in Egyptian children living in rural areas. Journal of Infectious and Public Health 11(2): 203-208. https://doi.org/10.1016/j.jiph.2017.07.025

El-Nawawi FA, Tawfik MA and Shaapan RM, 2008. Methods for inactivation of *Toxoplasma gondii* cysts in meat and tissues of experimentally infected sheep. Foodborne Pathogen and Disease 5: 687-690. https://doi.org/10.1089/fpd.2007.0060

Fricker-Hidalgo H, Brenier-Pinchart MP, Schaal JP, Equy V, Bost-Bru C and Pelloux H, 2007. Value of Toxoplasma gondii detection in one hundred thirty-three placentas for the diagnosis of congenital toxoplasmosis. Pediatric Infectious Disease Journal 26(9): 845-846. https://dx.doi.org/10.1097/INF.0b013e318123e8d3

Ghazy AA, Shaapan RM and Abdel-Rahman EH, 2007. Comparative serological diagnosis of toxoplasmosis in horses using locally isolated Toxoplasma gondii. Veterinary Parasitology 145(1-2): 31-36. https://doi.org/10.1016/j.vetpar.2006.11.010

Hassan SE, Shaapan RM, Ghazy AA and Abdel-Rahman EH, 2012. Evaluation of purified horse derived antigen in the diagnosis of human toxoplasmosis. Global Veterinary 9: 630-634.

Hassan SE, Toaleb NI, Shaapan RM, Abdel-Rahman EH and Elmahallawy EK, 2016. Diagnosis of toxoplasmosis using affinity purified fraction of tachyzoites local isolate. Research Journal Parasitology 11: 13-19. https://dx.doi.org/10.3923/jp.2016.13.19

Hassanain MA, Elfadaly HA, Shaapan RM, Hassanain NA and Barakat AM, 2011. Biological assay of *Toxoplasma gondii* Egyptian mutton isolates. International Journal of Zoological Research 7(4): 330-337. https://dx.doi.org/10.3923/ijzr.2011.330.337

Hassanain NA, Hassanain MA, Ahmed WM, Shaapan RM, Barakat AM and El-Fadaly HA, 2013. Public health importance of foodborne pathogens. World Journal of Medical Science 9(4): 208-222.

Hassanain NA, Shaapan RM and Hassanain H, 2018. Associated Antenatal Health Risk Factors with Incidence of Toxoplasmosis in Egyptian Pregnant Women. Pakistan Journal of Biological Sciences 21(9): 463-468. https://dx.doi.org/10.3923/pjbs.2018.463.468

Krainer FW, Darnhofer B, Birner-Gruenberger R and Glieder A, 2016. Recombinant production of a peroxidase-protein G fusion protein in Pichia pastoris. Journal of Biotechnology 219: 24-27. https://doi.org/10.1016/j.jbiotec.2015.12.020

Mahmoud MA, Ghazy AA and Shaapan RM, 2021. Review of diagnostic procedures and control of some viral diseases causing abortion and infertility in small ruminants in Egypt. Iraqi Journal of Veterinary Science 35(3): 513–521. http://dx.doi.org/10.33899/ijvs.2020.127114.1461

Mikaeel FB and Al-Saeed AT, 2020. Molecular detection and seroprevalence of Toxoplasmosis in free range local chickens (*Gallus domesticus*) in Duhok province. Iraqi Journal of Veterinary Science 34(2): 247-252. http://dx.doi.org/10.33899/ijvs.2019.125885.1173

Puchades R and Maquieira Á, 2013. ELISA tools for food PDO authentication. Comprehensive Analytical Chemistry 60: 145-193. https://doi.org/10.1016/B978-0-444-59562-1.00007-4

Sakban FM and A'aiz NN, 2020. Investigate the Toxoplasma gondii infection in the consumed beef in Al-Diwaniyah province. Iraqi Journal of Veterinary Science 34: 95-99. https://doi.org/10.1016/j.ijpara.2009.02.003

Saraiva JA, Nunes CS and Coimbra MA, 2007. Purification and characterization of olive (Olea europaea L.) peroxidase–Evidence for the occurrence of a pectin binding peroxidase. Food Chemistry 101(4): 1571-1579. https://doi.org/10.1016/j.foodchem.2006.04.012

Shaapan RM and Ghazy AA, 2007. Isolation of *Toxoplasma gondii* from horse meat in Egypt Pakistan Journal of Biological Sciences 10(1): 174-177. https://dx.doi.org/10.3923/pjbs.2007.174.177

Shaapan RM, 2016. The common zoonotic protozoal diseases causing abortion. Journal of Parasitic Diseases 40(4): 1116-1129. https://doi.org/10.1007/s12639-015-0661-5

Shaapan RM, Abd El Wahb WM, Abd El Had ME, Elfadaly HA, and Ahmed HD, 2018. *Toxoplasma gondii* infection and associated sociodemographic and behavioral risk factors among blood donors. Asian Journal of Epidemiology 11(2): 52-58. https://dx.doi.org/10.3923/aje.2018.52.58

Shaapan RM, Abo-ElMaaty AM, Abd El-Razik KA and Abd El-Hafez SM, 2012. PCR and Serological Assays for Detection of *T. gondii* Infection in Sport Horses in Cairo, Egypt. Asian Journal of Animal and Veterinary Advances 7(2): 158-165. https://doi.org/ajava.2012.158.165.

Shaapan RM, Al-Abodi HR, Alanazi AD, Abdel-Shafy S, Rashidipour M, Shater AF and Mahmoudvand H, 2021. Myrtus communis essential oil; anti-parasitic effects and induction of the innate immune system in mice with Toxoplasma gondii infection. Molecules 26(4): 819. https://doi.org/10.3390/molecules26040819

Shaapan RM, El-Nawawi FA and Tawfik MA, 2008. Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. Veterinary Parasitology 153(3-4): 359-362. https://doi.org/10.1016/j.vetpar.2008.02.016

Shaapan RM, Hassanain MA and Khalil FA, 2010. Modified agglutination test for serologic survey of Toxoplasma gondii infection in goats and water buffaloes in Egypt. Research Journal of Parasitology 5(1): 13-17. https://doi.org/10.3923/jp.2010.13.17

Shaapan RM, Toaleb NI and Abdel-Rahman EH, 2015. Significance of a common 65 kDa antigen in the experimental fasciolosis and toxoplasmosis. Journal of Parasitic Diseases 39(3): 550-556. https://doi.org/10.1007/s12639-013-0394-2

Shaapan RM, Toaleb NI and Abdel-Rahman EH, 2021. Detection of *Toxoplasma gondii*-specific immunoglobulin (IgG) antibodies in meat juice of beef. Iraqi Journal of Veterinary Science 35(2): 319–324. http://dx.doi.org/10.33899/ijvs.2020.126829.1390

Toaleb NI, Shaapan RM and Abdel-Rahman EH, 2014. Adoption of immuno-affinity isolated fasciola gigantica fraction for diagnosis of ovine toxoplasmosis. Global Veterinaria 12(1): 140-145.

Toaleb NI, Shaapan RM, Hassan SE and El Moghazy E, 2013. High diagnostic efficiency of affinity isolated fraction in camel and cattle toxoplasmosis. World Journal of Medical Science 8(1): 61-66. http://dx.doi.org/10.5829/idosi.wjms.2013.8.1.72161

Yan C, Liang LJ, Zheng KY and Zhu XQ, 2016. Impact of environmental factors on the emergence, transmission and distribution of *Toxoplasma gondii*. Parasites and Vectors 9(1): 1-7. https://doi.org/10.1186/s13071-016-1432-6

Zeedan GS, Abdalhamed AM, Shaapan RM and El-Namaky AH, 2022. Rapid diagnosis of Toxoplasma gondii using loop-mediated isothermal amplification assay in camels and small ruminants. Beni-Suef University Journal of Basic and Applied Sciences 11(1): 1-10. https://doi.org/10.1186/s43088-021-00184-x

Zhang N, Wang S, Wang D, Li C, Zhang Z, Yao Z, Li T, Xie Q, Liu S and Zhang H, 2016. Seroprevalence of Toxoplasma gondii infection and risk factors in domestic sheep in Henan province, central China. Parasite 23: 53. https://doi.org/10.1051/parasite/2016064