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ORIGINAL ARTICLE

Evaluation of *Toxoplasma gondii* propagated in specific pathogen free embryonated chicken egg, for diagnosis of toxoplasmosis in equids and human

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Abstract *Toxoplasma gondii* (*T. gondii*) is a worldwide distribution infects a wide variety of mammals, including humans. The present study aimed to detect the efficacy of soluble and whole *T. gondii* antigens propagated in specific pathogen-free of embryonated chicken egg (SPF-ECE) used to improve the potency of serological assays for diagnosis of toxoplasmosis in equids and human. Total of 220 serum samples from 170 equids (90 donkeys and 55 horses and 25 mules) and 50 humans were collected from different governorates in Egypt during the period from October 2017 to March 2018. Crude *T. gondii tachyzoites* antigens from low or high passages propagated in mice or SPF-ECE was used for modifying some serological tests. The experiment showed that the mortality rate of *T. gondii* for 10^3 and 10^4 low passages were 6/8 (75%) and 7/8 (88%) dead embryos but, lower mortality rate in high passage *T. gondii* were 4/8 (50%) and 5/8 (63%) dead embryos, respectively. No mortality or inflammatory signs were observed in control of negative groups. In equids sera were examined by S-ELISA using soluble *T. gondii* antigen propagated in SPF-ECE showed the highest positive results 26 (28.8%), followed by LAT 37 (22%) and MAGPT 36 (21.17%). While, W-ELISA and IFAT used whole *T. gondii* antigen prepared in SPF-ECE were 35 (20.58%) and 28 (19.41%) showed highly positive results than the same test used the whole antigen prepared in mice. The highest seroprevalence of *T. gondii* in human and donkeys were 19/50 (38%). and 26/90 (28.88%), more than mules were 6/25 (24%) and horses were 9/55 (16.3%) examined by S-ELISA respectively. SPF-ECE is considered an appropriate experimental model for isolation and propagation of *T. gondii tachyzoites*, and their soluble antigens used in serological tests (S-ELISA, LAT, and MAGPT) have sensitivity and specificity more than the whole antigen and provided reliable diagnostic tools for detection of *toxoplasmosis* in human and equids.

Keywords Equids · *T. gondii* · SPF-ECE · ELISA · I FAT · LAT · MAGPT

Introduction

Toxoplasmosis is one of the common zoonotic diseases worldwide distribution caused by obligatory intracellular parasite *T. gondii* infecting all mammals including human (Boyle and Radke 2009). Although *T. gondii* infection in different animals host is asymptotic diseases, it can cause abortions and many complications in both pregnant women and different animals' females, which has a significant effect in socioeconomic and public health problems (Ghoneim et al. 2010) and (Shaapan 2016). *T. gondii* transmitted by the oral route by Oo-cysts contaminated raw undercooked meat, consumption of raw unwashed food and water contaminated with Oo-cysts shed by infected felids (Tenter et al. 2000). Serological surveillance is a valuable tool for assessing the spread of infection in farms animals (Papini et al. 2015). For detection of antibodies to *Toxoplasma,* various techniques of analysis can be used (DT, IFAT, MAT, ELISA) (Rahbari et al. 2012). An accurate

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serodiagnosis of *T. gondii* using serological tests needs additional extensive researches to improve the potency of different serological assays using different *T. gondii* antigens. In the veterinary field indirect immunofluorescence (IFAT) is considered the reference test, although it appears to be time-consuming, expensive and difficult to interpret being related to the subjectivity of the operator (Pishkari et al. 2017). Evaluation of serological assays for detection of toxoplasmosis in equids using locally isolated *T. gondii* strain from Egypt proved that the indirect ELISA has a better diagnostic potency than other serological (Ghazy et al. 2007). To improve the potency of serological tests involved by using crude soluble antigens prepared in mice lead to increase sensitivity and specificity of tests (Khodakaram-Tafti et al. 2012). Recently, the embryonated chicken egg (ECE) introduced is an alternative animal model for studying protozoal parasites pathogenicity; *Trypanosoma cruzi* (*T. Cruzi*), *Neospora caninum* (*N. caninum*) *caninum tachyzoites*and *T. gondii tachyzoites* (Mello and Deane 1976; Que et al. 2004; Namavari et al. 2012). Also, *T. gondii* is propagated in chorioallantoic membrane of embryonated chicken egg (CAM-ECE) (Setasimy and Namavari 2015). *T. gondii* and *N. caninum* are similar parasites of the phylum *Apicomplexa* by comparing genome (Reid et al. 2012). The present study aimed to evaluate the soluble and whole *T. gondii* antigens propagated in specific pathogen-free of embryonated chicken egg (SPF-ECE) for diagnosis of toxoplasmosis in human and equids sera by different serological methods.

Materials and methods

Sera preparation

A total of 220 serum samples collected from 170 equids include (90 donkeys, 25 mule, and 55 horses) and 50 humans (male and female) from different governorates (Giza, Cairo, Beni Suef and El Fayoum) in Egypt during the period from October 2017 to March 2018. Samples collected by venipuncture in sterile free clotting vacuum tubes, allowed blood to clot and the sera separated by centrifugation 3000 r.p.m./10 min and after centrifugation sera were collected and stored at − 20 °C until used. We obtained the consent form person and animals owners for sample collection.

Toxoplasma gondii strains

Toxoplasma gondii virulence strain was kindly obtained from a colony maintained in Zoonotic Disease Department, Veterinary Research Division (VRD); National Research Centre (NRC), Egypt.

Inoculation of *T. gondii* into SPF-ECE

Forty SPF-ECEs on 1st day were used for *T. gondii* adaptation and propagation experiments. Eggs were incubated at 37.7 °C, with 55% humidity, the eggs were checks daily by Ovoscope Candler for embryo viability. SPF-ECE inoculation with the *T. gondii tachyzoites* at 9th day embryos age followed the methods described by Mansourian et al. (2013). Briefly, SPF-ECE with 9th days of incubation were randomly distributed into five groups eight embryos for each group, 1st group-1, were inoculated with 0.2 ml from 1×10^3 *T. gondii* low passage, 2nd group-2, inoculated by 0.2 ml 1×10^4 *T. gondii* low passage strain, 3rd group-3, with 1×10^3 *T. gondii* high passage strain, 4th group-4, inoculated with 1×10^4 *T. gondii* high passage strains and 5th group-5, kept as negative control inculcated with phosphate buffer saline sterile PBS (pH 7.2). After inoculation, the SPF-ECEs were incubation and examined daily. The dead embryos before 72 h after inoculation were discarding in different experimental groups.

Preparation of whole and soluble *T. gondii tachyzoites* antigen

Preparation of whole T. gondii tachyzoites antigen

The virulent strain of *T. gondii* strains were serial passages in White Swiss mice according to the method described by Klun et al. (2017). *T. gondii tachyzoites* were collected from the peritoneal fluid of white Swiss mice. Following euthanasia of mice, used 5 ml of sterile PBS (pH 7.2) into mice abdominal cavity and retrieved wash was centrifuged at 3000 rpm for 15 min. The pellet was washed for three successive times. In the end, the pellet was re-suspended in PBS. The *tachyzoites* counted to set the appropriate amounts used for the different experimental protocol.

Preparation of soluble *T. gondii tachyzoites* antigen

Soluble antigens were prepared from *T. gondii tachyzoites* in mice peritoneal exudates and SPF-ECE. The *T. gondii tachyzoites* pellet was suspended in PBS and disrupted with a sonicator (15–20 puls for 15 s). Examination of sonicated *T. gondii tachyzoites* were observed by light microscopy for the absence of whole parasites. The suspension was centrifuged 16.000 rpm/45 min at 4 °C, after centrifugation supernatant collected and the density of *T. gondii tachyzoites* protein suspension was determined by Nanodrop apparatus as the methods described by Bradford method (1976) standardized to 10, µg/ml, and stored at − 20 °C until use.

Detection of *T. gondii* antibodies by different serological tests

Indirect enzyme-linked immunosorbent assay (ELISA)

Indirect Enzyme-Linked Immunosorbent Assay was performed according to the methods described by Al-Kappany et al. (2018). To obtained appropriate dilution of sera and amount of antigen soluble and whole antigens were checked by checkerboard for optimal amounts of antigen and serum dilution and the optical density values equal or more than cut off were considered as positive results.

Indirect immune fluorescent test (FAT)

The whole *T. gondii tachyzoite* prepared in mice or SPF-ECE was prepared according to Walls and Barnhart (1978), briefly, washed *T. gondii tachyzoite* with PBS pH 7.2 and fixed in the formalin solution. The drop of suspended *T. godii tachyzoites* was dropped on the microscopic glass slide with circle zone, microscopic glass slide with a suspension let too dry at 37 °C. Equids and human sera were dilution 1: 64 (cut-off). Positive sera were subjected to twofold serial dilutions were tested again up to the endpoint titer. Positive results showed whole *T. godii tachyzoites* with diffused or continuous peripheral outer membrane fluorescence as in Fig. 2a, b, but apical shape of fluorescence alone was negative results as (Fig. 2).

Latex agglutination test (LAT)

It was performed according to the methods described by Holliman et al. (1989). LAT was performed by adding a drop of latex solution mixed with soluble antigen of *T. gondii tachyzoite* prepared in mice or SPF-ECE into a drop of serum sample on a microscope glass slide or white circular plate then mixed by side circular rotation. The agglutination flakes appeared after 2–3 min in positive cases.

Micro-precipitation agar gel test (MAGPT)

It was carried out using soluble antigen of *T. gondii tachyzoite* prepared in mice or SPF-ECE. Briefly, 1.5 g of Agarose and 1.5 g of Glycine were added to 100 ml distilled water, the mixture was boiled in a water bath or in microwave until dissolving, left at room temperature until reaching 45 °C then poured in Petri dishes, the plates were prepared with cutter rosette groups of 6 wells in a circle surrounding a center well. The peripheral wells were filled with the sera to be tested, while the central well was filled with soluble antigen of *T. gondii tachyzoite* prepared in mice or SPF-ECE. The plates were incubated at humidity chamber at room temperature and the plate was examined at 24, 48, and 72 h under diffused light. The observations, positive serum samples showed a line of precipitation between the positive serum and antigen wells, while negative serum samples showed no line of precipitation according to (Brandon-Mong et al. 2015).

Statistical analysis

The results were analyzed using Statistical Package for Social Sciences (SPSS, version 16 (Chicago, Illinois, USA). The different between serological tests used in serodiagnosis of toxoplasmosis in human and equids serum samples were calculated by the Chi square or Fisher's exact test at 95% confident interval (CI) and ($P \leq 0.05$).

Results

The mortality rate in ECE groups inoculated with 10^3 and 10^4 low passages of *T. gondii tachyzoites* were 6/8 (75%) and 7/8 (88%) dead embryos, respectively. In the groups with 10^3 and 10^4 high passage *T. gondii tachyzoites*, 4/8 (50%) and 5/8 (63%) dead embryos, respectively. Grossly, mild inflammatory signs were observed in the dead embryos or live embryo inoculated with 103 high passages *T. gondii tachyzoites*. Sever inflammation signs were observed in the dead or live embryos inoculated with *T. gondii tachyzoites* 10^3 or 10^4 low, no mortality or inflammatory signs were observed in control negative groups (Fig 1). Microscopically, examination revealed that the *T. gondii tachyzoites* were observed in suspension fluids from AF of dead or live embryos of any groups with the high or low passage. Immunofluorescence images of *T. gondii tachyzoites* were detected with equine positive sera samples by direct immunofluorescence as in Fig. 2.

The highest positive percentage of *T. gondii* antibody in donkeys sera examined by soluble antigen ELISA (S-ELISA) which using soluble *T. gondii* antigen propagated in SPF-ECE were 26 (28.8%), followed by LAT and MAGPT. While both W-ELISA and IFAT were used whole *T. gondii* antigen prepared in SPF-ECE showed highly positive results than the same test used the whole antigen prepared in mice.

The highest seroprevalence of *T. gondii* in donkeys were 26/90 (28.88%), more than mules were 6/25 (24%) and horses' were 9/55 (16.3%) by S-ELISA respectively. The results from Table 1 revealed that the highest reactivity in serological assays using soluble than serological assays used the whole antigen in equids sera as shown in (Table 1 and Fig. 2).

The examined human sera were found the highest prevalence of *Toxoplasma* antibody in women at risk

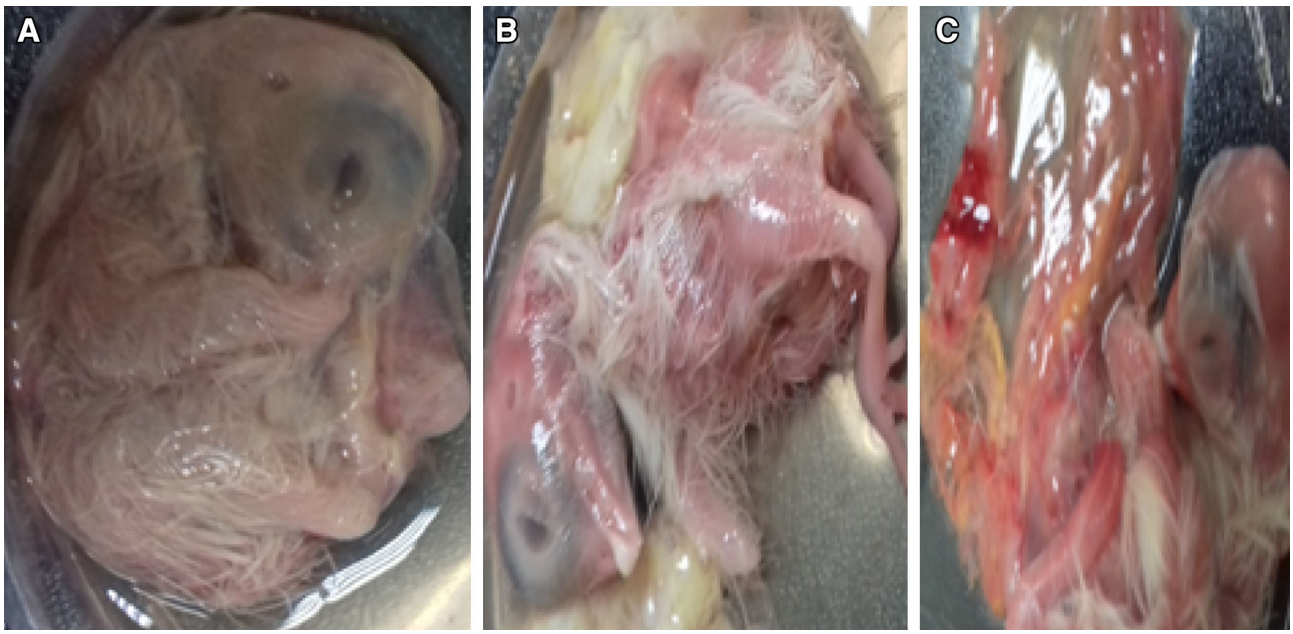

Fig. 1 macroscopic examination of embryonated Chicken egg; Control negative groups with no inflammations (**a**), mild inflammation in the dead embryos or alive embryo (**b**) severe inflammation observed in the dead or alive embryo inoculated with *T. gondii* tachyzoites **(c)**

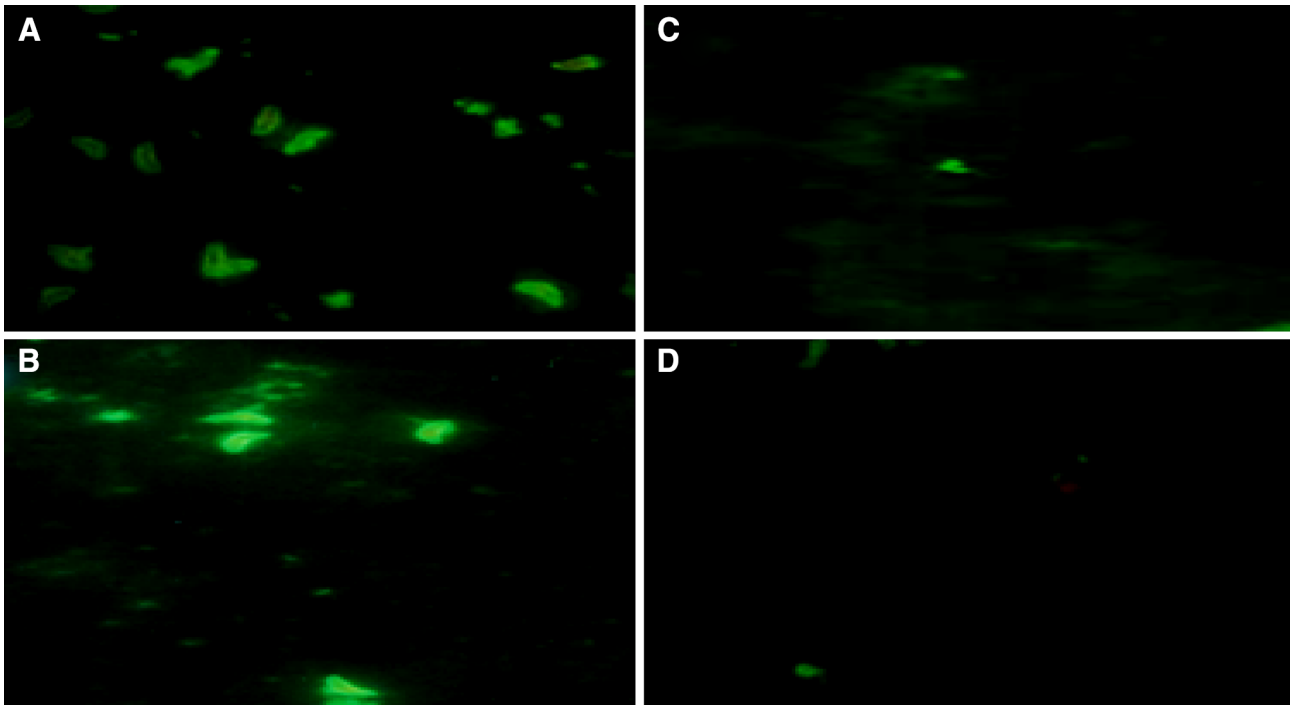

Fig. 2 **a**, **b** Positive IFAT for *Toxoplasma gondii* antigen prepared in SPF-ECE detected by positive equine and human sera. **c**, **d** negative equine sera for *Toxoplasma gondii* antigen prepared in SPF-ECE

groups were 13 of 30 (43.3%) by S-ELISA used *T. gondii tachyzoites* prepared in SPF-ECE followed by AGPT and LAT soluble antigen SPF-ECE were 12 out of 30 (40%) and 10 out of 20 (33.33%) women sera respectively. The lowest prevalence of anti-*T. gondii* antibody in man sera by soluble ELISA was (30%). Although, differences were notes between male and female with no difference was noticed as in (Table 2).

The sensitivity of the different serological test (ELISA, MAGPT, and LAT) used soluble ranged from 76.2 to

Table 1 Detection of *Toxoplasma gondii* antibody in serum samples of equids by different serological tests using soluble and whole antigen

Species	W-ELISA %		IFAT %		S-ELISA %		MAGPT		LAT %	
	WT in SPF-ECE	WTA in mice	WTA in SPF-ECE	WT in mice	S. antigen SPF-ECE	S. antigen in mice	S. antigen SPF-ECE	S. antigen in mice	S. antigen SPF-ECE	S. antigen in mice
Donkeys (n:90)	23 (25.55)	22 (24.44)	21 (23.33)	20 (22.22)	26 (28.88)	26 (28.88)	23 (25.55)	22 (24.44)	20 (22)	18 (20)
Mules (n:25)	5 (20)	5 (20)	4 (16)	4 (16)	6 (24)	6 (24)	7 (28)	6 (24)	7 (28)	7 (28)
Horses (n:55)	7 (12.72)	6 (10.9)	3 (5.45)	3 (5.45)	9 (16.3)	9 (16.36)	6 (10.90)	7 (17.72)	10 (18.18)	10 (18.18)
T equids (n:170)	35 (20.58)	33 (19.41)	28 (19.41)	27 (16.47)	41 (24.11)	41 (24.11)	36 (21.17)	35 (20.35)	37 (22)	35 (21)

The levels of *Toxoplasma gondii antibodies* detected by different serological tests using soluble and whole antigen were not significantly different ($P > 0.005$)

F human female/women, *M* human male/man, *T* total number of human male and female sera. S-ELISA and W-ELISA for measuring its specificity and sensitivity and the following formula: *W-ELISA* whole *T. gondii tachazoites* antigen coated Enzyme-Linked Immunosorbent Assay, *FAT* Indirect immune fluorescent test, *S-ELISA* soluble *T. gondii tachazoites* antigen coated Enzyme-Linked Immunosorbent Assay, *MAGPT* Micro-precipitation agar gel test, *LAT* Latex Agglutination Test, *WT-SPF-ECE* whole *T. gondii tachazoites*

Table 2 Comparative between different serological assays using soluble and whole toxoplasma tachzoites antigen for detection of antitoxoplasma antibody in human sera

Species	W-ELISA %		IFAT %		S-ELISA %		MAGPT		LAT %	
	WT in SPF-ECE	WT in mice	WT in SPF-ECE	WT in mice	S. antigen SPF-ECE	S. antigen in mice	S. antigen SPF-ECE	S. antigen in mice	S. antigen SPF-ECE	S. antigen in mice
Females (n = 30)	12 (40)	11 (37)	12 (40)	12 (40)	13 (43.33)	12 (41)	10 (33.33)	11 (36.66)	11 (36.7)	11 (36.7)
Males (n = 20)	6 (30)	5 (25)	6 (30)	5 (25)	6 (30)	6 (30)	5 (25)	5 (25)	8 (40)	8 (40)
Total (n = 50)	18 (36)	16 (32)	18 (36)	17 (34)	19 (38)	17 (34)	15 (30)	16 (32)	19 (38)	19 (38)

The levels of *Toxoplasma gondii antibodies* detected by different serological tests using soluble and whole antigen were not significantly different ($P > 0.005$)

F human female/women, *M* human male/man, *T* total number of human male and female sera

92.59% while specificity ranged from 71.45.8 to 78.59% but the sensitivity ranged from 76.2 to 78.6 for whole antigens assays W-ELISA and FAT detected anti-*T. gondii* antibodies in equine and human and specificity ranged from 71.45 to 78.26. Our results showed that the soluble antigen used in serological assays gave higher sensitivity and specificity more than the whole antigen for detecting anti-*T. gondii* antibodies in equids and human. The highest diagnostic potency of soluble ELISA followed by LAT, whole IFAT and soluble MAGPT. Moreover, soluble ELISA is able to detect early *toxoplasma* antibody in equine and human (Table 3).

Discussion

Introduced an animal model ECE to propagate *T. gondii* enhanced successfully and presented appropriate antigen as well as the availability of ECE, the important reason is easy in calculation lethal dose fifty (LD50 dose) beside less incubation time and easy handling (MacFarlane and Ruchman 1948). The present study showed well propagation of *T. gondii* in SPF-ECE without reducing the virulence at different stage levels of infection. Therefore inoculating ECE with the high-passage compared to low-passage has nearly the same survival and mortality rates as in Fig. 1 (and this result is in agreement with Warren and Russ (1948), determined virulence of *T. gondii tachyzoites*

Table 3 Sensitivity, specificity, of different serological tests using soluble and whole *T. gondii* antigens for detection of anti-toxoplasma antibodies in sera of human and equids

Species	Assays evaluation	W-ELISA %		IFAT %		S-ELISA %		MAGPT %		LAT %	
		WT in SPF-ECE	WT in mice	WT in SPF-ECE	WT in mice	S SPF-ECE	S. antigen in mice	S. antigen SPF-ECE	S. antigen in mice	S. antigen SPF-ECE	S. antigen in mice
Equids (n = 170)	Sensitivity %	87.56	79.26	86.21	82	91.27	84.58	82.3	81.5	84.58	84.58
	Specificity %	77.39	72	73.68	72.97	78.23	72.48	72.26	71.77	72.48	72.48
Human (n = 50)	Sensitivity %	87.26	83.1	87.5	76.2	92.59	84.65	85.2	84.3	84.65	84.65
	Specificity %	77.6	72.8	78.26	76.6	78.2	78.6	71.45	72.7	78.6	78.6

W-ELISA % IFAT % S-ELISA % MAGPT % LAT % WT in SPF-ECE WT In mice WT in SPF-ECE WT in mice

Sensitivity = [TP/ (TP + FN)] × 100 where TP = true-positive and FN = false-negative

Specificity = [TN/(TN + FP)] × 100, where TN = true negative, FP = false positive result

in ECE after continuous high and low high-passage in Vero cell line.

Accurate laboratory diagnostic test of Toxoplasma infection is vital and significant to the control and treatment of Toxoplasmosis in man and animals. Among the different serological methods that have been used for detection of toxoplasmosis in human and animals, ELISA can be modified by soluble and whole antigen for coated ELISA plate (Dubey 2010).

Modification of serological tests for improving the potency of rapid diagnosis of toxoplasmosis in human and equids. Although, preparation of whole *T. gondii tachyzoites* antigen is easy, fast and low cost, it has the disadvantage of high false negative and positive levels, and no need to special equipment such as sonicated and Nano-drop (Chen et al. 2008).

In the present study the prevalence of *T. gondii* infection in equids (donkey, mule, and horse) was (25.88%, 24.11%, and 21.17%), that examined by S-ELISA respectively. However, the lowest prevalence by FAT was (19.41%), difference between the results of serological tests may be attributed to the differences in the sensitivity and specificity of the serological tests used, host-parasite relationship which depends upon the virulence of *T. gondii* strain, time of exposure to infection, biology of the parasite and the immune status of the infected equine (Miao et al. 2013). Prevalence of *T. gondii* infection in the horse was lower (16.3%) than (24%) in mule and (28.88%) donkey as Table 1: These results agree with previous literature. The prevalence of *T. gondii* was 30–50% in horses from Egypt and the prevalence was lower in Arabian than Paint and Thoroughbred horses (Shappan et al. 2012). Similar results found in other countries 28% in Turkey (Razmi et al. 2014) and 31% in Saudi Arabia (Alanazi and Alyousif 2011). In addition, the seroprevalence of *T. gondii* antibodies were 13/30 (43%) in human female and 6/20 (30%) in human male by S-ELISA nearly similar results in different serological tests as in Table 2: These results agree with previous prevalence in Alexandria was 46.2%, El-Fayoum was 45.8%, Gharbia was 42.8% and Dakhalia governorate was 44% (Saleh et al. 2014).

The present study revealed that the soluble ELISA and whole IFAT have a higher sensitivity and specificity for the diagnosis of *T. gondii* antibodies (Table 3). Regarding MAGPT has a similar reactivity among all serological tests for detection of *T. gondii* infection in naturally infected human and equine is easy to perform and does not require sophisticated equipment (Wilson et al. 1990). Moreover, soluble ELISA and whole IFAT have the ablility to distinguish IgG and IgM, than MAGPT and LAT According to Piergili Fioretti (2004). ELISA and IFAT gave better results than LAT for detecting of *toxoplasmosis* in human and equids.

The highest percentage of *T. gondii* antibody in donkey were (28.88%) followed by mules (24%) and the horses were (16.3%) as in Table 1. Regarding the risk factors and *T. gondii* positivity in women, a significant increase was recorded in the women due to contact with equids and/or cats using different serological tests. Many previous studies reported that ownership of cat or contact with equids or cats was associated with positivity of *T. gondii*. Cats which act as the definitive host and considered the major source of toxoplasmosis to animals and humans through excreting the environmentally resist oocysts for years (Torrey and Yolken 2013).

Conclusions

The specific pathogen free embryonated chicken eggs (SPF-ECEs) are suitable for assessment and propagated *T. gondii* with uniform mortality occurred between 3 and 4 days after inoculation. The present study proved that the *T. gondii* propagated in SPF-ECE is considered an appropriate experimental model for isolation or propagation of *T. gondii tachyzoites*, and their soluble antigens used for improving potency and reactivity of serological tests (S-ELISA, LAT, and MAGPT).

In Egypt Equids are not slaughtered for human consumption, but a small number of unbarred dead donkeys, old horses and mules increased the risk of transmission to the final host Felida which leading to increase the risk of human infection with *T. gondii.* The present study proved to increase the diagnostic potency of serological tests by using soluble and whole antigens of *T. gondii* propagated in SPF-ECE.

Authors contribution Dr. GSGZ: Research idea, planned the study design, performed data, and, field animals samples collection, serological and propagation of toxoplasma in SPF-ECE, laboratory work, and drafting the paper. Dr. AMA: sharing in the conception of the research idea, field animal's samples collection, sharing designed work, identification of *T. gondii*, sharing serological test and and participated in drafting the manuscript. Dr. MAH and Dr. RMS: involved in samples collection, laboratory work, interpreted the data results, and helped in manuscript preparation and designed work and participated in drafting the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The study was approved ethically by Medical Research Ethical Committee Research, National Statement on Ethical Conduct in Human and animals Research at National Research Centre, Egypt under registration number # 19-030#.

References

Alanazi AD, Alyousif MS (2011) Prevalence of antibodies to *Toxoplasma gondii* in horses in Riyadh Province, Saudi Arabia. J Parasitol 97(5):943–945

Al-Kappany YM, Abbas IE, Devleess chauwer B, Dorny P, Jennes M, Cox E (2018) Seroprevalence of anti-*Toxoplasma gondii* antibodies in Egyptian sheep and goats. BMC Vet Res 14(1):120. https://doi.org/10.1186/s12917-018-1440-1

Boyle JP, Radke JR (2009) A history of studies that examine the interactions of *Toxoplasma* with its host cell: emphasis on in vitro models. Int J Parasitol 39(8):903–914

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1–2):248–254

Brandon-Mong GJ, Che Mat Seri NA, Sharma RS et al (2015) Seroepidemiology of toxoplasmosis among people having close contact with animals. Front Immunol 6:143

Chen S, Lou D, Lin A, Zeng X, Ding Z, Wen L, Ohta N, Wang J, Fu C (2008) Evaluation of a rapid ELISA technique for detection of circulating antigens of *Toxoplasma gondii*. Microbiol Immunol 52(3):180–187. https://doi.org/10.1111/j.1348-0421.2008.00020.x

Dubey JP (2010) Toxoplasmosis of animal and humans, vol 2. CRC Press, Boca Raton, p 313. https://doi.org/10.1186/1756-3305-3-112

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

Ghoneim NH, Shalaby SI, Hassanain NA, Zeedan GS, Soliman YA, Abdalhamed AM (2010) Comparative study between serological and molecular methods for diagnosis of toxoplasmosis in women and small ruminants in Egypt. Foodborne Pathog Dis 7:17–22. https://doi.org/10.1016/j.vetpar.2006.11.010

Holliman RE, Johnson JU, Duffy K, New L (1989) Discrepant toxoplasma latex agglutination test results. J Clin Pathol 42(2):200–203

Khodakaram-Tafti A, Mansourian M, Namavari M, Hosseini A (2012) Immunohistochemical and polymerase chain reaction studies in *Neospora caninum* experimentally infected broiler chicken embryonated eggs. Vet Parasitol 188(1–2):10–13. https://doi.org/10.1016/j.vetpar.2012.03.013

Klun I, Uzelac A, Villena I, MercierA BB, Nikolić A et al (2017) The first isolation and molecular characterization of *Toxoplasma gondii* from horses in Serbia. Parasit Vectors 10(167):2–9

MacFarlane JO, Ruchman I (1948) Cultivation of *Toxoplasma* in the developing chick embryo. Exp Biol Med 67:1–4

Mansourian M, Namavari M, Khodakaram-Tafti A, Rahimian A (2013) Experimental *Neospora caninum* infection in domestic bird's embryonated eggs. J Parasit Dis 39(2):241–244. https://doi.org/10.1007/s12639-013-0327-0

Mello MN, Deane MP (1976) Patterns of development of *Trypanosoma cruzi* in the embryonated chicken egg. Ann Trop Med Parasitol 70(4):380–388

Miao Q, Wang X, She LN et al (2013) Seroprevalence of *Toxoplasma gondii* in horses and donkeys in Yunnan Province, Southwestern China. Parasit Vectors 6:168

Namavari M, Mansourian M, Tafti AK, Hosseini MH, Rahimiyan A, Khordadmehr M, Lotfi M (2012) Application of chicken embryonated eggs as a new model for evaluating the virulence of *Neospora caninum* tachyzoites. Comp Clin Path 21(6):1665–1668

Papini RA, Buzzone G, Nardoni S, Rocchigiani G, Mancianti F (2015) Seroprevalence and genotyping of *Toxoplasma gondii* in horses slaughtered for human consumption in Italy. J Equine Vet Sci 35:657–661. https://doi.org/10.1016/j.jevs.2015.06.012

Piergili Fioretti D (2004) Problems and limitations of conventional and innovative methods for the diagnosis of Toxoplasmosis in humans and animals. Parassitologia 46:177–181

Pishkari S, Shojaee S, Keshavarz H, Salimi M, Mohebali M (2017) Evaluation of *Toxoplasma gondii* soluble, whole and excretory/secretary antigens for diagnosis of toxoplasmosis by ELISA test. J Parasit Dis 41(1):289–291

Que X, Wunderlich A, Joiner KA, Reed SL (2004) Toxopain-1 is critical for infection in a novel chicken embryo model of congenital toxoplasmosis. Infect Immun 72(5):2915–2921

Rahbari AH, Keshavarz H, Shojaee S, Mohebali M, Rezaeian M (2012) IgG avidity ELISA test for diagnosis of acute toxoplasmosis in humans. Korean J Parasitol 50:99–102

Razmi GR, Abedi V, Yaghfoori S (2014) Serological study of *Toxoplasma gondii* infection in Turkoman horses in the North Khorasan Province, Iran. J Parasit Dis 40(2):515–519

Reid AJ, Vermont SJ, Cotton JA, Harris D, Hill-Cawthorne GA, Könen-Waisman S et al (2012) Comparative genomics of the *apicomplexan* parasites *Toxoplasma gondii* and *Neospora caninum*: coccidia differing in host range and transmission strategy. PLoS Pathog 8(3):e1002567

Saleh AM, Ali HA, Ahmed SA, Hosny SM, Morsy TA (2014) Screening of *Toxoplasma gondii* infection among childbearing age females and assessment of nurses' role in prevention and control of toxoplasmosis. J Egypt Soc Parasitol 240(1416):1–4

Setasimy A, Namavari M (2015) Use of chicken embryonated eggs for evaluating the virulence of *Toxoplasma gondii*. J Parasit Dis 40(4):1223–1225

Shaapan RM (2016) The common zoonotic protozoal diseases causing abortion. J Parasit Dis 40(4):1116–1129

Shappan RM, Abo-Eimaty AM, Abd-Eirazik KA, Abd-Eihafez SM (2012) PCR and serological assays for detection of Toxoplasma gondii infection in sport horses in Cairo, Egypt. Asian J Anim Vet Adv 7:158–165. https://doi.org/10.3923/ajava.2012.158.165

Tenter AM, Heckeroth AR, Weiss LM (2000) *Toxoplasma gondii*: from animals to humans. Int J Parasitol 30(12–13):1217–1258

Torrey EF, Yolken RH (2013) Toxoplasma oocytes' as a public health problem. Trends Parasitol 29(8):380–384

Walls KW, Barnhart ER (1978) Titration of human serum antibodies to *Toxoplasma gondii* with a simple fluorometric assay. J Clin Microbiol 7(2):234–235

Warren J, Russ SB (1948) Cultivation of *Toxoplasma* in embryonated egg An antigen derived from choriolallantoic membrane. Exp Biol Med 67:85–89

Wilson M, Ware DA, Juranek DD (1990) Serologic aspect of toxoplasmosis. J Am Vet Med Assoc 196:277–280