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Comparison of PCR and Serologic Survey for Diagnosis of Toxoplasmosis in Sheep

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ABSTRACT

Comparative diagnosis of *Toxoplasma gondii* infection in sheep were carried out with PCR and some serological assays using 200 blood and serum samples collected from sheep of different ages and sexes slaughtered in the main abattoir in Cairo and Giza, Egypt. PCR showed the higher prevalence of toxoplasmosis (48.5%) followed by the Modified Agglutination Test (MAT) (45.5%) and the Latex Agglutination Test (LAT) (41.0%), while the lowest prevalence was detected with the Indirect Hem-Agglutination Test (IHAT) (38.5%). When the data of the serological tests were compared with that of the PCR, as a reference test for toxoplasmosis, MAT had the highest sensitivity (95.9%) followed by LAT (90.7%) and the lowest sensitivity by IHAT (80.4%). On the other hand IHAT had the highest specificity (91.3%) followed by MAT (88.3%) and the lowest specificity was by LAT (84.5%). The present study adopt PCR and serological survey of *T. gondii* antibodies in sheep by using more sensitive and specific antigens prepared from isolated *T. gondii* local strain and the obtained results suggests that MAT alone or with LAT can be used as a highly sensitive screening test followed by PCR as a specific confirmatory test for diagnosis of toxoplasmosis in sheep. Consequently, the high prevalence of sheep toxoplasmosis detected by this study scopes the public health significance of sheep's meat as source of human infection.

Key words: *Toxoplasma gondii*, sheep, PCR, serological assay

INTRODUCTION

Toxoplasmosis is one of the most important infectious and zoonotic diseases worldwide, caused by an obligatory intracellular protozoan parasite; *Toxoplasma gondii* which can infect humans and almost all warm-blooded animals (Vaz *et al*., 2010). The parasite distributed on regions and weather condition of the environment where oocysts survive and is responsible for major economic losses in humans and most classes of livestock including sheep through abortions, still birth and neonatal losses. It causes mental retardation and loss of vision in children and abortion in pregnant women (Raeghi *et al*., 2011).

Cats are main reservoir of *T. gondii* because they are the only hosts that can excrete the resistant stage (oocyst) of the parasite in the feces. Humans become infected by eating undercooked meat from infected animals and food and water contaminated with oocysts (Sreekumar *et al*., 2005). Besides vertical infection during pregnancy, humans and animals can get infected either by oral uptake of sporulated *Toxoplasma* oocysts or by ingestion of tissue cysts upon consumption of raw or undercooked meat of infected slaughtered animals (Zhou *et al*., 2012).

Among livestock, toxoplasmosis causes great losses in sheep. *Toxoplasma gondii* may cause embryonic death and resorption, fetal death and mummification, abortion, stillbirth and neonatal death in these animals (Dubey, 2009). Most sheep acquire the infection post-natally by ingestion of oocysts from contaminated environment or ingestion of infected tissues of animals and the disease which cause serious economic losses among sheep industry all over the world especially at lambing time (Shaapan, 2015). Also, sheep are important to the economy of many countries because they are a source of food for humans; the infected sheep meat is a source of *T. gondii* infection for humans and animals (Ragozo *et al*., 2008).

Many studies investigate the *T. gondii* incidence in sheep from different regions in Egypt which were 45% using Sabin Feldman tests (Michael and El-Refaii, 1977), 18.30 and 14.8% by using SFT and IHAT, respectively (El-Menyawy, 1987), 47 and 50% for ELISA and IFAT, respectively (El-Ghaysh and Mansour, 1994), 49.5 and 52% in slaughtered sheep in Tanta abattoir using IHAT and IFAT, respectively (Ibrahim *et al*., 1997), 55.9 and 54.1% with IHAT and IFAT (Aal and Barakat, 2000) and 37% by ELISA by mean of patent Ag-coated plates from specific kit (Kandil and Abou-Zeina, 2000) and recently, comparative serological examination revealed that, MAT showed the higher prevalence of toxoplasmosis (43.7%) followed by ELISA (41.7%), using antigen prepared from isolated *T. gondii* local strain (Shaapan *et al*., 2008).

Molecular biology methods have been increasingly used in diagnosis of ovine toxoplasmosis. Polymerase Chain Reaction (PCR) amplification has become the preferred method. Most PCR assays used for *Toxoplasma* identification use primers targeting the B1 gene. It is a 35-fold-repetitive gene that is highly specific and conserved among strains of *Toxoplasma* (Burg *et al*., 1989), the P30 surface antigen (single copy) the ribosomal RNA (110 copies), both the small subunit rRNA gene (Tenter *et al*., 1994) and the 529 bp DNA fragment (Homan *et al*., 2000). Moreover, PCR had been shown to be useful and reliable confirmative technique for diagnosis of *T. gondii* infection in ovine and caprine placenta and aborted fetuses (Hurtado *et al*., 2001; Masala *et al*., 2003).

The present work was aiming to studying the prevalence of *T. gondii* infection among sheep slaughtered in Cairo and Giza municipality abattoirs using IHAT, LAT and MAT serological tests and followed by the confirmatory PCR screening assay and also the sensitivity, specificity, advantages and disadvantages of each test were be determined.

MATERIALS AND METHODS

Blood and serum samples: Blood samples were collected from 200 apparently healthy sheep slaughtered at the main abattoir of Cairo (El-Bassatin), Giza (El-Moneeb) and sera were obtained, labeled in serial number and stored at -20°C until used for serological and PCR testing.

***Toxoplasma gondii* strain:** Virulent RH strain of *T. gondii* used for PCR and local *T. gondii* local strain tachyzoites used for antigens preparation for serological assays were obtained from colony kept in Department of Zoonoses, National Research Center. Both strains maintained by serial passage in mice according to the procedures described by Shaapan and Ghazy (2007).

Polymerase Chain Reaction (PCR)

DNA extraction: Genomic DNA was extracted from *T. gondii* RH strain (positive control) using a commercially available kit (Dneasy blood and Tissue kit, Qiagen Co., Cat. No. 69504). DNA was purified through the columns according to the manufacturer's protocol and eluted in 50 μL of the supplied AE buffer and then stored at -20°C. Concerning DNA extraction from blood samples, the

samples were collected in test tubes containing heparin as an anticoagulant then digested using the same kit using the blood protocol recommended by the manufacturer prior to assay by PCR.

PCR amplification: Primers used for PCR were targeting the repetitive 35-fold B1 gene (Burg *et al*., 1989). The outer primers were only used in this study. The reactions were set up to a final volume of 25 μL containing 2 μL of DNA sample (standard controls) or 5 μL of blood specimen DNA, 1 μL of each primer (100 pmol) and 12.5 μL Pyro-Start™ Fast PCR Master Mix (Fermentas Co., Cat. No. Ko211). The PCR was performed in a PTC-100 Peltier Thermal Cycler (MJ Research, Incline Village, USA). The first cycle included 1min of denaturation at 95°C. This first step was followed by 39 cycles of 2 sec of denaturation at 94°C, 5 sec of annealing at 48°C and 25 sec of primer extension at 72°C and a final extension step of 72°C for 10 min. A 10 μL aliquot of the amplified product was analyzed on 1% agarose gel and stained with Ethidium bromide. Every PCR run included positive and negative controls.

Serological assays

Indirect Hem-Agglutination Test (IHAT): The indirect hem-agglutination test was adopted using soluble tachyzoites antigen coated in tanned red blood cells and the procedures were carried out according the serology manual prepared by Palmer *et al*. (1976).

Latex Agglutination Test (LAT): The antigen prepared through sensitization of latex particles with *T. gondii* sonicated tachyzoites as procedures of Lunde and Jacobs (1967). The latex agglutination test was adopted as methods of Holliman *et al*. (1989), using negative and positive reference serum samples and reactions were performed by adding a drop of latex solution into a drop of serum on a microscope glass slide and mixed by side movement of slide. The agglutination appears within 2-3 min in positive cases.

Modified Agglutination Test (MAT): The formalized killed whole tachyzoites of *T. gondii* was prepared according to the method described by Desmonts and Remington (1980). The procedures were carried out according to the method described by Dubey and Desmonts (1987) at a dilution of 1:25.

Data analysis: The data of the various serological tests were analyzed. The sensitivity and specificity of each test were determined by comparing its results with that of PCR as a reference test according to the method described by Waltman *et al*. (1984) and Dubey *et al*. (1995).

RESULTS

Prevalence of *T. gondii* infection in sheep using PCR and serologic assays: Examination of the 200 serum samples of sheep by PCR, MAT, LAT and IHAT revealed that 97 (48.5%), 91 (45.5%), 82(41.0%) and 77 (38.5%) were positive reactors with *T. gondii* respectively which at the same time was considered the percentage of infection (Table 1 and Fig. 1).

Comparison between the PCR and serological tests: The sensitivity and specificity of LAT, MAT and IHAT serological tests were calculated by comparing their results with those of PCR test and reveled that MAT had the highest sensitivity (95.9%) followed by LAT (90.7%) and the lowest sensitivity by IHAT (80.4%). On the other hand IHAT had the highest specificity (91.3%) followed by MAT (88.3%) and the lowest specificity was by LAT (84.5%) (Table 2).

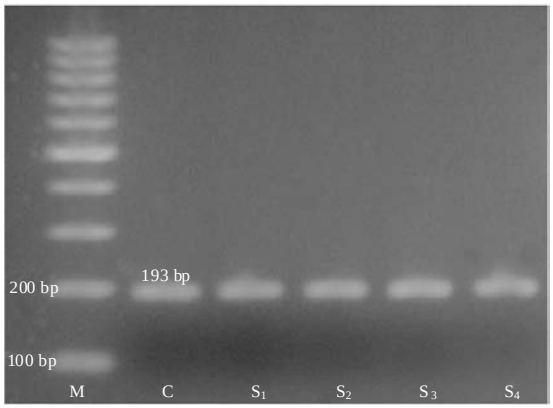

Fig. 1: Detection of *T. gondii* in sheep blood samples by PCR, M: Mol. wt. marker (100 bp ladder), C: Positive control, S_1-S_4: Positive PCR *T. gondii* samples at 193 bp

Table 1: Prevalence of *T. gondii* antibodies among sheep sera using PCR and different serological tests*

	Positive		Negative	
Test	No.	%	No.	%
PCR	97	48.5	103	51.5
MAT	91	45.5	109	54.5
LAT	82	41.0	118	59.0
IHAT	77	38.5	123	61.5

*No. of samples examined by each test were 200, PCR: Polymerase chain reaction, MAT: Modified agglutination text, LAT: Latex agglutination text, IHAT: Indirect hem-agglutination

Table 2: Comparison between the PCR and serological tests used for detection of *T. gondii* infection

	PCR and compared serological test		PCR				
Test	Positive (+v)	Negative (-v)	Positive (+ve)	Negative (-ve)	Sensitivity (%)	Specificity (%)	Total
MAT	93	91	97	103	95.9	88.3	200
LAT	88	87	97	103	90.7	84.5	200
IHAT	78	94	97	103	80.4	91.3	200

PCR: Polymerase chain reaction, MAT: Modified agglutination text, LAT: Latex agglutination text, IHAT: Indirect hem-agglutination

DISCUSSION

A few studies analyzed risk factors associated with *T. gondii* seropositivity in sheep, the presence of cats on the farm, using surface water for drinking water, altitude and farm size were factors associated with infection rates; prevalence was higher at low altitudes and on large farms (Vesco *et al*., 2007). Diagnosis of toxoplasmosis by demonstration of *T. gondii* in tissue is too much difficult and also the disease is manifested by mild clinical symptoms and develops an in-apparent infection. Therefore Malik *et al*. (1990) pointed out that the detection of antibody response by screening of slaughtered sheep sera serologically appears to be the conclusive tool for proper diagnosis of toxoplasmosis.

The present reported prevalence rate of *T gondii* infection in our study using PCR, MAT, LAT and IHAT were 48.5, 45.5, 41.0 and 38.5%, respectively. Higher incidence of toxoplasmosis (64 and 62.5%) were recorded by Dubey *et al*. (1986) and Malik *et al*. (1990) in adult sheep in USA, respectively; while lower incidence of toxoplasmosis (28.5 and 3.6%) were detected by Savio and Nieto (1995) and Dubey and Foreyt (2000) in adult sheep, respectively.

The difference between the obtained results of PCR and serological tests during the present study and those reported by other investigators might be attributed to the host-parasite relationship which depends upon the virulence of *T. gondii* strain; the immune status of the different infected sheep, the age and management of sheep in different localities and the time of exposure to infection and biology of the parasite. This is agreed with that concluded by Shaapan *et al*. (2008) who added that the prevalence of infection may vary strongly in some country from one locality to another due to difference in certain ecological factors and breeding system in these areas.

Generally the higher infection rate among sheep with *T. gondii* found in this study may be attributed to the feeding habit of sheep which usually graze short grasses and lick soil around them thus, are liable to contract the infection with *T. gondii* oocysts. Moreover, stray cats may easily enter to the environment of sheep. This agreed with that obtained by El-Menyawy (1987), Malik *et al*. (1990) and Lunden *et al*. (1993).

It is highly significant to determine the specificity and sensitivity of serological diagnosis of *T. gondii* infection in sheep. Highest sensitivity (95.9%) by MAT, highest specificity by IHAT (91.3%) and lowest specificity (84.5%) by LAT were obtained in this study when the data of the serological tests were compared with that of the PCR, as a reference test for toxoplasmosis. Also Dubey, (2010) concluded that detection of *Toxoplasma* DNA by PCR was considered the most specific and standard test by which all other tests should be judged. LAT revealed lowest specificity, is frequently produce false positive LAT titers and so technical considerations involving accuracy of reading weakly positive reactions could account for some of the differences (Holliman *et al*., 1989). On other hand, IHAT demonstrates great specificity, is quantitative, low cost and may be easily adopted but requires further refinement with regard to procedures and standardization of the antigen used (Dubey *et al*., 1995). Regarding MAT, Dubey (1997) and Shaapan *et al*. (2008) found that MAT has the highest sensitivity among all serological tests for detection of *Toxoplasma gondii* infection in naturally infected sows and sheep, respectively which is easy to perform and does not require sophisticated equipment.

CONCLUSION

The present report revealed high prevalence of this zoonotic parasite infection with *T. gondii* in Egyptian sheep which suggests that the risk of humans contracting infection through consumption of sheep meat. In addition this study suggested that MAT alone or with LAT can be used as a highly sensitive screening test followed by PCR as a specific confirmatory test for diagnosis of toxoplasmosis in sheep.

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