

## Serological and Molecular Diagnosis of Toxoplasmosis in Human and Animals

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**Abstract:** Blood samples were collected from 56 aborted women, 127 asymptomatic occupational personnel and 630 slaughtered animals (280 sheep, 230 swine, 32 buffalo and 88 cow). Seroprevalence of toxoplasmosis was detected in the investigated human and animal samples by ELISA IgG assay. Nested PCR was performed on all DNA of ELISA positive human and animal samples to amplify a fragment from the B1 gene. ELISA results of human samples showed that 34 out of 56 aborted women (60.7%) and 48 out of 127 (37.8%) asymptomatic occupational personnel were positive for Toxo IgG. Concerning animals; 172 (61.4%), 185 (80.4%), 11 (34.4) and 17 (19.3%) out of 285 sheep, 230 swine, 32 buffaloes and 88 cow, respectively were positive for Toxo IgG. Results of PCR revealed that 26.5, 35.4 and 19.7% of the examined 34 aborted women, 48 asymptomatic occupational personnel and 385 animals were infected with *T. gondii*. In the present study, all ELISA IgG results in human and animals were higher than those of PCR. This indicates that the presence of Toxoplasma-specific antibodies was an insufficient criterion for identifying Toxoplasma infection. It can be concluded that ELISA combined with the PCR technique is a recommended tool for accurate diagnosis of Toxoplasmosis.

**Key words:** *Toxoplasma* • PCR • ELISA • Animals • Human

### INTRODUCTION

*Toxoplasma gondii* is one of the most successful coccidian protozoans with potential zoonotic impact among humans, animals and birds. This protozoan infects up to a third of the world's population with an obligate intracellular tissue cyst-forming characters, with subsequence ranks among the 10 most commonly occurring opportunistic pathogens that cause latency in human and animal's hosts, however represent the third leading cause of death among food-borne diseases in the immune compromised individuals. Although the course of disease is generally benign, during unfit host immune condition and according to the virulent types, the protozoan stimulates serious affection and severe neurological disorders. Also, induce spontaneous abortion, still birth, or child mentally and/or physically retarded, with significant morbidity and mortality including humans with Acquired Immunodeficiency Syndrome or submitted to corticosteroids and cancer chemotherapy [1].

Food-borne toxoplasmosis in humans may result from exposure to different stages of *Toxoplasma gondii*, in particular from the ingestion of tissue cysts or tachyzoites contained in meat, primary offal (viscera) or meat-derived products of many different animals, or the ingestion of sporulated oocysts that are contained in the environment and may contaminate food and water [2]. Up to 50% of *T. gondii* infections are transmitted by ingesting undercooked meat, making toxoplasmosis one of the most clinically significant food-borne diseases in pregnant women [3].

There are many different serological techniques for diagnosis of toxoplasmosis, for example, the Sabin-Feldman dye test (SFDT) [4] and ELISA [5]. Polymerase chain reaction (PCR) is frequently used to detect *T. gondii* DNA in clinical samples [6]. It is performed by direct detection of parasite DNA and the results do not depend on the immunological status of the patient. The presence of Toxoplasma DNA in the maternal blood most probably indicates a recent infection [7].

Hussein *et al.* [8] evaluated IgM-ELISA and PCR in diagnosis of recent *T. gondii* infection. The results revealed that PCR detected very recently infected Egyptian cases (23 out of 70 suspected cases) than IgM-ELISA (only 18 cases). They concluded that combination of both tests may help to improve the sensitivity of diagnosis (45% in suspected group) more than either PCR (32.8%) or IgM-ELISA alone (25.7%).

Chiabchalard *et al.* [9] studied the sensitivity and specificity of PCR to detect *T. gondii* DNA and they were able to detect the specific gene from purified DNA samples containing as few as 0.25 parasites per 100,000 human leukocytes. These results had an impressive initial 100% specificity but later it decreased because of false-negative data.

Ghoneim *et al.* [10] carried out a comparative study between serological and molecular methods for diagnosis of toxoplasmosis in women and small ruminants in Egypt and recommended that each sample should be checked with both ELISA and PCR methods.

Therefore, the aim of the present investigation was the application of serological (ELISA IgG) with molecular (PCR) tests for diagnosis of toxoplasmosis in human and animals.

## MATERIALS AND METHODS

**Sampling:** Blood samples were collected from 56 aborted women, 127 asymptomatic occupational personnel and 630 slaughtered animals (280 sheep, 230 swine, 32 buffalo and 88 cow).

### ELISA Assay

**In Human:** Serum samples were tested for the presence of specific IgG by using Clinotech Toxo ELISA IgG kits (Clinotech Diagnostics and Pharmaceuticals, Richmond, Canada) as recommended by the manufacturer.

**In Animals:** ELISA was carried out according to Voller *et al.* [11]. Whole soluble tachyzoite antigen of RH strain (maintained in the Zoonotic Diseases Dep., NRC, Egypt) was prepared as described by Waltman *et al.* [12]. Protein content of the antigen was determined as outlined by Lowry *et al.* [13]. The optimal antigen (soluble tachyzoites antigen preparation) concentration, antibody and conjugate dilutions were chosen after preliminary checkerboard titration. 1 mg o-Phenylenediamine was dissolved in 1mL substrate buffer. The absorbance of the colored reaction

was read within 30 minutes at 405nm using a titertek multiskan ELISA reader. All incubation steps were carried out at 37°C in a moist chamber. The positive threshold value was determined to be two fold the mean cut-off value of negative sera.

**DNA Extraction:** DNA was isolated from the collected human and animal blood samples using a commercial purification system (Dneasy blood and tissue kit, Qiagen Co., Cat. No. 69504) following the manufacturer's instructions. Final pellets were re suspended in 30 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2) and stored at -20°C until used.

**Nested PCR Assay:** The nested PCR was performed on all DNA samples to amplify a fragment from the B1 gene, which is present in 35 copies and is conserved in the *T. gondii* genome, as described by Burg *et al.* [14].

The primers used in the first round of the PCR were (5'-GGAAGTGCATCCGTTTCATG AG-3') and (5'-TCT-TAAAGCGTTCGTGGTC-3'), which correspond to nucleotides 694-714 and 887-868, respectively at 193 bp.

The primers used in the second round were (5'-TGCATAG GTT-GCAGTCACTG-3' and (5'-GGCGACC-AATGTGC-GAATAGACC-3'), which correspond to nucleotides 757-776 and 853-831, respectively at 94 bp. Three microliters of template DNA were added to a final volume of 50 µL of PCR mixture consisting of 5 µL of 10 x PCR buffer (50 mM Tris-HCl, pH 9.1, 3.5 mM MgCl<sub>2</sub>), 8 µL of 1.25 mM deoxynucleoside triphosphates, 0.5 µL of *Taq* DNA polymerase (5 units/µL) and 1.5 µL (20 pmol) of each of the outer primers. The amplification was performed in Hypaid thermal cycler PCR. The cycling conditions for both PCRs were 95°C for five minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 90 seconds and 72°C for one minute and a final extension at 72°C for 10 minutes. 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.

## RESULTS

ELISA results of human samples showed that 34 out of 56 aborted women (60.7%) and 48 out of 127 (37.8%) asymptomatic occupational personnel were positive for Toxo IgG. Concerning animals; 172 (61.4%), 185 (80.4%), 11 (34.4) and 17 (19.3%) out of 285 sheep, 230 swine, 32 buffaloes and 88 cow, respectively were positive for Toxo IgG (Table 1).

Table 1: ELISA and PCR results of human and animal blood samples

Tested samples	NO. of samples	ELISA Sero-positive titer 1:80 to $\geq$ 1:640 No. (%)	PCR amplified samples No. (%)
Sheep	280	172 (61.4%)	23 (13.4%)
Swine	230	185 (80.4%)	46 (24.9%)
Buffaloes	32	11 (34.4%)	2 (18.2%)
Cows	88	17 (19.3)	5 (29.4%)
Occupational personnel	127	48 (37.8%)	17 (35.4%)
Aborted women	56	34 (60.7%)	9 (26.5%)

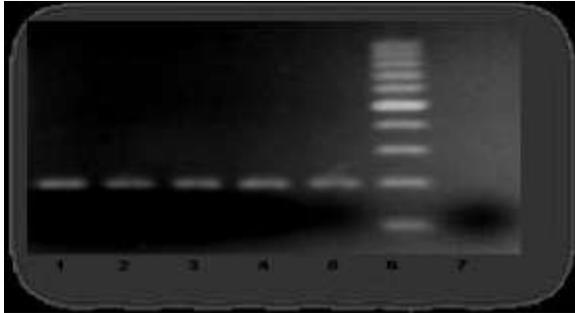


Fig. 1: Detection of *T. gondii* tachyzoites DNA by PCR. Lane 6: Mol. wt. marker (100 bp ladder); Lane 1: Positive control; lane 2, 3, 4 and 5: Positive *T. gondii* Egyptian isolates DNA at 94 bp.; Lane 7: negative control.

Results of the PCR revealed that 9 (26.5%), 17 (35.4%) and 76 (19.7%) of the examined 34 aborted women, 48 asymptomatic occupational personnel and 385 animals were infected with *Toxoplasma* (Table 1 and Fig. 1).

## DISCUSSION

Recent ecological and etiological investigation concerning risk factors and new sources of human toxoplasmosis must be updated to solve the unexplained equation of high prevalence in human and meat-producing animals. The incidence of final and intermediate hosts completely differs from country to other according to the socio-economic pattern of housing cats and the feeding behavior of human and animals. Where, the environmental factors in various communities indicate the important roles of eating and culture habits and contribute to the socio epidemiological aspects thought to be important factors for the spread of this zoonosis [15].

Crude *T. gondii* tachyzoites antigen represents raw material used to prepare fractions to be employed in different serologic tests for the diagnosis of toxoplasmosis, including IgM and IgG antibodies. The actual antigenic molecules of the parasite are complex and diverse. The extremely wide range of hosts

susceptible to *T. gondii* is thought to be the result of interactions between *T. gondii* ligands and receptors on its target cells [16].

In the present study, 172 out of 285 sheep (61.4%), 185 out of 230 swine (80.4%), 11 out of 32 buffaloes (34.4%) and 17 out of 88 cow (19.3%) were positive for Toxo IgG. Antibodies to *T. gondii* were found in 16.97% (141/831) of slaughter pigs [17]. Lower seroprevalence (8.8%) was recorded in buffaloes examined by Zhang *et al.* [18]. Higher seroprevalence of *Toxoplasma* IgG (98.4%) in sheep was recorded by Ghoneim *et al.* [10].

In the present study, the tested animals sera was positive for *Toxoplasma* IgG antibodies and regarded as high risk animal group for both public and animals' health, referred to the pattern of raising sheep on oocysts dirty unsanitary circumstance and reflect the requisite of restrict control measures opposite to stray cats in the locality of sheep subsist [19]. Older animals, found to have a higher prevalence of the organism, are routinely used in the production of sausages, salami and cured meats. Moreover, such products often contain meat from multiple animals in a single serving. Together these factors result in a higher potential risk of infection after consumption unless these foodstuffs are very well cooked [20].

In the current work, ELISA results showed that 34 out of 56 aborted women (60.7%) were positive for Toxo IgG. Yildiz *et al.* [21] reported nearly similar seroprevalence (51.49%) in pregnant women. On the other hand, lower seroprevalence of Toxo IgG was found in 23.7 and 17.2% of the investigated pregnant and aborted women, respectively by Ghoneim *et al.* [10].

In the present work, 48 out of 127 (37.8%) asymptomatic occupational personnel were positive for Toxo IgG. Most human infections are subclinical, but infection during pregnancy can be fatal for the fetus or newborn. Chronic infections persist for years and can reactivate causing severe disease in immunocompromised patients [22].

Nimri *et al.* [23] stated that diagnostic serology of *T. gondii* infection in early pregnancy could be confirmed by a positive *Toxoplasma*-specific PCR result in blood samples, even in the presence of serological results

difficult to interpret due to the lack of sequential follow-up during pregnancy. On the other hand, Guy and Johnson [7] mentioned that a negative PCR result does not exclude recent infections because the PCR sensitivity, in which a single trophozoite can be detected in a clinical sample, has potential problems for some types of specimens.

Results of the PCR revealed that 76 (19.7%) of the examined 385 ELISA positive slaughtered animals were infected with *Toxoplasma*. Aspinall *et al.* [24] found that 27 out of 71 meat samples (38%) obtained from UK retail outlets gave the expected polymerase chain reaction products when amplified with primers specific for the species from which the meat originated. 23 out of 172 (13.4%) ELISA positive sheep samples were positive for PCR. Higher PCR result in sheep (67.7%) was obtained by Ghoneim *et al.* [10].

In the present study, 9 (26.5%) and 17 (35.4%) of the examined ELISA positive 34 aborted women and 48 asymptomatic occupational personnel were PCR positive for *Toxoplasma*. Ghoneim *et al.* [10] reported PCR positivity of 32.2 and 27.6% in pregnant and aborted women, respectively.

In the present study, all ELISA IgG results in human and animals were higher than those of PCR. This indicates that the presence of *Toxoplasma*-specific antibodies was an insufficient criterion for identifying *Toxoplasma* infection. Accordingly, some women will be falsely identified as being infected and undergo unnecessary diagnostic amniocentesis and anti parasitic treatment.

In conclusion, the present study showed that serological tests can detect higher rate of toxoplasmosis than PCR, so ELISA combined with the PCR technique is a recommended tool for accurate diagnosis of Toxoplasmosis.

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