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Molecular and serological prevalence of *Toxoplasma gondii* in pregnant women and sheep in Egypt

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ABSTRACT

Objective: To investigate molecular and serological prevalence of *Toxoplasma gondii* (*T. gondii*) in pregnant women and sheep in Egypt.

Methods: Blood samples collected from healthy 364 pregnant women and 170 sheep were investigated for *T. gondii* antibodies and parasitemia using highly specific and sensitive surface antigen (*Tg*SAG2) based enzyme linked immunosorbent assay (ELISA) and real time-polymerase chain reaction (RT-PCR).

Results: Overall prevalence of *T. gondii* was 51.76%, 17.65% in sheep, 33.79%, 11.81% in pregnant women, using ELISA and RT-PCR respectively. Significant differences in *T. gondii* prevalence were observed on the basis of contact with cats or soil in pregnant women using either RT-PCR or ELISA. In pregnant women, a significant increase was detected in aged and those eating under-cooked mutton using simultaneous ELISA/RT-PCR.

Conclusions: Consumption of under-cooked infected mutton is an important source of human infection and the combination of the two assays provide accurate and precise data during infection.

1. Introduction

Toxoplasmosis is a zoonotic disease spread worldwide and caused by intracellular protozoan parasite *Toxoplasma gondii* (*T. gondii*) that infects humans and wide range of animals [1,2]. In the host, as it caused abortion, it could result in significant reproductive failures, economic and public health problems, since consumption of infected meat could facilitate zoonotic transmission [3]. In sheep, *T. gondii* infection could result in stillbirth, miscarriage and neonatal mortality [4,5]. In human, this disease is asymptomatic in healthy individuals during primary infection. On the other hand, toxoplasmosis can be fatal for congenitally infected fetus and immunodeficient patients (transplant recipients or HIV-infected patients), as a result of either reactivation of the parasite or acute recent infection [6–8]. *T. gondii* become very important during pregnancy, especially when occurred with early gestational age, as it can spread to the developing fetus across the placenta and thereby cause hydrocephalus, intracranial calcifications, chorioretinitis, and even stillbirth [9–11].

In Egypt, antibodies against *T. gondii* have been detected in pregnant women using the IgG avidity assay, indirect hemagglutination test (IHAT), direct agglutination test, indirect immunofluorescence test [12,13], enzyme linked fluorescent assay [14,15], latex agglutination test and enzyme linked immunosorbent assay (ELISA) [16–18]. Moreover, *T. gondii* has been identified in sheep by microscopy [19], IHAT, Latex agglutination test, modified agglutination test, ELISA, indirect immunofluorescence test [20–23]. Proper diagnosis of the zoonotic diseases through sensitive and specific surveillance is required to monitor and improve the public health status. An epidemiological prevalence using *T. gondii* specific surface antigen (*Tg*SAG2) based ELISA/real time-polymerase chain reaction (RT-PCR) approach to detect toxoplasmosis in important hosts like sheep and pregnant women will therefore provide highly desirable data for adequate control and prevention of the disease in Egypt.

Previous reports considered the consumption of under-cooked infected meat products as the main risk and suspected mutton, to be a major source of human infection [1,24–26]. Mutton is traditionally consumed throughout Egypt [27].

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Considering the big distribution of sheep in the study regions and the zoonotic burden of *Toxoplasma* infection, the current study aimed to investigate *T. gondii* prevalence in sheep and pregnant women in Menoufia and Gharbiya Provinces of Egypt through cross-sectional prevalence depend on specific ELISA and RT-PCR.

2. Materials and methods

2.1. Ethical statement

The current study was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice and approved by the Review Board of the National Liver Institute, Menoufia University, Egypt (approval number 00109/2015). The procedures and purpose involved in the current study were explained and written informed consent was obtained from all participants in this study. Moreover, the sheep sampling was started after consent of the Institutional Animal Ethical Committee, Menoufia University, Egypt (approval ID: MUFS/F/IM/1/15).

2.2. Parasite genomic DNA and TgSAG2t antigen

Parasite genomic DNA, *T. gondii*, RH strain, the glutathione *S*-transferase (GST) and GST-*Tg*SAG2t were received as gift from Prof. Dr. Xuenan Xuan and Dr. Yoshifumi Nishikawa, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan, and were used in RT-PCR and ELISA, respectively.

2.3. Sample collection and questionnaire

The sampling was carried out during January–December, 2015. Blood samples were collected from the brachial vein of 364 pregnant women, (20–35 years of age, 6–18 wk of gestation). A structured questionnaire was used to assess risk factors, which included such as age, residential area, pregnancy status, stage of pregnancy, previous abortion, contact with cats, contact with other animals, and consumption of undercooked mutton, and exposure to soil. Based on locations human and sheep samples were obtained from Menoufia and Gharbiya provinces in the Delta of Egypt. Blood samples were collected from the jugular vein by local veterinary practitioners from 170 sheep obtained from public markets (with consideration of sex). Sheep from 1 to 4 years old were divided into two groups based on their age, young sheep (2 years or less) and aged sheep (more than 2 years). Each blood sample was divided into two tubes, one was mixed with EDTA and the other was permitted to clot. Blood was incubated at room temperature for 1 h, and then centrifuged at 1 000 × *g* for 10 min, and the serum was collected and stored at −20 °C for further use.

2.4. ELISA

ELISA was done according to the modified procedure described previously [25,28,29]. The recombinant antigens (GST-*Tg*SAG2t, or GST, 5 μg/mL) in a coating buffer (50 mM carbonate/bicarbonate) were coated in the 96 well plates and incubated overnight at 4 °C. The plates were washed one time with washing buffer (phosphate buffer saline plus 0.05% Tween 20), blocked with blocking solution (phosphate buffer saline plus 3% skim milk) at 37 °C for 2 h. After washing the plates one time with washing solution, 50 μL of serum diluted one hundred times in blocking solution was added to duplicate wells for each sample and kept at 37 °C for 1 h. Then the plates were washed six times and incubated with 50 μL of horseradish peroxidase-conjugated rabbit anti-sheep IgG – H&L (Abcam, Cambridge, MA, USA), and horseradish peroxidase-conjugated goat anti-human IgG (Sigma, St. Louis, MO, USA) diluted in blocking solution four thousand times per well at 37 °C for 1 h. After washing six times, the 96 well plates were incubated with one hundred microliter substrate 2,2′-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid (ABTS)] in an ABTS buffer solution (0.1 M citric acid, 0.2 M sodium phosphate) per each well at room temperature for 1 h. The absorbance was detected at 405 nm using a microplate reader (Seac, Radim Company, Italy). The ELISAs data were calculated on the base of the mean optical densities at the value of 405 nm (OD_{405}) for the recombinant antigen (GST-*Tg*SAG2t) subtracted from those of the GST protein. The cutoff values were estimated as the OD_{405} value for *T. gondii* negative sera plus three standard deviations; which were 0.096 and 0.039 in sheep and pregnant women sera, respectively, ($n = 20$). The negative sera from sera stock were tested and confirmed negative by western blot and RT-PCR of its corresponding whole blood samples.

2.5. DNA isolation and real time-PCR

DNA was extracted from the whole blood samples and chemically purified by phenol-chloroform extraction and ethanol precipitation [30]. Amplification of *Toxoplasma* DNA was done using designed primers specific for the *T. gondii B1* gene (5′-AAC GGG CGA GTA GCA CCT GAG GAG A-3′ and 5′-TGG GTC TAC GTC GAT GGC ATG ACA AC-3′) that widely occurred in all strains the parasite [31]. The reaction mixture (20 μL) contained 1 × SensiFAST SYBR Lo-ROX Mix (SensiFAST™ SYBR Lo-ROX Kit, Bioline, France), 3 mM $MgCl_2$, 0.5 μ moles of each primer and 50 ng of genomic DNA was prepared. Amplification was performed by following a standard protocol recommended by the manufacturer (2 min at 50 °C, 2 min at 95 °C, then 40 cycles of 95 °C for 5 s, 60 °C for 10 s and 72 °C for 10 s). Amplification, data acquisition, and data analysis were performed using an ABI7500 Fast Real-Time PCR System (AB Applied Biosystems), and cycle threshold values were exported to Microsoft Excel for further analysis. Melting curve analyses were utilized to confirm positive sample specificity of each amplified PCR product. Positive and negative controls were used with each PCR run.

2.6. Assays agreement percentage calculation

Agreement between recombinant protein *Tg*SAG2 based ELISA and RT-PCR was calculated according to Ibrahim *et al.* [28,29].

$$\text{The percentage of agreement between the RT}-\text{PCR assay and ELISA} = \text{O}/\text{T} \times 100.$$

where the output (O) that represents the agreement between the two tests (ELISA & RT-PCR) in both the positivity and the

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