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Original Article

# Seroprevalence and epidemiology of *Toxoplasma gondii* in farm animals in different regions of Egypt

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ABSTRACT

Toxoplasmosis is a cosmopolitan protozoan disease that has been recorded in a wide range of vertebrate hosts, including humans. In response to the paucity of available data, this study was undertaken to comprehensively establish the seroprevalence of *Toxoplasma gondii* among various farm animals in different localities of Egypt. The latex agglutination test and TgGRA7-based enzyme-linked immunosorbent assay were used to screen the investigated animals for anti-*T. gondii* IgG antibodies. When only samples with simultaneously positive results for both the latex agglutination test and the TgGRA7-based ELISA were considered positive, 174 (26.7%) of 652 serum samples from different animals were seropositive. The prevalence of antibodies according to species was: sheep 38.7%, goats 28.7%, cattle 23.6%, and donkeys 22.6%. Thus, prevalence rate was significantly higher in sheep than in cattle or donkeys. The prevalence was also significantly higher in Kafr El Sheikh than in the other governorates investigated (Qena, Sohag, Minoufiya, and Matrouh). No significant differences were observed in age, sex, locality, or breeding system when evaluated as predisposing factors for *T. gondii* infection in cattle. In conclusion, this study demonstrates the high prevalence for *T. gondii*-specific antibodies among different animal species in southern and northern localities of Egypt, and provides valuable new data on the prevalence of *T. gondii* in donkeys, which are used as a food for carnivorous animals, particularly in the feline family, at Giza Zoo, Egypt.



## 1. Introduction

*Toxoplasma gondii* is a protozoan parasite that infects virtually all warm-blooded animals, including humans, livestock, birds, and marine mammals. Sheep, goats, and cattle are intermediate hosts of *T. gondii*, and are infected by the ingestion of food or water contaminated with oocysts shed by cats. The raw or undercooked meat from these animals is potentially hazardous if ingested by humans or other animals (Dubey, 2010). Toxoplasmosis has a severe economic impact on the sheep and goat industries because it induces abortion, still birth, and neonatal losses (Tenter et al., 2000).

Previous studies that estimated the seroprevalence of anti-*T. gondii* antibodies in Egypt focused predominantly on human surveillance. These studies have shown that 59.6% of asymptomatic blood donors (Elsheikha et al., 2009), 51.5% of pregnant women (Ibrahim et al., 2009), 67.5% of pregnant women (El Deeb et al., 2012) and 46.1% of women suffering spontaneous abortion (Tammam et al., 2013) were seropositive, and that 45.8% and 41.4% of these pregnant and nonpregnant women, respectively, had been in contact with animals (Ghoneim et al., 2010). These results imply the strong presence of *T. gondii* in Egypt, which may present a risk to pregnant women.

In farm animals in Egypt, anti-*T. gondii* antibodies were detected in 10.8% of the cattle sera tested by enzyme-linked immunosorbent assay (ELISA)-based on truncated surface antigen 2 (TgSAG2t) (Ibrahim et al., 2009), and in 43.7% or 41.7% of sheep sera, when a modified agglutination test or ELISA was used, respectively (Shaapan et al., 2008), and in 98.4% of

*Abbreviations:* HRP, horseradish peroxidase; iELISA, indirect enzyme-linked immunosorbent assay; IgG, immunoglobulin G; LAT, latex agglutination test; MAT, modified agglutination test; PBS, phosphate buffered saline; PBS-T, Tween 20 formulated in PBS; TgGRA7, dense granule protein 7 of *Toxoplasma gondii*; SM, skim milk.

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sheep and 41.7% of goats when an ELISA was used (Ghoneim et al., 2010). A high seroprevalence of 65.6% was recorded in donkeys (El-Ghaysh, 1998) and 48.1% in horses (Ghazy et al., 2007). Anti-*T. gondii* antibodies were detected in 17.4% of 166 camels (Hilali et al., 1998). When poultry were tested, 47.2% of chickens, 59.5% of turkeys, and 50% of ducks were positive for anti-*T. gondii* antibodies (El-Massry et al., 2000), and in another study, 40.4% of chickens and 15.7% of ducks (Dubey et al., 2003). Therefore, in Egypt, the seroprevalence of *T. gondii* antibodies is high, not only in highly susceptible animals such as sheep and goats, but also among other animals, such as cattle, donkeys, horses, camels, and domestic birds. Among farm animals in Egypt, sheep and goats are considered the most highly susceptible hosts of toxoplasmosis. The high rate of transmission from dams to offspring, remarkable fetal losses and abnormalities, and the high viability of cysts in the meat of infected animals are characteristic of *T. gondii* infections in sheep and goats (Buxton, 1998; Buxton et al., 2007; Dubey, 2009; Innes et al., 2009). However, *T. gondii*-infected cattle and donkeys are of negligible importance because the infections are not clinically significant in these animals and they have no severe complications. However, specific anti-*T. gondii* antibodies have been detected in serum samples and parasite DNA has been detected in the meat and milk from these infected cattle and donkeys, so they play an important role in the epidemiology of the infection (Dubey, 1986; Dubey and Thulliez, 1993, Opsteegh et al., 2011; Alvarado-Esquivel et al., 2015).

The latex agglutination test (LAT) is widely used as a reference test for the seroprevalence of toxoplasmosis in different animal species (Matsuo and Husin, 1996; Shahiduzzaman et al., 2011; Kyan et al., 2012; Matsuo et al., 2014). However, the TgGRA7-based ELISA shows higher potency, sensitivity, and specificity than other reference serodiagnostic tests, including LAT, the direct agglutination test, the modified agglutination test, and the indirect fluorescent antibody test, which are used to detect anti-*T. gondii* antibodies in serum samples from different animals (Terkawi et al., 2013; Wang et al., 2014a, 2014b; Gu et al., 2015; Ichikawa-seki et al., 2015). The main aim of this study was to establish a comprehensive record of the seroprevalence of *T. gondii*-specific antibodies in Egypt using several animal hosts at different locations and to identify the risk factors associated with toxoplasmosis, using a cross-sectional epidemiological study. Moreover, LAT and TgGRA7-based ELISA were used for further field validation of these detection systems against various animal species in Egypt.

## 2. Materials and methods

### 2.1. Animals and geographic distributions

Serum samples ($n = 652$) were collected in the period between May 2014 and June 2015. Cattle ($n = 301$), sheep ($n = 111$), goats ($n = 94$), and donkeys ($n = 146$) from different geographic locations in Egypt were screened for anti-*T. gondii* antibodies in this study. The availability of sampling animals with the adequate relevant data and the cooperation of animal owners determined the current animal grouping and distribution in this study. The cattle were divided into four groups: group 1 - randomly sampled male and female cattle of different ages from individual owners (less than five cattle per owner) and smallholder farms (5–20 cattle per farm), in different villages in the Qena governorate; group 2 - adult cows (over 3 years of age) that were bred in an intensive farming system (>2000 cattle) in Qena governorate; group 3 - adult bulls (over 3 years of age) that were admitted to the Qena slaughter house from individual owners and smallholder farms; and group 4 - randomly sampled cattle of different ages and genders from individual owners and smallholder farms from different villages in the Sohag governorate. Because serum samples from investigated governorates except cattle samples from Qena were collected from animals from individual owners and smallholder farms located in a limited geographical area with similar environmental and husbandry conditions, they were categorized as one group. The data for the different ages, sexes, breeding systems, and localities of the cattle sampled were used in a risk factor analysis for *T. gondii* infection.

The governorates investigated in this study are representative of all the regions in Egypt, including diverse climatic and ecological features. Qena and Sohag are located in the southern part, characterized by hot and dry weather. Giza and Minoufiya are in the middle region, where the weather is usually humid and temperate. Kafr El Sheikh, in the far northern area, is located in a coastal region and the weather is humid, rainy, and temperate for most of the year. Although all the governorates investigated are rural areas, Matrouh is a coastal semi-desert area, with predominantly heavy rains and cold climate in the winter, but dry and temperate weather in the summer. Details of the animal species investigated, their locations, and the numbers of samples collected are shown in Table 1 and Fig. 1.

### 2.2. Blood sampling

Blood samples were collected from each animal in the field with venal puncture, into glass tubes without anticoagulant. These samples were kept in an icebox, then sent to the laboratories at University of South Valley for samples of Qena and Sohag, University of Cairo for samples of Giza Zoo, University of Sadat City for samples of Minoufiya and Matrouh and University of Kafr El Sheikh for samples of Kafr El Sheikh. These serum samples were centrifuged to harvest the sera and kept at −20 °C until used.

### 2.3. Latex agglutination test (LAT)

The sera were tested with LAT to detect *T. gondii* infections using Toxocheck-MT (Eiken Chemical, Tokyo, Japan), according to the manufacturer's instructions. Samples were considered positive when agglutination was observed at a dilution of 1:32.

### 2.4. Recombinant protein expression

The recombinant TgGRA7 was expressed with previously described methods (Terkawi et al., 2013), with slight modifications. The purity and quantity of the proteins were confirmed with the detection of single bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by staining with Coomassie Brilliant Blue R250 (MP Biomedicals Inc., Illkirch-Graffenstaden, France). The protein concentration was measured with a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc., Rockford, IL, USA).

### 2.5. Indirect ELISA (iELISA)

Purified antigen (50 μl) at a final concentration of 0.1 μM was coated onto ELISA plates (Nunc, Roskilde, Denmark) overnight at 4 °C in a carbonate–bicarbonate buffer (pH 9.6). The plates were washed once with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T) and blocked with PBS containing 3% skimmed milk (PBS-SM) for 1 h at 37 °C. The plates were washed once with PBS-T, and 50 μl serum samples, diluted 1:100 with PBS-SM, were added to the wells. The plates were incubated at 37 °C for 1 h. After the plates were washed six times with PBS-T, they were incubated with HRP-conjugated anti-

**Table 1**
Geographic distributions and numbers of animal samples tested in this study.

| Geographical regions | Sampling area | Cattle | Sheep | Goat | Donkey |
|---|---|---|---|---|---|
| Southern region | Qena | 225 | 37 | 27 | – |
| | Sohag | 76 | – | – | – |
| Middle region | Giza | – | – | – | 58 |
| | Minoufiya | – | 28 | 37 | 43 |
| Northern region | Kafr El Sheikh | – | 46 | 30 | – |
| North western region | Matrouh | – | – | – | 45 |
| Total number | | 301 | 111 | 94 | 146 |