Title

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

Running title

Toxoplasma gondii antibodies in farm animals in Egypt

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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.

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.

Keywords:

Toxoplasma gondii; Toxoplasmosis; Seroprevalence; 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, at 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 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 (more than 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 Figure 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-IgG antibody of the corresponding species (Bethyl Laboratories, Montgomery, TX, USA), diluted 1:4000 for the sheep, goat, and cattle sera and 1:6000 for the donkey sera with PBS-SM and incubated at 37 °C for 1 h. The plates were washed six times and 100 µl of substrate solution (0.1 M citric acid, 0.2 M sodium phosphate, 0.003% H_2O_2, 0.3 mg/ml 2′,2-azino-bis[3-ethylbenzothiazoline-6 sulfonic acid]); Sigma-Aldrich) was added to each well. After incubation for 1 h at room temperature, the absorbance at 405 nm (A_{450}) was measured with an Infinite® F50/Robotic ELISA reader (Tecan Group Ltd, Männedorf, Switzerland). The cut-off point was determined as the mean A_{405} value for standard *Toxoplasma*-negative sera

kept in our laboratory (cattle, n = 10; sheep, n = 5; goat, n = 5; donkey, n = 10) plus three standard deviations. The negative serum samples were confirmed with both LAT and the TgGRA7-based iELISA.

2.7. Statistical analysis

The significance of the differences in the prevalence rates of different species and risk factors was analyzed with a chi-square (χ^2) test. A P value of < 0.05 was considered statistically significant. The kappa values, specificity, sensitivity, and 95% confidence intervals were calculated with www.vassarstats.net. The strength of agreement was graded with kappa values of fair (0.21–0.40), moderate (0.41–0.60), and substantial (0.61–0.80). χ^2 values and odds ratios were calculated with the GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA).

Results

In total, 652 animal sera were tested in a comprehensive survey of specific anti-*T. gondii* antibodies in different localities in Egypt among cattle, sheep, goats, and donkeys. The overall seropositive rate in all investigated animals was 26.7%. The seroprevalence among sheep was 47.8%, 51.4%, and 38.8% according to LAT, iELISA, and both tests, respectively, and the highest positive rates were in Kafr El Sheikh, at 69.6%, 69.6%, and 56.5%, respectively (Table 2). In goats, the overall seroprevalence was 35.1%, 39.4%, and 28.7% according to LAT, iELISA, and both tests, respectively, which were lower than those in sheep (Table 3). The highest seropositive rates for goats were also in Kafr El Sheikh (66.7%, 66.7%, and 60.0% according to LAT, iELISA, and both tests, respectively). The prevalence of anti-*T. gondii* antibodies in less susceptible hosts, such as donkeys and cattle, were 22.6% and 23.6%, respectively, in the animals positive on both LAT and iELISA (Tables 4 and 5).

To examine the predisposing factors for *T. gondii* infection, the animal species, locality, and climatic conditions were analyzed. The seroprevalence rates were significantly higher in sheep than in cattle or donkeys (Table 6). The seropositive rates in Kafr El Sheikh were higher than those in other locations (Table 6). However, no obvious difference was observed according to climate (Table 6). The influence of age (< 3 and > 3 years of age), sex (cow or bull), location (Qena or Sohag), and type of breeding (individual breeding or mass farming system) were analyzed to identify the potential risk factors for *T. gondii* infection in cattle in the southern part of Egypt (Table 7). The seroprevalence of anti-*T. gondii* antibodies did not differ significantly between animals of different ages, sexes, localities, or breeding systems (Table 7).

We compared the efficacy of the TgGRA7-based iELISA with that of LAT (reference test) to validate the iELISA (Table 8). The recorded kappa values were 0.645, 0.682, 0.649,

and 0.726 for sheep, goats, cattle, and donkeys, respectively. Therefore, there was substantial agreement between the TgGRA7-based iELISA and LAT in all the animals tested. High levels of sensitivity, specificity, and concordance were also observed for all the tested animals.

3. Discussion

Cattle, sheep, goats, and donkeys are the main components of the agricultural sector in Egypt, and are used for different purposes. Cattle, sheep, and goats are the main sources of animal protein and play a crucial role in maintaining the food security and health status of Egypt. Our study provides the first record of the serological prevalence of *T. gondii* antibodies in Qena, Sohag, and Matrouh governorates for all the animals investigated.

We demonstrated a high prevalence of *T. gondii* infection among all investigated animals in the south, middle and the far north regions of Egypt, indicating that the distribution of toxoplasmosis is ubiquitous across the Egyptian governorates. This result might be attributed to the frequent transportation of animals between different locations and the relatively similar condition for animal husbandry and environment across the Egyptian regions. The significantly higher prevalence rates of anti-*T. gondii* antibodies in the Kafr El Sheikh governorate than in the other governorates might be attributable to its highly favorable environmental conditions for the development of oocysts, particularly its humidity and temperature (Dubey, 1998), because as a coastal agricultural region, this governorate has humid temperate weather for most of the year. Kafr El Sheikh is also famous for its large animal populations, which are bred in large groups.

We also present valuable data on the sera collected from donkeys, which are slaughtered and used as food for carnivorous animals at Giza Zoo, the largest and oldest zoo in Egypt. Because at least 27.6% of the donkey sera tested were positive for *T. gondii* antibodies, the meat of the donkeys supplied to the zoo animals might be an important source of infection. The transmission of *T. gondii* infection via this route may also facilitate human infections through the handling of meats from infected animals. Stray cats and rodents exposed to the infected meats might be another source of infection.

The highest seroprevalence rates were observed in sheep and goats, rather than in cattle or donkeys, indicating that they are susceptible hosts of *T. gondii* infection. A further epidemiological investigation was conducted in cattle to assess whether age, sex, locality, or breeding system is a predisposing factor for *T. gondii* infection. Although the greatest difference was reported between the cattle sera from an individual breeding system (25.6%) and from intensive farming systems (18.8%), it was not significant. These results are similar to those reported in previous studies (Klun et al., 2006; Qiu et al., 2012; Zhou et al., 2012), although Berger-Schoch et al. (2011) and Lopes et al. (2013) reported that the age of the animal is a risk factor for *T. gondii* infection.

We compared our results with previous reports of the seroprevalence of *T. gondii* infection in Egypt. Ghoneim et al. (2010) demonstrated 98.4% and 41.7% seroprevalence in sheep and goats, respectively, in the Al-fayium governorate in the middle region of the country using an ELISA, which are higher than those in our study. In Dakahlia, nearby governorate to Minoufiya, Younis et al., (2015) revealed that 41.7% and 62% for sheep, 49.4% and 50.6% for goat and 44.3% and 68.4% for donkey as seropositive samples using LAT and ELISA, respectively. Ibrahim et al. (2009) reported 10.7% seropositive cattle in Sharkia in northern Egypt using a TgSAG2t-based ELISA, whereas our study showed 23.6% seropositive cattle in southern Egypt with both LAT and the TgGRA7-based ELISA. In the study of El-Ghaysh (1998), 65.6% of donkeys from Minoufiya were seropositive when tested with ELISA, indicating a higher infection rate than we observed (22.6%) and 45.0% positive reported by Haridy et al., (2010). These variations might be attributable to differences in the times and places of sampling, the numbers of animals tested, and/or the detection systems used.

In the same context, regional and global comparison of our results for *T. gondii* prevalence was additionally applied for more comprehensiveness of this study. Regarding the

prevalence in sheep, 38.74% seropositive, highest prevalence rate among screened animals in our study, was lower than 57.5% seropositive using LAT in Sudan (Khalil and Elrayah, 2011) and 53.3% seropositive in Brazil using modified agglutination test (MAT) (Cosendey-Kezenleite et al., 2014). On the other hand, 23.4% seropositive using LAT in Saudia Arabia (Sanad and Al-Ghabban, 2007) and 17.8% seropositive by indirect haemagglutination test in Kuwait (Alazemi 2014) showed a markedly lower prevalence than we recorded. Dumetre et al., (2006) showed 38% seropositive using direct agglutination test in France, which is similar result to our study. For seroprevalence in goat, lower prevalence rates in China (17.6%, indirect haemagglutination test, Zou et al., 2015) in Myanmar (11.4%, LAT, Bawm et al., 2016) were reported compared with our result (28.7% seropositive). Otherwise, Hamilton et al., (2014) recorded markedly higher seropositive rates using ELISA in Dominica and Grenade of Caribbean islands as 58% and 57% seropositive, respectively. The 23.6% seropositivity against *T. gondii* in cattle of this study was higher than the seropositivity 9.4% by LAT and 17% by ELISA in Thailand (Inpankaew et al., 2010), 13.3% by ELISA in Sudan (Elfahal et al., 2013), 7.3% by LAT in Japan (Matsuo et al., 2014) and 7.4% by TgGRA7-based ELISA in Indonesia (Ichikawa-Seki et al., 2015). Compared with our result, higher seropositive was observed in Sudan using LAT as 32% (Khalil and Elrayah, 2011). Except for ruminant animals, the seropositive rate in donkeys in this study was 22.6%, which was markedly higher than that in different regions of the world such as 8% using LAT in Italy (Machacova et al., 2013), 6.4% using MAT in United States of America (Dubey et al., 2014), 10.9% using MAT in Mexico (Alvarado-Esquivel et al., 2015). A similar result with our study was seen in northeastern China as 23.6% seropositive using MAT (Yang et al., 2013) and markedly higher seropositive was reported in Brazil as 43.3% seropositive using indirect fluorescent antibody test (de Oliveira et al., 2013). Collectively, all these data

concerning the seroprevalence of *T. gondii* in different animal species in multiple regions indicates the worldwide distribution of *T. gondii* infection and toxoplasmosis in farm animals.

Although LAT is widely used as a reference test (Matsuo et al. 2014) and the iELISA based on TgGRA7 has been validated for the serodiagnosis of toxoplasmosis in different countries throughout the world (Gu et al., 2015; Ichikawa-seki et al., 2015; Wang et al., 2014a, 2014b), further field validation studies are required to confirm the success of both tests in most animal species and in different regions of the world. Our study is the first to use the TgGRA7-based iELISA to examine the seroprevalence of *T. gondii* antibodies in Egypt. As shown in Table 8, the high performance of LAT and the TgGRA7-based iELISA was confirmed, with reliable kappa values and concordance, indicating their excellent potential for use in seroprevalence studies in the field. Given the high serological prevalence of *T. gondii* in several animals and at different localities in Egypt, specialists must establish new control and preventive policies to reduce the risk of toxoplasmosis in both the medical and veterinary contexts.

Conflict of interest

All the authors declare that they have no financial or personal conflicts that could improperly influence their contributions to this study

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Figure legend

Fig. 1: Geographic distribution of the sampling sites in Egypt used in this study. Dark-colored areas with different numbers indicate the investigated governorates. 1 Qena, 2 Sohag, 3 Giza, 4 Minoufiya, 5 Kafr El Sheikh, and 6 Matrouh.

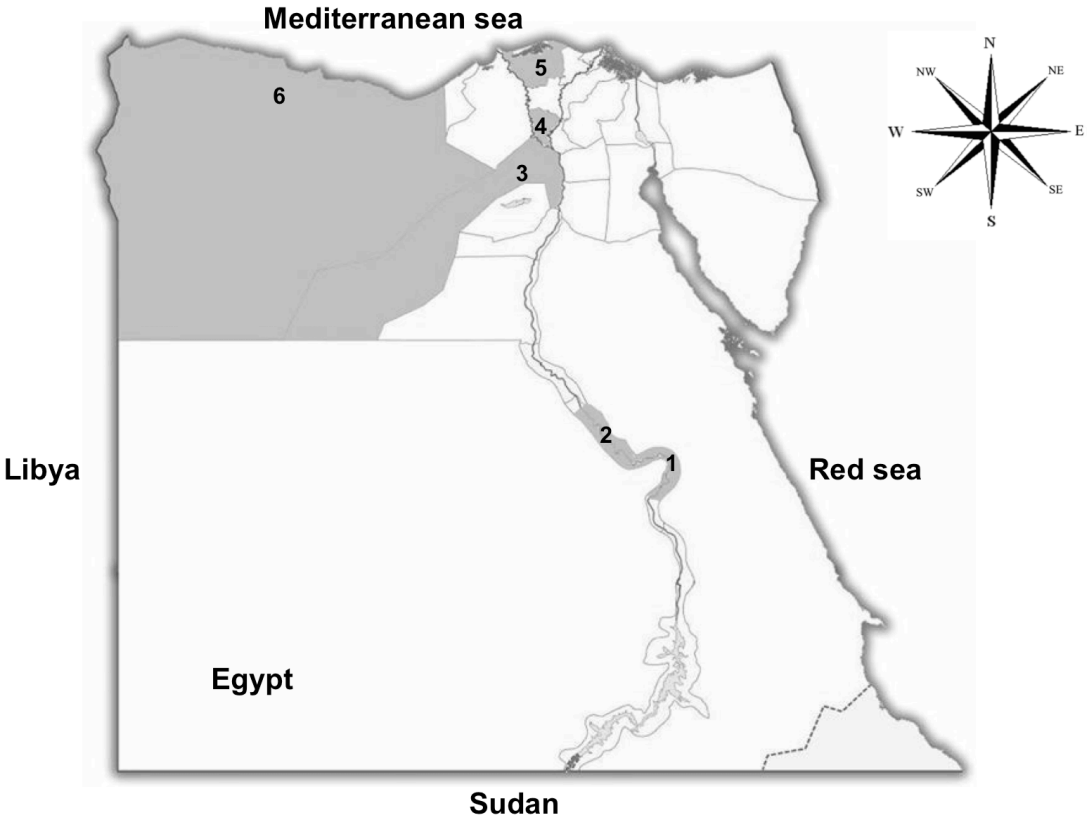

Table 1

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

Table 2

Seroprevalence of *T. gondii* in sheep in different governorates of Egypt

Governorate	LAT		ELISA		LAT/ELISA positive	
	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)
Qena	37	18 (48.7)	37	21 (56.8)	37	16 (43.2)
Kafr El Sheikh	46	32 (69.6)	46	32 (69.6)	46	26 (56.5)
Minoufiya	28	3 (10.7)	28	4 (14.3)	28	1 (3.6)
Total	111	53 (47.8)	111	57 (51.4)	111	43 (38.7)

LAT = Latex agglutination test, CI = Confidence interval

LAT/ELISA positive = samples that were simultaneously positive on both tests

95% CI calculated according to method descibed by (http://vassarstats.net/)

Table 3

Seroprevalence of *T. gondii* in goats in different governorates of Egypt

Governorate	LAT		ELISA		LAT/ELISA positive		
	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	95% CI
Qena	27	10 (37.0)	27	13 (48.2)	27	7 (25.9)	11.8 - 46.6
Kafr El Sheikh	30	20 (66.7)	30	20 (66.7)	30	18 (60)	40.7 - 76.7
Minoufiya	37	3 (8.1)	37	4 (10.8)	37	2 (5.4)	0.9 - 19.5
Total	94	33 (35.1)	94	37 (39.4)	94	27 (28.7)	20.1 - 39.1

LAT = Latex agglutination test, CI = Confidence interval

LAT/ELISA positive = samples that were simultaneously positive on both tests

95% CI calculated according to method descibed by at http://vassarstats.net/

Table 4

Seroprevalence of *T. gondii* in donkeys in different governorates of Egypt

Governorate	LAT		ELISA		LAT/ELISA positive		
	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	95% CI
Giza	58	16 (27.6)	58	22 (37.9)	58	16 (27.6)	17 - 41.1
Minoufiya	43	13 (30.2)	43	11 (25.6)	43	11 (25.6)	14 - 41.4
Matrouh	45	10 (22.2)	45	9 (20)	45	6 (13.3)	5.5 - 27.4
Total	146	39 (26.7)	146	42 (28.8)	146	33 (22.6)	16.2 - 30.4

LAT = Latex agglutination test, CI = Confidence interval

LAT/ELISA positive = samples that were simultaneously positive on both tests

95% CI calculated according to method descibed at http://vassarstats.net/

Table 5

Seroprevalence of *T. gondii* in cattle in different governorates of Egypt

Cattle group	LAT		ELISA		LAT/ELISA positive		
	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	95% CI
Group1 Qena	100	34 (34)	100	31 (31)	100	29 (29)	20.5 - 39
Group2 Qena	90	22 (24.4)	90	23 (25.6)	90	17 (18.9)	11.7 - 28.8
Group3 Qena	35	10 (28.6)	35	11 (31.4)	35	9 (25.7)	13.1 - 43.5
Group4 Sohag	76	22 (29.0)	76	20 (26.3)	76	16 (21.1)	12.8 - 32.2
Total	301	88 (29.2)	301	85 (28.2)	301	71 (23.6)	18.9 - 28.8

Group 1, 2, 3 were collected from Qena governorate but from different locations.

LAT = Latex agglutination test, CI = Confidence interval

LAT/ELISA positive = samples that were simultaneously positive on both tests

95% CI calculated according to method descibed by at http://vassarstats.net/

Table 6

Analysis of the influence of animal species, localities and climates on the distribution of *T. gondii* in Egypt

Analyzed factor	No. of tested	No. of positive (%)	OR (95% CI)	*P -value*
Animal species				
Sheep	111	43 (38.7)		
Goat	94	27 (28.7)	1.56 (0.87-2.81)	0.13
Cattle	301	71 (23.6)	2.04 (1.28-3.26)	0.002
Donkey	146	33 (22.6)	2.16 (1.25-3.73)	0.004
Localities				
Qena	289	78 (27.0)	3.71 (2.2-6.28)	<0.0001
Sohag	76	16 (21.1)	5.15 (2.52-10.54)	<0.0001
Giza	58	16 (27.6)	3.3 (1.73-7.52)	<0.0004
Minoufiya	108	14 (13.0)	9.23 (4.48-19.02)	<0.0001
Kafr El Sheikh	76	44 (57.9)		
Matrouh	45	6 (13.3)	8.93 (3.37-23.64)	<0.0001
Climate				
Hot and dry	365	94 (25.8)	0.897 (0.633-1.27)	0.54
Temperate and humid	287	80 (27.9)		

OR = Odds ratio, CI = Confidence interval

χ2 test was used to detect the difference between variables

χ2 test and odds ratio were calculated by GraphPad Prism 5 software

Table 7

Univariable analysis of risk factors associated with *T. gondii* infection in cattle in Egypt

Analyzed factor	No. of tested	No. of positive (%)	OR (95% CI)	*P -value*
Age				
< 3 years old	61	16 (26.2)	1.19 (0.62-2.27)	0.58
> 3 years old	240	55 (22.9)		
Gender				
Male	61	16 (26.2)	1.19 (0.62-2.27)	0.58
Female	240	55 (22.9)		
Location				
Qena	225	55 (24.4)	0.98 (0.52-1.84)	1
Sohag	76	16 (21.1)		
Breeding				
Individual	211	54 (25.6)	1.47 (0.8-2.72)	0.2
Mass Farming	90	17 (18.8)		

OR = Odds ratio, CI = Confidence interval

χ2 test was used to detect the difference between variables

χ2 test and odds ratio were calculated by GraphPad Prism 5 software

Table 8

Specificity and sensitivity of TgGRA7-based iELISA in detecting IgG to *T. gondii* infection in the field animal sera compared with the reference test LAT

Animal	Kappa value	Sensitivity (%)	Specificity (%)	Concordance (%)	PPV (%)	NPV (%)
Sheep	0.645	83	83	83	75	88
Goat	0.682	82	88	86	75	92
Cattle	0.65	84	88	86	68	94
Donkey	0.726	85	91	90	75	95

PPV = positive predictive value

NPV = negative predictive value