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Seroprevalence and associated risk factors for Toxoplasma gondii in water buffaloes (Bubalus bubalis) in Egypt

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ABSTRACT

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii* and affecting all warm-blooded animals. The available data about the epidemiological situation of *T. gondii* in water buffaloes in Egypt are scarce. Thus, a cross-sectional study was conducted to determine the seroprevalence of *T. gondii* in water buffaloes in three Egyptian governorates and to evaluate the associated risk factors for the infection. A total of 430 sera samples were examined using commercial Indirect ELISA Multi-species kit. The overall seroprevalence rate of *T. gondii* in examined water buffaloes was 7.4 %, and the highest rate (9.3 %) was found in Kafr ElSheikh governorate. Multivariate logistic regression analysis showed that adult buffalo (OR = 7.10; 95 % CI: 0.87–57.68; P = 0.067) and small herds (OR = 8.42; 95 % CI: 1.07–66.02; P = 0.043) were more likely than young buffalo and large herds to become infected with *T. gondii*. Moreover, the risk of buffaloes contracting *T. gondii* infection was higher in winter and especially among animals contacted with cats. It is necessary to identify risk factors in order to determine what mitigation, control, and prevention strategies to implement in order to reduce, control, and prevent *T. gondii* infection in domestic animals, which will in turn reduce human infection with the disease.

1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan that infects humans as well as many other warm-blooded animal species including buffaloes; it has a negative impact on public health and animal productivity [1,2].

Domestic cats are the sole definitive hosts, whereas many other species act as intermediate hosts [3]. The susceptible hosts contract the parasite through contaminated water and meat with infective oocysts [4]. In addition, the oocyst-infected products are a common way for farm animals to become infected. *Toxoplasma gondii* is primarily transmitted to humans through ingestion of undercooked or raw meat, and meat products containing tachyzoites and bradyzoites [5,6].

Human infections with *T. gondii* are usually asymptomatic, but severe complications may occur if congenital infection occurred, such as stillbirth, mortality, abortion, and hydrocephalus, or retinochoroidal lesions that can cause chronic ocular disease and lymphadenopathy, retinitis, or encephalitis [7,8].

Toxoplasmosis causes economic losses in livestock, particularly goats and sheep, mostly owing to reproductive problems such as miscarriage, stillbirth, and the delivery of weak animals [9,10]. Toxoplasmosis has an economic impact includes treatment expenses as well as a loss in predicted output production [11]. Buffaloes are often thought to be resistant to clinical illness, hence studies only report serological evidence of spontaneous infection in these animals [12].

Several studies have been conducted to determine the seroprevalence and assess the risk factors for *T. gondii* infection in buffaloes, which varied mainly according to environmental condition [13]. The higher seroprevalence of *T. gondii* was associated with high relative humidity, rainfall, and temperature, these are excellent circumstances for sporulation of oocysts in the environment [14]. Moreover, the seroprevalence was higher in adult buffalo as compared to calves and juveniles,

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probably because older animals are more likely to be exposed to the parasite as age increased [15,16].

In Egypt several studies have been performed to determine the prevalence of *T. gondii* in human [17], equine [18], camels [19], cattle [20], and sheep and goats [21]. However, few studies have been conducted to evaluate the prevalence and assessment the risk factors associated with *T. gondii* infection in water buffaloes in Egypt.

Therefore, the present study aimed to evaluate the seroprevalence of *T. gondii* infection and its associated risk factors in water buffaloes from three Egyptian governorates.

2. Materials and methods

2.1 Study area

A cross-sectional study was conducted in Kafr ElSheikh, Gharbia, and Qalyubia governorates in the Nile Delta of Egypt, Fig. 1. Those governorates represent the most agricultural region because it situated between two branches of Nile and have largest and highest pastures in Egypt. In the Delta region, the temperature is highs rarely exceeding 35 °C in the summer and lows often around 12 °C in the winter. In addition, rainfall in the delta area averages between 100 and 200 mm per year, with the vast majority falling during the winter months.

2.2 Sample size and sampling

We calculated sample size using Thrusfield's formula, based on an expected prevalence of 50 %, 5 % precision, and 95 % level of confidence. As a result, the sample size was 384 but was raised to 430 to increase the precision. A total of 430 buffaloes from three Egyptian governorates were sampled for the seroepidemiological survey during January–December 2021. Blood samples were collected by aseptic puncture of the jugular vein on each of the selected properties using a random sampling method. After centrifugation of the samples for 10 min at 3000 × *g*, the sera were separated, labeled, and stored at − 20 °C for subsequent serological analysis.

2.3 Questionnaire

At the time of sampling, a questionnaire was created to collect data from each inspected animal. In accordance with the information gathered, animals were divided based on their age into three groups (2, 2–4, and > 4 years), sex (male and female), herd size (small, middle, and large), season (spring, summer, autumn, and winter), and presence of cats (yes or no).

2.4 Serological testing

Toxoplasmosis Indirect ELISA Multi-species kit (ID Screen, ID.VET. Innovative Diagnostics, Montpellier, France) was used to screen the sera for the presence of antibodies against *T. gondii* in water buffaloes. This ELISA kit has a sensitivity of 100 % and a specificity of 96 % according to the manufacturer data.

The 96-well plate was coated with P30 *T. gondii* antigen, and the antigen-antibody complex produced with the assistance of the peroxidase conjugate, which was added subsequently. The manufacturer provided positive and negative controls, which were utilized to validate each test. The data were represented as optical density (OD); absorbance was measured at 450 nm using AMR-100 ELISA Plate reader (AllSheng, China). The positivity % computed as following = 100 × $OD_{sample}/OD_{Positive\ control}$ according to manufacturer instruction. The samples were regarded positive if they had a value of ≥ 50 %, doubtful if it was between 40 % and 50 %, and negative if it was ≤ 40 %.

2.5 Statistical analysis

Microsoft Excel spreadsheets were used for data collection and analysis using SPSS software Ver. 24 (SPSS Inc, IBM, Chicago, Illinois, USA). Cross tabulation was used to examine the relation between risk variables and the dependent variable. Univariable logistic regression analysis and Chi-square tests were used to assess the strength of the correlation between risk variables and *T. gondii* infection. Multivariate logistic regression was applied for all variables with $P < 0.05$ in the univariate analysis [22–31]. The Hosmer and Lemeshow goodness-of-fit

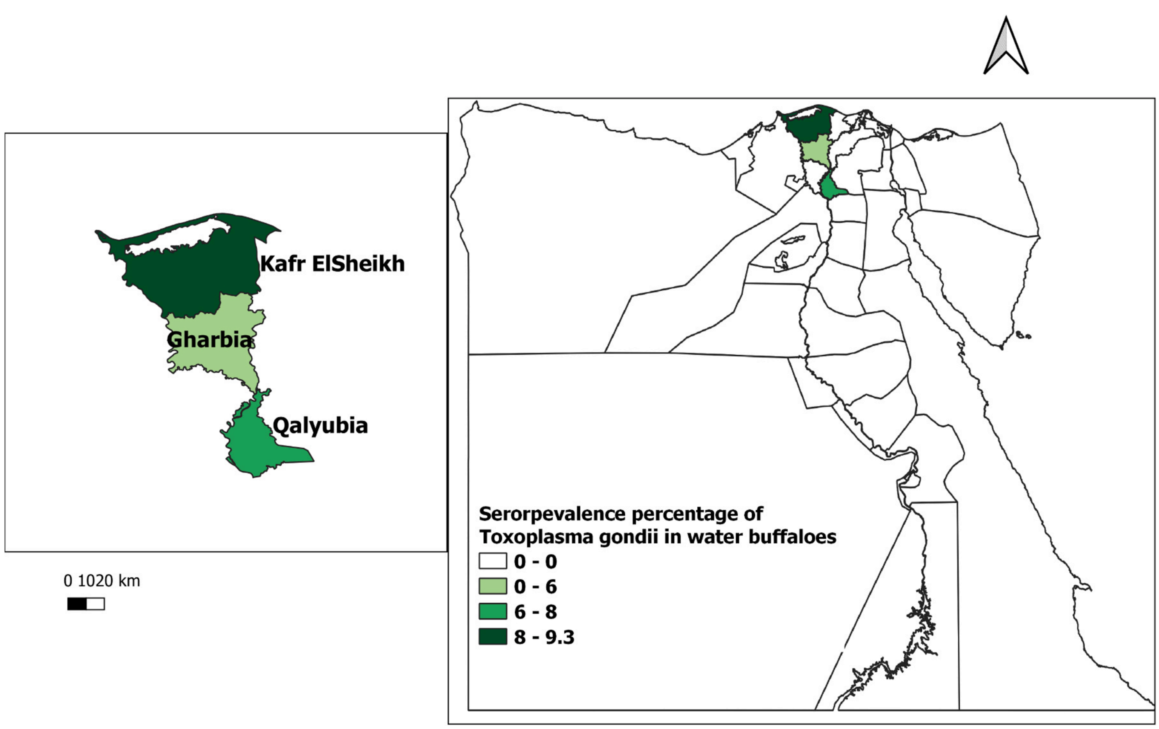

Fig. 1. Map showed the governorates under the study.

test was used to evaluate model fit.

3. Results

In general, antibodies against *T. gondii* were detected in 32 (7.4 %) of the 430 serum samples tested using ELISA test. The highest prevalence rate for *T. gondii* was found in water buffaloes raised in Kafr ElSheikh (9.3 %), while the lowest prevalence rate was recorded in Gharbia governorate (5.7 %). Furthermore, the prevalence rate in males was 2.9 %, while it was 7.8 % in females.

For calves under 2 years of age, only 1.9 % tested positive for *T. gondii* infection, but for buffaloes aged 2–4 years and over 4 years, it was 5.5 % and 13.6 %. According to the size of the herd, there was significant difference in the seroprevalence of *T. gondii* ($P = 0.009$); the small herd had the greatest prevalence rate (10.8 %), compared to the medium herd (3.8 %) and large herd (1.7 %), Table 1.

T. gondii seroprevalence varied significantly across seasons, The prevalence was higher in winter (13.6 %) and spring (8 %) than summer or autumn. Moreover, cats in contact with buffaloes increased the prevalence rate of toxoplasmosis (10.3 %) more than other animals (3 %), Table 1.

Based on multivariate logistic regression analysis, adult buffalo (OR = 7.10; 95 % CI: 0.87–57.68; $P = 0.067$) and small herds (OR = 8.42; 95 % CI: 1.07–66.02; $P = 0.043$) had a higher risk of infection with *T. gondii* compared to young buffalo and large herds, Table 2. In addition, the likelihood of *T. gondii* infection was four times higher in winter (OR = 4.64; 95 % CI: 1.42–15.16; $P = 0.011$) and three times higher in buffaloes contacted with cats (OR = 3.34; 95 % CI: 1.22–9.15; $P = 0.019$) when compared to other animals, Table 2.

4. Discussion

Water buffaloes play an important role in Egypt's economy since their meat and milk are utilized on a daily basis in Egyptian markets, particularly in the Nile delta. Although *T. gondii* infection causes abortion and stillbirth in adults and neurological problems in calves, there is little information available on the prevalence of this parasite in Egyptian buffaloes. Therefore, the aim of this study was to detect anti-*T. gondii* antibodies in buffaloes in three governorates as well as to identify potential risk factors for the infection.

Table 2
Multivariate logistic regression analysis for risk factors associated with *T. gondii* infection in water buffaloes.

Variables	B	S.E.	OR	95 % CI for OR	*P* value
Age					
2–4 years	0.797	1.070	2.22	0.27–18.07	0.046
> 4 years	1.960	1.069	7.10	0.87–57.68	0.067
Herd size					
Small (< 20)	2.130	1.051	8.42	1.07–66.02	0.043
Median (20–50)	0.900	1.125	2.46	0.27–22.31	0.042
Season					
Spring	1.096	0.655	2.99	0.83–10.81	0.094
Autumn	0.174	0.705	1.19	0.29–4.75	0.080
Winter	1.535	0.604	4.64	1.42–15.16	0.011
Presence of cats					
Yes	1.207	0.514	3.34	1.22–9.15	0.019

B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval.

In the present survey, the overall seroprevalence rate of *T. gondii* in water buffalo was 7.4 % which come in line with previous rate reported in Thailand 6.8 % [32] and in China 7.5 [33]. This seroprevalence rate was lower than those reported in Pará, Brazil 41.6–42.99 % [34,35], in Iran 14 % [36], in China 18 % [37] and 14 % in Pakistan [38]. However, the prevalence rate of the present study is higher than rates reported in different areas in China as in Qinghai Province (3.4 %) [39], Xinjiang Province (5.1 %) [40] and Shanghai City (2.5 %) [41].

The difference in prevalence is likely due to a variety of factors, including geographical location, ecological conditions, feeding conditions, and animal welfare, in addition to the number of samples tested [42,43].

Previous studies found that *T. gondii* transmission and dispersion in water buffaloes might be influenced by environmental variables such as rainfall and temperature [10,12,25,31,44–52]. Overall, the global frequency of *T. gondii* infection is higher in humid tropical locations than in hot and dry places and temperate areas [53,54].

According to the present findings, females showed a higher seroprevalence compared to males, and adults had a higher seroprevalence than young buffaloes. These results were consistent with previous studies of Fengcai et al. [33], de Oliveira et al. [35] and Inpankaew et al. [32].

Table 1
Seroprevalence of *T. gondii* in water buffaloes in relation to different variables.

Factor	Total examined buffaloes	No of positive	No of negative	% of positive	95 % CI	Statistic
Locality						
Qalyubia	140	10	130	7.1	3.92–12.65	$\chi2 = 1.404$ df = 2 $P = 0.496$
Kafr ElSheikh	150	14	136	9.3	5.64–15.05	
Gharbia	140	8	132	5.7	2.92–10.86	
Sex						
Male	34	1	33	2.9	0.52–14.91	$\chi2 = 1.086$ df = 1 $P = 0.297$
Female	396	31	365	7.8	5.57–10.9	
Age						
< 2 years	52	1	51	1.9	0.34–10.12	$\chi2 = 10.519$ df = 2 $P = 0.005$*
2–4 years	253	14	239	5.5	3.32–9.07	
> 4 years	125	17	108	13.6	8.67–20.7	
Herd size						
Small (< 20)	240	26	214	10.8	7.5–15.4	$\chi2 = 9.353$ df = 2 $P = 0.009$*
Median (20–50)	130	5	125	3.8	1.66–8.69	
Large (> 50)	60	1	59	1.7	0.3–8.86	
Season						
Spring	100	8	92	8.0	4.11–15	$\chi2 = 9.979$ df = 3 $P = 0.011$*
Summer	120	4	116	3.3	1.3–8.25	
Autumn	100	5	95	5.0	2.15–11.18	
Winter	110	15	95	13.6	8.44–21.29	
Presence of cats						
Yes	263	27	236	10.3	7.16–14.53	$\chi2 = 7.842$ df = 1 $P = 0.005$*
No	167	5	162	3.0	1.28–6.81	
Total	430	32	398	7.4	5.32–10.31	

* The results with *P* value less than 0.05 considered significant.

This high prevalence with age might be attributed to older animals being exposed to the parasite for a longer period, particularly in integrated farming where buffaloes freely graze on polluted grasslands or water sources [15,55]. Female buffaloes are routinely bred for calf, milk, and meat production in Egypt's integrated systems, and then slaughtered at the end of their productive lives. As a result, females are generally older than males, which may contribute to a general propensity for female buffaloes to be more highly diseased than males [8, 18,19,56,57].

In addition, females may have a higher sensitivity due to their lowered immune response during certain periods of their lives [58], lactation and pregnancy stress impair their immune system and make them more likely to contract *T. gondii* [59].

The herd size, particularly when it is small, is regarded as the most significant risk factor in the current study, regardless of the species investigated. *T. gondii* seroprevalence in buffaloes was higher in small herds than in other herds, which is consistent with findings of Abdallah et al. [60]. This may be due to the fact that small herds are traditionally managed, because the livestock's food supply is readily accessible to cats, and the animals is usually grazing as well as a lack of zoo-hygienic measures, such as organizing feeding, cleaning, etc [60,61].

Concerning to season, the present findings revealed significant variation in prevalence of *T. gondii* in different season, where the prevalence increased significantly in winter compared to summer season. This was consistent with findings of Ibrahim et al. [20]. This explained by wet environment generated by rain allows sporulated oocysts to survive in the soil and grass for several years, increasing the chances of buffaloes becoming exposed to oocysts when eating on pasture [54].

The presence of cats was evaluated as a risk factor in our study since it is partly widespread in practically all farms analyzed or those close. The high prevalence of antibodies against *T. gondii* in this study might be due to oocyst clearance and environmental contamination, which caused by the presence of resident or stray cats. The high prevalence rate of toxoplasmosis in domestic ruminants supports the significance of cats as a reservoir for *T. gondii* infection [62–64].

5. Conclusion

The findings of present study revealed that presence of antibodies against *T. gondii* in buffaloes which considered as risk source for infection in human. The multivariate logistic regression for significant variables confirmed that the age, herd size, season and contact of cat with susceptible animals are potential risk factors for *T. gondii* infection. To prevent and manage *T. gondii* infection in Egyptian buffaloes in the Nile delta, integrated and effective approaches are necessary.

Consent for publication

Not applicable.

Funding

Non.

Ethical statement

The handling, collection of blood samples and experiments were approved by ethical committee of faculty of medicine, the Benha University (Approval no.: RC17-8-2023). Animal owners provided informed consent for sample collection. The ARRIVE criteria were followed throughout the study procedure.

CRediT authorship contribution statement

Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, A.S., H.S.G., M.H.A., G.A.R., S.S. and A.A.; Writing – review & editing, A.S., H.S.G., M.H.A., G.A.R., S.S. and A.A.; Project administration, A.S., H.S.G., M.H.A., G.A.R., S.S. and A.A.; Funding acquisition, A.S., H.S.G., M.H.A., G.A.R., S.S. and A.A. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

There are no conflicts of interest declared by the authors.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

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