

# Seropositivity and Other Determinants Associated with Toxoplasmosis in Local Buffalo in Iraq

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## Abstract

A cross-sectional study was conducted to determine the Seropositivity of *Toxoplasma gondii* in water buffalo in three Iraqi governorates (Baghdad, Dhi Qar, and Maysan) and to estimate the risk aspects related to infestation throughout the period from January to December 2019. A total of 430 serum samples were inspected with a commercial ELISA (Enzyme linked immunosorbent assay) kit. Indirect multi-species kit. The overall Seropositivity of *T. gondii* in the examined local buffalo was 7.4%, and the highest rate (9.3%) was in Baghdad Governorate. A multivariate regression analysis revealed that adult buffalo (OR = 7.10; 95% CI: 0.87 - 57.68; P = 0.067) and young herds (OR = 8.42; 95% CI: 1.07 - 66.02; P = 0.043) were more subject to infestation from young buffalo and large herds. Furthermore, the hazard of toxoplasmosis was increased in winter especially among animals in contact with cats. It is therefore requisite to determine risk aspects to evaluate which mitigation, control, prevention and procedures should be carried out to diminish, control and prevent infestation with *T. gondii* and its propagation.

## Keywords

Toxoplasmosis, Buffalo, Local, Seropositivity, Iraq

## 1. Introduction

Toxoplasmosis (in English: *Toxoplasma gondii*) is an obligate intracellular parasite that infects humans in addition to many other ruminants, including buffalo, and has a harmful effect on health and productive performance of animals [1]. Domiciliary cats are the known host [2] [3]. Animals subjected to the causative agent become infested by contaminated water and meat [4]. The parasite is prin-

cially diffused to humans through ingestion of bloody or fresh meat, and its products comprising infectious stages of the parasite [4]. Infestation of human with toxoplasmosis is usually symptomless, and thoughtful difficulties may happen leading to fetal anomalies, such as amplified mortality and miscarriages [5].

The *Toxoplasma gondii* parasite can cause economic losses in livestock, particularly goats and sheep, due to amplified reproductive complications such as miscarriages, stillbirths, and the birth of weak animals, and a financial influence that comprises increased treatment expenditures as well as a loss in commercial incomes [6]. Buffalo are supposed to be a more resistant to parasites, and therefore experiments only reveal serological indication of Self-infection in exposed animals [7]. Many experiments have been attained to assess the Seropositivity of toxoplasmosis in buffalo [8], which differs mostly in relation to the environmental factors. The high Seropositivity of *causative agent* has been related to high temperature and humidity, which are favorable circumstances for the parasite to grow in the environment [9].

Infestation is a higher in adult buffalo in comparison to young calves, perhaps since older animals are more vulnerable to infestation with growing age [9] [10] [11]. Many experiments have been directed in Iraq to investigate the occurrence of Toxoplasmosis in humans, horses, camels, cattle, [12] [13] [14]. Nevertheless, additional researches are desirable to assess the prevalence and risk factors associated with *Toxoplasma gondii* infestation in Iraqi buffalo.

## 2. Materials and Methods

A study was achieved in local private buffalo breeding stations of Baghdad, Dhi Qar and Maysan governorates in Iraq using record of mentioned stations. These governorates signify the most shelter areas since they are located near the Tigris and Euphrates rivers and are famous for raising buffalo. Maximum temperatures exceed 40°C in summer and often drop to around 12°C in winter. Furthermore, average precipitation in these areas ranges between 100 and 200 mm per year, and most of the precipitation falls during winter.

The sample size was 430. The samples were tested using Thrusfield's formula [15], depending on a predictable occurrence of 50%, a precision of 5%, and a confidence level of 95%. Samples were reserved from three Iraqi governorates for the serological study throughout the period from January to December 2019. The samples were located in a centrifuge for 10 minutes at 3000 × g, the sera were separated, numbered, and stored at a temperature of -20 degrees in preparation for consequent serological tests using a random sampling method. Variables that been studied are (age, sex, herd size, season and existence or non-existence of cats). Animals were separated into three groups depending on their age (2, 2 - 4, and >4 years), sex (males and females), herd size (small, medium, and large), season (spring, summer, fall, and winter), and existence or non-existence of cats.

An indirect toxoplasmosis multiplex ELISA kit (ID Screen, ID.VET. Innova-

tive Diagnostics, Montpellier, France) was used to test sera for the existence of antibodies in counter to the parasite in blood of local buffalo. The ELISA kit had a sensitivity of 100% and specificity of 96% depending on the manufacturer's information. The 96-well plate was flooded with p30 *Toxoplasma gondii* antigen, and the antigen-antibody complex was formed with the assistance of a peroxidase conjugate, which was added later. The manufacturer supplied positive and negative standard solutions (controls), which were accustomed to investigate each test. Data were represented by optical density (OD); Absorbance was measured at 450 nm using AMR-100 ELISA Plate reader (AllSheng, China).

The positive percentage was calculated as follows:

$$\text{Percent positive} = 100 \times \text{OD}_{\text{sample}} / \text{OD}_{\text{positive control}}$$

when their value is  $\geq 50\%$  Samples are accounted positive, dubious if they are between 40% and 50%, and negative if they are  $\geq 40\%$ .

### 3. Statistical Analysis

Data were analyzed using SAS software to inspect the relation between risk variables and the dependent variable. Univariate logistic regression analysis and Chi-square tests were used to inspect the strength of the relation between seropositivity and causative agent infestation.

### 4. Results

It was found that antibodies against *Toxoplasma gondii* in 32 (7.4%) of the total serum samples. The highest occurrence rate of the parasite was found in local buffalo elevated in Baghdad (9.3%) than in Dhi Qar Governorate (7.4%), whereas the lowest occurrence rate was documented in Maysan Governorate (5.7%).

The incidence among males was 2.9%, compared to 7.8% among females. Only 1.9% tested positive for infestation in young calves less than 2 years of age, but it was 5.5% and 13.6%, respectively, for adult buffaloes 2 - 4 years of age and >4 years of age. In concerning with herd size, there was a substantial difference in the seropositivity of *T. gondii* ( $P = 0.009$ ); the small herd had the highest incidence (10.8%), compared to the medium herd (3.8%) and the large herd (1.7%), **Table 1**. The seropositivity of causative agent varied significantly across seasons, and the incidence was higher in winter (13.6%) and spring (8%) than in summer or fall. Moreover, cats in contact with buffalo had an elevated incidence of the disease (10.3%) more than other animals (3%), **Table 1**.

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 greater hazard to infestation compared to small buffalo and large herds, **Table 2**. Furthermore, the likelihood of infestation with the parasite was four times higher in winter (OR = 4.64; 95% CI: 1.42 - 15.16;  $P = 0.011$ ) and three times higher in buffalo that had contact with cats (OR = 3.34; 95% CI: 1.22 - 9.15,  $P = 0.019$ ) compared to other animals, **Table 2**.

**Table 1.** Seropositivity of the parasite in local buffalo and its association with other factors.

Variables	Total investigated buffaloes	No. of positive	No. of negative	% of positive	95% CI	Statistic
<b>Location</b>						
Dhi Qar	140	10	130	7.1	3.92 - 12.65	$\chi^2 = 1.404$ df = 2 P = 0.496
Baghdad	150	14	136	9.3	5.64 - 15.05	
Maysan	140	8	132	5.7	2.92 - 10.86	
<b>Sex</b>						
Male	34	1	33	2.9	0.52 - 14.91	$\chi^2 = 1.086$ df = 1 P = 0.297
Female	396	31	365	7.8	5.57 - 10.9	
<b>Age</b>						
<2 years	52	1	51	1.9	0.34 - 10.12	$\chi^2 = 10.519$ 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	
<b>Herd size</b>						
Small (<20)	240	26	214	10.8	7.5 - 15.4	$\chi^2 = 9.353$ 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	
<b>Season</b>						
Spring	100	8	92	8.0	4.11 - 15	$\chi^2 = 9.979$ 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	
<b>Presence of cats</b>						
Yes	263	27	236	10.3	7.16 - 14.53	$\chi^2 = 7.842$ 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	

\*Different letters between the groups horizontally indicate the presence of significant differences  $P \leq 0.05$ .

**Table 2.** Multivariable logistic regression analysis of seropositivity accompanied with the parasite in local buffalo.

Variables	B	S.E.	OR	95% CI for OR	p-Value
<b>Age</b>					
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
<b>Herd size</b>					
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

Continued

	Season				
<b>Spring</b>	1.069	0.655	2.99	0.83 - 10.81	0.094
<b>Autumn</b>	0.174	0.705	1.19	0.29 - 4.75	0.080
<b>Winter</b>	1.535	0.604	4.64	1.42 - 15.16	0.011
	Existence of cats				
<b>Yes</b>	1.207	0.514	3.34	1.22 - 9.15	0.019

\*Different letters between the groups horizontally indicate the presence of significant differences  $P \leq 0.05$ .

## 5. Discussion

Local buffalo play a significant role in the Iraqi economy, as their products are consumed on a daily basis in local markets. While the infestation can cause miscarriage and stillbirth in adult animals and neurological problems in newborns, there is little evidence accessible about the degree of the spread of this disease. Consequently, the aim of this study was to detect antibodies to *Toxoplasma gondii* in local buffalo in three provinces as well as to recognize possible risk factors for infestation.

The seropositivity of parasite in local buffalo was estimated to be 7.4%, which is in link with the rate informed in Thailand as 6.8% [16] and in China as 7.5 [17]. In this study, the seropositivity rate was lower than that reported in Pará, Brazil 41.6% - 42.99% [18], in Iran 14% [19], in China 18% [20] and 14% in Pakistan [21]. Nevertheless, the incidence of this study is higher than the rates stated in many areas in China for example in Qinghai Province (3.4%) [21], Xinjiang Province (5.1%) [22] and Shanghai City (2.5%) [23].

The variance in the occurrence rate is due to numerous reasons, including: geographical location, height above sea level, and environmental conditions [24] [25].

The infestation rate has been found to be higher in humid tropical areas than in hot, dry and temperate areas [25] [26]. In concerning to the present results, females exhibited a higher incidence rate related to males, and adults had a higher incidence rate than young buffaloes. These results were harmonious with studies directed by [16] [27] and [28].

This higher occurrence with age may be due to those older animals being infested with *T. gonadii* for a longer period, particularly in open rearing, where buffaloes graze freely on contaminated grasslands or water sources [29]. Furthermore, females may have higher incidence due to diminished immune response during certain periods of their lives [30], and stress during lactation and pregnancy declines their immune systems and increase susceptibility to infection with the pathogen [31]. The herd size is an importance in the spread of the parasite, nonetheless of the species examined. It has been shown that the seropositivity of pathogen in buffaloes is higher in small herds than in other herds, which is reliable with the findings of [32]. Small herds are managed habitually, because

feed is easily available to cats, and animals regularly graze lacking hygienic measures for animal housings, such as feeding, cleaning, etc. [33].

Concerning the season, the present results presented a significant difference in the occurrence of the parasite in different seasons, as the incidence increased meaningfully in the winter season compared to the summer season. This was reliable with the results of [34]. This clarifies that the humid environment permits the parasite to persist in the soil and grass for numerous years, which rises the likelihoods of buffaloes being infested when during feeding [26].

The existence of cats is a hazard issue in this study because it is prevalent on most farms analyzed or those adjacent to them. The high prevalence of antibodies against the causative agent may be owing to environmental pollution, which is triggered by the presence of endemic cats. The high incidence of the disease in farm animals has proven the role of cats as a reservoir for pathogen [35]. The relation of the *T. gondii* with any secondary infection or causative agent in Iraqi local buffaloes must be investigated to find out other hazardous factors.

## 6. Conclusion

The results of the present study exhibited that the existence of antibodies to *Toxoplasma gondii* in buffalo is a risk cause for disease in humans. Other factors (Age, herd size, season, and cats' contact with susceptible animals) have been revealed to be possible risk aspects for the infestation. For prevention and transmission of pathogen in Iraqi buffalo, integrated and operative protective methods are essential.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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