



## Seroprevalence and Risk factors of *Toxoplasma gondii* among children in Al-Qadisiyah Province – Iraq

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### 1. Introduction

Toxoplasmosis is one of the common Zoonotic diseases. *T. gondii* (which causes this disease) belonging to Coccidian from Apicomplexa phylum, all its members are obligated endo-parasites<sup>[1]</sup>. It infects many intermediate hosts such as sheep, rats, mice and humans<sup>[2]</sup>, and records many infections in marine mammals such as whales and dolphins<sup>3</sup> and affects many organs and tissues, eye<sup>[4]</sup>, central nervous and endothelial systems<sup>5</sup> as well. The pathogenesis of this parasite depends on several factors, including parasite virulence factors, strain and dose size, in addition to the host sensitivity, the degree of acquired immunity against the parasite, age and sex<sup>[1]</sup>.

The incidence rate is between 30% and 60% among the world's population<sup>[6]</sup>, the disease is most prevalent in warm and humid areas, as well as the nature and intensity of the population and the healthy culture in addition to food habits<sup>[7]</sup> and factors such as gender, age and domestic area have a significant impact on the prevalence of the disease<sup>[8]</sup>, the disease is transmitted in two ways directly with food or drink contaminated with parasite Oocyst, eating uncooked meat and dealing with soil contaminated with these cysts<sup>[9]</sup>, indirectly (congenitally) from the affected mother to the fetus<sup>[10]</sup>.

Infection with *T. gondii* stays Asympatic in adults, but it causes many health problems when transmitted from mother to her feotus<sup>[11]</sup>. Clinical signs are insufficient to diagnose the infection, so it depends on histological, serological and molecular tests to diagnosis<sup>[12]</sup>, where it stimulates the

presence of a humoral immune response by forming antibodies from B lymphocytes (IgM, IgA, IgE, IgG) that, in cooperation with complement proteins, contributes to eliminating the free parasite phase (Tachyzoite) in body fluids<sup>[13]</sup>.

ELISA test was the first tested in the Netherlands by Van-waeman and Schurs, this test is one of the most accurate methods currently used to detect toxoplasmosis, to determine immunoglobulin IgA, IgE, IgM and IgG<sup>[14]</sup>, Immunoglobulin (IgE, IgA and IgM) can be identified early during the acute stage of the infection, when IgE first appears, while IgA appears when the parasite enters the intestinal lining and constitutes 80% of the intestinal immunity<sup>[15]</sup>, the emergence of IgM begins after the parasite crosses the intestinal lining and spreads to other parts of hosts body in the first and the second week and reaches its peak in the fourth week, its level decreases in less than 3 months be reduced faster than IgG, which appears after 2-3 weeks of the appearance of IgM and up to the peak in about two months, and may last for a long time or a life spine<sup>[16]</sup>.

## 2. Materials and method

### 2.1 Population and the study area

This study was prepared from 6/1/2019 to 25/12/2019. It was included children from the age of thirteen and under, whose living in Al-Qadisiyah Governorate – Iraq. Al-Qadisiyah poor agricultural governorate. Located in the southeast of the capital Baghdad, between 31° 51 ' N and 45° 3'E. It consists of four districts Diwaniyah, Shamiya, Hamza and Afak (Fig. 1) <sup>[17]</sup>.



Figure.1: Geographical map of Qadisiyah Province, Iraq

### 2.2. Samples and study community

The current study examined 463 children. The information associated with the disease, such as gender, age, dealing with animals and the domestic environment, which were recorded. According to the

recommendations of the Ethics Committee at Sciences College of Al-Qadisiyah University, 2-mm blood from each child was drawn, samples left until coagulated, thereafter, it was placed in a centrifuge at 3000 rpm for five minutes, then, the serum was withdrawn by a pipette and placed in tubes kept at  $-20^{\circ}\text{C}$ <sup>[18]</sup>.

### 2.3 Enzymatic tests

In the Parasitology Laboratory of the College of Sciences at Al-Qadisiyah University, antibodies against the parasite were revealed using the kit produced by Demeditec Diagnostics GmbH, Germany, were used according to the manufacturer's instructions. In brief:

1. The diluted serum was added to the purified Toxoplasma antigen in encapsulated pits (12 x 8 holes).
2. All non-bound materials were washed with ELISA washing machine.
3. HRP-conjugate was washed and TMB reagents were added.
4. Stopping the catalytic reaction associated with the enzyme at a specific time.
5. The intensity of the colour generated is proportional to the amount of specific IgG or IgM antibodies in the sample.
6. Optical density (OD) reading at 450 nm within 15 min by a microwell reader, which distinguishes the acuteness from the chronic infection.

### 4.2. Statistical analysis

The Chi-square value, SD, and the results of this study was calculated by SPSS software version 20 (SPSS, Chicago, IL, USA).

have mentioned this bird species within the Iraqi bird checklists. This paper, along with the detailed description and confirmation, represents the first confirmed observation of Shikra for Iraq.

## 3. Results

### 1.3. Epidemiology and seroprevalence

The study recorded a total infection rate (23.3%), when it was found infection of (108) children. (4.1%) of the samples indicated an acute infection after the presence of the IgM antibody in these samples, while the IgG antibody was present in (17.9%), where indicated the chronic infection. The study recorded the presence of two types of antibodies (IgG and IgM) at (1.3%) (Table 1).

**Table.1: seroprevalence as immunoglobulin type.**

	Antibodies	Positive	Persent(%)
P.value = 0.1	IgM	19	4.1
	IgG	83	17.9

Total samples	IgM+IgG	6	1.3
463	Total	108	23.3

### 2.3. Patient characteristics

The study examined 463 children at the age of thirteen and under, distributed among the districts of the governorate as follows: Al-Diwaniyah (29.2%), Al-Shamiya (26.3%), Al-Hamza (24.2%), and Afak (20.3%) (Table 2). The results appeared that (43.8%) of them were males and (56.2%) were females and divided into three age groups (2-5) years, at a rate of (24%), (6-9) years (25%), (10-13) years (51%) at a mean and SD ( $7.5 \pm 3.6$ ). (65%) lived in a rural and (35%) in an urban. The percentage (75.8%) contacted with animals and (24.2%) did not as shown in Table 3.

**Table.2: seroprevalence as province districts.**

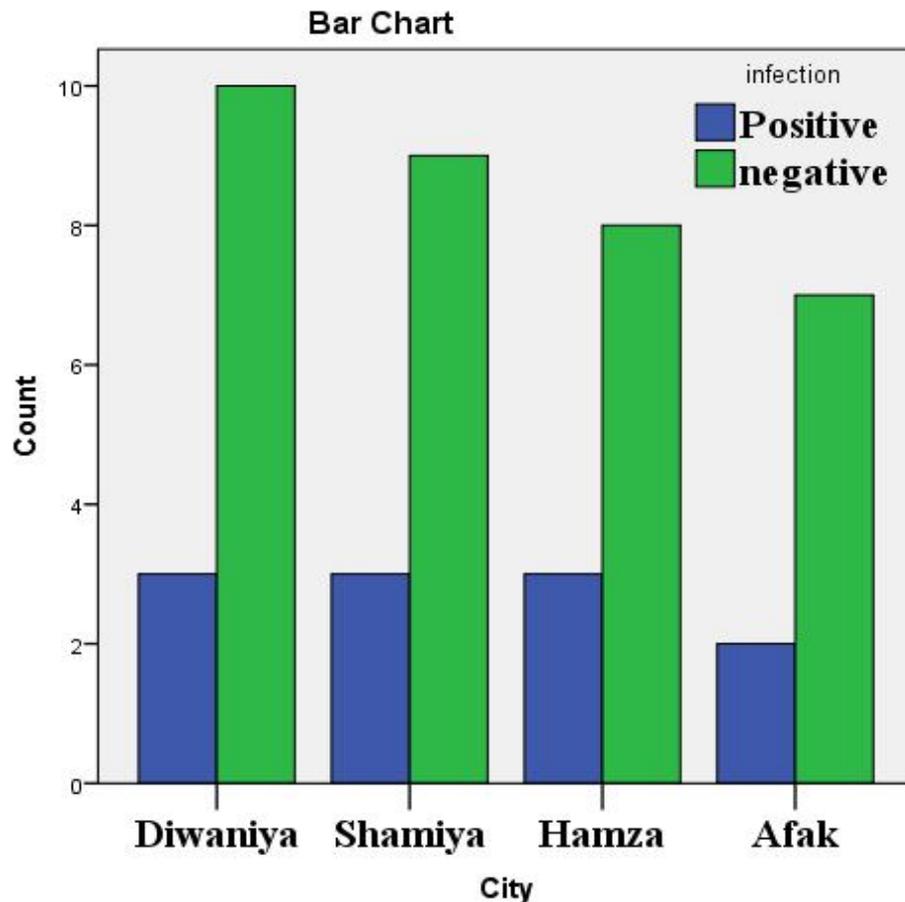
Region	Samples(%)	Positive(%)	P.ratio	
Diwaniya	135(29.2%)	26(24%)	0.82	p.value 0.63
Shamiya	122(26.3%)	29(26.9%)	1	
Hamza	112(24.2%)	31(28.7%)	1.2	
Afak	94(20.3%)	22(20.4%)	1	
total	463(100%)	108(23.3%)		

### 3.3 Risk factors for *Toxoplasma gondii*

Our study indicated that the highest incidence of infection occurred in Al- Hamza district (28.7%), and the lowest incidence was in Afak district (20.4%) without a significant difference as shown in Table (2) and Figure (2). Table 3 shows the risk factors in this study, the highest occurrences of IgG antibody were among (10-13) years (11.2%), male (10.8%), rural (14.9%), contact with animals ( 16.4%). The highest occurrences of IgM antibody also were in the category (2-5) years (2.8%), females (4.1%), while the rural population and contacted with animal were (4.5%, 5.4%) respectively.

### 3.4 Statistical results

The results of the study indicated the significance of differences according to gender, age, housing and animal contact parameters, at  $P.value \leq 0.05$ .



**Figure .2:** seroprevalence of *T.gondii* in Al-Qadisiyah province.

**Table.3:** risk factors of *Toxoplasma gondii* infection.

Variables	Samples(%)	IgM(%)	IgG(%)	Total	P.ratio	P.value
Age						0.01*
2-5	111(24%)	13(2.8%)	14(3%)	26(5.6%)	0.23	
6-9	116(25%)	8(1.7%)	23(5%)	30(6.5%)	0.26	
10-13	236(51%)	4(0.9%)	52(11.2%)	52(11.2%)	0.22	
Gender						0.01*
Male	203(43.8%)	6(1.3%)	50(10.8%)	52(11.2%)	0.25	
female	260(56.2%)	19(4.1%)	39(8.4%)	56(12.1%)	0.22	
Domestic						0.01*
Rural	301(65%)	21(4.5%)	69(14.9%)	85(18.4%)	0.28	
urban	162(35%)	4(0.9%)	20(4.3%)	23(4.9%)	0.14	
Contact						0.01*
Yes	351(75.8%)	25(5.4%)	76(16.4%)	96(20.7%)	0.27	
no	162(24.2%)	0(0%)	13(2.8%)	12(2.6%)	0.02	
total	463(100%)	25(5.4%)	89(19.2%)	108(23.3%)		

\*Significant difference at P.value  $\leq$  0.05.

#### 4. Discussion

The spread of *T. gondii* parasite increases with the presence of many important factors including the geographical and environment nature, the socio-economic structure and the cultural people, and the variety of food consumed<sup>[19]</sup>, in addition to, the presence of hosts (intermediate and final). Toxoplasmosis is a common disease among humans and animals, and it can be transmitted between them<sup>[20]</sup>. Also, it depends on the soil as a site for saving Oocysts, and transmits into the intermediate host<sup>[21]</sup>.

The serological diagnosis of either acute or chronic infections depends on the presence of the parasite antibody in the samples (IgG, IgM, IgA and IgE)<sup>[15]</sup>, as the initial infection urges to produce high titres of IgM that continue to be presented in the blood of the affected person for several weeks<sup>[22]</sup>. Its presence continues for (18) months<sup>[23]</sup>, the level of immunoglobulin reaches the peak within 1–3 weeks of infection: this period is considered the best time to diagnose the presence of the parasite, although, it is possible to ascertain the presence of IgM after 6–9 months from infection<sup>[24]</sup>.

The presence of IgM after this long period can indicate an acute infection, thus, gives a wrong serological examination, as the infection during this period turns into a chronic infection and the parasite turns into the latent phase, so to ascertain the type of infection this test should be attached to IgG test<sup>[23]</sup>.

The study indicated a high rate of infection among children in Al-Qadisiyah Governorate, where the overall infection rate was (23.3%), local studies on this age group are almost non-existent. By comparing the results of this study with the available data we found that the percentage of infection is less much of what was recorded in Najaf among children under the age of six by using VIDAS technique<sup>[25]</sup>, and less than that recorded in Najaf as well among newborns<sup>[26]</sup>. The results were closed to what was recorded among children in Kirkuk, when recorded infection rate (24.7%)<sup>[27]</sup>, and what was recorded among children in Basrah (24%)<sup>[28]</sup>.

The infection rate recorded in this study remains less than the infection rate among children in Turkish city (Hatay), where the percentage of infection is (32.4%)<sup>[29]</sup>, in Iranian city (Isfahan) (41.4%)<sup>[30]</sup>, while was higher than that in the city of Dhamar in Yemen (20.43%)<sup>[31]</sup> and other cities (17.7%)<sup>[32]</sup>, China (15.13%)<sup>[33]</sup>.

The highest infection rate was in Al-Hamza district (28.7%), the lowest percentage was in Afak district (20.4%). This is due to the rural nature in this city (dominant in the province, whole), because the study focused on individuals in this environment where poverty and lack of a healthy culture, animal contact, drinking water and milk un-boiled or unsterilized, all these factors facilitate the spread of infection<sup>[34]</sup>.

The study registered an increase in the rates of chronic infection (the presence of IgG) with increasing age (3%), (5%), (11.2%) in (2-5), (6-9),(10-13) years, respectively. According to the studied age groups, these ratios agree with<sup>[35]</sup>. [36] and [37] indicated that The incidence rates increase with increasing age, which does not indicate that the elder are more susceptible to infection than the youngest, but may be due to the repeated exposure to the pathogens<sup>[38]</sup>. The highest proportion of the

IgM antibody was (2.8%) in the age group (2-5) years; the reason may be that children of this age have a lot of movement and playing, and therefore more exposure to pathogens.

The present study showed an increase in the rate of IgG in males (10.8%), whereas IgM in females was the highest (4.1%), these results do not correspond to<sup>[39],[40]</sup>, but they correspond with<sup>[41]</sup>. The reason corresponds with culture and customs in the area of this study where girls are more concerned with household matters and animal husbandry than boys who are not assigned many tasks at such ages.

The incidence rate in the rural area is greater than the urban in terms of IgM, IgG (4.5%, 14.9%) respectively, because the demographic nature of the governorate is rural. This study focused on the rural more than the urban, where animals exist and more people interact with them, in addition to the polluted soil and water, the absence of many healthy services makes the population depending on drinking the polluted water and taking advantage of animal products that may be infected<sup>[42]</sup>. These results are consistent with<sup>[43],[44]</sup>, the rate of disease increases with an increase that contact with animals, consuming contaminated food, undercooked meat and other foods containing the latent phase and drinking water, milk or contact with soil contaminated with Oocytes<sup>[45]</sup>.

## Conclusion

The current study indicates a high prevalence of the *Toxoplasma gondii* (23.3%) in the regions of Al-Qadisiyah governorate. So, the significant differential association is directly related with age, gender, location and environment. Therefore, the current study also represents a database that benefits researchers in the field of health and scientific research.

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