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Research Article

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN FREE-RANGE LOCAL BIRDS IN SUMEL DISTRICT, DUHOK PROVINCE, IRAQ

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ABSTRACT: In the present study, Direct Latex Agglutination Test (DLAT), Modified Agglutination Test (MAT) and enzyme-linked immunosorbent assay (ELISA) were used to detect the immune responses to *Toxoplasma gondii* (*T. gondii*) among free-range local birds (chicken, turkey, geese and ducks) in Sumel District of Duhok province, Iraq. The data revealed the overall seropositive rates of 62.2%, 33.3% and 21.1% by DLAT, MAT and ELISA, respectively. Non-significant differences were found between the prevalence rates among the tested birds indicating that all birds' species to be susceptible for infection. The highest antibodies titers in response to *T. gondii* were found in chicken sera which was significantly higher ($p < 0.05$) than that in ducks. The data indicated that free-range local birds could be considered as important sources of toxoplasmosis for the locals in Duhok area. Preventative measurements should be taken to contain the further prevalence of the disease among the locals, namely pregnant women.

Key words: Chicken, Duck, Geese, Iraq, Seroprevalence, *Toxoplasma gondii*, Turkey.

INTRODUCTION

Toxoplasmosis is a cosmopolitan infection caused by an apicomplexan protozoan parasite *Toxoplasma gondii* (Sonar and Brahmbhatt 2010) affecting wide range of animal species including birds (Prestrud *et al*. 2007). The avian species are considered as an important indicator for the epidemiological studies on prevalence of *T. gondii* due to their ground-feeding nature which can be used as indicators of soil contamination by infective stage of the parasite, the oocysts (Dubey *et al*. 2010). *T. gondii* in birds may occur through ingestion of the oocysts of the parasite secreted within the feces of infected definitive hosts, cats (Hill and Dubey 2013, Mirza Alizadeh *et al.* 2018).

The disease has been reported in different species of free-range birds from various geographical locations *viz*. geese (Verma *et al*. 2016); ducks (Yang *et al.* 2012); chicken, turkeys, sparrows and other birds (Dubey 2010, Alkhaled 2012, Mohammed 2013, Hadi *et al*. 2019). Various serological techniques have been used for epidemiological studies on toxoplasmosis in human and animals using rapid test cassette (Toxo IgG/ IgM) (Hadi *et al.* 2019), latex agglutination test (LAT) (direct and indirect), modified agglutination test (MAT) (Huang *et al*. 2019, Mohammed, 2013) and ELISA (IgM / IgG) (Andiappan *et al.* 2014, Kader and Al-Khayat 2013). The LAT is a reliable test for screening of toxoplasmosis among animals (Sroka *et al.* 2008) with high accuracy rates (Tagwireyi *et al.* 2019).

Further, ELISA and MAT are considered as highly sensitive tests for qualitative and quantitative detection of acute and chronic toxoplasmosis (Dubey *et al.* 2016), as no significant differences have been found between these tests in serodiagnosis of animal toxoplasmosis (Mosallanejad *et al.* 2017).

Tissue cysts of *T. gondii* (bradyzoites) in raw or undercooked meat of different sources including birds are considered as important sources for human infection (Hussain *et al.* 2017). The popularity of consuming free-range local birds, namely geese and turkey by locals in

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Duhok province is greatly increasing, meanwhile toxoplasmosis among human within the area is exacerbated (Hussien *et al.* 2018, Salih *et al.* 2020). Sumel district reported the highest prevalence rate of toxoplasmosis at 33% among the free ranged local chicken compared to other districts of Duhok province (unpublished data). The present study planned to employ LAT, MAT and ELISA for detection of seroprevalence of toxoplasmosis among free-range birds in Sumel district of Duhok province, Iraq and to detect the most susceptible species of the bird within the study area.

MATERIALS AND METHODS

Study area and blood collection

From September 2019 to January 2020, a total of 90 blood samples were randomly collected via wing vein from four species of free-range local birds *viz*. turkey (26 samples), chicken (21 samples), the grey geese (23 samples) and Muscovy duck (20 samples)) from Sumel district, Duhok province, Iraq. The collected bloods were transported in cold box to the laboratory of Clinical Pathology at College of Veterinary Medicine, University of Duhok, Iraq where these were centrifuged at 5000 rpm for 5 min for isolation of the sera. The collected sera were decanted into micro tubes and stored in deep freezer at -35ºC until used.

Serological examination

Direct Latex Agglutination Test (DLAT): A commercial kit PLSMATEC (UK) was used for rapid screening to determine antibodies against *T. gondii* (IgG and/or IgM class). The test was performed according to the manufacturer's instructions. Agglutination and changing of colour (in the case of positive reaction) or lack of agglutination and change in colour (for negative reaction) were observed. The reactions were determined visually under a high-intensity incandescent light.

Modified Agglutination Test (MAT): To determine the IgG responses to *T. gondii* infection in sera of the free-range local birds, Modified Agglutination Test (MAT) as described previously (Kader and Al-Khayat 2013) was used with some modifications. Briefly, 30µL of the collected sera were added to the "U" bottom of 96-well microtiter plates followed by equal volume of 2-mercaptoethanol (2-ME) at 0.1 mol/L in phosphate buffer (pH 7.2) to inactivate the IgM (Ortega-Mora *et al*. 2007) and incubated for 30 min. The incubated sera were tested with latex agglutination kit as mentioned before. Positive and negative sera samples were recorded and the suspicious results were retested. Where, 2ME selectively destroys IgM antibodies whereas IgG antibodies are unaffected. Agglutination after 2ME treatment indicates presence of IgG antibodies (chronic infection). Non-agglutination after 2ME treatment indicates presence of IgM antibodies (acute infection).

ELISA

Commercial ELISA kit (ID Screen® Avian Toxoplasmosis Indirect, France) was used to investigate the antibodies responses to *T. gondii* from the collected sera. Indirect ELISA was used to detect IgG according to the manufacturer's instructions; the reactions absorbances were measured at 450 nm using a micro plate reader (Bio-Tek- USA). The results are expressed as a percentage of Sample/positive control (S/P %), percentage of the mean absorbance calculated according to the following formula S/P% = (optical density of sample (OD sample)-Optical density of negative control (ODNC)/ (optical density of positive control (ODPC) - Optical density of negative control ODNC) ×100. Depending on manufacturer's recommendation sera were regarded negative when S/P% = 50% and positive when S/P% = 50%.

Table1. Prevalence rates of *T. gondii* among four different free-range local birds based on DLAT, MAT and ELISA in Sumel district, Duhok province, Iraq.

Type of birds	No. of tested samples	DLAT		MAT		ELISA	
		No. of positive samples	% of positive	No. of positive samples	% of positive	No. of positive samples	% of positive
Turkeys	26	15	57.6%	10	38.4%	6	23.1%
Chickens	21	17	80.9%	9	42.8%	7	33.3%
Geese	23	13	56.5%	6	26.1%	4	17.4%
Ducks	20	11	55%	5	25.00%	2	10.0%
Total	90	56	62.2%	30	33.3%	19	21.1%

Ethical approval

This study was carried out with the approval of Research Ethics Committee of Duhok Research Center at the College of Veterinary Medicine, Duhok University, Iraq under protocol reference number: DR2020919CV.

Statistical analysis

Graph pad prism was used to carry out the statistical analysis of the data. Chi squared test was used to determine the statistical significance between the variables. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Studies confirmed that human infection with *T. gondii* may occur through ingestion of tissue cysts (bradyzoites), which is not only restricted to consumption of red meat, but also through infected chickens; undercooked or raw meat and eggs from the infected birds with *T. gondii* are reported as important sources for human toxoplasmosis (Dubey 2010). Besides, studies found that *T. gondii* is localized and persist within different organs such as brain, heart, and leg muscles in infected birds for a considerable period of time (Mendez and Koshy 2017).

Studies have been done to determine the sources of human infection with *T. gondii* in Duhok area; prevalence rates of toxoplasmosis in Duhok province among different species of animals including sheep and goats have been reported (Issa 2017, Issa and Omer 2011, Mikaeel *et al*. 2015). In Duhok province, human toxoplasmosis has greatly increased since past few years (Hussien *et al*. 2018, Salih *et al*. 2020). Local birds are considered as an indicator for regional contamination with the oocysts of *T. gondii* was shed within the feces of the definitive host, cats (Konell *et al*. 2019). Little was known about avian toxoplasmosis; it was unknown to what extent the disease was prevalent among free-range local turkey, ducks and geese. Besides, the susceptibility of each of the tested birds to the infection was unknown; the importance of the birds' toxoplasmosis in food safety, community health and animal husbandry was uncertain. Therefore, immune assays were used to detect anti- *T. gondii* antibodies in sera of locals' chickens, turkeys, geese and ducks were reared under the same feeding, management and ecological systems within the Duhok province, namely in Sumel district.

The data revealed by using DLAT in tested samples indicated an overall prevalence rate was 62.2%; with the highest prevalence (at 80.9%) among chickens (Table 1). However, the data was non-significantly higher at ($p>0.05$) than that reported in turkeys, geese and ducks

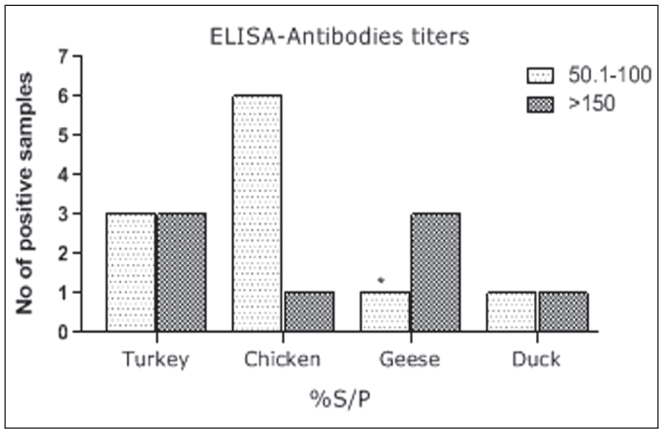

Fig. 1. **The ratio of S/P (%) of anti-*T. gondii* (IgG) antibodies detected by indirect ELISA test in sera of free-range local birds in Sumel district, Duhok province, Iraq.**

at rates of 57.6%, 56.5% and 55%, respectively. These findings are in agreement with that reported by Mohammed (2013) who found the prevalence rate at 60% by latex agglutination test in domestic chickens in Sulaimani Province, Iraq. In mid Euphrates area of Iraq, *T. gondii* infection by using LAT in free-range chicken and duck was reported at rates 67% and 56%, respectively (Alkhaled 2012). Presence of high prevalence rates of *T. gondii* among free-range local birds is indicative of high levels of environmental contamination with oocysts of the parasite.

To differentiate between the recent and the past infection (acute and chronic infection), MAT was used to detect the IgG responses to *T. gondii* infection. The data revealed that the overall prevalence rate among the tested animals was 33.3% (Table 1), the highest prevalence rates were reported among chickens (at 42.8%) which was also non significantly higher ($p>0.05$) than that reported in turkeys, geese and ducks at 38.4%, 26.1%and 25.0%, respectively. The data revealed that about half of infected birds were in acute stage of infection; these findings are further supportive for the contamination of the area with infective stages of the parasite, oocysts. Acute toxoplasmosis in animals and humans is likely due to fresh contact with oocysts of *T. gondii* found within the contaminated area with feces of cats (Dumètre and Dardé 2003), or it might be due to the stress factors which might adversely affected the immune system of the host leading to rapidly replicating tachyzoites of the parasite from a latent, life-long stage bradyzoites persisting inside tissue cysts in chronic infection (Egorov *et al*. 2018).

The prevalence rates of *T. gondii* in turkeys, chickens, geese and ducks based on indirect ELISA test for IgG detection revealed of 21.1%. However, non-significant

differences were found between the prevalence rates in tested birds, the highest prevalence rate was found among free-range local chickens at 33.3% which was followed by 23.1%, 17.4% and 10% reported in turkeys, geese and ducks, respectively (Table 1). Besides, the study also revealed bird to bird differences within the % S/P positive titers which were ranging from 52-345, the highest titer was among the chickens where six samples were higher than 150 which was significantly higher ($p<0.05$) than that reported in geese (Fig.1). The higher prevalence rates in chickens is due to their feeding habits where the chickens during the daytime scavenge and scratch the ground searching for feed; chickens have greater contact with feces of cats containing oocysts of the parasite (Stelzer *et al*. 2019). The lowest prevalence rates in ducks might be due to the infective doses of *T. gondii* required to cause infection in ducks; ducks need infective dose of more than $1x\ 10^{5.7}$ oocysts of *T. gondii* K21 strain compared to chicken and turkey where developed severe signs with a lower doses at $1x10^3$ (Bártová *et al*. 2004).

To sum up, the presented data indicated that the study area is heavily contaminated with oocysts of the parasite; high prevalence rates of *T. gondii* were found among free-range local birds; seropositivity values among the tested birds, suggesting that all species are susceptible to the infection and could be important sources for human infection. Therefore, to minimize the zoonotic impact of the disease, the locals' namely pregnant women must be screened periodically, more preventive and biosecurity measures should be adopted.

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