

Survey of *Toxoplasma gondii* antibodies in retail red meat samples in Erbil governorate, Kurdistan Region, Iraq

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Abstract

Since livestock meat, has been demonstrated to be a potential source of human infection, a careful evaluation of the prevalence of infection with *T. gondii* in these animals' meat is needed to protect public health. Latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA) were performed on meat juice from 380 red meat samples (125 Beef, 135 Mutton, and 120 goats' meat) that are sold in Erbil governorate, Kurdistan region, Iraq. The current results demonstrated that the overall prevalence of *anti-T. gondii* antibodies among red meat was 18.7% and 16.3% according to LAT and ELISA, respectively. The highest rate was found in October (25.8% and 22.6%) by using LAT, and ELISA, respectively. While, the lowest rate was recorded in August (12.5% and 9.4%) by both assays, respectively. Both tests performed similarly without significant difference between their performances as diagnostic tests. Moreover, no significant differences between meat types were found in terms of toxoplasmosis antibodies. In conclusion the current survey provides significant evidence about risk of human exposure to *T. gondii* through the consumption of raw or undercooked red meat potentially contaminated with infectious tissue cysts.

Keywords: Toxoplasmosis, Latex, ELISA, Meat juice, Prevalence.

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INTRODUCTION

Toxoplasmosis is a worldwide significant food-borne zoonotic disease that results from the infection with an obligate intracellular protozoan parasite named *Toxoplasma gondii*. It is a major public health problem producing a wide range of clinical syndromes in humans, particularly pregnant women and immunosuppressed individuals, land and sea mammals, and various bird species (McLeod et al., 2020). *T. gondii* has been recovered from all locations throughout the world, except Antarctica, and is described as a 'Silent threat' in most of the Asian countries (Sroka et al., 2019; Simon et al., 2020). Felidae are the only definitive hosts that excrete million oocysts of *T. gondii* in their feces and thus contaminate the environment.

T. gondii has a complex life cycle that consists of two stages; sexual and asexual phases. Sexual reproduction takes place in cats where oocysts are formed and excreted with feces. Meiosis of oocysts in the environment leads to formation of sporozoites that are infectious to the intermediate hosts (rodents and livestock animals). In the intermediate hosts, rapidly replicating tachyzoites are disseminated throughout the body forming tissue cysts containing bradyzoites (Dubey, 2020). Ingestion of tissue cysts by carnivorous or omnivorous animals leads to transmission to other intermediate hosts or cats repeating the sexual phase of the life cycle. The asexual stage takes place in the intermediate hosts where rapid intracellular growth of the parasite as tachyzoite takes place. The tachyzoites are spread throughout the body leading to development of tachyzoites to cysts mainly in neural and muscular tissues that can persist for long time (Dubey et al., 2012; Hosseini et al., 2020; Paneth et al., 2019).

Human acquire the infection by consumption of insufficiently cooked or raw meat containing the tissue cysts or water contaminated with oocysts or contaminated food utensils. Trans-placental infection route is another significant way of transmission which often leads to severe and lifelong disabilities in the infected infant. However, food-producing animals may represent a real risk for transmission of the disease to humans, either directly or through farming (Dubey & Jones, 2008; Ranucci et al., 2020). Furthermore, there is no available data about the proportion of the infection among red meat animals in Erbil governorate, Iraq. Therefore, this work aimed to determine the prevalence of anti-*T. gondii* antibodies among red meat using two serological assays; Latex agglutination test (LAT) and ELISA techniques.

MATERIALS AND METHODS

Study design and sampling

A total of 380 fresh steak red meat samples (125 Beef, 135 Mutton, and 120 Goats meat) were randomly and aseptically collected from retail shops in city center and outskirts of Erbil city during the period from July to December 2019 according to previously published method (Al-Mashhadany, 2019b). About 200-250g of each sample were placed in separate sterile polyethylene bags within cold container and transported to Department of Medical Lab Science (DMLS), College of Science (CSCN), Knowledge University (KNU). Meat juice was prepared according to previously published protocols (Al-Mashhadany, 2019a; Wallander et al., 2015). Briefly, after one week of deep freezing, samples were thawed at 20-25°C. About 5 ml of meat juices were collected in an Eppendorf tube and separated by centrifugation into two parts; for LAT and ELISA assays. For each different meat type,

a number of 19 - 24 samples were tested on monthly basis.

Detection of T. gondii antibodies by LAT

Meat juice collected from beef, sheep, and goat meats were screened for antibodies to T. gondii using Latex agglutination test (Toxocell-latex® 3000-4525, Biokit Company, Spain) according to the manufacturer's instructions. Briefly, this serological test is based on the principle of antigen-antibody complex formation. If specific anti-Toxoplasma antibodies exist in examined sera, they will react with soluble Toxoplasma antigen in the latex reagent and will be visualized by latex particles as whitish granules after 5 minutes of turning of the slide.

Detection of T. gondii antibodies by ELISA

The extracted meat juice samples were also assayed for the presence of IgG antibodies to T. gondii by ELISA test using antigen and other reagents from commercial kit (BioCheck BC-1085 kit, California, USA) according to manufacturer's instructions. Negative and positive controls were run in parallel by kits provided by the same manufacturer. The optical density (OD) values were read at 450nm using a microplate spectrophotometer Thermo Scientific TM Multiskan TM GO (Fisher Scientific, Delaware, USA).

Statistical analysis

Data were analyzed using the SPSS software version 25. Confidence intervals of prevalence were estimated using Clopper-Pearson method at 0.95 confidence level. Chi square test was employed to test the difference between groups. To evaluate the agreement between the two tests, inter-rater reliability (kappa) was calculated and kappa values (κ) were considered as

follows: poor agreement ($\kappa < 0.20$); fair agreement ($\kappa = 0.21 - 0.40$); moderate agreement ($\kappa = 0.41 - 0.60$); good agreement ($\kappa = 0.61 - 0.80$); and very good agreement ($\kappa = 0.81 - 1.00$).

RESULTS

Surveillance of T. gondii antibodies

The obtained results showed that anti-T. gondii antibodies were found in 71 out of the 380 meat samples (18.7%) with the LAT test, whereas 62 (16.3%) samples of meat juices were found to be seropositive for toxoplasmosis by ELISA. There was no significant difference between the two assays for detection of T. gondii antibodies in meat juice samples ($p = 0.373$). The distribution of positive samples among different meat types for anti-T. gondii antibodies showed that the higher prevalence was recorded among mutton meat (21.5% and 19.3%) as compared to goat (19.2% and 15.8%) and beef meat (13.6% and 15.8%) as depicted in Table (1). However, there was no significant difference between meat types in terms of harboring anti-T. gondii antibodies ($p = 0.411$). Agreement between the two tests using Kappa analysis revealed a very good agreement ($\kappa = 0.918$, with 95% CI of 0.865 to 0.97%).

Table 1. Prevalence of T. gondii antibodies among red meat samples (n=380).

Meat type	No. examined	LAT positive		ELISA positive	
		n (%)	95% CI	n (%)	95% CI
Beef	125	19 (15.2)	9.41-22.71	17 (13.6)	8.13-20.88
Mutton	135	29 (21.5)	14.88-29.37	26 (19.3)	12.98-26.93
Goats meat	120	23 (19.2)	12.56-27.36	19 (15.8)	9.81-23.62
Total	380	71 (18.7)	14.89-22.97	62 (16.3)	12.74-20.42

* No significant difference was found between meat types in terms of T. gondii prevalence ($p = 0.411$).

Prevalence of *T. gondii* antibodies according to sampling location

The present survey illustrated that the highest prevalence was found in suburban area (18.3% - 24.3%) as compared to urban area (12.3% - 17.1%). A statistically significant difference was found between anti-*T. gondii* prevalence and screened regions by ELISA (p=0.03) and LAT tests (p=0.05) respectively, as shown in Table (2).

Table 2. Prevalence of *T. gondii* antibodies (ELISA) according to sampling location.

Meat type	Urban		Suburban	
	No. tested	No. of positive (%)	No. tested	No. of positive (%)
Beef	65	6 (9.2)	60	11 (18.3)
Mutton	70	11 (15.7)	65	14 (21.5)
Goats meat	60	7 (11.7)	60	13 (21.7)
Total	195	24 (12.3)	185	38 (20.5)

Temporal variations of *T. gondii* prevalence during study period

In regard to monthly dynamics, the current results showed that the highest rate of anti-*T. gondii* antibodies was detected in October (25.8%) and September (20.0%), while the lowest rate was found in August (12.5%) according to LAT. However, the highest rate of anti-*T. gondii* antibodies according to ELISA results was detected in October (22.6%) and December (19.4%), with the lowest rate in August (9.4%). According to the two assays, there was only a weak association ($r^2 = 0.254$ & 0.348 for LAT & ELISA, respectively) between

progress of summer-autumn months and increase in prevalence of anti-*T. gondii* antibodies (Table 3 & Figure 1).

Table 3. Prevalence of *T. gondii* antibodies in different red meat at time scale.

Month	Total examined *	LAT positive n (%)	ELISA positive n (%)
July	63	10 (15.9)	9 (14.3)
August	64	8 (12.5)	6 (9.4)
September	65	13 (20.0)	11 (16.9)
October	62	16 (25.8)	14 (22.6)
November	64	12 (18.8)	10 (15.6)
December	62	12 (19.4)	12 (19.4)
Total	380	71 (18.7)	62 (16.3)

*Monthly examined samples comprised approximately equal proportions of beef, mutton, and goat meat.

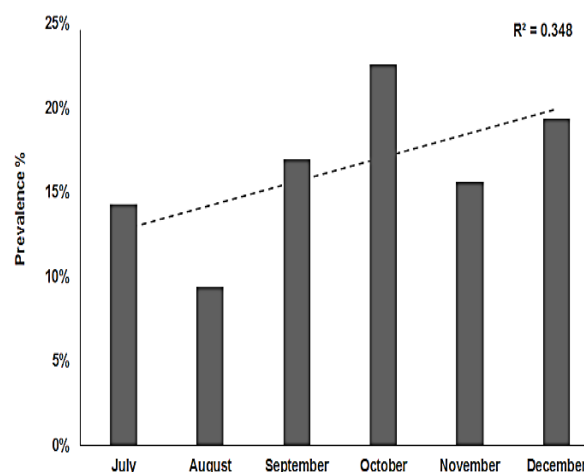


Figure 1. Temporal variations of *T. gondii* prevalence during study according to ELISA assay.

DISCUSSION

Seroprevalence is a good indicator of the presence and tracking of *T. gondii* in meat introduced into market especially when nation-wide surveillance programs are scarce. This results of total prevalence are consistent with data published by Tonouhewa and colleagues who reported that the prevalence of anti-*T. gondii* antibody among cattle, sheep, goats from different African countries was 12.0%, 26.1%, and 22.9%, respectively (Tonouhewa et al., 2017).

On the other hand, an Algerian study documented the presence of anti-Toxoplasma antibodies in cattle, sheep, and goats were 3.92%, 11.59%, and 13.21% (Dechicha et al., 2015). However, our results are lower than prevalence found in Mosul governorate (Iraq) (27%) (Zakaria, 2011), but higher than recently documented in Al-Diwaniyah province (Iraq) (17.5%) (Sakban & A'aiz, 2020). In contrast, significantly higher prevalence findings were reported from Ethiopia (24%) (Tilahun et al., 2018), Czech Republic (28%) (Lorencová et al., 2016), Tunisia (28.37%) (Amdouni et al., 2017), Yemen (28.94%) (Al-Shaibani et al., 2018), Poland (37.7%) (Kornacka et al., 2020), Lebanon (38%) (El Safadi et al., 2019), and Pakistan (42.80%) (Ullah et al., 2018).

Such variations are mostly attributed to difference in geographical locations, rearing practice, farm hygiene, and different diagnostic tests (serological, molecular, and bioassays) (Peyron et al., 2019). Indeed, the common method of rearing sheep and goats increases the likelihood of contact with environments polluted by oocysts, such as pastures, water, soil, and thereby increases the hazard of infection among farm animals (The European Food Safety Authority, 2013; Guo et al., 2015).

Urban areas tend to have lower prevalence due to less contact with wildlife and availability of veterinary care. It was reported that animals from farms visited by a veterinarian were less infected than farms without any veterinary care (Dahourou et al., 2019). In the study conducted by Sroka and associates, the highest prevalence of *T. gondii* DNA was among raw meat products obtained for samples originating from south-east regions of Poland-Podkarpackie (17.9%) and Małopolskie (12.6%) (Sroka et al., 2019). These are mountainous regions with numerous small farms, where pigs are often free-range reared and might have direct contact with cats, wild animals, and other potential sources of *T. gondii* infection.

Results of temporal variations (Table 3) are in good agreement with a very recent study in Al-Diwaniyah province, Iraq, a study confirmed that the autumn season (September, October, and November) was associated with increased prevalence and recorded significantly higher rates of infection in beef (Sakban & A'aiz, 2020). Moreover, another study confirmed that the overall *T. gondii* prevalence in retail fresh meat was higher in spring collection months, particularly during April and May, than in the summer or fall (Iqbal et al., 2018). Furthermore, the seroconversion rates varied from 0.42 to 0.96 seroconversions per year and were higher in autumn and winter than in spring and summer (Simon et al., 2018).

In Erbil, temperature decreases gradually during the second half of the year. This may provide chances for the oocysts to sustain viability and increase in infections. Indeed, it has been suggested that toxoplasmosis decreases during warmer, drier seasons because of the decreased number of viable oocysts in the environment (Logar et al., 2005).

Additionally, similar observations were reported from Iran where intermediate hosts were found to be more infected in the summer season (34.48%), while prevalence in other seasons (winter, spring, and autumn) was 30.44%, 18.18%, and 19.05%, respectively (Mosallanejad et al., 2012).

CONCLUSION

The results of this study provide baseline information on the occurrence of toxoplasmosis in red meat in the regions of Iraq and refer to an important human health and hygienic risk associated with the consumption of raw and undercooked meat from these animal species. It is vital to raise awareness of people regarding *T. gondii* infection. Consequently, national preventive strategies should be applied to avoid and control *T. gondii* transmission between food-producing animals and humans.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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