Evaluation of commercial Linked immune-sorbent assay (ELISA) for detecting sero-prevalence of *Toxoplasma gondii* antibodies in Iraqi women

Rasha Amer Noori Al-Tufaili

University of Kufa/Faculty of Science/Department of Laboratory Investigations

ABSTRACT

(147) women were investigated about IgG and IgM serum antibodies for Toxoplasma parasite by Eliza method, during the period January 2020 to May 2020. For the qualitative and quantitative detection of IgM and IgG Toxoplasma gondii antibodies it has been ELISA (enzyme immunoassay) Test Kit.

The result of present study showed that (35) of (147) samples were positive Toxoplasma gondii by ELISA of IgM and IgG. With respect to age parameter, the highest rate of infection was in (31-40) years old range, while the lowest rate of infection was in (51-60) years old range. The results ensured that the rate of infection in rural (21) residence more than that in urban. The results of present study showed that infection rate increased in private workers more than that of governmental workers. with respect to blood group parameter, the highest rate of infection was in (B+) years old range, while the lowest rate of infection was in (O-, A-, and AB-) years old range. The results according to weight ensured that the rate of infection increased in group (91-100 kg) more than the rest groups.

INTRODUCTION

The protozoan mammalian parasite toxoplasma gondii is ingested if oocytes excreted in cats or present in inadequate meat are transmitted. Invasive forms penetrate the bloodstream to the brain , heart and pulm, creating cystic aggregates which remain latent, but which can be reactivated during their lives. The majority of people in many cultures have been diagnosed with early infancy, but healthy individuals do not develop clinically obvious diseases. However, toxoplasmosis has significant consequences for HIV-infected patients (1).

In particular in low- and middle-income countries (LMICs), toxoplasmosis represents a major public health problem. More than 29 percent of the world 's population give serological evidence of T. Infection with gondii (2).

The parasite is transmitted horizontally to humans mainly by ingesting, handling and treating water , food or soil that has oocyst contamination or raw or undercooked cyst-containing meat. Infection in much more than 80% of immunocompetents is symptomless or flu-like in over 80 percent (3).

Although T. Gondii Parasite is kept at a dormant stage is called bradyzoites and will be an active form by weakening the immune system leading to clinical manifestations known as toxoplasmosis. Infections such as HIV, fatty liver diseases, or physiologic reaction shifts, such as in the case of pregnancy, can be due to reduced immunity (2).

If during pregnancy primary infection occurs, T. Crossing the placenta, gondii may be transmitted vertically to the fetus (congenital toxoplasm). Congenital toxoplasmosis may lead to abortions, death or significant sequelae in the eyes and neurology ranging from slightly less visual to serious disorders, including retinochoroiditis, hydrocephalus and intracerebral calcification (4). The risk of infecting child and of harming fetus depends on age of gestation when a mother is infected (5). This may lead to serious damage to the brain of the fetus, stroke, hydrocephalus, chorioretinitis, and mental delay (2).

During pregnancy there are 20 to 50 percent risk of congenital infections from primary T. Gondii infection if

untreated. The detection of nonimmune women at the start of pregnancy, details about the way infection is to be avoided, and serological follow-up will prevent congenital toxoplasmosis. Repeated testing of the specific IgG and IgM is based on the serological follow-up to identify acute and chronic infections in case of positivity (3).

Toxoplasma gondii seroprevalence rates tend to increase; even so, infection rates depend on dietary habits, health standards and socioeconomic standards vary widly among countries and regions. In most industrialized countries, improvements in hygiene conditions and farming systems and increased socio-economic levels have led to a decline in seroprevalence (5).

Human persons are infected by intake in raw or under-cooked meat of sporulated oocysts, contact with cat feces, soil, and by ingesting contaminated food and water. The transmission to the fetus and organ donation from infected donors involved other unusual causes of the transmission (6). Each stage of the life cycle involves multiple virulence factors, allows the immune system to be compromised and a chronic infection developed, and no human toxoplasmosis vaccine has until now been approved (7).

MATERIALS AND METHODS

Samples:

147 women were investigated about IgG and IgM serum antibodies for Toxoplasma parasite by Eliza method, during the period January 2020 to May 2020. Blood samples were obtained from the different clinical labs of Baghdad province, with recommended ethics and patient ask. The ages of women were 21-60 years. Weight of women were ranging between 51-100 kg. Other parameters had been reordered (residence, occupation, and blood group).

Collection of samples:

Approximately 4-5 ml of blood samples have been obtained from pregnant females in plane tubes. Estimated blood samples have been collected. Blood samples were centrifugated at 3000 xg for 15 min after 15 minutes at the room temperature. For IgG and IgM anti-toxoplasma

antibodies serum samples were separated and stored at - 40 C °.

Determination of IgG and IgM Toxoplasma antibodies

For the qualitative and quantitative detection of IgM and IgG Toxoplasma gondii antibodies it has been used two types kits are Toxoplasma IgM ELISA(enzyme immunoassay) Test Kit and Toxoplasma IgG ELISA(enzyme immunoassay) Test Kit manufactured by ACON Laboratoies, Inc. San Diego, USA.

Statistical analysis

The SPSS software package (version 12 for Windows) was used for statistical analysis.

RESULTS AND DISCUSSION

The result of present study showed that (35) of (147) samples were positive for Toxoplasma gondii by ELISA of IgM and IgG.

The result of the samples distribution according to their characteristics (no=147) was revealed in table (1).

Table (1) Samples distribution according to their characteristics (no=147)

Characteristics of Subjects	Groups	N
Residence	Urban	89
	Rural	58
Occupation	private workers	102
	governmental workers	45
	O+	53
	O-	4
Blood groups	A+	34
	A-	3
	B+	28
	B-	3
	AB+	21
	AB-	1
Age groups	21-30	84
	31-40	41
	41-50	12
	51-60	7
weight groups	51-60	95
	61-70	11
	71-80	10
	81-90	6
	91-100	25

The prevalence of infection with *T. gondii* according to age group

Sero- positivity for (147) samples were only (35) samples. With respect to age parameter, the highest rate of infection was in (31-40) years old range, while the lowest rate of infection was in (51-60) years old range, as shown in table (2).

These results accepted with Abamecha F, *et. al.*, 2016; Arce-Estrada GE, *et. al.*, 2017; and Madinna Mustafa *et. al.,* 2019(8, 9, 10), who ensured that people who aged 21–30 years having the highest frequency of T. gondii infection. Juliana Boaventura Avelar, *et. al.,* 2018 (11) results also accepted with recent study results.

Table (2) Sero-positivity of *T. gondii* with respect to age

Rate of infection with respect to age	Rate of Infection				
	Overall Seropositive	21-30	31-40	41-50	51-60
	35	10	21	3	1

The major risk factors for this infection include: age, level of education and diet habits. Sero-positivity is much more significant in older people after 31 years; due to the longer time of exposure, T. gondii's seroprevalence is higher in older people than youth; they are therefore more likely to get contaminated by various forms of infection (11).

In Iraq, Erbil, women suffered the highest toxoplasmosis Between the ages of 26 and 30. The explanation may be that these ages are most involved and that they are likely to have contact with one of many infection routes as they age (12).

The prevalence of infection with *T. gondii* according to Residence group

Table (3) showed the relationship of the infection with the residence of infected samples (no=35). The results ensured that the rate of infection in rural (21) residence more than that in urban (14).

Table (3) Sero-positivity of *T. gondii* with respect to Residence

Residence	Rate of Infection (no=35)
Urban	14
Rural	21

The results of this study was agreed with Rizgar Rashid Al- Bajalan, *et. al.*, (2015) and Juliana Boaventura Avelar, *et. al.,* 2018, (11, 12).

Data obtained from Suhad H. Mahmood *et. al.*, 2013 showed substantially high percentage of toxoplasmosis in inhabited rural area compared to urban residents (13).

These results can be attributed to people living in crowded conditions and to those working in land-related employment. All in, agricultural activities are a major source, including for other areas, of infection in the rural area, in particular farm buildings and their surroundings which house cats and intermediary host. In particular, T. Gondii is open to rural areas and farming to circulate between the wild and domestic environment , which makes it possible for T. gondii to mix domestics and sylvatic cycles with their spatial distribution, management and levels of biosecurity. (12).

The association between actions, food habits and toxoplasmosis positivity and current or past dog ownership, and meat intake were not defined as risk factors raw or undercooked meat (11).

The difference in seropositivity between rural and urban areas may depend on the hygienic and socioeconomic condition of oocyst removal by cats and people who are

in contact with the soil; in particular, the pavement that is widely seen in urban areas is considered to contribute to the reduced oocyst removal period of cats (13).

The prevalence of infection with *T. gondii* according to Occupation group
The results of present study showed that infection rate increased in private workers more than that of governmental workers, as shown in table (4).

Table (4) Sero-positivity of *T. gondii* with respect to Occupation

Occupation	Rate of Infection (no=35)
private workers	18
governmental workers	17

The results above accepted with Suhad H. Mahmood *et. al.,* 2013 work.
The cause of these findings may be that farmers are more likely than others to have low levels of education or access to soil. Communication with soil, infected vegetables and fruits was found to be a risk factor for infection by Toxoplasma, which has been reported as having significantly higher seroprevalence Toxoplasma antibodies for individuals keeping cattle and slaughter workers and inhabitants who consume raw or cooked meat and petting, particularly the cats, are more risk factors than other occupational groups and suggested exposure to T. gondiy infection (13) .
Human infected with toxoplasmosis by inhalation or ingestion of oocyst-contaminated dust particles. In addition, ineffectual hygiene, feeding and adequate weather factors may have led to high seroprevalence for sporulation and survival of oocysts in the area. Attributed to differing in cultural patterns and climatic factors affecting oocysts, the main objective of risks is relatively different from country to country. The reporting seroprevalence may be different from the study area, population of study samples, size, age of the sample, serological technology sensitivity of the cats used, cat densities in the regions, and access to oocyster contaminated feed and water (14) .

The prevalence of infection with *T. gondii* according to blood group
This study tried to explain the relationship between ABO blood groups and toxoplasmosis, with respect to blood group parameter, the highest rate of infection was in (B+) years old range, while the lowest rate of infection was in (O-, A-, and AB-) years old range, as shown in table (5).

Table (5) Sero-positivity of *T. gondii* with respect to blood group

Blood groups	Rate of Infection (no=35)
O+	9
O-	1
A+	6
A-	1
B+	12
B-	2
AB+	3
AB-	1

The results of this study agreed with that of Radhia Hussain Fadel, *et. al.,* (2018) (15).
Also the results above agreed with that obtained by Kolbekova P *et. al.* (2007) and Midtvedt T, Vaage L (1989) (16, 17).
Also these results shared the same findings of Fatemeh Talebi Meymand *et. al.,* (2015) (18).
Natural resistance to many infections was known to depend to some degree on an individual's blood group. Blood group A, B and O are classified on the surface of red blood cells by the existence of, or absence of A and B carbon antigens (19).
This establishes natural susceptibility to certain cell surface antigens similar to antigens of various blood groups in humans. This mechanism may partly explain the greater vulnerability of people with type AB in multiple infections because of a lack of naturally occurring antibodies in the blood of these individuals. The potential association between the grouping ABO system and anti-T. gondii antibodies is investigated in previous studies. Their findings are contradictory to four studies that have shown a linkage between B and AB infections (20). These studies have suggested that B-antigen might be a potential T.-gondii receptor. However, there was no evidence of this connection in two other similar investing initiatives. B antigen is proposed to represent a receptor of T. gondii in human demographics. There may be several factors in the debate in these results and other studies. For Iraqi patients, the molecular heterogeneity of strains may have been detected by the study's patients only male patients or AB antigens may have a significant influence on gastrointestinal mucosa adhesion and their contribution is evident from the high prevalence of Iraqi common infections T. gondii's adherence to the gastroindestinal mucosa (19).

The prevalence of infection with *T. gondii* according to weight group
Table (6) showed the relationship of the infection with the weight of infected samples (no=35). The results ensured that the rate of infection increased in group (91-100 kg) more than the rest groups.

Table (6) Sero-positivity of *T. gondii* with respect to weight group

weight groups	Rate of Infection (no=35)
51-60	5
61-70	4
71-80	6
81-90	2
91-100	18

Our results show that overweight and obese study participants have an increased chance of becoming seropositive.

There is excellent evidence that increased consumption of meat is linked to an increase in body weight, which in turn results in an increased chance of ingestion of contaminated meat (21). On the other hand, consuming vegetarian is adversely linked to seropositiveness and reports that eating vegetables infected with oocysts is not an necessary driver of seroconversion. These data are in total coordinated with most foodborne infections (22).

Seroprevalence of T. gondiiin pateints using ELISA test

It gave positive result by using tow types of ELISA test, the IgG positive was found in 35 case (45.16%), IgM positive 35 case (26.07%), from total of 147 examined samples, showed in table (7).

Table (7) Sero-positivity of *T. gondii* with respect to IgG and IgM

Ig percentage %	Age group (y)			
	21-30	**31-40**	**41-50**	**51-60**
IgG	23.9	45.16	3.65	6
IgM	5	26.07	9.62	0

In many instances, serological methods may not be reliable or inconclusive, including patients with underlying diseases which cause repressed anticorps, patients on immunosuppressive treatments and some congenital toxoplasmosis cases. Approximately one week after infection and for months or years, IGM antibodies can be detected. The IgM antibody titer for antimicrobials therefore does not necessarily imply that the patient is infected recently. A marker for acute infection developed earlier than IgM, IgA antibodies are known to survive for several months. Fast and rapidly, specific IgE antibodies are also generated, providing a higher indication of current infection. The presence of IgG antibodies does not provide details about the timing of infection, however indicates that infections occur (25).

In relation to age group the results showed high positive percentage samples in ELISA IgG and ELISA-IgM test at age group of (31-40) years. Whereas the lowest one was noticed at the age group of (51-60) years.

These results agreed with results obtained by Suhad H. Mahmood, *et. al.,* (2013) (19), and Kadihm, M. A. (2006). (23).

In young women adults, high prevalences of T-gondii infections were reported, due to more frequent contact with toxoplasms in children and adolescents, via cat contact, soil touch. Such discrepancies between previous findings and current results may be attributable to variations in methods specificity and sensitivity for diagnosing and reacting to parasite strain from individual host (23,24).
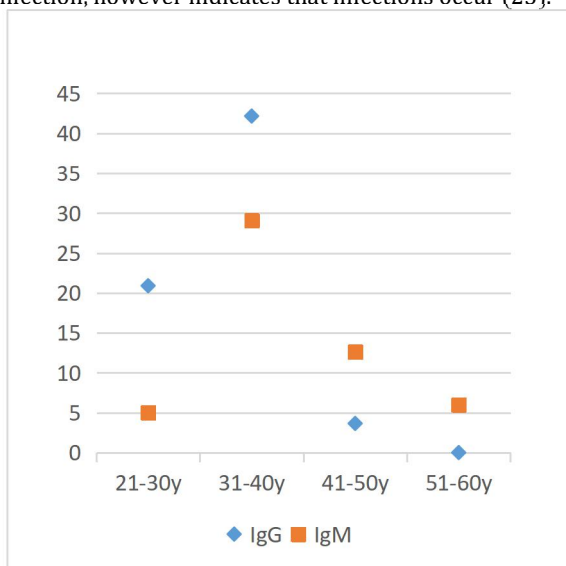

Figure (1) The mean percentage of IgM and IgG in the sera of the studied infected with toxoplasmosis using ELISA according to the age groups
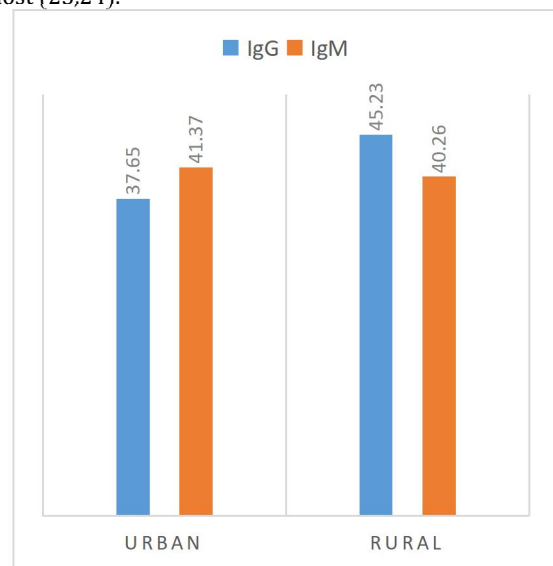

Figure (2) The mean percentage of IgM and IgG in the sera of the studied infected with toxoplasmosis using ELISA according to the Residence and Occupation

REFERENCES

1. Mosab Nouraldein Mohammed Hamad, (2018). TOXOPLASMOSIS OVERVIEW. Faculty of Health Sciences Elsheikh Abdallah Elbadri University, Sudan. April 2018 DOI: 10.13140/RG.2.2.30318.92488.

2. Madinna Mustafa, Fatima Fathy, Abubaker Mirghani, Mona A. Mohamed1, Mohamed S. Muneer, Abdallah E. Ahmed5, Mohamed Siralkhatim Ali, Rihab A. Omer,

Emmanuel E. Siddig, Nouh S. Mohamed and Amjed M. Abd Elkareem, (2020). Prevalence and risk factors profile of seropositive *Toxoplasmosis gondii* infection

3. among apparently immunocompetent Sudanese women. Mustafa et al. BMC Res Notes (2019) 12:279 https://doi.org/10.1186/s13104-019-4314-0.

4. Daniela Fanigliulo, Serena Marchi, Emanuele Montomoli, and Claudia Maria Trombetta, (2020). Toxoplasma gondii in women of childbearing age and during pregnancy: seroprevalence study in Central and Southern Italy from 2013 to 2017. Parasite 27, 2 (2020) _D. Fanigliulo et al., published by EDP Sciences, 2020 https://doi.org/10.1051/parasite/2019080.

5. Zaid Hamid Mahmoud, Nuha Farhan Abdul Kareem, Aklas Ahmed Abdul Kareem., Effect of solvents on size of copper oxide nanoparticles fabricated using photolysis method. ASIAN J CHEM., 30(1): 223-225, 2018.

6. Robert-Gangneux F, Darde ML. 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. Clinical Microbiology Reviews, 25(2), 264–296.

7. Mohamed Alkhatim Alsammani, (2016). Sero-epidemiology and risk factors for Toxoplasma gondii among pregnant women in Arab and African countries. J Parasit Dis (July-Sept 2016) 40(3):569–579 DOI 10.1007/s12639-014-0558-8.

8. Iltefat Amer Al-Tameemi , Basim Hashim Abdullah and Shaymaa Jabbar Raisan, (2018). SEROPREVALENCE OF TOXOPLASMA GONDII AMONG CANCER PATIENTS IN BASRAH PROVINCE/IRAQ. World Journal of Pharmaceutical Research SJIF Impact Factor 8.074 Volume 8, Issue 1, 193-199. Research Article ISSN 2277– 7105.

9. Mohammed Alwan Farhan, Zaid Hamid Mahmoud, Marwa Sabbar Falih., Synthesis and characterization of TiO2/Au nanocomposite using UV-Irradiation method and its photocatalytic activity to degradation of methylene blue., ASIAN J CHEM., 30(5): 1142-1146, 2018.

10. Arce-Estrada GE, Gⓞmez-Toscano V, Cedillo-Pelⓙez C, Sesman-Bernal AL, Bosch-Canto V, Mayorga-Butrⓞn JL, et al. Report of an unusual case of anophthalmia and craniofacial cleft in a newborn with Toxoplasma gondii congenital infection. BMC Infect Dis. 2017;17(1):459.

11. Madinna Mustafa, Fatima Fathy, Abubaker Mirghani, Mona A. Mohamed, Mohamed S. Muneer, Abdallah E. Ahmed, Mohamed Siralkhatim Ali, Rihab A. Omer, Emmanuel E. Siddig, Nouh S. Mohamed* and Amjed M. Abd Elkareem, (2019). Prevalence and risk factors profile of seropositive Toxoplasmosis gondii infection among apparently immunocompetent Sudanese women . Mustafa et al. BMC Res Notes (2019) 12:279, https://doi.org/10.1186/s13104-019-4314-0.

12. Juliana Boaventura Avelar, Marcos Gontijo da Silva, Hanstter Hallison Alves Rezende, Heloisa Ribeiro Storchilo, Waldemar Naves do Amaral, Isolina Rodrigues Xavier, Mariza Martins Avelino and Ana

Maria de Castro, (2018). Epidemiological factors associated with Toxoplasma gondii infection in postpartum women treated in the public healthcare system of Goiânia, State of Goiás, Brazil. Rev Soc Bras Med Trop 51(1):57-62, January-February, 2018 doi: 10.1590/0037-8682-0112-2017.

13. Rizgar Rashid Al- Bajalan, Fatima S. Al- Nasiri1 and Saadi M. Mahmood, (2015). Detection Toxoplasma gondii by Latex and ELISA Test in Infertile and Fertile Men in Kalar City, Kurdistan Region, Iraq. Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 570-585.

14. Suhad H. Mahmood, Ban N. AL-Qadhi and Khawla H. Zghair, (2013). Prevalence of Toxoplasmosis of Males Blood Donors in Baghdad. Iraqi Journal of Science, 2013, Vol 54, No.4, pp:832-841.

15. Gebremedhin, E.Z., Abebe, A.H., Tessema, T.S., Tullu, K.D., Medhin, G., Vitale, M., Marco, V.D., Cox, E., Dorny, P. (2013). Seroepidemiology of Toxoplasma gondii infection in women of child-bearing age in central Ethiopia. Infec. Dis., 13(101): 1 9.

16. Noor Sabah Al-Obaidi, Zaid Hamid Mahmoud, Ahlam Ahmed Frayyih Anfal S Ali, Farah K Ali., Evaluating the electric properties of poly aniline with doping ZnO and α-Fe2O3 nanoparticles. Pharmacophore., 9(5):61-67, (2018).

17. Kolbekova P, Kourbatova E, Novotna M, Kodym P, Flegr J (2007).Newand old risk-factors for Toxoplasma gondiiinfection: prospectivecross-sectional study among military personnel in the CzechRepublic. Clin Microbiol Infect. 2007; 13(10): 12-7.

18. Midtvedt T, Vaage L. Relationship between Toxoplasma gondii antibodies and blood group. Eur J Clin Microbiol Infect Dis.1989; 8(6): 575-6.

19. Fatemeh Talebi Meymand, Shahnaz Shirbazou, Laila Abasian1 and Ali Delpisheh, (2015). Prevalence of Toxoplasma gondii infection in blood donors. Int.J.Curr.Microbiol.App.Sci (2015) 4(1): 500-504.

20. Suhad H. Mahmood, Ban N. AL-Qadhi and Khawla H. Zghair (2013). Prevalence of Toxoplasmosis of Males Blood Donors in Baghdad. Iraqi Journal of Science, 2013, Vol 54, No.4, pp:832-841.

21. Midtved, T. and L. Vaage. 1989. Relationship between Toxoplasma gondii antibodies and blood group .Eur journal clin. Microbiol. infect.Dis. 8(6) , pp:575-6.

22. HendrikWilking, MichaelThamm, Klaus Stark, ToniAebischer & Frank Seeber, (2016). Prevalence, incidence estimations, and risk factors of Toxoplasma gondii infection in Germany: a representative, cross-sectional, serological study. Published: 03 March 2016. Scientific Reports | 6:22551 | DOI: 10.1038/srep22551.

23. Jones, J. L. & Dubey, J. P. Foodborne toxoplasmosis. Clin. Infect. Dis. 55, 845–851 (2012).

24. Kadihm, M. A. (2006).Study of some immunological parameters of women sera infected with toxoplasmosis .M. Sc. Thesis. College of science .Baghdad University .pp:130 (in Arabic).

24. Spalding; S.M., M. R. R.; Amendoeira, C. H. Klein and L. C. Ribeiro. **2005**. Serological screening and toxoplasmosis exposure factors among pregnant women in south of Brazil. *Rev. Soc. Bras. Med. Trop.*, 38, pp:173-177.

25. Hatham I. Khalil,Maysoon A. Merdaw,Arwa M. Abdullah, Waleed K. El-Hashimi, Nada M. Al-Bashier, Huda J. Mohemmad, (2016). Estimation of Toxoplasma gondii infection by Serological and Immunohistochemical methods in Baghdad City-Iraq. International Journal of Advanced Research (2016), Volume 4, Issue 4, 272-278.