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

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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 (85) 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 (23) 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 (0-, A-, and 4B+) 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.

Keywords: Evaluation of commercial Linked immune-sorbent assay (ELISA), sero-prevalence of Toxoplasma gondii, antibodies in Iraqi women

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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 toxoplasmosis). Congenital toxoplasmosis may lead to abortions, death or significant sequelae in the eyes and neurology ranging from slightly less visual to serious disorders, including retinocochoroiditis, 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 widely 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>
<thead>
<tr>
<th>Characteristics of Subjects</th>
<th>Groups</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residence</td>
<td>Urban</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>58</td>
</tr>
<tr>
<td>Occupation</td>
<td>private workers</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>governmental workers</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>0+</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>0-</td>
<td>4</td>
</tr>
</tbody>
</table>

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.

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>
<thead>
<tr>
<th>Rate of Infection with respect to age</th>
<th>Overall</th>
<th>Seropositive</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of Infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>35</td>
<td>10</td>
<td>21</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

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

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

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 domestic 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

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Rate of Infection (no=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>private workers</td>
<td>18</td>
</tr>
<tr>
<td>governmental workers</td>
<td>17</td>
</tr>
</tbody>
</table>

The results above accepted with Suhad H. Mahmoud 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. gondii* 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 oocyst 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

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>Rate of Infection (no=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O+</td>
<td>9</td>
</tr>
<tr>
<td>O-</td>
<td>1</td>
</tr>
<tr>
<td>A+</td>
<td>6</td>
</tr>
<tr>
<td>A-</td>
<td>1</td>
</tr>
<tr>
<td>B+</td>
<td>12</td>
</tr>
</tbody>
</table>

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 gastrointestinal 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

<table>
<thead>
<tr>
<th>weight groups</th>
<th>Rate of Infection (no=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>51-60</td>
<td>5</td>
</tr>
<tr>
<td>61-70</td>
<td>4</td>
</tr>
<tr>
<td>71-80</td>
<td>6</td>
</tr>
<tr>
<td>81-90</td>
<td>2</td>
</tr>
<tr>
<td>91-100</td>
<td>18</td>
</tr>
</tbody>
</table>
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 seropositivity 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. gondii patients 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>
<thead>
<tr>
<th>IgG percentage</th>
<th>Age group (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21-30</td>
</tr>
<tr>
<td>IgG</td>
<td>23.9</td>
</tr>
<tr>
<td>IgM</td>
<td>5</td>
</tr>
</tbody>
</table>

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).

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

**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


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