Comparison of PCR with ELISA in Diagnosis of recent Toxoplasmosis in Pregnant women

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Abstract: Background: Maternal infection with toxoplasmosis during pregnancy is frequently associated with transplacental transmission to the fetus. Early diagnosis of toxoplasmosis in pregnant women is necessary to get effective treatment and prevent fetal complications. Objective: The present study aimed to assess the use of PCR and compare it with enzyme-linked immunosorbent assay (ELISA) for detection of Toxoplasma recent infections in sera of pregnant women. Methodology: one hundred and thirty pregnant women were included in this study ranging in age from 17-38 years selected from patients attending outpatient clinics of the Obstetric & Gynecology Department at Zagazig University Hospital. They were divided into 2 groups, control group of 30 healthy pregnant women and case group of 100 complicated pregnancy states. Both groups were tested for the presence of Toxoplasma DNA in their blood by PCR and specific antibodies to Toxoplasma by ELISA. The results obtained were recorded to evaluate the best technique to detect recent infection. Results: Out of 130 subjects of the study, 62 (47.7%) & 47 (36.15%) cases had positive results for ELISA anti-T. gondii (IgG) and (IgM). In the control group, five (3.85%) & two women (1.5%) had positive results respectively with statistically highly significant difference (P < 0.001). 73 cases (56.1%) had positive PCR results, 27 cases (20.8%) and all controls were negative. with highly significant increase of PCR positivity in patient compared with controls (P<0.001). Sensitivity & Specificity of ELISA IgG were 71. % & 63% respectively with accuracy of 69% and of IgM was 54.7% & 74.1% respectively with accuracy of 60% when compared with PCR. There is significant association between women in contact with soil or cats and positive ELISA & PCR. Conclusion: this study highlights the need for a confirmatory test to detect primary acute toxoplasmosis in pregnant women. It demonstrates the possibility of defining and selecting the high-risk cases for mother-to-child transmission of infection by combining specific serology and PCR tests to formulate a specific approach. Tox-IgG indicated catching Toxoplasma infection but not enough to determine recent infection, while PCR besides, being valuable in diagnosing it.


Key words: Toxoplasma gondii, PCR, ELISA and Complicated pregnancy.

1. Introduction:
Primary maternal infection during pregnancy is frequently associated with transmission of T. gondii to the fetus. Transplacental transmission of T. gondii may lead to severe congenital infection including abortion (Zargar et al., 1999, Vado-Solis et al., 2013 and Gebremedhin et al., 2013), fetal death, or neurological or ocular damage of the fetus (Tenter et al., 2000). Neonatal loss or congenital defects depending on the stage of gestation when the infection occurs (Dubey and Beattie, 1988). Early maternal infections are less likely to result in congenital infection but the sequelae are more severe, maternal infection occurs in the latter half of pregnancy is more common, but fetal injury is usually less severe (Gange, 2001).

Although serological testing has been one of the major diagnostic techniques for toxoplasmosis, it has many disadvantages, for example, it may fail to detect specific anti-toxoplasma immunoglobulin G (IgG) or IgM during the active phase of T.gondii infection, because these antibodies may not be produced until after several weeks of parasitemia. Therefore the high risk of congenital toxoplasmosis of a fetus may be undetected because the pregnant mother might test negative during the active phase of T.gondii infection. Several PCR – based techniques (Lee et al., 1999 and Pujo-Mique et al., 1999) which have been developed for the diagnosis of Toxoplasma using various clinical specimens, including amniotic fluid (Romand et al., 2004 and Schaefer et al., 2011), blood (Bergstrom et al., 1998, Joss et al.,1999 and Bin Dajem & Almushait, 2012), cerebrospinal fluid (Roberts et al., 2001), and tissue biopsy (Su et al., 2010). Among these techniques, nested PCR followed by hybridization of PCR products has been the most sensitive method. The present study aimed to assess the use of PCR and compare it with ELISA for detection of Toxoplasma recent infections in sera of pregnant women.

2. Material and Methods
Type of study: case control study.
Study design:
This study was carried out during the period from November 2011 to November 2012 all of the laboratory techniques were performed in Parasitology and Microbiology Departments, Faculty of Medicine, Zagazig University.

This study was carried out on 130 pregnant women ranging in age from 17-38 years which selected randomly from patients attending Outpatient Clinics of the Obstetric & Gynecology Department at Zagazig University Hospital. These women were divided into 2 groups, control group of 30 healthy pregnant women and case group of 100 complicated pregnancy states had to fulfill one of the following criteria; Women with repeated abortion (>two times), Premature delivery, Congenial anomaly, Intrauterine fetal death (IUFD), Still birth (S.B). These data were obtained from the pregnant women who were interviewed using a structured questionnaire. Demographic and other relevant information were recorded for each woman. For ELISA and PCR techniques, 10 ml of venous blood were collected from each participant in the study. Each blood sample was divided into two tubes, one heparinized tube were used for PCR and the second one serum for ELISA.

**Detection of Toxoplasma (IgG, IgM) by ELISA technique according to Turunen et al. (1983).**

**Principle of ELISA test**

The test is based on the passive adsorption of the soluble antigen on the surface of a solid phase polystyrene 96 wells microtitration plates. The addition of the serum containing specific antibodies results in formation of specific antigen antibody complex fixed to the plates. Enzyme could be coupled to antigen antibody complex by adding antibodies against the specific immunoglobulin labeled with enzyme. Quantity of the enzyme linked to antigen – antibody complex could be measured through its effect on the specific substrate that gave color proportional to enzyme activity.

Detection of anti-Toxoplasma IgG & IgM in sera was done using commercially available ELISA-kit: IgG& IgM Antibody – Pishtaz Teb Diagnostics - MA Toxoplasma IgG& IgM _48_01, Catalogue No. PT- Toxoplasma –IgG& IgM _48_, Iran).

**Detection of Toxoplasma DNA by PCR technique according to Grover and Thulliez (1990).**

**DNA extraction**

PCR amplifications:

Isolation of DNA.

DNA was isolated from blood samples using a commercial purification system (Wizard Genomic DNA Purification Kit; Intron Biotechnology, Inc. (Korea). following the manufacturer’s instructions for DNA purification from blood. Final pellets were resuspended in 30 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2) and stored at –20°C until used.

The PCR was performed on all DNA samples to amplify a fragment from the B1 gene, which is present in 35 copies and is conserved in the *T. gondii* genome, as described by Burg et al. (1989).

The primers used in the round of the PCR were (5′-CCG CCT CCT TCG TCC GTC GTA -3′), and (5′- TGA AGA GGA AAC AGG TGG TCG -3′), which correspond to nucleotides 694-714 and 887–868, respectively at 193 bp.

Three microliters of template DNA were added to a final volume of 50 μL of PCR mixture consisting of 5 μL of 10x PCR buffer (50 mM Tris-HCl, pH 9.1, 3.5 mM MgCl2), 8 μL of 1.25 mM deoxynucleoside triphosphates, 0.5 μL of Taq DNA polymerase [5 units/μL], and 1.5 μL (20 pmol) of each primers. The amplification was performed in Biometra T Gradient thermal cycler PCR. The cycling conditions for PCR were 95°C for 10 minutes, followed by 30 cycles at 94°C for one minute, 55°C for one minute and 75°C for one minute, and a final extension at 72°C for five minutes.

DNA extracted from RH strain of *T. gondii* from the collection of the Service de Parasitologie-Mycologie (Grenoble, France) was used as a positive control. The PCR mixture without DNA and with DNAase-free water were used as negative controls to monitor for cross-contaminations.

Controls were loaded last to avoid contamination of the sample. To ensure the reliability of the results and detect any possible contamination, all samples were processed in duplicate. The test result was considered positive if the amplified DNA fragment was clearly visible in both samples.

Five microliters of the PCR product were subjected to electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized using transilluminator (Burg et al., 1989).

**Ethical consideration:**

Ethical approval was obtained from the Committee of Research, Publications and Ethics of the college of Medicine, Zagazig University, Egypt. All procedures were explained to patients in the local language, and written or thumb-printed informed consent was obtained.

**Statistical analysis:**

All data were subjected to statistical analysis using SPSS win statistical package version 11 using Chi-square test to examine the relation between qualitative variables. Significance was defined as *P* < 0.05.

**3. Results:**

The results are presented in the following tables and figures.
Table (1): Demographic data of the subjects included in the study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n=100)</th>
<th>Control (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>77</td>
<td>16</td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>Contact with cats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26</td>
<td>7</td>
</tr>
<tr>
<td>No</td>
<td>74</td>
<td>23</td>
</tr>
</tbody>
</table>

Table (2): Results of ELISA anti-Toxoplasma (IgG) among tested groups.

<table>
<thead>
<tr>
<th>Serum IgG - ELISA</th>
<th>Cases</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>62</td>
<td>(47.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>38</td>
<td>(29.3)</td>
</tr>
</tbody>
</table>

P < 0.01

Chi-square test used

Table (3): The Results of ELISA anti-Toxoplasma (IgM) among tested groups.

<table>
<thead>
<tr>
<th>Serum IgM ELISA</th>
<th>high risk cases</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>47</td>
<td>(36.15)</td>
</tr>
<tr>
<td>Negative</td>
<td>53</td>
<td>(40.7)</td>
</tr>
</tbody>
</table>

P < 0.01

Chi-square test used

Table (4): Comparison between results of ELISA (IgM) and (IgG) among high risk cases.

<table>
<thead>
<tr>
<th>IgM –ELISA</th>
<th>(IgM)</th>
<th>(IgM)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG-ELISA</td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
<td>24</td>
<td>47</td>
</tr>
<tr>
<td>Negative</td>
<td>39</td>
<td>14</td>
<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>38</td>
<td>100</td>
</tr>
</tbody>
</table>

P < 0.01

Table (5): Results of PCR technique among tested groups.

<table>
<thead>
<tr>
<th>Serum PCR</th>
<th>High risk cases</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>73</td>
<td>(56.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>27</td>
<td>(20.8)</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td></td>
</tr>
</tbody>
</table>

P < 0.01

Table (6) accuracy of ELISA IgG compared to PCR:

<table>
<thead>
<tr>
<th>IgG</th>
<th>PCR</th>
<th>+ve (n=73)</th>
<th>-ve (n=27)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n=62)</td>
<td></td>
<td>52</td>
<td>10</td>
<td>71.2</td>
<td>63</td>
<td>83.8</td>
<td>44.7</td>
<td>69</td>
</tr>
<tr>
<td>Negative (n=38)</td>
<td></td>
<td>21</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (7) accuracy of ELISA IgM compared to PCR:

<table>
<thead>
<tr>
<th>IgM</th>
<th>PCR</th>
<th>+ve (n=73)</th>
<th>-ve (n=27)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n=47)</td>
<td></td>
<td>40</td>
<td>7</td>
<td>54.7</td>
<td>74.1</td>
<td>85.1</td>
<td>37.7</td>
<td>60</td>
</tr>
<tr>
<td>Negative (n=53)</td>
<td></td>
<td>33</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity (%) = TP/ (TP+FN) x 100 and specificity (%) = TN/ (TN+FP) x 100 (TP: true positive, FN: false negative, TN: true negative and FP: false positive values). The positive predictive value of a diagnostic test is the proportion of total positive test results that are true positives. The negative predictive value of a diagnostic test is the proportion of total negative results that are true negatives. These were calculated using the following formulas: positive predictive value (%) = TP/ (TP+FP) x 100 and negative predictive value (%) = TN/ (TN+FN) x 100. Accuracy were also calculated as the accuracy% = (TP+ TN)/ all cases examined x 100.
control, specificity or sensitivity of many commercial serologic test kits in the market (Shin et al., 2009).

Physicians, responsible for care of pregnant women are confused when faced with conflicting results. This often leads to incorrect information being provided by the laboratories to the physicians as well as by the physicians to their patients. In recent years, a major effort has been made toward improving the ability to detect recently acquired infection in the pregnant women and congenital infection in the fetus and newborn by using PCR (Montoya, 2002).

In the present work 100 cases of complicated pregnancies were selected as highly suspected cases of toxoplasmosis. Their demographic data were represented in (Table 1) as 77 women of the high risk cases were in contact with soil, 31 cases were eating undercooked meat and 26 of them were in contact with cats.

The ideal situation for the diagnosis of recent T. gondii infection in pregnancy depends on having an antibody-negative serum sample collected at the beginning of pregnancy or before conception but this is usually not possible. Usually, the first serum sample is taken at the first antenatal health care visit confirming pregnancy which is usually between the eighth and thirteenth weeks of gestation. The used tests in antenatal screening should be able to determine if the infection occurred after conception or not (Vimercati, 2000).

In the present study, ELISA anti-Toxoplasma IgG was positive in 62 % cases of complicated pregnancy group and it is statistically highly significant in relation to 5 cases in the control group ($P < 0.01$) (Table 2). This result agrees with Markovich et al. (2013) who reported 60.4% incidence of IgG-positive cases. However, Bouhamdan et al. (2010) demonstrated 67 % incidence of IgG positive cases in private laboratories in their study.

Lower results of IgG-positive cases were reported by many authors. Saeedi et al. (2007) reported (48.3 %) and Galvan et al. (1995) reported (44.9 %) in cases with abortions. Abu-Madi et al. (2010), in Qatar, reported (35.1%) IgG positive results in their study.

A lower incidence of IgG-positive cases had been reported by other authors. In comparison to the present results of anti-Toxoplasma IgG antibodies, previous studies recorded lower incidence rates to be 0.8% in Korea (Song et al., 2005), 11% in U.S. (Jones et al., 2007), 20.45% in Egypt (Maysa and Awadallah, 2010). According to Ieni and Kaya (2009), they found a prevalence of (33.4%) with the microparticle enzyme immunoassay (MEIA). Moreover, Khurana et al. (2010) reported only (15.13%) in uncomplicated screened pregnant women.

4. Discussion

Toxoplasmosis caused by Toxoplasma gondii is usually asymptomatic in immunocompetent individuals; however, it may cause severe disorders in immunocompromised patients and in the pregnant women because of the higher risk of transplacental transmission and the occurrence of multiple congenital lesions in the fetus (Foulon and Villena, 1999).

A rapid and accurate diagnosis is required in order to start the relatively efficient antiparasitic treatment and to prevent the risk assessment of fetal transmission of infections (Bastion, 2002).

Although the diagnosis of patients with toxoplasmosis has been faced by a number of problems, the most frequent challenge encountered by physicians all over the world is how to determine if pregnant women essentially are not at risk to get infected infants unless they are immunocompromised (Shin et al., 2009).

The high prevalence and lifelong persistence of anti-Toxoplasma IgG antibodies among healthy individuals in many geographical areas prevent their use to reflect the recent infection (Remington and Joes, 2004). Another problem is the frequent lack of reliability when anti-Toxoplasma IgM antibodies test results are used to discriminate between recent and distant infection. In addition, there is lack of quality
using IgG-avidity. These different results may be due to different environmental and hygienic conditions which may play a role in epidemiology of toxoplasmosis. The discrepancy between their results and ours may be also due to the use of different immunoassay tests.

Jenum et al. (1997) reported that the best serological means of diagnosis of acute acquired toxoplasmosis is by using ELISA- anti Toxoplasma (IgM) test. Generally, most clinicians determine an active Toxoplasma-IgM antibodies or by detecting a threefold increase in IgG antibodies in pregnant women during the first trimester. However, relying only on IgM or IgG test to detect an acute infection may results in unnecessary interventions in pregnant women (Iqbal, 2007).

In our study, out of 100 patients with complicated pregnancy cases, 47 % were positive by ELISA- IgM test which was statistically highly significant result in relation to two positive cases in the control group (P < 0.01) (table 3). This result was in agreement with that recorded by, De Paschale et al. (2008) who reported 45.89 % in a study in Italy. Swai and Schoonman (2009) reported 46% seropositive cases in Tanzania.

Higher results were obtained by Wilson (1997) who recorded 77.5% positive IgM cases by using Toxo-IgM kit in a study on IgG positive cases.

Lower result was recorded by Galvan et al. (1995) who found 33.4 % IgM positive result in cases of repeated abortions. Lower incidence (19.3%) was reported also by Gebremedhin et al. (2013) in Ethiopia. Abdel-Hameed and Hassanien (2004) reported that 13.2% of their patients were positive for ELISA- IgM. This difference is attributed mainly to the type of selection of the studied samples; most of the previous studies were performed on uncomplicated cases.

The present study was performed to highlight the possible role of PCR test in the accurate diagnosis of toxoplasmosis where infection was suspected in complicated pregnancy states. Romand et al. (2004) concluded that, the PCR protocol appears to be the most sensitive protocol in the detection of T. gondii DNA. The high sensitivity combined with the high specificity offered by PCR led us to investigate the presence of the T. gondii genome in the blood of pregnant women suspected to be infected with this parasite.

In this study, out of 130 pregnant women, PCR detected T. gondii DNA in 73 (56.1%) cases, while all pregnant women in the control group were negative by PCR (Table 4). In agreement to our results, Higa et al. (2010), reported (47.2%) of blood samples were positive PCR. On the other hand, Nimri et al. (2004), who used nested PCR on cases of repeated abortions, recorded only (13.5%) positive cases of PCR but they gave similar results of negative PCR of the all control subjects.

Some authors evaluated different techniques in the diagnosis of toxoplastic encephalitis including detection of antibodies, circulating antigens and parasite DNA by PCR. Menotti (2010) concluded that, the detection of T. gondii DNA by PCR was the most useful test especially in untreated patients with a sensitivity of 81%.

Using PCR analysis to detect Toxoplasma DNA to confirm the recent infection represents an additional confirmatory method in IgM-positive or IgM-negative women. Moreover, the use of PCR decreases the need for follow-up sera and for unnecessary therapeutic intervention in pregnant women (Iqbal, 2007).

Previous studies have documented that PCR can actually detect T. gondii in blood samples of women before or during pregnancy. Wahab et al. (2010) concluded that, the detection of T. gondii DNA by PCR amplification has advantages over other methods. Their results showed that a single T. gondii parasite could be directly detected by PCR using the 35-fold-repetitive B1 gene as target for amplification.

Among the factors influencing the PCR outcome, the choice of the DNA target is generally considered essential (Reischl et al., 2003). Few DNA target loci have been described for Toxoplasms PCR. But more than 25 different primer pairs have been used in different assays, most of them targeting the repetitive 35-copy-number B1 gene (Buchbinder et al., 2003) which already had been used in this work.

In the present work, by comparing the IgG-ELISA results with serum PCR we found that, among 62 positive samples by IgG-ELISA, only 52 samples were positive by PCR and the remaining 10 samples were negative (table 6). On the other hand, out of 38 samples negative by IgG-ELISA, 21 were positive by PCR. So, PCR test detect more positive cases than IgG-ELISA. In relation to PCR, IgG-ELISA had sensitivity of 71.2 % and specificity of 63 %.

Remington and Klein (1995) concluded that IgG ELISA may give false positive result. The explanation of this is that IgG antibodies usually appear within 1-2 weeks of infection, peak within 1-2 months, declined at various rates and usually persist for life. IgM antibodies may appear earlier and decline more rapidly than IgG antibodies. Also, Press et al. (2005) recorded that the presence of IgG and IgM Toxoplasma antibodies in a single serum sample drawn during gestation can't be used to define whether the infection was recently acquired or chronic.

According to these results, in comparison of IgM ELISA results and PCR we detected that, out of 47 cases positive by IgM ELISA, only 40 were positive by PCR and the remaining 7 were negative. This
serves that the 40 positive cases by both the IgM ELISA and PCR are recent infections (table 7). The
cause of the previous complicated pregnancy outcomes in these women was not clear, and it cannot be related
bit to previous maternal Toxoplasma infections.

In the present work, true- positive IgM antibody
test results were in 40 cases while seven cases were
false positive for this test (Table 7). False-positive
ELISA, IgM results have been reported by other
investigators (Howe and Sibley 1995). In addition,
even true positive results must be interpreted with
cautions because IgM antibodies may persist for more
than one year after acute infection. The use of
appropriate conjugate to measure only IgM antibodies
to T. gondii has increased the sensitivity and
specificity of the test (Gras et al., 2004). The false
positive IgM results may be due to rheumatoid factor
or antinuclear antibodies (Liesenfeld et al., 2001).
Also, some authors reported that, IgM antibodies
could be detected as long as 12 years after the acute
infection (Bobić et al., 1991).

In the present study, in relation to PCR, IgM-
ELISA test had a sensitivity of 54.7 % and a
specificity of 74.1 % (Table 7). Nearly, the same
sensitivity of IgM was reported by Pinon et al. (2001)
that have proved poor sensitivity (54 %) of IgM
detection. In agreement of our results, Beaman et al.
(1994) concluded that, the presence of IgM was poor
indicator of primary infection.

Lower results were reported by other authors.
Abu-Madi et al. (2010) compared IgM -ELISA with
PCR test, they reported that, PCR test was positive in
44% of cases in Qatar while IgM-ELISA was positive
only in 11% of cases. Ramadan et al. (2000) found
that, among children with malignancy, the incidence
of toxoplasmosis was estimated at 1.4% using IgM
ELISA, 8.2 % by IgG ELISA and 12.3 % by using
PCR. They concluded that, PCR was the most useful
diagnosing toxoplasmosis with malignancy.

Abdel-Hameed and Hassanein (2004)
evaluated the semi quantitative PCR and ELISA-IgM
in the diagnosis of toxoplasmosis in females with
repeated abortions. They found Toxoplasma antibodies
in (35 %) of cases while PCR detected toxoplasmosis
in (38%). Furthermore the sensitivity of ELISA
compared to PCR was 81.75 % and specificity was
93.5%.These results were in agreement of our results.

Bin Dagem (2012) reported Toxoplasma DNA in
(41%) of pregnant women by nested PCR. IgM-
ELISA assay detected 9 (6.5%) of these cases. The
results of IgG detection were positive in 53 (38.6%)
of the patients.

Other investigators evaluate using Western blot
diagnosis of toxoplasmosis in maternal serum. Western blot proved (86.9%) sensitivity and its
specificity were 96.1% when this test was used with
ELISA (Pinon et al., 2001).

Also, Peng et al. (2011) reported that, although
serological testing has been one of the major
diagnostic techniques for toxoplasmosis, it has many
limitations. For example, it may fail to detect specific
anti- Toxoplasma IgG or IgM during the active phase
of T.gondii infection because these antibodies may not
be produced until after several weeks of parasitemia.
Therefore, the high risk of congenital toxoplasmosis
of fetus may be undetected because the pregnant mother
might test negative during the active phase of T.gondii
infection.

Moreover, serological tests may fail to detect
T.gondii infection in certain immunocompromised
patients due to the fact that, the titers of specific anti-
Toxoplasma IgG or IgM may fail to rise in this type of
patients (Robert et al., 2001).

James et al. (1996) speculated that, serological
investigations is time consuming and may lack
sensitivity. Also, PCR produces more positive
reactions than isolation of the parasite by means of cell
culture or animal inoculation. Similar conclusions
were reported by Franck et al. (2001). They
concluded that, PCR was more sensitive, more specific
and rapid than animal culture and no cross reactions
were observed in samples containing various
opportunistic pathogens.

In the present work, the relation of the results of
the diagnostic tests and the risk factors were recorded.
The risk factor strongly associated with acute infection
in the positive cases was the direct contact with soil
(Figure 1). The same result was reported by Pinon et al.
2001. Also, Lebech and Peterson (1992) found
higher incidence in villages than in cities. These
results due to that in the rural areas there are more
environmental pollution, poor hygiene, low socioeconomic condition and high humidity that might
be responsible for oocyte transmission.

Moreover, in the present study, PCR-positive
results were in 20(77%) cases of women who were in
contact with cats (Figure 2). Higa (2010) in Barazil
reported that cat contact was significant risk factor for
infection for toxoplasma in pregnant women. However,
Ennegro et al. (2013) reported that contact with cats was found to be not associated with
seropositivity of toxoplasmosis. The difference
between them and our results referred to the difference
of the environmental factors that affect the
epidemiology of toxoplasmosis.

Romand et al. (2004) reported that it is likely
that the use of real- time PCR, especially on amniotic
fluid, will result in a decrease in the laboratory
variations observed with the conventional three-stage
PCR. In addition, real-time PCR can be used to
estimate the concentration of parasites in amniotic
fluid, which may be helpful for physicians to assess neonatal outcome (i.e., maternal infections acquired before 20 weeks with a parasite of load of >100/ml of amniotic fluid to have the highest risk of severe fetal outcome.

Cresti et al. (2001) reported that the PCR of urine or any body fluid may be valuable in diagnosing congenital toxoplasmosis. The clearance time for *Toxoplasma* DNA from the blood of patients with acute toxoplasmosis lymphadenopathy was estimated to be 5.5-13 weeks. Based on this, the presence of *Toxoplasma* DNA in the maternal blood most probably indicates a recent infection or an indicator of apparent parasitemia, which is likely to be clinically significant (Iqbal, 2007).

Serological assays of serial blood samples from acutely infected pregnant women were insufficient for identifying *Toxoplasma* infection in early pregnancy because some acute infections will not be detected (Jenum et al., 1997). Also, some women will be falsely identified as being infected and undergo unnecessary diagnostic amniocentesis and antiparasitic treatment (Liesenfeld et al., 2001). So, PCR is a good confirmatory assay in addition to serological assays to detect recent infection and we recommend national implantation of these molecular diagnostic tools.

The patient's physicians must be informed of the test results to offer treatment to the PCR- positive cases to prevent congenital infection and to suggest follow up of the fetuses.

**Author contribution:** R L El Gamal shared in the study design and research topics. M A Selim shared in the study design, initiated the research idea and reviewed the manuscript. S MA Mohamed, GM Fathy & S A Abdel Rahman shared in performing the laboratory work, interpretation of the results and collecting references, and wrote the manuscript.

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