Molecular Detection Of Toxoplasmosis In The Intermediate Hosts By Using B1 Gene Characterization

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Abstract
The goal of this research was to detection of *Toxoplasma gondii* by the molecular technique and comparison with the conventional methods such as rapid test and identification the group of woman that more susceptible to infection. The study included 55 blood samples from pregnant women and 30 samples from small ruminants (sheep and goats) suspected with the infection *Toxoplasma gondii* during the period from January, 2021 to May, 2021, which was confirmed by a rapid test examination. The results was revealed that 34 (40%) positive samples and 51 negative samples, which were considered a control group. On the other hand, the results of PCR technique for detection of B1 gene revealed that 35(63.6 %) of samples were found positive, the primers that amplify the outer and inner primers that had molecular weight 580bp and 531bp respectively.

Keywords: Toxoplasmosis, pregnant, small ruminants, B1 gene

Introduction

Toxoplasma gondii is an obligate intracellular parasite. It is observed in reticuloendothelial cells and many other nucleated cells. It is world large distribution and human infections are common, although most infections are benign or asymptomatic (Jones *et al.*, 2003), but sometimes the parasite can causes damaging disease. Infection may additionally be congenitally or after birth acquired (Majeed *et al.*, 2020). Only in cases where a woman becomes infected during pregnancy may congenital infection occur. Congenital infections that begin in the first trimester at some point are more severe than those that begin in the second and third trimesters (Hill and Dubey,2020) .Toxoplasma organisms are only found inside cells. The sex segment is found in cats. The time it takes for excreted oocysts to develop into infectious forms is 1 to 5 days. Tachyzoites actively replicate in all types of cellphones. There are sometimes cysts within the body that harbor bradyzoites, which can keep an organism alive in a latent state of infection (Al-Jebouri *et al.*, 2013).

Tachyzoites are able to replicate well in tissue culture, and this is responsible for a wide range of scientific manifestations during most important infections or reactivations of latent infections (Al-Qurashi *et al.*, 2001). Scientists have hypothesized that, with timely detection of predominant maternal Toxoplasma infection, chemotherapy can minimize every transmission of the parasite to offspring, as well as the morbidity and mortality associated with congenital infection (Mohammed *et al.*, 2015). This year has been very different from past years. *T. gondii* can cause serious diseases in the developing fetus and people who are immunocompromised.

T. gondii parasite is spread to humans and animals by eating undercooked meat that has T. gondii tissue cysts on it., by means of eating water or meals contaminated with *T. gondii* oocysts (Al-Dubbag et al., 2021), or via transplacental transmission. Given the significance of toxoplasmosis, particularly in immunocompromised individuals and seronegative pregnant women, as well as the high consumption of meat in diets and the danger of infection transmission. The most recent information on the significance of fitness in pregnant women and their progeny is learned by detecting this parasite using molecular techniques.

Materials and methods

Samples collection

From January 2021 to May 2021, 30 blood samples were collected from pregnant women in the early months of pregnancy who went to hospitals in the province of Al-Anbar, such as the Al-Ramadi Gynecology and Pediatrics Educational

Hospital, L 'Al-Fallujah educational hospital of gynecology and Pediatrics, then examined using anger and takes the facts of each afflicted person by questioning the patient. In addition, 30 samples from small ruminants (sheep and goat) were collected from slaughtered house at Al_Muthanna province during the same study times.

Extraction of DNA and PCR Assay

The genomic DNA was isolated from the blood of the infected human and small ruminant with *T. gondii* by using (bioner Kit, Korea), following the manufacturer's instructions, then it was extracted. The DNA samples were stored at -20C. The PCR reaction were conducted by using two specific primers to detect the T. gondii forward primer (5-TGT TCT GTC CTA TCG CAA CG-3), and reverse primer (5-ACG GAT GCA GTT CCT TTC TG-3), forward primer (5-TCT TCC CAG ACG TGG ATT TC-3), and reverse primer (5-CTC GAC AAT ACG CTG CTT GA-3), which amplify 580 bp and 531bp respectively, region of B1 gene from *T. gondii (*Al-Ani et al., 2020).

The PCR reactions actually takes place in a total volume of 20 µl, and contained 12.5µl of Green master mix (Promega / USA), 5 µl of genomic DNA, 1 µl of each primer, and 5.5 µl of nucleus free water. An initial denaturation stage at 94 C° for 2 min was followed by 40 cycles, each lasting 1 min at 94 C°, 57 C°, and 72 C°, with a final extension period of 5 min at 72 C°. Following the amplification stage, all PCR data were analyzed. Five l from amplification samples were immediately loaded on a 1.5% agarose gel electrophoresis, and the results were seen using a UV transilluminator.

Result and discussion

When our findings were agreed, Al-Ramadi University Hospital of Obstetrics and Gynecology and Pediatrics and Al-Fallujah University Hospital of Obstetrics and Gynecology and Pediatrics confirmed the compensation for infection in women who assessed 30 (54.5%) infections. Following PCR, the Rabid test showed the most environmentally friendly PCR effect with 35 (63.6%). This is consistent with PCR, as it is based on predictions of parasitic genetic material (Al-Rawazq, 2020). Improving simple, sensitive, and rapid methods for detecting and identifying toxoplasmosis is essential for predictive and epidemiological studies of toxoplasmosis zoonosis (Cong et al., 2016). Over the past two decades, molecular strategies have been developed based on a variety of genetic markers, each with advantages and limitations (Qin *et al.,* 2015). The software of these methods provided useful facts for improving the assessment of the epidemiology of *T. gondii,* population genetics and phylogeny (Kadir *et al.,* 2011).However, previously obtained data were limited, as most studies focused entirely on genetic traits, rather than full identification of *T. gondii* (Zheng *et al.,* 2019). Of the 55 samples investigated, 30 were mass screened for the *T. gondii* B1 gene by PCR enhancement. Recent studies have shown that humans can share the same genotype of *T. gondii* as wild, domestic, and lilac. Several genotypes of T. gondii have been described in China, but genotypes I (ToxoDB # 10) and rare (ToxoDB # 9)(Qin *et al.,* 2015), with other places, Although markers have been evaluated and used to identify and classify *T. gondii,* the SAG2 gene locus by the fragment size limited polymorphism (RFLP) method has been widely used for this reason(Howe *et al.,* 1997), but the B1 gene is the most common widely used for identification (Lv *et al.,* 2021). *T. gondii* was completely analyzed with three genotypes based on RFLP (I, II, III).

Molecular characteristics and T genotypes. gondii not only helps to study the appearance and identification of T. gondii genotypes (Al-Ani *et al.,* 2020), it is the most common source and is currently widespread in certain populations and regions and how transmission. develops medicines and vaccines and manages diseases. Our findings provide a basis for the future monitoring and management of these human parasites in Iraq and confirm the importance of the one health approach to the management of toxoplasmosis. The results of our study show that the prevalence in the 25-30 age group is 16 (29.09%) with significance differences as showed in table (1).

Table (1): the distribution of *Toxoplasma gondii* according to the Age groups according to the results of rabid test.

Age groups(years)	Positive	Negative	Total
25-30	16(29.09%)	39(70.9%)	55(100%)
30-35	10(18.18%)	45(81.8%)	55(100%)
35-40	4(7.27%)	51(92.7%)	55(100%)

On the other hand, according to PCR results, the overall infection rate 35 (36.6%) for *T. gondii* is high in the 25-30 age group and remains the same, but only the infection rate increases in the age group (35-40), where 9 (16.3%) of rabid test 4 (7.2%) may be due to the incorporation of a specific inhibitor by DNA extraction with protein and salt to prevent DNA from leave the cell as showed in figure (1).The PCR reaction were conducted by using two specific primers to diagnosis T.gondii in the intermediate host, which amplify 580 bp and 531bp respectively, region of B1 gene from *T. gondii* as showed in figure (1and 2).

These results are consistent with those of (Al-Shikhly, 2010; Aqeely *et al.,* 2014; Mohammed *et al.,* 2015), which have the highest infection rates in age group 35 and over in these countries. In addition, the study present was revealed that the PCR result was shown only 4 from 30 (13.3%) sample were positive to *T. gondii* in small ruminant as showed in table (2).

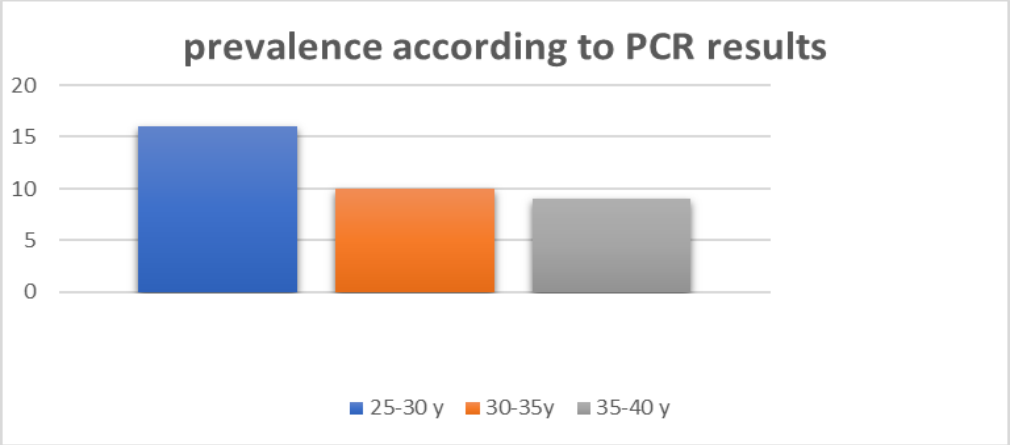

Figure (1): displayed the prevalence of T. gondii in women pregnant, according to the results of PCR.

Figure (2): 1.5% agarose gel electrophoresis for detection PCR product showed 531 bp fragment amplification w1-w2. DNA ladder 1000 bp.

Figure (3): 1.5% agarose gel electrophoresis for detection PCR product showed 580 bp fragment amplification w1-w2. DNA ladder 1000 bp.

Table (2): Showed the distribution of *T. gondii* in small ruminant, according to the results of PCR.

Animals	Positive	%	Negative	%
Sheep	4	26.6	11	(73.3%)
Goats	0 (0%)	0	15	(100%)
Total	4	7.27	30	(13.3%)

These differences may be due to differences in the topography, behavior and diet of the study area, the presence or absence of cats and differences in study methods, as well as differences in the quantity and quality of the sample in all these differences. Not just in the world, but in a geographical area. In addition, Table (3) shows the prevalence of parasites in residential areas with a prevalence of 26 (47.27%) in rural areas and 4 (7.2%) in urban areas as a result of the Rabid test. The infection rate increased during PCR. . In urban areas, there is a significant difference of up to 9 (16.3%). Our study was consistent with (Aqeely *et al.*, 2014), but not with the results (Mohammed *et al.*, 2015), due to dietary differences and increased contact with cats in rural areas. Lack of healthy habits and places in the house to prevent cats from returning home, food hygiene and places where cats often go.

Table (3) shows the prevalence of parasites in residential areas according to rabid test

Residency	Pos.	Neg.	Total
Rural areas	26(47.27%)	29(52.72%)	55(100%)
Urban areas	4(7.2%)	51(92.72%)	55(100%)

Conclusion

In this study, we conclude the nested PCR is more efficiency in the detection of *B1 gene* of *Toxoplasma gondii* , therefore detection of the causative agent of the abortion in the pregnant woman in Al-Anbar province, as well as the results of our study showed the higher rate of the infection in the young woman due to the many factors are mention before distribution of parasite according to Residency , seasonal variation showed the differences results, the negative samples in PCR may be due to the interference some inhibitors during the DNA extraction.

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