

Epidemiological study and Molecular characterization of *Toxoplasma gondii* infection in Aborted Women in Al-Diwanyah Province, Iraq

Hind Al-shabani¹, Marwa sami alwan², Lubna.A.Al-ibrahimi³

^{1,2,3}Department of Biology / College of Education /University of Al-Qadisiyah,Iraq

DOI: <https://doi.org/10.5281/zenodo.8211269>

Published Date: 03-August-2023

Abstract: *Toxoplasma gondii* is a protozoan parasite that infected broad range of animals, This study was aimed to detection of *T. gondii* from aborted placenta of infected women. A total of 65 placenta samples were collected from A borted women from Al-Diwaniya hospital for women and children city, during the period October 2022 until June 2023. The current study showed the infection with the *T. gondii* parasite was 60.00% (39 of 65 Samples) when examining the placenta microscopic examination by Impression method, and the infection rate was 67.69% (44 of 65 Samples) by Nested-PCR. The results of this study showed that the number and rate of infection in rural was 26 (59.09%) higher than in urban 18 (40.90%) infection was found among the age group 41-50 years 15(34.09 %). The isolation and identification of *T. gondii* were done using microscopic visualization followed by confirmation using a polymerase chain reaction (PCR) technique targeting the ssuRNA gene, All samples appeared to be contained this gene show one distinct band (313 bp) when electrophoresed on agarose gel. The results of this study indicated that the PCR technique had a high specify in the detection of *T. gondii* especially this species that encoded to ssuRNA gene isolated from aborted women.

Keywords: *Toxoplasma gondii*, Placenta, ssuRNA gene, Toxoplasmosis.

1. INTRODUCTION

T. gondii is an obligate intracellular protozoan parasite. Infection with *T. gondii* parasite is a major public health problem worldwide and it can be transmitted from mother to fetus [1]. The parasite can reproduce sexually and complete its life cycle only in felids, and they are the final hosts that are able to secrete massive numbers of oocysts with feces [2]. People become infected postnatally mainly by ingesting tissue cysts from undercooked meat, or consuming food or water contaminated with oocysts [3].

Tissue cysts are most likely viable during the life of the host. During infection, the parasite invades of immune cells and later spreads throughout the body, crossing biological barriers to reach with immune privileges such as the brain [4].

2. MATERIALS AND METHODS

Isolation of Parasite

A total of 65 placenta samples were collected from The hospitals in Al-Diwaniyah city for detect the parasite, during the period October 2022 until June 2023. making sure of the presence of the parasite in those samples using the direct smear method (Impression Smear), the placenta tissue was cut into small pieces.

PCR

The DNA was extracted using Genomic DNA extraction kit (Geneaid, china) and following the kit instructions. The DNA was estimated for quantity and quality using a Nanodrop. A characterizing and confirming step was done using a PCR technique targeting the *ssuRNA* gene. The piece targeted was at 313 bp of length. The primers used were from (5), and following the instructions accompanied with the kit using 10pmol from each primer. For the PCR conditions, the denaturation was at 95°C for 3min, 35 cycles were for the (main denaturing at 95°C for 1min, annealing at 55°C for 1min, and extension at 72°C for 1min), and the ending extension at 72°C for 10min. PCR products were run on 1.5% agarose gel pre-treated with ethidium bromide. The product separation was visualized using a UV imager.

STATISTICAL ANALYSIS

According to the results of the study, the statistical test used the Chi-square test and the T test to determine the differences between the study groups. Confidence interval is 95% and the probability level is less than 0.05 ($P < 0.05$) [6].

3. RESULT AND DISCUSSION

Microscopically

The microscopic results revealed the presence of Tissue cysts, figure 1,2.

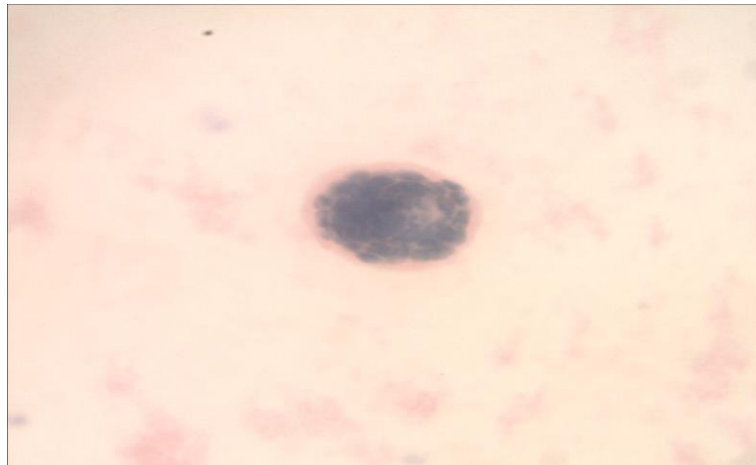


Figure 1: Tissue cyst of the *T. gondii* parasite in placental prints Giemsa stain, (100X).

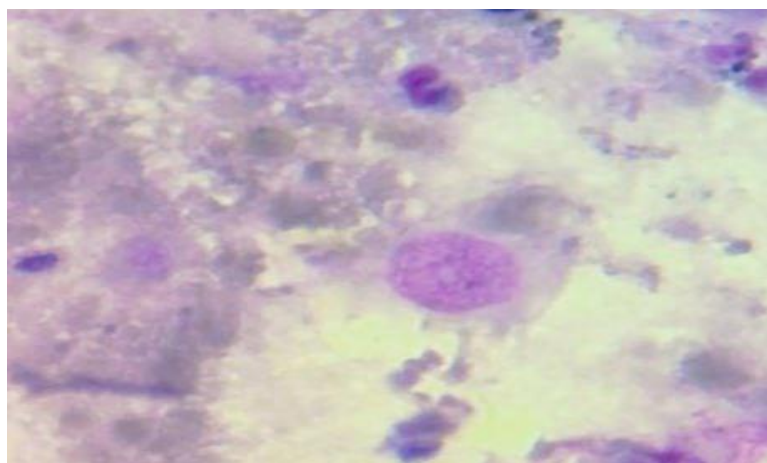


Figure 2: Tissue cyst of the *T. gondii* parasite in placental prints Giemsa stain, (40X).

Table (1) shows the percentage of infection in aborted women of 65 patients according to their age groups. It was found that the age group 41-50 years were 15 (34.09%) being the highest rate compared to other age groups, while the lowest percentage infect 8(18.18%) in the age groups (≥ 51) of patients, table 1.

Table: 1 Prevalence of infection with Toxoplasmosis according to age

Age Group	No. of aborted women	Percentage %
20-30	9	20.45%
31-40	12	27.27%
41-50	15	34.09%
≥ 51	8	18.18%
Total	44	67.69%

Table (2) shows the percentage of *T.gondii* among 44 patients according to their residence, the result showed that, 18 (40.90%) were lived in areas inside the city (urban), while the others 26 (59.09%) were in areas around or outside the city rural.

Table 2: Prevalence of infection with Toxoplasmosis according to their residence.

Residence Group	No. of aborted women	Percentage %
Urban	18	40.90%
Rural	26	59.09%
Total	44	67.69%

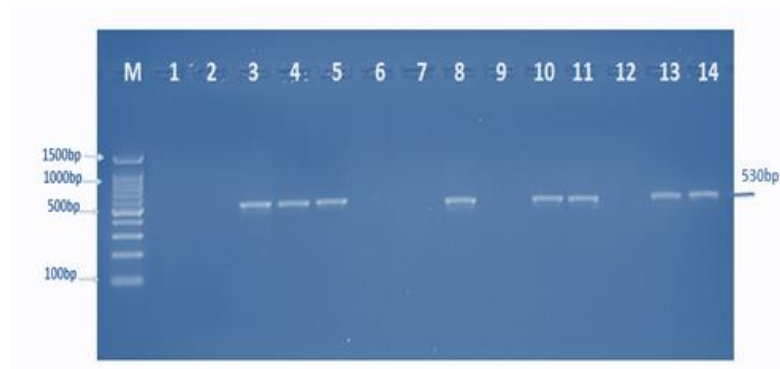


Figure 3: agarose gel electrophoresis of the *T. gondii* PCR products (first round) for the *ssuRNA* gene from placenta samples. Where M is the ladder.

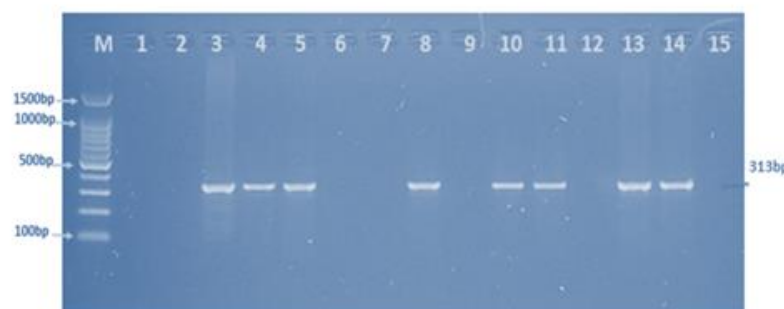


Figure 4: agarose gel electrophoresis of the *T. gondii* PCR products (second round) for the *ssuRNA* gene from placenta samples. Where M is the ladder.

The high prevalence of *T.gondii* in Rural region of the country could be attributed to several factors. This region has a high population of cats (the final host of the parasite), so there is a high risk of infection to intermediate hosts .(7) These cats live near human dwellings, Living in close contact with host animals and vehicles of oocyst transmission are important risk factors for the infection.(8) Other intermediate hosts of *T. gondii* infections are sheep and chickens, which represent another important risk factor in toxoplasmosis distribution, as these are the major meat sources of this region.(9)

T. gondii is frequently described as one of the most successful parasites, due to its ubiquitous distribution, the wide range of host species it is able to infect and its high prevalence rates around the world.(10)

Contamination with this parasite is usually long-lasting and asymptomatic.(11) shows the percentage of infection in aborted women of 65 patients according to their age groups. It was found that the age group 41-50 years were 15 (34.09%) being the highest rate compared to other age groups. Our results are compatible with NOWAKOWSKA study. Mean prevalence of IgG antibodies between 2004 and 2012 in pregnant women was 40.6% and increased with age with a yearly seroconversion rate of 0.8% (12). Risk for *T. gondii* infection increased with age and was higher among persons with a lower educational level, those who lived in crowded conditions, and those who worked in soil-related occupations. Most women of childbearing age in the United States are susceptible to acute infection and should be educated about ways to minimize exposure to *T. gondii*.(13)

Difference in infection rates between microscopic examination and PCR reaction, Although microscopy remains the most appropriate method for *T. gondii* diagnosis, molecular diagnostics such as real-time PCR offer a more reliable means to detect parasites, particularly at low levels.(14)

REFERENCES

- [1] Delgado, I.L.S.; Zúquete, S.; Santos, D.; Basto, A.P.; Leitão, A.; Nolasco, S. The Apicomplexan Parasite *Toxoplasma gondii*. Encyclopedia 2022, 2, 189-211. <https://doi.org/10.3390/encyclopedia2010012>.
- [2] Dubey, J. and J. Frenkel, Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. The Journal of protozoology, 1976. **23**(4): p. 537-546.
- [3] Grigg, M.E. and N. Sundar, Sexual recombination punctuated by outbreaks and clonal expansions predicts *Toxoplasma gondii* population genetics. International journal for parasitology, 2009. **39**(8): p. 925-933.
- [4] Barragan, A. and L.D. Sibley, Transepithelial migration of *Toxoplasma gondii* is linked to parasite motility and virulence. The Journal of experimental medicine, 2002. **195**(12): p. 1625-1633.
- [5] Jenum, P.A., Holberg-Petersen M, Melby KK, Stray-Pedersen B.(1998). Diagnosis of congenital *Toxoplasma gondii* infection by polymerase chain reaction (PCR) on amniotic fluid samples. AI'MIS.,106:680–686.
- [6] Motulsky, H. J., Prism 4 Statistics Guide-Statistical Analyses for Laboratory and Clinical Researchers, GraphPad Software Inc., San Deigo CA (2003).
- [7] Dubey, J.P.(2009). History of the discovery of the life cycle of *Toxoplasma gondii*. International Journal for Parasitology. 2009;39(8):877–882
- [8] Mason S, Quinnell RJ, Smith JE. Detection of *Toxoplasma gondii* in lambs via PCR screening and serological follow-up. Vet Parasitol. 2010;11:258–63
- [9] S. Stelzer, W. Basso, J. Benavides Silva, L.M. Ortega-Mora, P. Maksimov, J. Gethmann, F.J. Conraths, G. Schares, *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact, Food and Waterborne Parasitology, Volume 15, 2019,
- [10] Jiménez-Ruiz, E.; Wong, E.H.; Pall, G.S.; Meissner, M. Advantages and disadvantages of conditional systems for characterization of essential genes in *Toxoplasma gondii*. Parasitology 2014, 141, 1390–1398.
- [11] Silva CH, et al. Early diagnosis of congenital toxoplasmosis in newborn infants using IgG subclasses against two *Toxoplasma gondii* recombinant proteins. Memórias do Instituto Oswaldo Cruz 2012; 107: 342–347.
- [12] D. NOWAKOWSKA,^{1,*} W. WUJCIKA,¹ W. SOBALA,² E. ŚPIEWAK,³ Z. GAJ,¹ and J. WILCZYŃSKI¹.(2014).Age-associated prevalence of *Toxoplasma gondii* in 8281 pregnant women in Poland between 2004 and 2012.;Epidemiol Infect.; 142(3): 656–661.
- [13] Jeffrey L. Jones and others, *Toxoplasma gondii* Infection in the United States: Seroprevalence and Risk Factors, American Journal of Epidemiology, Volume 154, Issue 4, 15 August 2001, Pages 357–365,
- [14] Quan, Liu.; Ze-Dong, Wang, Si-Yang Huang and Xing-Quan Zhu.(2015). Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii* . Parasites Vectors 8, 292 .