Journal of Gynecology and Obstetrics
2022; 10(4): 196-202
http://www.sciencepublishinggroup.com/j/jgo
doi: 10.11648/j.jgo.20221004.16
ISSN: 2376-7812 (Print); ISSN: 2376-7820 (Online)

Molecular Diagnosis of Toxoplasma Gondii in Fetoplacental Tissue of Women with Miscarriage and Assessment of Risk Factors for Toxoplasmosis

Tamanna Ferdous Reza[1, *], Shahanara Chowdhury[1], Nasreen Banu[1], Abu Mohammad Abdul Momen Zonaed Siddiki[2]

[1]Department of Obstetrics and Gynecology, Chittagong Medical College and Hospital, Chattogram, Bangladesh

[2]Department of Pathology and Parasitology, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh

Email address:

dr.tamannareza@gmail.com (T. F. Reza), shahanacmc@yahoo.com (S. Chowdhury), drnasreenb@gmail.com (N. Banu), zsiddiki@cvasu.ac.bd (A. M. A. M. Z. Siddiki)

[*]Corresponding author

To cite this article:

Tamanna Ferdous Reza, Shahanara Chowdhury, Nasreen Banu, Abu Mohammad Abdul Momen Zonaed Siddiki. Molecular Diagnosis of Toxoplasma Gondii in Fetoplacental Tissue of Women with Miscarriage and Assessment of Risk Factors for Toxoplasmosis. *Journal of Gynecology and Obstetrics*. Vol. 10, No. 4, 2022, pp. 196-202. doi: 10.11648/j.jgo.20221004.16

Received: July 14, 2022; **Accepted**: July 26, 2022; **Published**: August 4, 2022

Abstract: Congenital toxoplasmosis is an important cause of miscarriage worldwide. There is high seropositivity of *T. gondii* among pregnant women in Bangladesh. The aims of the study were to estimate the presence of *T. gondii* in fetoplacental tissue among women with miscarriage and assess risk factors for toxoplasma infection in pregnant women. This cross-sectional study was performed for a period of one year on 138 patients. Obstetric parameters; cat, food, and soil related risk factors for toxoplasmosis were recorded and PCR analysis of fetoplacental tissue was performed for B1 and GRA6 gene in fetoplacental tissue by conventional PCR analysis. Comparisons were made between PCR positive and negative patients with regards to presence of risk factors for toxoplasmosis using Chi-square test or Fisher's exact for categorical data; and t test or Mann Whitney U test for continuous data where appropriate. Among a total of 138 patients with miscarriage, 38 patients' fetoplacental tissue (27.5%) were found to be positive for *T. gondii*. Age ranged from 18 to 37 years, mean 25 (±5) years. Living in a neighborhood with a cat was the only significant risk factor associated with PCR positivity (P=0.03). Other cat related factors and exposure to other animals were not related to PCR positivity. *T. gondii* was present in about one-fourth women with miscarriage at a tertiary hospital which demands further research.

Keywords: Fetoplacental, Miscarriage, Molecular Diagnostic Technique, Toxoplasmosis

1. Introduction

T. gondii is an obligate, intracellular parasite and about one third of the world's population is infected by *T. gondii* [1, 2]. The natural cycle of *T. gondii* transmission occurs between cats and between cats and their prey [3]. The infection is acquired by ingesting tissue cysts from undercooked or raw meat, consuming food or drink contaminated with oocysts shed by felids, or by accidentally ingesting oocysts from the environment [4].

Several studies have reported strong association between presence of IgM antibody against *T. gondii* and miscarriage [5-8]. However, it has been suggested that detection of *T. gondii* in fetoplacental tissue is more suggestive of being associated with miscarriage than detection in serum sample [9, 10]. But very few studies have been found searching literature on detection of *T. gondii* in human fetoplacental tissue. The aims of the study were to detect presence of *T. gondii* DNA by using PCR in fetoplacental tissue of women with miscarriage presenting at CMCH, assess the magnitude of it in this region and find out the risk factors among them.

2. Materials and Methods

2.1. Study Design and Setting

A cross sectional study was conducted among women with miscarriage admitted in the Department of Obstetrics and Gynecology, Chittagong Medical College hospital (CMCH) from March 2018 to March 2019. CMCH is the largest tertiary level government referral center for the South East part of Bangladesh which constitutes about one fifth of the population of the country. The general objective of the study was to detect presence of *T. gondii* infection in fetoplacental tissue of woman with miscarriage. The specific objectives were to detect presence of *T. gondii* DNA in fetoplacental tissue by PCR analysis among women with miscarriage and to investigate the high-risk behavior in this population for *T. gondii* infection, such as: contact with cat or cat faces, food related practice, and exposure to soil. Patients of induced abortion; patients with other diagnosed causes of miscarriage such as uterine abnormalities, cervical incompetence, drugs; and patients with systemic illness such as, diabetes mellitus, thyroid disorders, and hypertension were excluded from the study.

2.2. Data Collection

A pretested case record form was used to record findings. The case record from used structured and open-ended interview schedule to record demographic factors, relevant history and risk factors; and the findings of PCR analysis. It gathered information on obstetric parameters (trimester of pregnancy, bad obstetric history, number of pregnancies, duration of marriage), cat related behavior (ownership of cat, involvement in close contact and care of cat, exposure to surrounding cats), milk and meat related practice (eating undercooked meat, unpasteurized milk), and exposure to soil (involved in works related to soil contact).

2.3. PCR Analysis

Fetoplacental tissue was obtained for PCR analysis. After collection of fetoplacental tissue, it was refrigerated at -20°C in a deep freezer in the Department of Pathology and Parasitology of Chittagong Veterinary and Animal Sciences University where the PCR analyses were performed. A tiny piece of (25 mg) tissue was cut with a sterile BP blade, weighed and kept in 1.5 ml Eppendorf tube. DNA extraction was done from the tissue by Favorprep^{TM} FFPE Tissue DNA Extraction microkit (Favorgen) in accordance with the manufacturer's instructions. Cells were lysed and digested with 200 µl of FATG1 buffer and 20 µl of proteinase K and mixed thoroughly by vortexing; incubated at 60 °C for three hours. Absolute ethanol (200 µl) was added and the mixture was transferred to mini column in a 2 ml collection tube and centrifuged for 1 min. The columns were washed twice, and the DNA was eluted from the columns with 50 µl of elution buffer. Amplifications were conducted in a final volume of a 20 µl reaction mixture that contained 10 µl Dream Taq Green PCR Master Mix-2X (by Thermo Fisher Scientific), 20 pmol of each primer, 5 µl of nuclease free water and 3 µl of template DNA. For nested PCR, 1 µl of the nested PCR product was used as template. Applied Biosystem 2720 thermal cycler was used to amplify the segments of the 35-fold repetitive DNA region of 183 bp length of B1 gene and 344 bp length of GRA6 gene of *T. gondii.* The forward; 5'-GAACTGCATCCGTTCATG- AG -3' and reverse; 5'-TCTTTAAAGCGTTCGTGGTC-3' primers were used to amplify the related target in B1 gene. Two primers were used for GRA 6 gene; AR6-F1: 5'-ATTTGTGTTTCCGAGCAGGT-3', R1: 5'-GCACCTTCGCTTGTGGTT-3'; GAR6-F2: 5'-TTTCCGAGCAGGTGACCT-3', R2: 5'-TCGCCGAAGAGTTGACATAG-3' [11-13].

Cycling conditions for B1 gene were: denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min and final extension at 72°C for 10 min. Cycling conditions for GRA6 gene were: denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 seconds, annealing at 59°C in the first round and 57°C in the nested PCR for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 10 min. A total of 5 µl of each PCR product along with a 100 bp DNA ladder was subjected to electrophoresis in agarose gel (favorprep ^{TM} Gel/PCR purification kit). The bands were then stained with ethidium bromide and visualized under ultra-violet trans-illumination.

2.4. Data Analysis

Data were analyzed using SPSS version 22. Chi-square or Fisher's exact test was used to analyses the association of categorical variables (obstetric, cat, food and soil related risk factors). Independent sample t test or Mann-Whitney U test was used to test the differences between two means or medians, respectively (age, gestational age between PCR positive and negative patients). Binary logistic regression model was used to explain the relationship of cat, food and soil related nominal or ordinal independent risk factors with the dependent variable PCR positivity. Regression analyses with factors that produced an odds ratio >1, were put in the final model for binary logistic regression analysis. Odds ratio (OR), P value and 95% confidence interval were used to see the association of the risk factors with PCR positivity. The levels of significance for all analyses were set at 0.05 and P value < 0.05 was considered as significant. This study was approved by Ethical Review Committee of Chittagong Medical College (memo no: CMC/PG/2019/508). All participants gave written informed consent before the study began.

3. Results

There was a total of 138 patients. Age ranged from 18 to 37 years (mean 25 ±5 years). 15.2% were less than 20 years, 62.3% between 20-29 years and 22.5% were between 30-39 years. Thirty-eight patients (27.5%) were found to be positive for *T. gondii* during PCR analysis for B1 gene and none were positive for GRA6 gene (Figure 1). There was no

significant difference in age between PCR positive and negative patients (mean 25.7 vs 25.0 years, respectively, p=0.47).

Gestational age ranged from 8 to 18 weeks and median gestation ages in both PCR negative and positive patients were 12 weeks (P=0.49). There was also no significant difference between PCR positive and negative women in terms of obstetric parameters such as, trimester of pregnancy (p=0.21), bad obstetric history (p=0.10), number of pregnancies (p=0.93) and duration of marriage (p=0.54).

Living in a neighborhood with a cat was the only cat related factor which was significantly related to PCR positivity (P=0.03) (Table 1). None of the patients had any knowledge about transmission of toxoplasmosis by cat. PCR positivity rates were 32.32% (32/99) for living in a neighborhood with cat, 31.18% (29/93) for neighbor's cat coming to their house, 30% (3/10) for having a cat, 50% (1/2) for housing for cat, and 25% (1/4) for letting cat sleep in their bed.

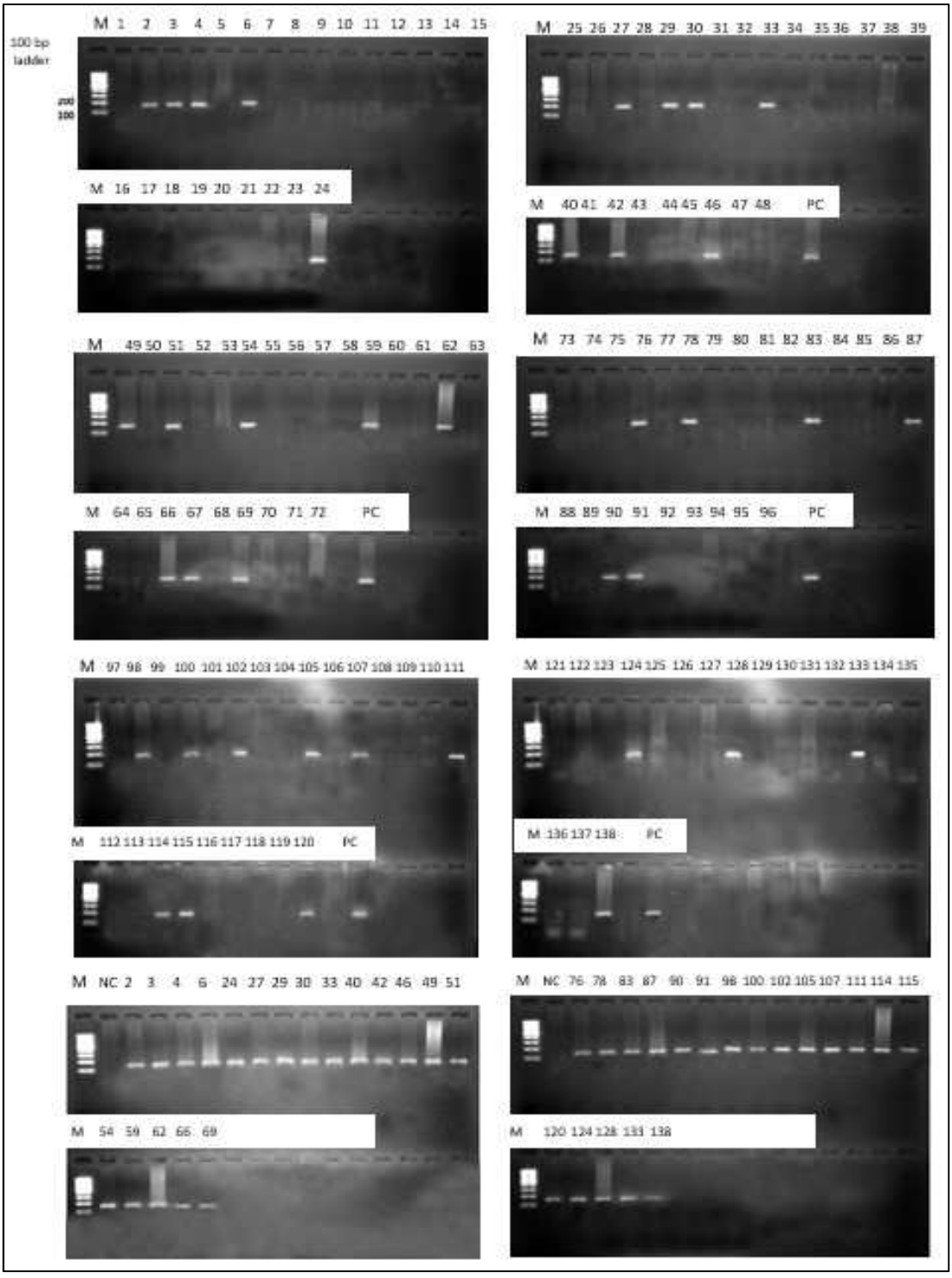

Figure 1. *PCR analysis for B1 gene. M=marker; PC=positive control; NC=negative control.*

Table 1. *Relation of contact with cat and PCR positivity (N-138).*

Contact with cat	PCR positive n (%)	PCR negative n (%)	P value*
Own a cat			
Yes	3 (2.2)	7 (5.1)	0.55
No	35 (25.4)	93 (67.4)	
Housing for cat			
Yes	1 (0.7)	1 (0.7)	0.48
No	37 (26.8)	99 (71.7)	
Let cat sleep in their bed			
Yes	1 (0.7)	3 (2.2)	0.70
No	37 (26.8)	97 (70.3)	
Neighbor's cat comes to home area			
Yes	29 (21.0)	64 (46.4)	0.12
No	9 (6.5)	36 (26.1)	
Lives in neighborhood with a cat			
Yes	32 (23.2)	67 (48.6)	0.03
No	6 (4.3)	33 (23.9)	
Knowledge about transmission			
Yes	0	0 (0.0)	†
No	38 (27.5)	100 (72.5)	
Cat uses litter box			
Yes	0	0 (0.0)	†
No	38 (27.5)	100 (72.5)	
Housing for cat			
Yes	1 (0.7)	1 (0.7)	0.48
No	37 (26.8)	99 (71.7)	
Involved in disposing cat litter			
Yes	3 (2.2)	6 (4.3)	0.47
No	35 (25.4)	94 (68.1)	
Manure handling			
Yes	1 (0.7)	2 (1.4)	0.62
No	37 (26.8)	98 (71.0)	
Wear gloves at manure handling			
Yes	0 (0.0)	0 (0.0)	†
No	38 (27.5)	100 (72.5)	

†Analysis not done when all data were in the same group.
*P values were derived from Fisher's exact test.

None of the milk and meat related practices were significantly related to PCR positivity (Table 2). Among the PCR positive patients, 39 (28.3%) used to drink milk, 36 (26.1%) pasteurized milk, washed their hands and knifes after handling and cutting meat, and 33 (23.9%) used to eat beef. Positivity rates for these factors were 28.26% (39/138), 27.27% (36/132), 26.87% (36/134), and 25.78% (33/128), respectively.

Table 2. *Relation of milk and meat related practice with PCR positivity (N=138).*

Milk and meat related practice	PCR positive n (%)	PCR negative n (%)	P value *
Milk consumption			
Yes	39 (28.3)	99 (71.7)	0.13
No	2 (1.4)	1 (0.7)	
Pasteurization/boiling of milk			
Yes	36 (26.1)	96 (69.6)	0.75
No	2 (1.4)	4 (2.9)	
Beef consumption			
Yes	33 (23.9)	95 (68.8)	0.10
No	5 (3.6)	5 (3.6)	
Undercooked meat consumption			
Yes	0	5 (3.6)	0.16
No	38 (27.5)	95 (68.8)	
Handwash after handling meat			
Yes	36 (26.1)	98 (71.0)	0.50
No	2 (1.4)	2 (1.4)	
Wash knife after cutting meat			
Yes	36 (26.1)	98 (71.0)	0.31
No	2 (1.4)	2 (1.4)	

*P Values were derived from Chi-square test.

Behavior and practice related to exposure to soil or other animals were not significantly related to PCR positivity (Table 3). Among the PCR positive patients, 18 (13%) had contact with soil by other means, 5 (3.6%) own other domestic animal, 4 (2.9%) were involved in manure handling and care of other animal, and 3 (2.2%) were involved in gardening. Positivity rates for these factors were 27.69% (18/65), 26.32% (5/19), 57.14% (4/7), 36.36% (4/11), and 50% (3/6), respectively.

Table 3. *Relation of exposure to soil or other animals to PCR positivity (N=138).*

Exposure to soil/other animals	PCR positive n (%)	PCR negative n (%)	p value*
Involved in gardening			
Yes	3 (2.2)	3 (2.2)	0.21
No	35 (25.4)	97 (70.3)	
Involved in agricultural work			
Yes	0 (0.0)	0 (0.0)	†
No	38 (27.5)	100 (72.5)	
Involved in manure handling			
Yes	4 (2.9)	3 (2.2)	0.07
No	34 (24.6)	97 (70.3)	
Contact with soil by any other means			
Yes	18 (13.0)	47 (34.1)	0.83
No	20 (14.5)	53 (38.4)	
Own other domestic animals			
Yes	5 (3.6)	14 (10.1)	0.57
No	33 (23.9)	86 (62.3)	
Care of anther animal			
Yes	4 (2.9)	7 (5.1)	0.36
No	34 (24.6)	93 (67.4)	

*P Values were derived from Chi-square test.
†Analysis not done as all values belonged to same group.

In univariate analysis, only living in a neighborhood with cat was found significant with OR 2.63, 95% CI (0.999-6.905), P=0.045. Regression analysis with factors that produced an odds ratio >1 also showed that living in a neighborhood with a cat was a significant risk factor for PCR positivity (P=0.02), OR=4.07, CI (1.26-13.16) (Table 4).

Table 4. *Regression analysis with factors with OR>1.*

Risk factors	OR	95% C.I.		P value*
		Lower	Upper	
Live in a neighborhood with a cat	4.07	1.26	13.16	0.02
Exposure to soil	1.26	0.56	2.86	0.58
Milk consumption	10.16	0.64	161.88	0.10
Beef consumption	1.97	0.48	8.19	0.35

* P values were derived from binary logistic regression analysis.

4. Discussion

This study reports a 27.5% prevalence rate of *T. gondii* in women with miscarriage in Chattogram through a hospital-based study on the fetoplacental tissue from women with miscarriage. Earlier, studies from serum samples reported 15- 38.5% seropositivity among pregnant women in this country [14-16].

A recent meta-analysis showed that the incidence of abnormal pregnancy outcome was five times higher in *T. gondii* infected pregnant women than the incidence in uninfected women [17]. Ghasemi et al. showed that the rate of PCR positive placental tissue in women with miscarriage and still birth was 3.7 times higher than the rate in women with normal delivery [9, 10]. Abdoli et al. performed nested PCR analysis targeting GRA6 gene on 210 fetoplacental tissues from women with recurrent spontaneous abortions and detected 3.8% positive samples [18]. Earlier, Asgari et al. performed semi nested PCR analysis targeting B1 gene on fetoplacental tissue from 524 women with miscarriage, and 14.4% were positive [11]. This study targeted both GRA6 and B1 gene on 138 women with miscarriage. However, only B1 gene was detected by conventional PCR in 27.5% samples and no GRA6 positive samples were found suggesting a regional variation.

Contact with cat has been widely implicated to the occurrence of toxoplasmosis. Only 10 patients (7.2%) in this study had owned a cat; three of them were PCR positive which was not significant. However, in 93 (67.4%) patients, neighbor's cat came to the patients' home area and 99 (90%) patients lived in a neighborhood with a cat. This suggests that majority had the risk of contamination of their environment by oocyst excreted by cat. Samad et al. reported that 11.2% pregnant women were seropositive in another region of Bangladesh and seropositivity rate was

significantly higher in the family members with seropositive cat owner (24%) in comparison to family members without cats (11.1%) (P<0.01) [19]. A study from Nigeria found that IgM seropositivity was significantly more among women who kept cat pets than those that did not (75% vs. 25%, p = 0.025) [20]. Although there are not many domestic cats in Bangladesh, the presence of large number of stray cats suggests that cats can contaminate the environment of this densely populated area and sporozoites containing oocysts excreted by cats are in abundance. These are very infectious and can remain viable for many years [2].

Several studies have stated that eating undercooked meat is a determining factor of toxoplasmosis [21]. Meat consumption was not related to PCR positivity in this study. Many other studies also failed to found any relation of meat or undercooked meat consumption with toxoplasmosis. Alvarado-Esquivel et al. hypothesized that pregnant women of low socio-economic condition could eat meat at a lower frequency and quantity than those with a better socioeconomic level due to lack of affordability which is why meat consumption was not a significant factor in toxoplasma infection in their study [22]. Singh et al. also did not find relation of meat with toxoplasmosis and concluded that type of food, social considerations and quality of water consumed were more likely factors for high prevalence (37.3%) of toxoplasmosis in South India [23].

Contact with soil has been implicated to toxoplasmosis by Alvarado-Esqiuvel et al. [22]. Twenty-eight of their 34 patients (82.35%) in Mexico who had contact with soil were seropositive for *T. gondii* (OR = 7.16; 95% CI: 1.39–36). Although involvement with manure handling were more among PCR positive patients in this study, involvement in gardening, agricultural work or contact with soil by other means failed to show any association.

In recent years, the prevention and treatment of congenital toxoplasmosis have raised interest among policy makers in the developed countries. However, except France, where this is highly prevalent, most countries have not yet started any screening program for toxoplasmosis mainly due to increased cost involvement [24]. Since the modes of contamination are related to living habits, it should be possible to reduce the risk of infection during pregnancy by adequate health education. It has been showed that adequate health education prevented congenital toxoplasmosis in France and it was less expensive than screening program. The lack of awareness suggests that toxoplasmosis is a neglected disease in this country.

This study has several limitations. The sample size was moderate and from a single center, there were no control group and no comparison with serological findings. However, this study highlighted the relation of *T. gondii* with miscarriage in this region and further studies should be carried out to find out their causal relations.

5. Conclusion

There was presence of *T. gondii* DNA in about one-fourth of the patients with miscarriage. Living in a neighborhood with a cat was significantly associated with PCR positivity for *T. gondii*. However, other cat related factors, along with food and water related hygiene were not significantly associated with presence of *T. gondii* in fetoplacental tissue.

References

[1] Hill, D. E., & Dubey, J. P. (2013). Toxoplasma gondii prevalence in farm animals in the United States. International Journal for Parasitology, 43 (2), 107–113. https://doi.org/10.1016/j.ijpara.2012.09.012

[2] Hill, D. E., Chirukandoth, S., & Dubey, J. P. (2005). Biology and epidemiology of Toxoplasma gondii in man and animals. Animal Health Research Reviews, 6 (1), 41–62. https://doi.org/10.1079/AHR2005100

[3] Tenter, A., Heckeroth, A., & Weiss, L. (2000). Toxoplasma gondii: from animals to humans. Int J Parasitol, 30 (12–13), 1217–1258.

[4] Dubey, J. P., & Jones, J. L. (2008). Toxoplasma gondii infection in humans and animals in the United States. International Journal for Parasitology, 38, 1257–1278. https://doi.org/10.1016/j.ijpara.2008.03.007

[5] Zargar, A., Waani, G., Laway, B., & Al, E. (1999). Seroprevalence of toxoplasmosis in women with recurrent abortions, neonatal death and its treatment outcome. Ind J Pathol Microbiol, 42 (4), 481–83.

[6] Yasodhara, P., Ramalakshmi, B., Lakshmi, V., & Krishna, T. (2004). Socioeconomic status and prevalence of toxoplasmosis during pregnancy. Ind J Med Microbiol, 22 (4), 241–43.

[7] Jamal, A. A. (2013). Congenital Toxoplasmosis : Prevention of Vertical Disease Transmission by Vaccination, 20 (1), 27–55. https://doi.org/10.4197/Med.

[8] Jumaian, N. F. (2005). Seroprevalence and risk factors for Toxoplasma infection in pregnant women in Jordan. Eastern Mediterranean Health Journal, 11 (1), 45–51.

[9] Ghasemi, F. S., Rasti, S., Piroozmand, A., Bandehpour, M., & Kazemi, B. (2015). Toxoplasmosis-associated abortion and stillbirth in Tehran, Iran. J Matern Fetal Neonatal Med, online, 1–4. https://doi.org/10.3109/14767058.2014.996127

[10] Ghasemi, F., Rasti, S., Bandehpour, M., Kazemi, B., Piroozmand, A., & Mousavi, G. (2015). Molecular Diagnosis of Toxoplasma gondii in Aborted Women. Jundishapur J Microbiol, 8 (1), 2014–2016. https://doi.org/10.5812/jjm.15925

[11] Asgari, Q., Fekri, M., Monabati, A., Kalantary, M., Motazedian, M. H., & Sarkari, B. (2013). Molecular Genotyping of Toxoplasma gondii in Human Spontaneous Aborted Fetuses in Shiraz, Southern Iran. Iranian J Publ Health, 42 (6), 620–625.

[12] Porta, A. R., & Enners, E. (2012). Determining Annealing Temperatures for Polymerase Chain Reaction. The American Biology Teacher, 74 (4), 256–260. https://doi.org/10.1525/abt.2012.74.4.9

[13] Carr, A. C., & Moore, S. D. (2012). Robust Quantification of Polymerase Chain Reactions Using Global Fitting. PLoS ONE, 7 (5), 1–8. https://doi.org/10.1371/journal.pone.0037640

[14] Margia, U., Begum, N., Hossain, M., Dey, A., & Alam, M. (2017). Seroprevalence of toxoplasmosis in women in Mymensingh and Rangpur in Bangladesh: a hospital survey. Bangl. J. Vet. Med, 15 (1), 81–85.

[15] Sultana, M., Hossain, S., Dewan, F., Sultana, J., & Maliha, R. (2014). Association of Toxoplasma Gondii Infection with Spontaneous Abortion. Bangladesh J Obstet Gynaecol, 29 (2), 87–93.

[16] Ashrafunnesa, Khatun, S., Islam, M. N., & Huq, T. (1998). Seroprevalence of Toxoplasma Antibodies among the Antenatal Population i n Bangladesh*. J. Obstet. Gynaecol. Res, 24 (2), 115–119.

[17] Li, X., Wei, H., Zhang, H., Peng, H., & Lindsay, D. S. (2014). A Meta Analysis on Risks of Adverse Pregnancy Outcomes in Toxoplasma gondii Infection A Meta Analysis on Risks of Adverse Pregnancy Outcomes in Toxoplasma gondii Infection. PLoS ONE, 9 (5), e977775. https://doi.org/10.1371/journal.pone.0097775

[18] Abdoli, A., Dalimi, A., & Soltanghoraee, H. (2017). Molecular Detection and Genotypic Characterization of Toxoplasma gondii in Paraffin-Embedded Fetoplacental Tissues of Women with Recurrent Spontaneous Abortion. Int J Feryil Steril, 10 (4), 327–336.

[19] Samad, M., Det, B., Chowdhury, N., Akhter, S., & AKMMR, K. (1997). Sero-epidemiological studies on Toxoplasma Gondii Infection in Man and Animals in Bangladesh. South Asian J Trop Med Public Health, 28 (2), 339–43.

[20] Abdullahi, I. N., & Adekola, H. A. (2015). Prevalence and Associated Risk Factors of Toxoplasma gondii Antibodies among Pregnant Women Attending Maiduguri Teaching. J. Med. Sci, 15 (3), 147–154. https://doi.org/10.3923/jms.2015.147.154

[21] Jacquemard, F. (2000). Clinical aspects of infection during pregnancy. Congenital toxoplasmosis: scientific background, clinical management and control, 10–13.

[22] Alvarado-esquivel, C., Sifuentes-álvarez, A., Narro-duarte, S. G., Estrada-martínez, S., Díaz-garcía, J. H., Liesenfeld, O., & Al., E. (2006). Seroepidemiology of Toxoplasma gondii infection in pregnant women in a public hospital in northern Mexico. BMC Infectious Diseases 2006, 6, e113. https://doi.org/10.1186/1471-2334-6-113

[23] Singh, S., Munawwar, A., Rao, S., Mehta, S., & Hazarika, N. K. (2014). Serologic Prevalence of Toxoplasma gondii in Indian Women of Child Bearing Age and Effects of Social and Environmental Factors, 8 (3). https://doi.org/10.1371/journal.pntd.0002737

[24] Berger, F., Goulet, V., Strat, Y. Le, & Desenclos, J. (2009). Toxoplasmosis among pregnant women in France : Risk factors and change of prevalence between 1995 and 2003. Revue d'E´pide´miologie et de Sante´ Publique, 57, 241–248. https://doi.org/10.1016/j.respe.2009.03.006