

THE INCIDENCE OF PARASITE TOXOPLASMOSIS IN PREGNANT WOMEN

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ABSTRACT

Congenital toxoplasmosis is a disease with severe clinical manifestations in newborns so screening of pregnant women is crucial. This study was aimed at evaluating the latest status of toxoplasmosis in pregnant women and its respective risk factors in Iraq. In this study, the results showed that all these factors were statistically significant in IgG-positive subjects. In IgM-positive subjects, contact with a cat, the degree of meat cooking, unwashed raw vegetables, fruits consumption, and history of miscarriage were among the more important risk factors. The new cases of toxoplasmosis are being occurred in the pregnant women in the region under the study and therefore these pregnancies

are uncertain.

KEYWORDS: Toxoplasmosis, pregnant women.

INTRODUCTION

Toxoplasma gondii is an obligate protozoan parasite that causes toxoplasmosis infection. About one-third of the world's human population is predictable to be infected with toxoplasmosis disease.^[1] Cats are the primary hosts and the parasite infects the central nervous system of animals and humans. Transmission starts by ingestion of food or contaminated water containing cysts of the parasite; it also can be transmissible through blood transfusion and organ transplant.^[2] Toxoplasmosis is an asymptomatic infection in immune competent individuals.^[3] However, only fewer case develop severe clinical symptoms, such as cerebral toxoplasmosis.^[4] It can cause severe symptoms in pregnant women which can lead to hurt the fetus, or in lesions which usually involve the eyes and brain. For immune compromised patients, such as with HIV or common immune deficiency diseases, this parasite can be a lethal pathogen.^[5] Toxoplasmosis concenter chronic condition

when affecting the central nervous system. *Toxoplasma gondii* infection can be diagnosed through different types of screening, directly through polymerase.

Toxoplasmosis is a worldwide zoonosis caused by the protozoan *Toxoplasma gondii*. Toxoplasmosis is an important cause of miscarriage or adverse fetal effects such as neurological and ocular diseases and may also lead to late sequelae in the life of the infected newborn.^[6] Toxoplasmosis prevalence varies among adult individuals depending on the studied population and on the age of the individuals. These variations occur due to the difference of exposure to the main sources of infection which are: soil, water or food contaminated with feces from infected cats that contain *T. gondii* oocysts; or raw or uncooked meat that contain bradyzoite cysts.^[7]

The ubiquity of the infection source and the differential exposure of the individuals to it, due to cultural and hygienic habits, may explain why the prevalence of toxoplasmosis is extremely variable between countries and even within different regions of the same country.^[8] One of the major causes of foodborne death in the United States is toxoplasmosis. The large variability of the toxoplasmosis prevalence described by the literature from studies performed in different regions characterizes the great regional variability of the incidence of this disease and also the specific characteristics of each studied population.^[9]

In spite of both maternal and congenital infections are frequently asymptomatic, the *T. gondii* infection is particularly severe when the primo-infection occurs during pregnancy. For instance, in developed countries such as the USA, only in 10% of the infected pregnant women the disease is symptomatic with unspecific signs. On the other hand, in developing countries such as Brazil, the prevalence of toxoplasmosis among pregnant women varies from 50 to 80% throughout the whole territory of this vast country.^[10]

BACKGROUND

The diagnosis of the gestational infection is based on serological tests that search for specific antibodies. Usually, these tests are used to confirm the infection due to their high sensitivity and specificity. It is important to highlight that serological tests for IgM may present persistently positive results for long periods; hence the IgG avidity test performed during the first trimester of pregnancy would help to determine whether it is a recent infection.^[11] Therefore, a positive IgM test result in a pregnant woman requires caution and further confirmation of acute infection. In fetuses and newborns, the diagnosis of toxoplasmic

infection is complex and performed through the union of serological and parasitological analysis. In newborns, anti-*T. gondii* IgM and IgA in any titer, anti-*T. gondii* IgG ascending titers or the detection of the parasite DNA in the blood of the patient confirm the infection.^[12]

For the fetus's infection confirmation, it is also recommended the amniocentesis to identify the parasite in the amniotic fluid by PCR or inoculation in mice if the maternal primary infection is diagnosed, if serologic testing cannot confirm nor exclude acute infection, or in the presence of abnormal ultrasound findings such as intracranial calcifications, microcephaly, hydrocephalus, ascites, hepatosplenomegaly, or severe intrauterine growth restriction.^[13] This confirmation is essential for the prevention of possible vertical transmission of *T. gondii* or the adequate treatment that may minimize the fetal sequelae.^[14] For the newborn infection confirmation besides the serological tests from newborn and mother sera, it is recommended to perform PCR on the cord blood, placenta and amniotic fluid collected at birth.

Toxoplasmosis is a zoonotic protozoal disease caused by tissue parasite, *Toxoplasma gondii*. *Toxoplasma* is a parasite of cosmopolitan distribution present in hot, humid countries able to develop in a wide variety of vertebrate hosts. Cats and other members of Felidae are the definitive hosts, while a human and wide range of animals, birds, and rodents act as intermediate hosts.^[15] Parental infections account for 2% to 3% of all congenital *Toxoplasma* abnormalities. The transmission of the disease to human is either by eating raw or uncooked meat, blood transfusion, and organ transplantation or through ingestion of oocysts introduced into the environment by cats or congenitally during pregnancy.^[16] Primary maternal *T. gondii* infection during pregnancy is frequently associated with its transmission to the fetus. The transmission rate of maternal infection to the fetus is estimated to be about 45%; of these, 60% are sub-clinical infections, 9% resulting in the death of the fetus and 30% have severe damages such as hydrocephalus, intracerebral calcification, chorioretinitis, and mental retardation. In most cases, the laboratory diagnosis of acute and latent toxoplasmosis relies on the detection of *T. gondii* specific IgG and IgM antibodies. And the avidity test of *T. gondii* specific IgG antibodies has also been very helpful in the diagnosis. Many serological tests such as the LATEX agglutination test, ELISA, indirect fluorescence antibody test (IFA) and hemagglutination test have been used for the detection of antibodies against *T. gondii* in pregnant women.^[17]

Recent epidemiological studies have identified risk factors for *T. gondii* infection: owning cats being in proximity to seropositive, cats in farming areas, cleaning the cat litter box. In humans, it is one of the most common parasites, serological studies estimate that up to a third of the global population has been exposed to and may be chronically infected with *T. gondii*, although infection rates differ significantly from country to country. The parasite prevalence in Iraq was 36.6% in Kirkuk, 29.2% Tikrit, 58% in Basrah in 2011. Infection in humans and animals is necessary to be evaluated, especially in married pregnant women and women about to marry to prevent the future complications that may occur, therefore the aim of this study was to detect *Toxoplasma* prevalence among human population (married and non-married women) and cats by using serologic and microscopic methods.^[18]

Toxoplasma gondii is an obligate intracellular protozoan parasite distributed globally in humans and other warm-blooded animals. It is estimated that approximately one-third of the world's human population has been exposed to *T. gondii*. Humans become infected by ingesting food or water contaminated with oocysts shed by cats; by eating undercooked or raw meat containing tissue cysts; or congenitally by transplacental transmission of tachyzoites.

Most infections are asymptomatic but, in some individuals, especially if immunocompromised, the parasite can become widely disseminated causing severe clinical signs including encephalitis. Primary infection during pregnancy can result in severe damage to the fetus manifested as mental retardation, seizures, blindness, or even death. The rate of congenital transmission and the degree of severity of toxoplasmosis in fetuses vary depending largely on the stage of gestation at the time of infection, the risk of transmission is lower in the first trimester and higher during the last trimester. Therefore, early diagnosis of *T. gondii* infection during pregnancy is very important for the prevention of congenital toxoplasmosis.^[19]

Risks associated with toxoplasmosis during pregnancy

- Mother-to-child transmission rates have been reported to range from 11.3% (Ricci et al 2003) to 18.5%. The risk of transmission increases with gestational age (from 5% at 12 weeks to 80% just before birth). However, babies infected early in pregnancy have a greater risk of congenital abnormalities.^[20]

- Congenital toxoplasmosis has been associated with stillbirth, intracranial abnormalities and/or developmental delay, ocular inflammation and impaired hearing. In a prospective cohort study (n=620), babies with congenital toxoplasmosis had lower gestational age but there was no significant association with low birth weight or small for gestational age.^[21]

There are two generally accepted reasons to evaluate a patient for toxoplasmosis. The first is a suspicion of maternal infection. The fetal diagnosis then should follow when there is serologic evidence of maternal infection. The second is in the setting of an abnormal fetal ultrasound with findings suspicious for congenital toxoplasmosis infection. The American College of Obstetrics and Gynecology (ACOG) recommends against routine screening for toxoplasmosis in pregnancy, except in the setting of maternal HIV infection. This is due to the relatively low prevalence of disease in the United States in conjunction with the high false positive rate of serologic screening.^[22]

Routine screening in the U.S. would lead to innumerable unnecessary interventions for very few true positive cases. For this reason, ACOG recommends testing only for those in whom a high risk of acute infection is suspected. This might include the unusual circumstance in which a pregnant woman has been changing the cat litter or having close contact with a cat that is new to the household and also spends time outdoors. Maternal HIV infection is another accepted reason for testing.^[23]

Ultrasonographic findings suspicious for fetal toxoplasmosis infection include ventriculomegaly, intracranial calcifications, microcephaly, ascites, and growth restriction. These findings may also be seen in other conditions, however, and these should also be included in a differential diagnosis. Conditions that can cause similar ultrasound findings and occur more commonly than congenital toxoplasmosis include various aneuploidies. Other common infectious etiologic agents include Cytomegalovirus or Parvovirus B19.^[24]

When maternal infection is suspected, serologic screening should be conducted by a reference laboratory. Testing for toxoplasma specific antibodies is conducted for detection of IgM and IgG. Because of the false positive rates, ruling out recent infection is much more reliable than testing to rule in acute infection. A negative IgM test is reliable to exclude a recent infection. However, a positive IgM test can be either a false positive or can persist following a prior infection for months to years. In the setting of a positive IgM and IgG, IgG avidity testing can be conducted. Again, a high avidity test is suggestive of past infection, and

in the early part of pregnancy essentially rules out recent infection. However, a low avidity can persist for months and thus cannot definitively diagnose recent infection but can prompt further evaluation. This further evaluation may take the form of serial serology for maternal infection or of fetal testing, via amniocentesis. Serial serology can be helpful to either identify IgG seroconversion or demonstrate an increase in IgG titer in someone with a recent illness.^[25]

The most common way to diagnose fetal infection is by amniocentesis. In the past, fetal blood sampling was used, but this method was associated with increased fetal risk and is not a superior diagnostic approach. Amniocentesis is conducted under ultrasound guidance and amniotic fluid can be evaluated for the presence of *T. gondii*. Polymerase chain reaction (PCR) testing should be performed but results should be interpreted with caution since both false positive and negative results have been reported.^[26]

Table 1: Multivariate analysis of selected characteristics of the participants and their association with *T. gondii* infection.

Characteristic ^a	Adjusted odds ratio ^b	95% confidence interval	P value
Residence area	1.55	1.15–2.18	0.010
Cats in home	3.45	2.40–4.91	<0.001
Dogs in home	1.08	0.87–1.49	0.48
Contact with cats and dogs	3.07	2.33–4.12	<0.001
Consumption of raw vegetables and fruits	1.03	0.81–1.31	0.64
Consumption of raw/undercooked meat	1.13	0.83–1.53	0.44
Exposure to soil	1.66	1.18–2.34	0.004

Neonates suspected of having an infection can also be tested by PCR techniques from blood or body fluids. Isolation of parasites is technically difficult, and require live parasites for culture. Detection of tachyzoites in tissue or body fluid smears indicates active pathology.

Table 2. Risk factors and their relation to IgM positive pregnant women

Risk factors	Number of subjects	Positive cases (%)	P-value
Age of pregnancy			
18-25	140	5 (3.6)	0.302 ^a
25-35	157	7 (4.5)	
35-45	53	0	
Residence area			
Urban	229	6 (2.6)	0.354 ^b
Rural	121	6 (5)	
Contact with cats			
Yes	196	12 (6.1)	0.002 ^b
No	154	0	
Degree of meat cooking			
Extra-rare or rare	17	0	<0.001 ^b
Well-done	333	12 (3.6)	
Unwashed raw vegetable or fruit consumption			
Yes	30	6 (20)	<0.001 ^b
No	320	6 (1.9)	
Raw milk consumption			
Yes	198	4 (2)	0.138 ^b
No	152	8 (5.3)	
History of miscarriage			
Yes	35	5 (14.3)	0.004 ^b
No	315	7 (2.2)	

^aP-value was calculated by chi-square statistical test^bP-value was calculated by Fisher's exact test

Table 3: Sociodemographic and risk factors associated with Toxoplasma seropositivity in pregnant women and controls by univariate analysis.

Characteristic	Pregnant women (N = 965)				Controls (N = 965)				Total (N = 1930)			
	Number tested	Number positive	%	P value	Number tested	Number positive	%	P value	Number tested	Number positive	%	P value
Age group (years)												
25 or less	339	47	13.9	0.17	252	44	17.5	0.49	591	91	15.4	0.03
26-35	467	68	13.7									
>35	159	32	20.1									
Location												
Qingdao	445	69	15.5	0.83	415	77	18.6	0.37	860	146	17.0	0.45
Weihai	520	78	15.0									
Residence area												
Urban	534	70	13.1	0.04	386	60	15.5	0.24	920	130	14.1	0.02
Rural	431	77	17.9									
Cat at home												
Yes	14	4	28.6	0.16	108	33	30.6	<0.001	122	37	30.3	<0.001
No	951	143	15.0									

RESULTS AND DISCUSSION

These results espouse the importance of Toxoplasma test prior to marriage, in addition, to performing other tests. Traditionally, screening for toxoplasmosis has been carried out in

France and Brazil. This study reveals that the most age group infected in married women was 34-37 years old. The highest rate of *Toxoplasma* seropositivity was found among the age group 19-35 years (38.3%).^[27] Comparable to our result is that stated by Rabiee. A significant ($P > 0.05$) relation showed in a current study between *Toxoplasma* prevalence rate and the mother's age,^[28] confirms the fact that seroprevalence of *Toxoplasma* is well known to increase with age; the greater the prevalence, the earlier the rise.^[29] This might be explained by the older the person the longer time being exposed to the causing agent and may retain a steady level of anti-*Toxoplasma* IgG in serum for years.

The effect of ABO blood group antigens on the distribution of *Toxoplasma* in the present study reveals that the A, AB blood groups were the most blood group infected with *Toxoplasma*. Previous studies investigated possible relationships between the ABO blood group system and the presence of anti-*T. gondii* antibodies, but their conclusions are conflicting. Four studies reported an association between infection by this parasite and B and AB blood groups.^[30] These studies proposed that the B antigen could act as a potential receptor for *T. gondii*. However, two other similar investigations did not find any evidence of this association. The IgG antibody rate which is detected in this study by ELISA in infected married women was 89.5%. The lower rate was recorded by Youssefi they stated a rate of 63.9% but the rate found by Studeničová^[31] was 24.4%. The IgG antibody present in non-married women was 81.8%. In the present study, the rate of IgM was 5.3% in married and 9.09% in non- married women. This was comparable with that studied by Al-Hindi and Lubbad.^[32]

In a study of Hajssoleimani the prevalence of recently acquired infections (IgM positive) was relatively low (1.4%). The IgG seropositive rate in his study increased with age. Women older than 30 years had a significantly ($P > 0.05$) higher seroprevalence (48%) compared to those who were 20 or less (28.7%). IgM antibodies are detected early in the acute infection. Because they may persist for prolonged periods, IgM antibodies may be detected in pregnant women.^[33]

The importance of toxoplasmosis prevalence among pregnant women determination is due not only to define the adequate primary attention measures towards the susceptible women but also to establish the correct therapy aiming the reduction of the fetal sequelae.^[34] The strikingly high prevalence of vertical transmission found in this study is related to risk factors

described previously for this same population by Silva et al.^[35] which are age, raw meat and in natural milk intake, schooling, work, and poor hygienic habits during the meal preparation.

Women health care, especially during pregnancy, is of utmost importance in public health policies. The antenatal care provides the adequate moment to the implementation of prophylactic measures against maternal-fetal transmission of several diseases, including toxoplasmosis which presents a high prevalence.^[36]

The toxoplasmosis prevalence in pregnant women of the European countries is estimated to be between 2-70% with the lowest prevalence in Italy and the highest in France.^[37] Studies in the African countries showed the prevalence of toxoplasmosis in pregnant women was approximately between 20-60%.

Based on the studies conducted in Asia, it has a prevalence of 3-35% in different countries.^[38] In Iraq, many studies have been conducted on this issue in different provinces and the result showed that the prevalence of toxoplasmosis in women at childbearing age is between 23-64% depending on IgG antibody.

CONCLUSION

Toxoplasmosis is caused by infection with the obligate intracellular protozoan parasite *Toxoplasma gondii*. It is one of the most prevalent chronic infections affecting one-third of the world's human population (Jones, et al, 2001). One of the major consequences of pregnant women becoming infected by *Toxoplasma gondii* is vertical transmission to the fetus. Although rare, congenital toxoplasmosis can cause severe neurological or ocular disease (leading to blindness), as well as cardiac and cerebral anomalies. *Toxoplasma gondii* infection acquired by pregnant women during gestation and their transmissions to the fetus continue to be the cause of tragic yet preventable disease in the offspring. In addition to the unfortunate outcome for infants and children are the emotional and economic burdens faced by the parents and society. Although the majority of infants appear to be healthy at birth, significant long-term sequelae may become obvious only months or years later. Toxoplasmosis is a widely-distributed zoonosis caused by *Toxoplasma gondii* protozoa, although there is a high prevalence of unapparent infections, toxoplasmosis can develop into a severe systemic illness when in its congenital form, in which the mother, when infected for the first time during pregnancy, can present a temporary parasite with focal lesions generated within the placenta, thereby infecting the fetus. The parasite reaches the fetus

transplacentally, causing various degrees of damage, depending on the virulence of the parasite, on the immune response of the mother and on the pregnancy period of the woman when infected, resulting in fetal death or in severe clinical symptoms.

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