The Importance of Use of the On-line Databases as a Source for Systematic Review of Toxoplasmosis Screening During Pregnancy

Chrysa Voyiatzaki¹, Christos Orovas², Maria Trapali¹, Dimitrios I. Chaniotis¹, Anastasios G. Kriebardis¹, Apostolos Beloukas¹, Nikolaos D. Thalassinos¹, Eirini Orovou¹, Georgios Iatrakis¹, Evangelia Antoniou¹
¹University of West Attica, Athens, Greece
²University of Western Macedonia, Kozani, Greece

Corresponding author: Evangelia Antoniou, Associate Professor, Department of Midwifery, University of West Attica, Tel: +30-6977960041, E-mail address: lilanton@uniwa.gr, ORCID ID: https://orcid.org/0000-0003-4494-3089

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SYSTEMATIC REVIEW

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ABSTRACT

Background: Infection with the parasite Toxoplasma gondii is a common infection in animals and humans worldwide. This infection can occur after ingestion of water or food contaminated with cat oocysts, ingestion of tissue cysts in mammalian and avian meat and congenitally. The prenatal infection can lead to Congenital Toxoplasmosis with miscarriage or stillbirth. After infection, laboratory tests are positive within 2-3 weeks and remain positive throughout life. However, testing for Toxoplasma infection during pregnancy is necessary in some countries, while in others it is not a mandatory “screening” test. Objective: The aim of this study was to review systematically the screening of toxoplasmosis in pregnancy in different countries worldwide. Methods: Cohorts, retrospective and cross-sectional studies were incorporated in our review, finally including 11 articles from an initial pool of 1532 related papers. Results: The seroprevalence of pregnant women varies from countries with low prevalence to regions with high prevalence and screening policies also differ. Most countries worldwide have control policies, while Germany and Mexico that do not have systematic screening for Toxoplasma during the prenatal period. Conclusion: Our results show that Congenital Toxoplasmosis is very rare in some countries and it is very difficult to find a balance between potential risk and benefit of a screening program. For this reason, some countries are limited to prenatal counseling to reduce CT. In addition, the reduction of major sources of contamination especially in developing countries is the most important prevention measure. Keywords: Toxoplasma gondii; prenatal infection; prenatal toxoplasmosis screening; toxoplasmosis screening worldwide; toxoplasmosis antibodies.

1. BACKGROUND

Toxoplasma gondii (TG) is an extremely potent parasite particularly due to its ability to remain within cells for the lifetime of the host. Its significance in human health was clarified with the first reports of congenital toxoplasmosis (CT) in the 1940s (1). TG, a polyxenous pathogenic protozoan parasite belonging to the Apicomplexa phylum, has a complex life cycle. The human infection named toxoplasmosis is a zoonosis transmitted by a) ingestion or manipulation of undercooked or raw infected meat consumption containing tissue cysts; b) intake of food/water contaminated with Toxoplasma oocysts from cat feces; c) an infected mother passing the infection to her fetus (congenital form). In addition to humans, dogs, pigs, cattle, sheep, and a wide range of vertebrate species are intermediate hosts for the parasite.

The cat (both domestic and wild) is the definitive (reservoir) host, which is infected by eating contaminated undercooked or raw meat. After a person is infected, the parasite remains dormant in muscle and nerve tissues and it is never eliminated (2).

1.1 Epidemiology

The prevalence of toxoplasmosis indicates that it is a very common human infection which increases in warm climates and at lower altitudes rather than cold climates and mountainous regions (3). It is assumed that one third of the general population is infected with TG with high heterogeneity between countries and regions.
(2). In some countries, such as France (mainly Paris), where meat is not well cooked, more than 50% of women had been already infected (4). The Consumption of undercooked meat (such as undercooked lamb) is one of the most important risk factors for Toxoplasma infection (5).

However, in the last decade in France, the incidence of infection has decreased significantly with seroconversion being found at a rate of 2 to 2.5 per 1000 seronegative women. As per recent data, the overall prevalence rate of CT in France was 3.3 per 10,000 live births and the incidence of the disease at birth was 2.9 for every 10,000 live births (6). In industrialized countries with a temperate climate, there was serological evidence of an older infestation from TG in 10 to 50% of women aged 15 to 45 years, with a declining trend in seropositivity (4). Indicatively, in the USA, at the previous age range, seropositivity in 1994 was higher than 14%, in 2001 it was 11%, and in 2009 it was 9% (7). In Europe, there is a variation in prevalence rates, with Slovenia, Austria and Spain having > 30%, while the United Kingdom (UK) and Norway have less than 10%. In fact, during the reproductive period, the annual infection occurs in 1 in 100 women, while initial infection during pregnancy occurs in about 1 in 200 women (8). Of those who become infected initially during pregnancy, 40% will give birth to congenitally infected children. Only women with parasitemia (primary infection) are at risk of transmitting the infection to the fetus (9, 10). In 2017, 194 confirmed cases of congenital toxoplasmosis were reported in the EU/EEA (8).

1.2. Screening and diagnosis

Worldwide, more than 211 million pregnancies occur each year. Without Toxoplasmosis screening and preventive care, each of these pregnancies is prone to neonatal complications (11, 12, 13). In most cases toxoplasmosis is asymptomatic. However, the mother may experience fever, resembling infectious mononucleosis. In women with AIDS, toxoplasmosis is the most common central nervous system infection, so there may be a corresponding clinical status (14, 15). After toxoplasmosis infection, laboratory tests are positive within 2-3 weeks and remain positive throughout life. The presence of anti-Toxoplasma gondii specific IgM during pregnancy does not necessarily mean acute infection and therefore, additional tests are necessary (16). According to the national recommendations, Toxoplasma infection during pregnancy is not a “screening” test on several countries (USA, UK) because the prevalence of the disease and the frequency of maternal infection are very low, and screening is expensive. A variety of surveillance schemes have been implemented on a worldwide scale for reporting the incidence of CT. More specifically, in USA estimates of infection prevalence are limited in scope and seroprevalence is estimated at approximately 11% for women of reproductive age (7). At the same time, data/evidence on the CT incidence was obtained from the neonatal screening program by the New England Regional Toxoplasmosis Working Group, displayed an estimated incidence ranging from 0.1 to 1 per 1000 live births in different areas (17). In Canada, low prevalence of the disease in the population and limitations in diagnosis and treatment restrain the effectiveness of screening strategies (18). In addition, the UK National Screening Committee concluded that there was insufficient evidence to recommend toxoplasmosis screening (19). Furthermore, in a European survey of 28 European countries which took place in 2004, it was found that only 14 countries had a toxoplasmosis monitoring system (congenital or not). Denmark, Germany, France and Italy were the only participating countries that had implemented a CT screening system and achieved to identify symptomatic and asymptomatic cases (20).

In the opposite screening policy, physicians often face the need to interpret a positive IgM test from a single sample. Unfortunately, this test does not make a reliable diagnosis of recent infection since in about 25% of women with a history of toxoplasmosis, the detection of IgM antibodies can persist for years (21). It should be mentioned that IgM antibodies (first) appear as early as two weeks after infection.

Today, the recommendations for (possible) screening are the following: Regardless of IgG, if IgM is positive or doubtful, the diagnosis should be confirmed in a reference laboratory. Indicatively, in the USA, this is referred to as “name” (Dr. Jack S. Remington Laboratory for Specialty Diagnostics) (22). If screening is carried out before 20 weeks of gestation, and especially during the first trimester, negative IgM and positive IgG antibodies show a previous infection, so no confirmation test is needed (23). Given that recent infection is particularly important for fetal infection, recent toxoplasmosis can be diagnosed when IgM and IgG seroconversion has been confirmed in sequential testing. So, it can be concluded from the above that the “law of probability” intervenes in the diagnosis. In countries with increased rates of toxoplasmosis, the search for toxoplasmosis during pregnancy is a screening test (7). Thus, in France, the monthly control was imposed (by law) (4). However, a meta-analysis of all available cohort studies found little evidence of reduced transmission to the fetus, even with monthly screening (24). Prenatal diagnosis of CT was based on Polymerase Chain Reaction PCR in the amniotic fluid and in the sequential monitoring of the fetus with ultrasound (25).

1.3. Infection during Pregnancy and Outcomes

TG along with other infectious agents has been associated in various ways with spontaneous abortions but it has not been shown to cause recurrent abortions (26). CT occurs predominantly after primary infection of a pregnant woman and the transmission to the fetus can lead to death, abortion and neurocognitive deficits (27). The frequency of CT varies depending on the trimester of pregnancy during which the maternal infection was acquired. The transmission rate is 25% in the first trimester, 54% in the second trimester and 65% during the third trimester, in untreated women (28). However, CT from primary maternal infection in advanced pregnancy is generally asymptomatic at birth with few exceptions (29). In general, the average fetal infection throughout pregnancy is about 40% and of these fetuses less than 20% will show moderate damage, while a percentage of 10% serious abnormalities. In general, IgM levels in umbilical cord blood may be useful in diagnosing endometrial infection. Since IgM
is not transmitted from the mother, it is the fetal IgM that is produced by it. Possible findings in the fetus-newborn are: residual development hepatosplenomegaly, chorioretinitis, microcephaly, cerebral calcifications, lethargy or convulsions (or both), deafness, mental retardation, etc. The performance of fetal MRI was considered a necessary test for seropositivization for toxoplasmosis to rule out brain damage, even if an ultrasound is negative (30, 31). In general, the prognosis of children with congenital toxoplasmosis is very poor and more than 10% dies. Of those who survive 80% have visual disturbances and more than 80% mental retardation. Newborns of mothers with certain or potential infection should be closely followed up and subjected to clinical and serological testing in order to diagnose and treat infection as soon as possible (32, 33, 34).

2. OBJECTIVE

The aim of this review was to systematically investigate the seroprevalence of prenatal toxoplasmosis in different countries, and how this is related to different screening policies.

3. MATERIAL AND METHODS

The research was carried out based on Medline/ PubMed, Google Scholar and Crossref databases. The keywords used were: “Toxoplasma gondii and pregnancy”, “Toxoplasma gondii and screening”, Toxoplasma gondii and screening methods”, “Toxoplasma gondii and seroprevalence in pregnancy”, “Toxoplasma gondii and European pregnant women”, “Toxoplasma gondii and pregnant women”.

The timeline was from 2005 to 2020 and out of 1532 research articles; only 11 were included in the review. The articles identified through the initial search strategy were first screened by abstract, issue and title. The full texts of appropriate research articles were examined against the inclusion and exclusion criteria and from a total of 1532 studies, a total of 528 reviews, systematic reviews and meta-analyses were rejected. After the next screening, 702 articles including other Toxoplasma-related issues, such as congenital Toxoplasma, Toxoplasma in animals, and Toxoplasma treatment, were rejected too (Figure 1).

The study also analyzed exposure to TG infection after appropriate tests (molecular and serology) in the mother’s blood serum and in some infected cases from amniotic fluid and neonatal blood serum. All seroprevalence mothers have been laboratory confirmed with laboratory diagnostic assays laboratory tests for detection in serum isotypes IgM and IgG against TG specific antibodies (Immunoenzymatic or Chemiluminescence tests, Indirect Immunofluorescence test (IIFT), immunoblot, immunosorbent agglutination assay (ISAGA), Sabin and Feldman test, avidity of specific IgG antibodies) and Toxoplasma-DNA identification by RT-PCR). As Toxoplasmosis screening we defined all the Toxoplasmosis laboratory tests that took place during the 3 trimesters of pregnancy. As an outcome, we define the seroprevalence and seroconversion of TG during pregnancy.

Nine criteria were used to evaluate the methodological quality of the systematic review. The first criterion, concerning the representative exposure sample was met by all studies except the two clinical ones. The second criterion (selection of non-exposed), is also met by all. All studies met the third criterion because the seropreva-
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<table>
<thead>
<tr>
<th>Authors/Year</th>
<th>Design</th>
<th>Start / Expiry</th>
<th>N</th>
<th>Country</th>
<th>Toxoplasmosis Screening</th>
<th>Seroprevalence</th>
<th>Seroconversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. De Paschale (2010) (35)</td>
<td>Retrospective study</td>
<td>2006-2008</td>
<td>4,694 pregnant women of Northern Italy</td>
<td>Italy</td>
<td>5–7 serological screenings (IgG, IgM) at intervals of 30–40 days during pregnancy</td>
<td>20.7%</td>
<td>1.8%</td>
</tr>
<tr>
<td>2. Prusa A, (2017) (36)</td>
<td>Retrospective study</td>
<td>1992-2008</td>
<td>1,387,680 Pregnant women and their infants</td>
<td>Austria</td>
<td>Mandatory prenatal screening (IgG, IgM, PCR) is performed on a bimonthly schedule, at 8, 16, 24, and 32 weeks of gestation</td>
<td>34.4%</td>
<td>–</td>
</tr>
<tr>
<td>3. Lange E (2016) (37)</td>
<td>Cohort study</td>
<td>2002-2008</td>
<td>5,402 Pregnant women</td>
<td>Germany</td>
<td>German state health insurance does not cover toxoplasmosis screening</td>
<td>34.4%</td>
<td>0.3%</td>
</tr>
<tr>
<td>4. Antoniou M (2005) (38)</td>
<td>Cohort study</td>
<td>1999-2003</td>
<td>5,532 pregnant women and their infants</td>
<td>Greece</td>
<td>Serological prenatal screening (IgG, IgM or PCR) is performed in every trimester</td>
<td>29.45%</td>
<td>3.34%</td>
</tr>
<tr>
<td>5. Robinson E (2021) (4)</td>
<td>Cohort study</td>
<td>2016</td>
<td>13,586 pregnant women</td>
<td>France</td>
<td>Monthly screening</td>
<td>31.3%</td>
<td>0.31%</td>
</tr>
<tr>
<td>6. Capretti M (2014) (39)</td>
<td>Cohort study</td>
<td>2009-2011</td>
<td>10,347 pregnant women</td>
<td>Italy</td>
<td>First screening in early pregnancy and then every 4-5 weeks with IgG, IgM</td>
<td>22.3%</td>
<td>0.77%</td>
</tr>
<tr>
<td>7. Esquivel A (2016) (40)</td>
<td>Cross-sectional study</td>
<td>2014-2016</td>
<td>338 pregnant women</td>
<td>Mexico</td>
<td>Laboratory tests for the serological diagnosis of T. gondii infection are not available in many hospitals in Mexico. For the needs of this research, serum samples were tested for IgG, IgM antibodies</td>
<td>6.2%</td>
<td>4.8%</td>
</tr>
<tr>
<td>8. Sert U, (2019) (41)</td>
<td>Cohort study</td>
<td>2008-2017</td>
<td>84,587 Pregnant women</td>
<td>Turkey</td>
<td>A serologic test for Toxoplasma (IgG, IgM) is free of charge for all pregnant women as part of routine antenatal care at their first prenatal visit</td>
<td>22.3%</td>
<td>0.64%</td>
</tr>
<tr>
<td>9. Teweldemedhin M(2019) (42)</td>
<td>Cross-sectional study</td>
<td>2018</td>
<td>360 Pregnant women</td>
<td>Ethiopia</td>
<td>Women were tested for IgG and IgM anti T.gondii specific antibodies. If women who were positive, a new sample was taken after 3 weeks</td>
<td>32.5</td>
<td>3.1%</td>
</tr>
<tr>
<td>10. Khurana S (2010) (43)</td>
<td>Cross-sectional study</td>
<td>2005-2006</td>
<td>300 Pregnant women</td>
<td>India</td>
<td>Anti-Toxoplasma gondii IgG, IgM, IgA anti and IgG avidity were assessed by ELISA. 2 samples were taken at least 3 weeks (one in each trimester)</td>
<td>15.33%</td>
<td>3%</td>
</tr>
<tr>
<td>11. Sakikawa M (2012) (44)</td>
<td>Cross-sectional study</td>
<td>1997-2004</td>
<td>4,466 Pregnant women</td>
<td>Japan</td>
<td>The antibodies were measured with latex agglutination (LA) microtiter, during the first, the second and the third trimester. The mean interval between antibody measurement in early and late pregnancy was 16.2 weeks</td>
<td>10.3%</td>
<td>0.25%</td>
</tr>
</tbody>
</table>

Table 2. The seroprevalence and seroconversion in global prenatal screening. Notes: Seropositive women were defined by the presence of specific anti-Toxoplasma IgG in the serum. Seroconversion is defined by the presence of anti-Toxoplasma IgM with IgA and/or IgG avidity antibodies.

4. RESULTS

The 11 articles included in this study have been carried out in various countries in Latin America, Europe, the USA, Africa and Asia (Figure 2), (Table 2). More specifically, the research of De Paschale et al., 2010 (35) investigated the implementation of screening of Toxoplasma gondii infection during pregnancy. The cases studied consisted of 4,694 pregnant women who gave birth between 2006 and 2008 in Italy. The current Italian legislation indicates a protocol for laboratory antibodies for Toxoplasma tests at the beginning of the gestation and every 30–40 days until delivery (5-7 screenings). In this study the 84.1% of women underwent their first screening during the first trimester; however, only a few were followed up in the second and in the third trimester. Although the management of screening is active in Italy, the monitoring of seronegative cases is lacking. Also, in a later survey of Capretti M et al., 2014 (39) the total seroprevalence was similar as in the previous survey. However, the seroprevalence among non-native women was significantly higher than that among native women (32.8% vs. 19.1%), while the incidence rate of primary infection was 0.77% and 7 infants were diagnosed with CT.

Systematic screening for TG in each trimester of pregnancy is also performed in Greece. Although a percentage of about 70% of women were at risk of developing acute toxoplasmosis during pregnancy, only 54% of women un-
toxoplasmosis screening. In this survey, a percentage of 25.8% of women were not tested and only 0.3% of women had active toxoplasmosis. However, the likelihood of participating in a second toxoplasmosis screening increased among women with a good level of education.

Toxoplasma screening also appears to differ between countries outside Europe. More specifically, Mexico is one of the countries that do not apply systematic screening for toxoplasmosis during pregnancy. The IgG seropositivity presented very low (6.2%) and the active toxoplasmosis 4.8%. Furthermore, seropositivity was significant higher in women with a history of hepatitis, memory impairment, white ethnicity and frequent abdominal pain (40).

Prenatal screening for Toxoplasma is a subject of debate in different countries. In Turkey with 22.3% IgG seropositivity, 0.64% seroconversion and no one case of CT, the antenatal screening is free of charge for all women as part of routine during their first prenatal visit (41). Also in India, a country with low seroprevalence (18.33%), the protocol prescribes at least 2 samples during pregnancy (43). In another Asian country, Japan, seroprevalence was very low (10.3%) and it was higher in women above 35 years of age. Furthermore, only a percentage of 0.25% of women exhibited seroconversion during pregnancy. The antibodies were measured with latex agglutination (LA) microtiter in the three trimesters (44).

In Africa the prevalence seems to be higher. More specifically in Ethiopia, pregnant women were tested for IgG and IgM anti TG specific antibodies with IgG seroprevalence (32.5%) and seroconversion (3.1%). The risk factors were mainly the low educational level, lack of hand washing, the presence and the history of a domestic cat and the eating habits (42).

5. DISCUSSION

To our knowledge, this is the first systematic review to evaluate the screening of TG during pregnancy in different countries. We estimated that the total prevalence of TG during pregnancy varies by region, with the lowest prevalence in American countries and the highest prevalence in Europe and Africa (Figure 2). Therefore, the routine universal screening differs according to the seroprevalence of each country.

In T urkey with 22.3% IgG seropositivity in women with a history of hepatitis, memory impairment, white ethnicity and frequent abdominal pain (40).

We see a significant lead in the implementation of TG screening protocol in most European countries (Table 2). According to the studies, Germany does not apply prenatal toxoplasmosis screening (37). Although the IgG seropositivity in Germany was quite high, the state health insurance does not cover toxoplasmosis screening.

The results according to the articles show that other countries with a high IgG seroprevalence during pregnancy perform systemic toxoplasmosis screening in each trimester of pregnancy to prevent CT, but the increased involvement was only in the first trimester (35, 38, 42). Also in some European countries, such as French and Austria, which have a high IgG seroprevalence, Toxoplasmosis screening is more frequent (4, 36). On the other hand, we did not have enough studies available about screening policies between American countries. More specifically, in Mexico, laboratory tests for the serolog-
tical diagnosis of TG infection are not mandatory, so they are not available in many hospitals. However, the seroprevalence was found for the research purposes (40). In Africa and Asia, prenatal screening is also available, even in countries with low seroprevalence such as India (43).

In our findings, all studies except one (36), investigated seroconversion during pregnancy and all had low rates ranging from 0.25% to 4.8% (Table 2, (Figure 3). Furthermore, a few studies present findings of CT, which are minimal compared to the sample number of each study (36, 39, ). It is worth mentioning that in 2 large cohort studies (Greek and Turkish) no case of CT was observed (38, 41).

Screening policies are also different between countries. Some countries perform only one laboratory test during the first prenatal visit (41, 42), other countries every trimester (38, 43, 44) and others more often (4, 35, 36).

The strength of this systematic review is that it is the first to study the seroprevalence, seroconversion and Toxoplasmosis screening policy during pregnancy in different countries of the world. In the present study, despite our broad inclusion criteria, it was not clear where the screening tests came from and this is the limitation of the study. The Sabin Feldman dye test, which measures IgG antibodies, is very difficult to implement outside a reference laboratory. These difficulties are based on distinguishing if there was an infection which occurred before pregnancy (46). In addition, there is a dissimilarity of studies due to the lack of similar surveys worldwide, and for this reason there is a chronological discrepancy between them.

According to the European World Health Organization (47), greater awareness is needed for Toxoplasmosis during pregnancy. However, given that prenatal screening is not routine or required in the majority of countries, the recommendations are based on a cost-benefit assessment of seroprevalence and the effects of toxoplasmosis in each country and the associated risks of screening.

5. CONCLUSION

The findings of this study revealed that the seroprevalence of pregnant women varies from countries / regions with low prevalence to regions with high prevalence. In addition, the serological diagnosis of toxoplasmosis in pregnant women is not subject to mandatory prenatal screening in some countries, resulting in complications in the fetuses or newborns.

Based on our analysis the use of immunoenzymatic or chemiluminescence test is the most frequently applied serological diagnostic method with high sensitivity and specificity detection of CT as well as the asymptomatic form of the infections. If screening for maternal toxoplasmosis is not initiated in the first weeks of pregnancy, it is very likely that a long time, a “blind period” of pregnancy, will pass, without having the opportunity to detect seroconversion, as the most reliable and definitive way to confirm an acute infection in the pregnant woman. Furthermore, a “blind period” in late pregnancy misses those infections that are easy to diagnose, while suspected infections in the first test in pregnancy are difficult to confirm even by reference laboratories. In case additional confirmatory testing shall be carried out (positive IgM and low IgG avidity), the diagnostic decision becomes more difficult for a reference laboratory, if the period between conception and the first serum test is quite long. “Blind periods” leave the fetus without early treatment, but also lead to under presentation and may even introduce bias into studies.

However, systematic screening for Toxoplasma during the prenatal period does not necessarily depend on the seroprevalence of each country and sometimes fails to fulfill its purpose. According to our results, the symptomatic CT is very rare to zero in some countries, as well as treatment in pregnancy has not been fully evaluated for its benefits. In addition, if the increased cost of the antenatal screening is calculated, it is very difficult to find a balance between potential risk and benefit of a screening program and for this reason some countries are limited to prenatal counseling to reduce CT. Also, the reduction of major sources of contamination such as contaminated drinking water and contaminated meat and its products, especially in developing countries, are important prevention measures. Therefore, further research is needed to determine the cost-benefit of screening programs, including the cost of the unnecessarily effective treatment.

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