iMedPub Journals
http://journals.imed.pub

INTERNATIONAL ARCHIVES OF MEDICINE
SECTION: LABORATORY MEDICINE
ISSN: 1755-7682

2015
Vol. 8 No. 57
doi: 10.3823/1656

Diagnosing congenital toxoplasmosis: where are we? A systematic review

REVIEW

Maria do Socorro Vieira dos Santos[1,2], Cláudio Gleidiston Lima da Silva[1,2], Pedro Neto Lima de Oliveira[1], Karla Denise Barros Ribeiro[1], Antonio Gilvan Teixeira Júnior[1,3], Matheus Felipe Aguiar Santos[1], Victor Hugo Gonçalves Lopes[1,3], Modesto Leite Rolim-Neto[1], Bianca Bianco[2], Anete Sevciovic Grumach[2]

1 Faculty of Medicine, Federal University of Cariri, Barbalha, Ceara, Brazil.
2 Faculty of Medicine of ABC, Santo André, São Paulo, Brazil.
3 Institutional Program for Scientific Initiation (PIBIC) / National Council for Scientific and Technological Development (CNPq) fellow at Faculty of Medicine, Federal University of Cariri, Barbalha, Ceara, Brazil.

Contact information:

Maria do Socorro Vieira dos Santos.

socorro.vieira@ufca.edu.br

Abstract

Purpose: Compile information on laboratory methods for diagnosis of congenital toxoplasmosis, considering the tests conducted since the gestational stage until the child period.

Methods: A systematic review of 01.01.2006 to 31.12.2013 was held by VHL (Virtual Health Library). The search was performed with the descriptors ''toxoplasmosis" and "diagnosis. The selected articles were indexed in MEDLINE. The information pertinent to the study was selected, categorized and analyzed. Of the 186 articles found, 41 met the eligibility criteria.

Results: Laboratory tests are based on the presence of antibodies IgM and IgG anti-*Toxoplasma gondii*, in this sense it is important to correctly interpret serology, because the detection of specific antibodies is often delayed by the presence of maternal IgG or late production of specific antibodies in newborns. Molecular techniques (PCR) have emerged as alternative due to its higher sensitivity and specificity in diagnosing instruments, given the ability to detect parasite DNA and non-dependence of the immune response of the patient, such as serological tests.

Conclusions: The need for early treatment of congenital toxoplasmosis in order to avoid sequelae justifies the search for more sensitive and specific laboratory tests in early detection of the parasite. The integration among the different levels of care in the public health system is essential for obtaining effective control of toxoplasmosis in pregnant women.

Keywords
Diagnosis, Pregnancy, Neonates, Toxoplasmosis

Introduction

Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii*, an intracellular protozoan of a het-eroxenic life-cycle that is capable of infecting a variety of warm-blooded vertebrates [1]. Primary infection is most often asymptomatic in healthy individuals, and a symptom free chronic infection is established. Nevertheless, essentially in two circumstances, a life-threatening disease may occur in reactivation in immunecompromised patients (HIV-infected patients or transplant recipients) and in primary infection during pregnancy, which is followed in about one-third of the cases by an infection of the fetus [2].

During the primary infection acquired during pregnancy, the effective parasite transmits to fetus through placenta, causes congenital infection and various sequels [3]. Placental transfer was the first known transmission way of *Toxoplasma gondii*. The fetus is usually infected by tachyzoites that cross the placenta from the maternal circulation during primary infection but dormant tissue cysts of past infection may restart the life cycle of the parasite in immunossupressed pregnant women and, in rare cases, in immunocompetent pregnant women. Reinfection has more recently been observed [4].

Primary maternal *Toxoplasma* infection may be health-threatening for the fetus, or may even cause death in utero, depending on the date of transmission, parasite burden, parasite genotype, and host susceptibility [5]. The chances of fetal infection by *T. gondii* increase with the stage of pregnancy, from 5-15% in the first half of gestation, to 60-80% in the second half. Conversely, the chances of serious lesions and death decrease, declining from 70-80% in the first half to less than 10% in the second half [6, 7]. The clinical picture is notably variable in congenital toxoplasmosis. While sometimes no symptoms or findings are seen, spontaneous abortions and stillbirth may occur. The most frequent findings are chorioretinitis, intracranial calcifications and hydrocephalus. In the babies born as asymptomatic, hearing and visual impairment, neurologic findings and mental retardation may develop after long years [3].

Neonates with congenital toxoplasmosis, even asymptomatic at birth, should be treated early to reduce long-term sequelae. Postnatal diagnosis of congenital toxoplasmosis is essential because prenatal diagnosis fails to detect approximately 15% of cases or cannot be performed when maternal infection is acquired in late pregnancy. Detection of parasites in the placenta is one diagnostic approach to the early neonatal diagnosis of congenital toxoplasmosis [8]. The need for an early treatment of congenital toxoplasmosis to avoid late sequelae justifies the search for more sensitive and specific assays to detect *T. gondii* infection as early as possible [9].

The objective of this review was to compile information on laboratory methods for diagnosis of congenital toxoplasmosis, considering the tests conducted since the gestational stage until the child period. In this context, it analyzes the interpretation of a preventive diagnosis in reducing clinical cases at a later age.

Methods

It was performed a qualitative systematic review of articles about diagnosis of toxoplasmosis published in electronic databases previously selected. It was conducted a search in the literature through the online databases of the Virtual Health Library (VHL), that hosts the base of MEDLINE, in August 2014, by limiting itself to articles published between January 1, 2006 to December 31, 2013.

The reason to limit the search between 2006 and 2013 was because before this period, the number of published works were little expressive and at the same time not addressed directly to diagnosis of toxoplasmosis.

The following descriptors were used, in Portuguese, for searching in the VHL:

#1." toxoplasmosis'' (Descriptors in Health Sciences [DeCS, in Portuguese]);
#2." diagnosis''(DeCS term);

A similar search strategy was held in the PubMed database, by using the same terms mentioned above.

The analysis of the article followed eligibility criteria previously determined. The survey was carried out in one phase: 1 AND 2. A search was conducted for those combinations by using the filter "title, abstract, subject".

It was adopted the following inclusion criteria: (1) written publications in English, in Spanish or in Portuguese. (2) studies about the diagnosis of toxoplasmosis; (3) subject page Congenital Toxoplasmosis human limit; (4) human limit; (5) original articles with full text accessible through the Portal de Periodicos CAPES (The Coordination of Improvement of Higher Education Personnel) a virtual library connected to the Brazilian Ministry of education with content restricted to authorized users; and (6) prospective or retrospective observational studies (descriptive or analytical, except for case studies), experimental or almost experimental. The exclusion criteria were: (1) other study designs, e.g. case reports, case series, literature reviews and comments; (2) non-original studies, including editorials, reviews, forewords, short communications and letters to the editor.

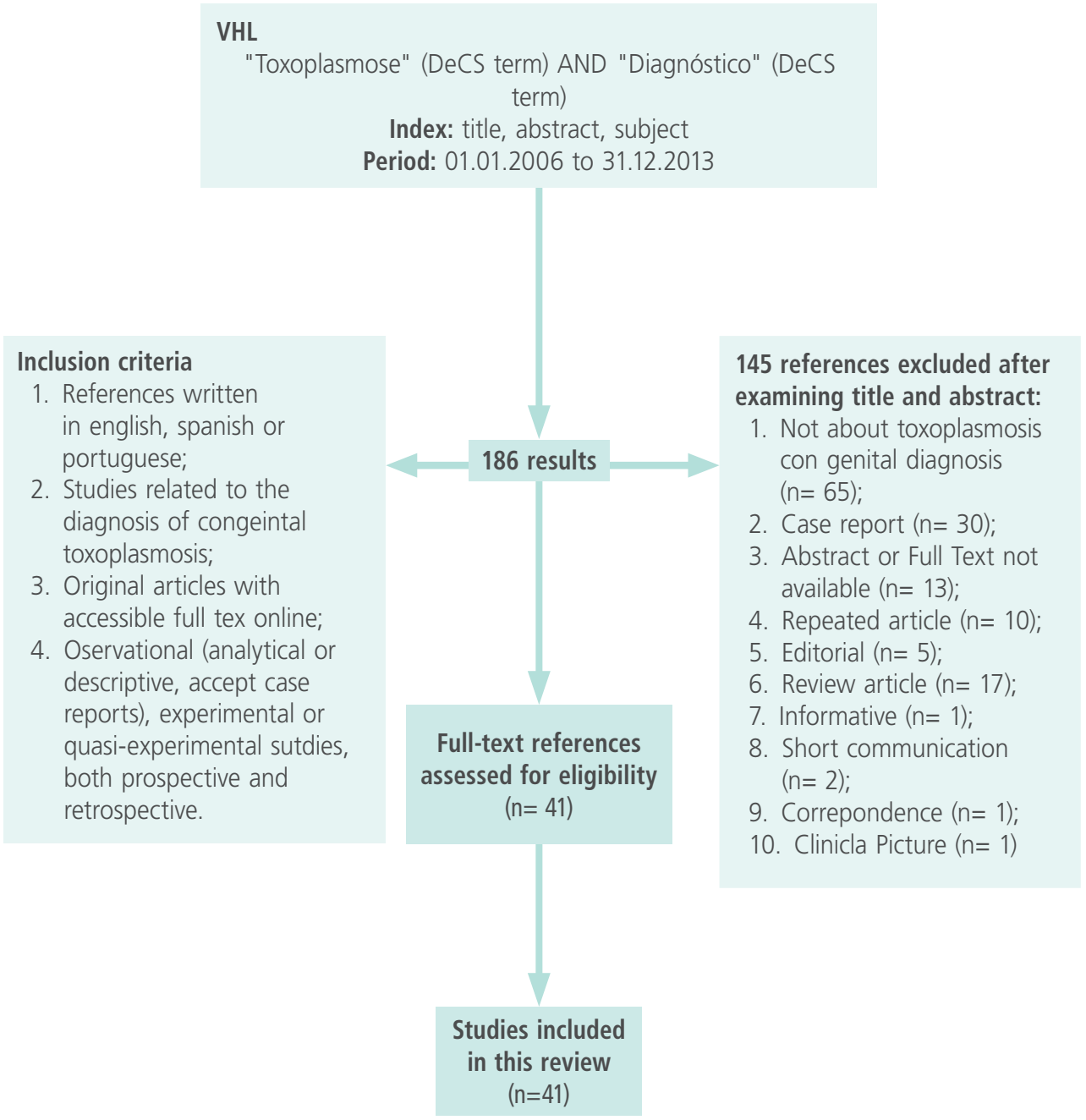
Each article was read in its entirety, and the information was entered in a spreadsheet that included authors, year of publication, the study sample description, key data, and databases. Some studies found about diagnosis of toxoplasmosis, treated them only the epidemiology of toxoplasmosis in certain regions, about treatment and costs, prognosis, adherence to screening for toxoplasmosis in pregnancy, toxoplasmosis in twins, eye, brain, auditory manifestations in neonates, risk factors for toxoplasmosis, toxoplasmosis caused by aftermath. To better analyze the data, the following stage involved the comparison between the articles and the division of the results obtained from the reading of each one of them in four categories: methods of diagnosis, diagnosis in pregnant women, neonatal diagnostics and infants diagnosis.

Results

Initially, the aforementioned search strategies resulted in 186 references. After browsing the title and abstract of the retrieved citations for eligibility based on study inclusion criteria, 145 articles were excluded and 41 articles were further retrieved and included in the final sample **(Figure 1)**. Articles from MEDLINE database matched the inclusion criteria of the present study.

Table 1 provides an overview of all studies included in the final sample and of all data elements used during the data analysis process. The 41 studies were distributed into the previously determined three categories as follows: DIAGNOSTIC METHODS [2, 4, 5, 8, 9, 12, 14, 16, 17, 20, 25, 27, 29, 32, 34, 35, 37, 41, 42 (nineteen studies)]; DIAGNOSIS IN PREGNANT WOMEN [3, 7, 10, 14, 25, 27, 35, 42, 46, 50, 51, 53, 59 thirteen studies)]; DIAGNOSIS IN NEONATES [7, 8, 11, 20, 61, 64, 65, 67, 68, 70, 71, 72 (twelve studies)] and DIAGNOSIS IN INFANTS [1, 3, 5, 9, 11, 12, 20, 27, 32, 42, 67, 68, 72, 78, 79, 85, 87 (seventeen studies)]. Among the 41 studies, some studies were referenced in more than one category. The categorization of studies aims to a better organizational quality systematic review and it is not compulsory that each article must be referenced only in their respective category

Figure 1: Flow chart showing study selection for the review.



Abbreviations: VHL: Virtual Health Library; DeCS: Health Sciences Descriptors

Table 1. Studies and main findings.

Authors	Journal	Sample	Main findings
Teixeira et al. (2013)	Rev Soc Bras Med Trop	One hundred pregnant women who seroconverted during pregnancy were included in the study. The definition of cases was based on a 12-month follow-up of the infants.	The conventional PCR assay detected 50 cases (9 false-negatives), nested-PCR detected 58 cases (1 false-negative and 4 false-positives), multiplex-nested-PCR detected 57 cases (2 false-negatives), and real-time-PCR detected 58 cases (1 false-negative). The real-time PCR assay was the best-performing technique based on the parameters of sensitivity (98,3%), specificity (100%), positive predictive value (100%), negative predictive value (97,6%), positive likelihood ratio (∞), negative likelihood ratio (0.017R) and efficiency (99%).
Costa et al. (2013)	J Clin Microbiol	Peripheral blood samples from 150 children diagnosed with congenital toxoplasmosis were collected during confirmatory tests, when children had an average age of 55.8 ± 15.8 days old.	We conclude that T. gondii qPCR positivity is higher in infants with ocular involvement, particularly in those with active retinochoroiditis.The parasite load in peripheral blood of congenitally infected infants within the first 2 months of life is low, with no association between degree of parasitemia and the presence of retinochoroidal lesions.
Torres et al. (2013)	Pediatr Infect Dis J	Peripheral blood samples were collected from mothers and children during the infants' first 3 months of life and were referred to the "Centro de Investigaciones Biomédicas" at the University of Quindio for confirmatory serological tests.	Twenty-three children were defined as infected congenitally by the persistence of IgG anti-Toxoplasma titers during follow-up or by the other criteria proposed by the European Network on Congenital Toxoplasmosis (ENCT). Thirty-one children were uninfected as determined by the disappearance of IgG during follow-up in the absence of treatment. Our results show that prenatal treatment significantly reduces the sensitivity of confirmatory assays.
Delhaes et al. (2013)	Diagn Microbiol Infect Dis	We evaluated the performance of three real-time polymerase chain reaction (PCR) assays on 73 samples from mothers and children with congenital toxoplasmosis.	In this study, we observed the better detection rate of PCR targeting the 529-bp repeat element than PCR targeting the B1 gene in the context of CT diagnosis. We showed a higher sensitivity of PCR in this diagnosis during the prenatal period than the birth period when testing AF and blood samples. This could account for the lower detection rate of PCR in children born to treated mothers and to a better performance of PCR on prenatal AF.
Wallon et al. (2013)	Clin Infect Dis	We analyzed data from pregnant women diagnosed with acute Toxoplasma infection in Lyon (France) from 1987 to 2008 and assessed how the risks of congenital toxoplasmosis and of clinical signs at age 3 years vary depending on GA at the time of maternal infectin.	These analyses demonstrated that introduction of monthly prenatal screening and improvement in antenatal diagnosis were associated with a significant reduction in the rate of congenital infection and a better outcome at 3 years of age in infected children.

Authors	Journal	Sample	Main findings
Uysal et al. (2013)	Rev Med Chile	To study the seroprevalence of Toxoplasma gondii infection among pregnant women living in Izmir, Turkey. A blood sample was obtained from 4651 women aged between 15 and 45 years, during their first trimester of pregnancy.	IgG antibodies were positive in 1871 (39.9%) participants. Of these, 48 (2.5%) also had positive IgM antibodies. In 41 of these 48 women, the IgG avidity test was performed and only one woman had a low avidity. Her offspring had an intrauterine growth retardation and oligohydramnios. A chorioretinitis was diagnosed in the offspring of other woman with both antibodies positive. In this series, the prevalence of congenital toxoplasmosis was low. However, women with positive antibodies against Toxoplasma Gondii should be further studied and followed during their pregnancy.
Prusa et al. (2013)	Neonatology	In this prospective observational study, 5.545 consecutive women were included over a 19-month period. Routine prenatal maternal toxoplasmosis serology screening was performed along with additional cord blood serology screening at delivery.	Based on the initial prenatal maternal screening serology results, there was evidence of a prior chronic infection manifest in 1,830 (33.0%) women and 3,708 (66.9%) were not infected. Seven (0.13%) were diagnosed with acute toxoplasma infection based on seroconversion. Identification of Toxoplasma gondii infection by prenatal maternal serological testing is significantly improved by the addition of maternal and/or fetal serological testing at birth.
Sterkers et al. (2012)	J Clin Microbiol	From a prospective cohort of 344 women who seroconverted for toxoplasmosis during pregnancy, 344 amniotic fluid, 264 placenta, and 216 cord blood samples were tested for diagnosis of congenital toxoplasmosis using the same PCR assay.	In total, our experience confirms the usefulness and accuracy of a high-performance-level molecular diagnosis to detect congenital toxoplasmosis, both prenatally and at birth. The novelty of our study relies upon the consideration of the curves of pre- and posttest risks obtained from this molecular diagnosis for estimation of the actual risk of congenital toxoplasmosis throughout the course of pregnancy.
Morelle et al (2012)	J Clin Microbiol.	This evaluation was based upon a T. gondii DNA serial dilution assay, three amniotic fluid samples spiked with T. gondii at different concentrations, and a clinical cohort of 33 amniotic fluid samples. The T. gondii DNA serial dilution assay showed a much lower sensitivity for the commercial kit.	We compared the performances of three molecular methods for the detection of Toxoplasma gondii DNA in amniotic fluid: a commercial method using nested PCR and two laboratory-developed methods, one using conventional PCR and the other one real-time PCR. This study emphasizes that commercial PCR diagnostic kits do not systematically perform better than carefully optimized laboratory-developed methods. There is a need for thorough evaluation of such kits by proficient groups, as well as for performance standards that commercial kits can be tested against to improve confidence in those selected by health care providers.
Prusa et al. (2012)	Clin Vaccine Immunol.	In a retrospective study, serum Toxoplasma gondii antibodies were measured in samples from 333 infants, including 212 noninfected infants and 121 infants with congenital toxoplasmosis. A total of 1.157 umbilical cord blood and peripheral serum samples were analyzed.	All noninfected infants were seronegative by Liaison IgG within the first year of life. The Liaison system showed a sensitivity of 81.8%, a specificity of 100.0%, a positive predictive value of 100.0%, a negative predictive value of 90.6%, and overall agreement of 84.4% by comparison with the dye test. Overall agreement of both IgM test systems was 96.0%. The Liaison system is a valuable tool to monitor the serologic course of infants at risk. A final serologic confirmatory test is recommended to improve the rate of detection of congenital toxoplasmosis at 1 year of life.

Authors	Journal	Sample	Main findings
L'Ollivier et al. (2012)	Clin Vaccine Immunol.	The study was performed on 236 mother-child serum pairs collected from 1999 to 2010 at the parasitology laboratories of the university hospitals of Lyon and Marseille.	Our findings demonstrate that taking high-molecular-mass bands into account greatly improves the sensitivity of the test without yielding false-positive results. The biologists who interpret immunoblot profiles should be particularly aware of the great diagnostic value of this three-IgM-band association at 75, 90, and 100 kDa, called the IgM triplet. In our series, this specific association markedly improved the performance of this test with a resulting sensitivity of 95.8% when combined with prenatal and conventional serological neonatal tests without loss of specificity. Despite the significant progress brought about by the analysis of these additional immunoblot bands, postnatal follow-up remains, however, necessary in the first year of life to fully identify infected children.
Faucher et al. (2012)	Eur J Clin Microbiol Infect Dis.	To optimize NucliSens easyMAG, we evaluated the addition of proteinase K pre-treatment and the increase of the amount of silica particles used for the extraction. The optimized method was then compared to QIAamp DNA minikit on samples containing less than 25 tachyzoites/ml.	The optimized method yielded more positive PCRs than the manual method, especially for samples containing 5 tachyzoites/ml or less (71% vs 26%, p<10-4). The DNA amount in samples found positive by PCR was higher after optimized automated extraction than after manual extraction (p<10-4). Proteinase K pre-treatment should be added to extract DNA from amniotic fluid using NucliSens easyMAG. Using this optimized automated method rather than manual methods would improve the sensitivity of Toxoplasma PCR and simplify the daily workflow.
Silva et al. (2012)	Mem Inst Oswaldo Cruz	Sera from 217 newborns initially testing positive for specific IgM in filter paper dried blood spots were tested for specific IgM and IgG by ELFA-VIDAS®. Congenital toxoplasmosis was confirmed in 175 and ruled out in 42 infants.	Congenital toxoplasmosis was confirmed in 175 and ruled out in 42 infants. The validity of the ELISA tests was determined using the persistence of IgG antibodies (ELFA-VIDAS® kit) at the end of 12 months, which is considered the reference test for the diagnosis of congenital toxoplasmosis. All of the antigens showed high sensitivity and low specificity in detecting anti-T. gondii IgGt and IgG1 and low sensitivity and high specificity in detecting IgG3 and IgG4. The combined detection of IgG antibody subclasses against recombinant toxoplasmic antigens may be useful for the early diagnosis of congenital toxoplasmosis.
Liu et al. (2012)	Foodborne Pathog Dis.	The recombinant antigen of ROP2186–533 was applied for diagnosis of both human and mouse serum samples using enzyme-linked immunosorbent assay (ELISA).	In summary, Toxo-IgM and -IgG antibodies in the sera of suspected Toxoplasma infection were examined and the antibody kinetics in mice infected with PRU strain of T. gondii with the rROP2186–533 antigen in ELISA were analyzed. The data showed that the rROP2186–533 was an efficient diagnostic antigen for detection of Toxo-IgM antibodies compared with WSA. It could be used as a specific antigen to capture Toxo-IgM antibodies for serodiagnosis of congenital toxoplasmosis and for differentiating between recent and latent infections.

Authors	Journal	Sample	Main findings
Said et al. (2011)	J Trop Pediatr	We prospectively studied 80 preterm neonates, recruited from neonatal intensive care units (NICUs) of Cairo University hospitals. Whose gestational age 34 weeks with (n=60) or without (n=20) CT risk. Serum samples for specific IgA, IgM antibodies and avidity of IgG toxoplasma antibodies were measured by ELISA then compared to PCR.	Of the 60 studied cases, 16 (26.7%) were positive for toxoplasmosis by PCR, of which 15 (25%) had low avidity of IgG antibodies (positive), 14 (23.3%) were positive for IgA and 10 (16.7%) were positive for IgM, with sensitivity for avidity of IgG, IgA and IgM: 93.2%, 87.5% and 62.5%, respectively. Determination of avidity of IgG toxoplasma antibodies and/or serological detection of specific IgA for toxoplasmosis offer, simple tests for diagnosis of congenital toxoplasmosis with (better sensitivity) than IgM.
Pessanha et al. (2011)	Rev. Paul. Pediatr	This cross-sectional retrospective study from 2003 to 2006 enrolled 98 pregnant women with positive IgM test for toxoplasmosis and 99 children. The follow-up of the children with or without congenital infection was reviewed, as well as the clinical presentation of those with congenital infection and the laboratory tests used to diagnose the infection by Toxoplasma gondii during pregnancy.	The results of this research suggest that, for the diagnosis of acute T. gondii infection in pregnancy should always consider holding the IgG avidity test in the first trimester in pregnant women with positive IgM serology. The presence of positive IgM serology as a test isolated, has limited value for detecting recent infection and should be used in combination with other complementary tests for the diagnosis of acute infection.
Sterkers et al. (2011)	Diagn Microbiol Infect Dis	In a cohort of 12 consecutive neonates, polymerase chain reaction (PCR) established the diagnosis of 5 of 6 cases of congenital toxoplasmosis and did so earlier than serologic methods.	In conclusion, with a low false-negative rate and no false positive result, we validated that PCR on neonatal peripheral blood i) can be performed routinely, ii) is able to affirm the diagnosis of CT, and iii) may be the first positive examination. Therefore, this unusual practice appears to be a useful alternative diagnostic approach.
di Carlo et al. (2011)	Acta Pharmacol Sin	Eighty five mothers with Toxoplasma seroconversion and their offspring were enrolled (among them, 2 spontaneous abortions were documented in the first trimester). Prenatal PCR diagnosis was carried out on 50 patients (60%), with 7 positive cases (14%). Morphological ultrasound scanning revealed anomalies in one fetus.	Western blot analysis may help to evaluate infection within the 6 months of life. The accuracy of ultrasound imaging to determine the brain damage in the fetus and newborns is doubtful, and should be combined with MR imaging. Multistep approaches can improve the timing of postnatal follow-up.
Gómez-Marin et al. (2011)	PLoS Negl Trop Dis	We collected 15,333 samples from umbilical cord blood between the period of March 2009 to May 2010 in 19 different hospitals and maternal-child health services from seven different cities. We applied an IgM ELISA assay (Vircell, Spain) to determine the frequency of IgM anti Toxoplasma	61 positive samples for specific IgM (0.39%) and 9 positives for IgA (0.5%) were found. 143 questionnaires were positive for a clinical diagnosis or treatment for toxoplasmosis during pregnancy. 109 out of the 218 children that had some of the criteria for postnatal confirmatory tests were followed. Congenital toxoplasmosis infection was confirmed in 15 children: 7 were symptomatic, and three of them died before the first month of life (20% of lethality). A significant correlation was found between a high incidence of markers for congenital toxoplasmosis and higher mean annual rainfall for the city.

Authors	Journal	Sample	Main findings
Magi, B and Migliorini, L. (2011)	New Microbiol	Fifty-six mother-child pairs were enrolled in the study, in the period 2001-2008. All pregnancies had a regular course, without clinical signs of pathology due to Toxoplasmainfection. None of the babies had clinical manifestations suggesting congenital toxoplasmosis.	We can therefore conclude that of the several serological tests used to detect congenital toxoplasmosis Western blot was the most sensitive, demonstrating IgM with a greater sensitivity than the ISAGA technique and being the only technique to differentiate IgG of maternal origin from that of fetal and neonatal origin.
Malinger et al. (2011)	Mem Inst Oswaldo Cruz	The aim of this study was to evaluate the utility of western blot (WB) analysis as a diagnostic tool for congenital toxoplasmosis in 215 newborn infants. The children were submitted to clinical examinations to assess macular, neurological and hearing signals.	This work showed that the WB assay is an effective tool for confirming the diagnosis of infants clinically suspected of congenital toxoplasmosis. The difference in the standard of antigen recognition observed in this work may indicate a differentiated profile of antibodies in response to the differential antigenic constitutions of the isolates predominating in Brazil. An association between WB results and clinical diagnosis of infants was demonstrated for the first time, with the IgM-WB test result likely being an indicator of macular lesions in newborn infants.
Roc et al. (2010)	Enferm Infecc Microbiol Clin	Toxoplasmosis seroprevalence was analyzed in 68.712 serum samples from 47.635 pregnant women living in the catchment area of Hospital Miguel Servet during the period of 1992 to 2008. Detection of toxoplasma-specific immunoglobulins (IgM, IgA, IgG) and IgG avidity studies were carried out in the microbiology laboratory.	The cases of congenital toxoplasmosis were detected by maternal seroconversion during pregnancy. IgA was the most sensitive marker for the detection of congenital infection in neonates.
Wallon et al. (2010)	Obstet Gynecol	This was a prospective cohort study of women with Toxoplasma infection identified by prenatal screening in three centers routinely carrying out real-time PCR for the detection of Toxoplasma gondii in amniotic fluid.	Polymerase chain reaction analysis was carried out on amniotic fluid for 261 of the 377 patients included (69%). Real-time PCR analysis significantly improves the detection of T. gondii on amniotic fluid. It provides an accurate tool to predict fetal infection and to decide on appropriate treatment and surveillance. However, postnatal follow-up remains necessary in the first year of life to fully exclude infection in children for whom PCR results were negative.
Souza-Júnior et al. (2010)	Sci. Med	A cross-sectional study included pregnant women with serological diagnosis of acute toxoplasmosis (presenting a positive Toxoplasma gondii-specific IgM test) attended at the outpatient unit for high-risk pregnancy of the Faculty of Medicine, Federal University of Mato Grosso do Sul, Brazil, in the period from November 2002 to November 2007.	In this study the rate of congenital infection in pregnant women diagnosed with acute toxoplasmosis was 4%. In pregnant women with positive Toxoplasma gondii-specific IgM, results of Toxoplasma gondii-specific IgG avidity test were associated with the presence or absence of congenital infection, with a high negative predictive value (no fetal/neonatal infection when avidity was high).

Authors	Journal	Sample	Main findings
Chapey et al. (2010)	J Clin Microbiol	Patient group 1 was used in a pilot study to check the validity of the technique before the testing of infants. It included 172 patients consulting in the outpatient department at Hospital de la Croix Rousse, Lyon, France, and pregnant women tested for toxoplasmosis serology as part of the French national program of prevention to PHA stimulation or spontaneous secretion of IFN- Group 2 consisted of 62 infants under 1 year of age who were born to mothers who seroconverted during pregnancy. Group 3 comprised 124 congenitally infected patients enrolled at ages 1 to 30 years.	We demonstrated that evaluation of the IFN-response after stimulation of whole blood by crude toxoplasmic antigen is a simple, easily performed test that is suitable for diagnosing congenital toxoplasmosis in newborns. This test could reliably rule out congenital infection at birth and avoid unnecessary anxiety and serological follow-up. The use of purified antigens or synthetic peptides to improve the performance of the test should be investigated.
Robert-Gangneux et al. (2010)	Pediatr Infect Dis J	We examined 102 placentas which were sent to our laboratory between February 2003 and April 2008, for T. gondii detection within the framework of neonatal screening performed routinely in France in cases of maternal Toxoplasma infection during pregnancy.	Parasites were detected more often when maternal infection was acquired during the third trimester of pregnancy ($P < 0.01$), regardless the type of treatment. The sensitivity of IgM detection appeared to be related to maternal treatment since IgM was positive in 43% and 75% when mothers were treated or not, respectively ($P < 0.01$). Placental examination is an efficient tool for the early diagnosis of congenital toxoplasmosis.
Rodrigues et al. (2009)	Mem Inst Oswaldo Cruz	In a prospective longitudinal study, 50 infants with suspected congenital toxoplasmosis were followed up in the ambulatory care centre of Congenital Infections at University Hospital in Goiânia, Goiás, Brazil, from January 1st, 2004 to September 30th, 2005.	The results showed that 28/50 infants were infected. During the neonatal period, IgM was detected in 39.3% (11/28) of those infected infants and IgA was detected in 21.4% (6/28). The presence of specific IgM and IgA antibodies during the neonatal period was not frequent, although it was correlated with the most severe cases of congenital transmission. The results indicate that the absence of congenital disease markers (IgM and IgA) in newborns, even after confirming the absence with several techniques, does not constitute an exclusion criterion for toxoplasmosis.
Okay et al. (2009)	Clinics (Sao Paulo)	A total of 467 amniotic fluid samples from T. gondii IgM- and IgG-positive Brazilian pregnant women being treated for 1 to 6 weeks at the time of amniocentesis (gestational ages of 14 to 25 weeks).	The use of PCR for the diagnosis of fetal Toxoplasma infections in Brazil should be targeted to the B1 gene when only one gene can be amplified, preferably by nested amplification with primers B22/B23.
Bessières et al. (2009)	Mem Inst Oswaldo Cruz	From 1994-2005, in Toulouse University Hospital, France, amniocentesis was performed on 352 pregnant women who were infected during pregnancy.	PCR performed on AF had the highest level of sensitivity and specificity for the diagnosis of CT. It permits an early diagnosis of most cases and should be recommended for identification of congenital infection. However, negative results cannot rule out fetal infection. Neonatal and postnatal screening identified these cases.

Authors	Journal	Sample	Main findings
Lago et al. (2009)	Sci. Med	Prospective cross-sectional study of consecutive mothers and their liveborn infants within the first 12 months of the implementation in a maternity ward of a routine consisting in screening for toxoplasmosis at delivery.	Maternal serologic screening at delivery was useful for the early detection of cases of congenital toxoplasmosis that would have otherwise gone undetected in the neonatal period, and allowed for earlier treatment of newborns with retinochoroiditis. The high prevalence of Toxoplasma gondii antibodies in pregnant women and of congenital toxoplasmosis justifies a prenatal screening program in this population.
Ferguson et al. (2008)	Ir Med J	This study sought to estimate the prevalence of Toxoplasma susceptibility in pregnant women. As detection of Toxoplasma antibodies in neonatal blood reflects maternal exposure history, maternal antibody seroprevalence was determined using anonymized residual blood from newborn screening cards. A total of 20,252 cards were tested in 1 year.	A total of 20 252 cards were tested which represented 40.2% of registered live births in 1 year. 4 991 (24.6%) cards tested positive for Toxoplasma antibody. The mean seroprevalence in Ireland was 25%, ranging by county from 19.9% to 41.3% indicating that a significant majority of women of child bearing age remain susceptible to Toxoplasma infection in pregnancy.
Carellos et al. (2008)	Cad Saude Publica	This cross-sectional study of 420 women in two public maternity hospitals from August 2004 to May 2005 evaluated the application of a prenatal toxoplasmosis serological screening protocol in Belo Horizonte, Minas Gerais State, Brazil, and the information provided to susceptible pregnant women.	The initial testing identified 163 women as susceptible to toxoplasmosis: 44% of these did not undergo repeat serological testing, and 42% of them did not remember having received information on the prevention of toxoplasmosis infection. Orientation on risk factors included: avoiding contact with cats (95%), not handling or eating raw meat (70%), and washing vegetables carefully before consumption (53%).
Ciardelli et al. (2008)	Pediatr Infect Dis J	T lymphocyte proliferation, interferon (IFN)-gamma production and lymphocyte activation antigens expression were evaluated in 23 infected and 65 uninfected neonates at different times, in the first year of life.	To the best of our knowledge, this is the first study reporting the concurrent evaluation of different immunologic assays for in vitro T cell responses to T. gondii antigens defining cut off values to improve accuracy and sensitivity. Our results underline the importance of evaluating cellular immunity to establish an early diagnosis of congenital toxoplasmosis.
Carral et al. (2008)	Medicina (B Aires)	Screening tests in pregnant women were done in nine different hospitals within the city of Buenos Aires and surroundings, where 19825 births between May 1st, 2006 and April 30th, 2007 were registered. Screening tests were done in 13632 pregnant women, using IgG and IgM determinations by ELISA.	The discovery of markers in blood of children and their absence in maternal blood is confirmatory of prenatal infection. Comparison of maternal and neonatal serology was useful in one case where IFI determinations ISAGA E and M were positive and negative in the child and in the mother, respectively. We conclude on the importance of serological screening during pregnancy and in the newborn, according to the experts' proposal explicit in the Argentine Consensus Congenital Toxoplasmosis. It was shown that the extension of the study allows more accurately diagnose acute infection, discard recent infections and avoid unnecessary treatments.

Authors	Journal	Sample	Main findings
Lago et al. (2007)	Paediatr Perinat Epidemiol	This prospective, cross-sectional study, conducted in 2002, tested a sample of 10 000 consecutive infants for T. gondii-specific IgM, using the same blood samples routinely obtained from all newborn infants treated by the public health system in Porto Alegre, in order to screen for phenylketonuria, congenital hypothyroidism and haemoglobinopathies.	In conclusion, in addition to finding a high prevalence of congenital toxoplasmosis in Porto Alegre, this study showed that neonatal screening identified cases of infection not detected by obtaining only one or two serum samples from pregnant women for T. gondii serology, mainly when infection was acquired and transmitted in late pregnancy. Maternal serology at delivery and neonatal screening were especially useful in the identification of infants with congenital toxoplasmosis when the mother did not receive regular prenatal serological testing or prenatal care.
Gilbert et al.(2007)	J Med Screen	In this report, we confined analyses to infants born in the 10 centres (in 3 countries) that operated prenatal screening. Neonatal screening centres could not be included as they did not follow up infants with negative results.	Poor performance of IgM and IgA tests in the newborn, particularly if the mother seroconverted in early pregnancy, casts doubt on the value of neonatal screening in industrialized countries where the risk of clinical manifestations during childhood is low. More accurate diagnostic tests are needed for newborns identified by prenatal screening.
Castilho-Pelloso et al. (2007)	Rev Saude Publica	A retrospective study was conducted with IgM-anti-Toxoplasma gondii reagent pregnant women and their children who attended the public health system in the state of Paraná, Southern Brazil, from January 2001 to December 2003. Information were obtained from clinical, laboratory (ELISA IgM/IgG) and ultrasonographic data and from interviews with the mothers.	The present study showed that the recommendations for monitoring this infection risk are not being systematically observed as some pregnant women with suspect laboratory results were not duly investigated, and some fetuses and newborns went without adequate monitoring for up to 12 months. Confirmed cases of congenital toxoplasmosis were detected only after birth in children with evident clinical abnormalities and the presence of IgM.
Isabel et al. (2007)	Sci. Med	The results of the serological tests for toxoplasmosis in the prenatal care routine were analyzed in two groups of patients. One consisting of pregnant women with negative T. gondii specific IgM (n=200) and the other consisting of pregnant women with positive T. gondii-specific IgM (n=33).	Even in the presence of a high specific T. gondii IgG avidity test in the first trimester of gestation, the antiparasitic treatment was instituted, demonstrating that the avidity test was not adequately utilized in the clinical practice.
Altcheh et al. (2006)	Diagn Microbiol Infect Dis	The reactivity values of Toxoplasma gondiiROP2, GRA4, and GRA7 recombinant antigens (rAgs) were analyzed by immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) in 23 congenitally infected (I) and 36 noninfected (NI) infants.	Our results indicate that rGRA4 and rGRA7 could have a potential to be used in the designing of novel and alternative diagnosis systems of CT. Between 1 and 4 months, the presence of antibodies antirGRA7 suggests a congenital infection, whereas the absence of reactivity against rGRA4 would be suggestive of no infection.

Authors	Journal	Sample	Main findings
Reis et al. (2006)	Rev. Bras. Ginecol. Obstet	Specific IgG and IgM determinations were performed using fluorometric tests, with IgM capture. A second sample within two to three weeks was requested from all IgM-positive pregnant women and IgG avidity was performed in IgM-positive pregnant women at the beginning of pregnancy. Neonatal IgM was obtained when the delivery occurred at the institution.	A high prevalence of infection and congenital toxoplasmosis was found in pregnant women, even without data on seroconversion. Most of the IgM-positive serologies were related to past infection. The cost-benefit ratio of prenatal care in isolated samples may be optimized analyzing the risk of mother-to-child transmission in IgM-positive pregnant women. When there is a risk, a neonatal IgM test must be requested and the newborn should be followed during the first year of life.

Discussion

Early diagnosis of congenital toxoplasmosis in pregnancy is crucial for proposing the most appropriate therapeutic approach in order to prevent late complications. Different time steps in the microbiological diagnosis of congenital toxoplasmosis, using different methods on different samples, may be individualized: during pregnancy, at birth, and during the first year of age [10].

In this way, CT diagnosis is confirmed if one of the following tests is positive: prenatal diagnosis based on the detection of *Toxoplasma gondii* in the amniotic fluid or neonatal diagnosis primarily based on the detection of neosynthesis of IgM and/or IgA specific antibodies in the infant serum [11]. Diagnosis of congenital toxoplasmosis is difficult due to the presence of maternal specific IgG anti-T. gondii antibodies in the newborn blood acquired through transplacental passage. Nevertheless, IgM and IgA antibodies do not cross the placental barrier and when they do so at birth, they have a lifespan of only five days, which allows them to be used as serological markers of vertical transmission [12].

Diagnostic methods

Serology

Serological tests are the fastest way to diagnose this infection since they can indicate the presence of two classes of immunoglobulins that do not cross placental barrier in the newborn or nursing infant serum: anti-*T. gondii* IgM and IgA [13, 12]. The diagnosis of congenital toxoplasmosis based on specific antibody detection is often delayed by the presence of maternal IgG and/or by the late production of specific antibodies in neonates as a consequence of immune system immaturity [9].

Serological tests that detect IgM class antibodies, present in recent infections are more commonly used for diagnosis of acute toxoplasmosis, but the most modern methods to detect minimal amounts for more than one year after the initial infection (residual IgM) [14]. Specific IgM is usually the first to be detected, in the first week post-infection. Its persistence for more than six months with great sensitivity testing as used is responsible for the observed low in the diagnosis of acute infection, extremely important in pregnancy specificity. Thus, an isolated positive result has not an absolute value, as IgM can be "residual" or a false positive [15, 4]. Therefore, when the first examination during gestation shows positive result, demonstrating the increase in antibody titers in samples with a minimum interval of three weeks is recommended [14].

It is important to correctly interpret confirmed positive serology for anti-T gondii IgM by assessing whether their presence reflects an acute, a past infection with residual IgM antibodies, or has no meaning infection, be due to a cross reaction, in other words, false positive [16]. When it has isolated sample or requested later in pregnancy, the levels of IgM must be linked to the levels of IgG

to the interpretation of serology consider both the dynamics of antibody formation as the space of a few months between conception and birth [4]. Postnatal serological diagnosis is usually performed by detecting specific IgM or IgA antibodies, but in a large percentage of congenitally infected children, these anti-bodies may be absent or produced in concentrations below the sensitivity thresholds of the methods available. Thus, in the absence of clinical signs, the diagnosis may be delayed until the persistence or increase in *Toxoplasma* specific IgG is observable [17].

Recently, it was shown that serum samples from infants with CT and from their mothers might recognize different parasite antigens, resulting in a differential profile by Western blot [18, 19, 20]. Fifty-six mother-child pairs were enrolled in the study, in the period 2001-2008. All mothers were tested during pregnancy for specific IgG and IgM and for IgG avidity and, on the basis of the results, they were considered at risk for transmission. Seroconversion was observed during the course of pregnancy or strongly suggested by the presence of specific IgM and/or a threefold elevation of specific IgG titers in two serum samples taken at a distance of three weeks and analysed in parallel. The several serological tests used to detect congenital toxoplasmosis Western blot was the most sensitive, demonstrating IgM with a greater sensitivity than the ISAGA technique and being the only technique to differentiate IgG of maternal origin from that of fetal and neonatal origin. In one case Western blot also proved useful for demonstrating active synthesis of IgG, as it showed bands of IgG directed against *Toxoplasma* antigens that were not present in the previous sample. The method enabled us to diagnose congenital toxoplasmosis in cases in which the infection had not been detected by classical serology techniques [17].

Demonstration of specific IgA anti-*Toxoplasma* antibodies have been the recommended antibody isotype for diagnosing neonatal infection because they can be as sensitive as, or more sensitive than, the demonstration of IgM antibodies [21, 12]. The IFI M is early positive and in most cases become negative in 6 to 12 months. The ISAGA is a more sensitive and specific technique for the detection of IgM and remains positive about 9 months to a year. With the same technique can be detected IgA and IgE. Both are characteristic of the acute stage, IgA become negative at about 7 months and IgE at 4-5 months [22, 23, 24, 25]. By contrast, the presence of specific IgG antibodies may only repre-sent transmission of maternal antibodies, which disappear in the first year of life [12]. The IgG antibody present in the newborn may reflect maternal infection due to passive transfer of antibodies. For this reason, tests for detecting IgA and IgM are commonly used for the diagnosis of infection in children [26, 27]. In general, in uninfected children, IgG levels decline gradually in the first months of life [28, 27].

IgM antibodies against *Toxoplasma* (Toxo-IgM) have been believed to be significant indicators for both recently acquired and congenital toxoplasmosis [29]. Recently, identified several antigens for detection of IgM antibodies using protein microarray displaying the polypeptides products of Toxoplasmic exons with well-characterized sera.[30, 29]. So far, however, there has not been any recognized protein of *T. gondii* that specifically reacts to IgM antibodies. Here, an antigen exclusively for detection of IgM antibodies screened by two-dimensional electrophoresis and mass spectrometry has been reported. The study identified 13 *Toxoplasma* proteins probed by IgG antibodies and one (rhpotry protein 2 [ROP2]) by IgM antibodies with human sera of Toxo-IgM–-IgG+ and -IgM+-IgG–, respectively, which had been prescreened by Toxo-IgM and -IgG commercial kits from the suspected cases. Following cloning, expression, and purification of the fragment of ROP2186–533, an enzyme-linked immunosorbent assay with rROP2186–533 to measure IgM and IgG antibodies was developed. As a result, 100%(48/48) of sera with Toxo-IgM+-IgG–showed

positive Toxo-IgM but none of them (0%) showed positive Toxo-IgG when rROP2186–533 was used as antigen. Neither Toxo-IgG nor Toxo-IgM antibodies were found when tested with 59 sera of Toxo-IgM–--IgG+. These results indicate that rROP2186–533 could be used as an antigen that specifically capture Toxo-IgM antibodies and may have a high potential in the serological diagnosis of both acute acquired and congenital toxoplasmosis [29].

Demonstrated that positive specific IgA is more indicative for acute *T. gondii* infection than specific IgM as it disappears earlier (between 3 and 9 months) while IgM remains at high levels for several months (up to 1 year), rendering inadequate sero-diagnosis of acute toxoplasmosis [31, 32]. It is important the joint detection of IgM and IgA in newborns because it can be positive for only one marker of prenatal infection macroglobulins [33, 25]. The discovery of markers in blood of children and their absence in maternal blood confirm prenatal infection. Comparison of maternal and neonatal serology was useful in one case where IFI determinations ISAGA E and M were positive to the child and negative to the mother [25]. Whenever maternal serology indicate risk, should be asked IgM to newborn and accompany him during the first year of life. Integration between different levels of care in the public health system is needed so that you can achieve maximum efficiency in controlling gestational and congenital toxoplasmosis [4].

Polymerase Chain Reaction - PCR

Conventional laboratory diagnosis of toxoplasmosis is based on the presence of IgM and IgG anti-*Toxoplasma gondii* antibodies; however, molecular techniques have emerged as alternative tools due to their increased sensitivity [5]. Performance variation among PCR systems in detecting *Toxoplasma gondii* has been extensively reported and associated with target genes, primer composition, amplification parameters, treatment during pregnancy, host genetic susceptibility and genotypes of different parasites according to geographical characteristics [34].

The superiority of PCR sensitivity in diagnosis of congenital toxoplasmosis refers to its ability in detection of the parasite DNA (even if single) in blood [32]. A positive *Toxoplasma gondii* polymerase chain reaction (PCR) assay on an amniotic fluid (AF) sample entails radical changes in pre and post-natal management. However, the follow-up of pregnant women may be irregular, or mothers infected during pregnancy may refuse amniocentesis. In these situations and when prenatal diagnosis was negative, biological neonatal diagnosis of CT could be performed by molecular testing of different samples (AF, placenta, cord blood, peripheral blood) as well as comparative mother-child serologic tests [35].

The extreme diversity of methods and performances of *Toxoplasma* PCR assays makes the use of commercial PCR kits an attractive alternative, as they offer a chance for standardization [2]. *Toxoplasma* PCR in amniotic fluid (AF) is the cornerstone of the antenatal diagnosis of congenital toxoplasmosis [36, 37]. Highly sensitive methods are necessary because up to 46% of infected AF contain less than ten tachyzoites per milliliter (T/ml) [38, 37]. We compared the performances of three molecular methods for the detection of *Toxoplasma gondii* DNA in amniotic fluid: a commercial method using nested PCR and two laboratory-developed methods, one using conventional PCR and the other one real-time PCR. This evaluation was based upon a *T. gondii* DNA serial dilution assay, three amniotic fluid samples spiked with *T. gondii* at different concentrations, and a clinical cohort of 33 amniotic fluid samples. The *T. gondii* DNA serial dilution assay showed a much lower sensitivity for the commercial kit than for the laboratory-developed methods. Moreover, out of 12 proven congenital toxoplasmosis cases, 91.7% were detected by the laboratory-developed assays, whereas only 50% were detected by the commercial kit. A lack of sensitivity of the method, partly due to the presence of PCR inhibitors, was the main drawback of the commercial method [2].

Antenatal diagnosis of congenital toxoplasmosis relies on PCR in amniotic fluid. Because parasitic load is often low, DNA extraction must be optimized. Manual methods remain widespread although automated methods appear more effective. To optimize NucliSens easyMAG, we evaluated the addition of proteinase K pre-treatment and the increase of the amount of silica particles used for the extraction. The optimized method was then compared to QIAamp DNA minikit on samples containing less than 25 tachyzoites/ml. NucliSens easy-MAG DNA yield was improved after proteinase K pretreatment ($p<0.01$), but not with a higher silica particle input. The optimized method yielded more positive PCRs than the manual method, especially for samples containing 5 tachyzoites/ml or less (71% vs 26%, $p<10-4$). The DNA amount in samples found positive by PCR was higher after optimized automated extraction than after manual extraction ($p<10-4$). Proteinase K pretreatment should be added to extract DNA from amniotic fluid using NucliSens easyMAG [37].

Quantitative PCR is a prognostic marker of fetal infection, since higher *T. gondii* concentrations in amniotic fluid are correlated with clinical signs in neonates [39, 8]. Congenital toxoplasmosis was diagnosed in 28 of the 102 cases. Specific IgM was detected in 57% of the babies at birth. A positive placental examination by PCR and mouse inoculation was the only evidence of infection in 3 neonates (11%) who were asymptomatic at birth. Parasites were detected more often when maternal infection was acquired during the third trimester of pregnancy (P 0.01), regardless the type of treatment. A better sensitivity of prenatal diagnosis when maternal infection was acquired at 17 to 21 weeks of gestation, and a lower sensitivity when seroconversion occurred during the first trimester, but the excellent negative predictive value of their PCR test supports its use [40, 8].

A total of 467 amniotic fluid samples from *T. gondii* IgM- and IgG-positive Brazilian pregnant women being treated for 1 to 6 weeks at the time of amniocentesis (gestational ages of 14 to 25 weeks). One nested-B1-PCR and three one-round amplification systems targeted to rDNA, AF146527 and the B1 gene were employed. The B1 gene PCR was far more sensitive than the AF146527 PCR, and the rDNA PCR was the least effective even though the rDNA had the most repetitive sequence. The use of PCR for the diagnosis of fetal *Toxoplasma* infections in Brazil should be targeted to the B1 gene when only one gene can be amplified, preferably by nested amplification with primers B22/B23 [34].

In a cohort of 12 consecutive neonates, polymerase chain reaction (PCR) established the diagnosis of 5 of 6 cases of congenital toxoplasmosis and did so earlier than serologic methods. In conclusion, with a low false-negative rate and no false positive result, we validated that PCR on neonatal peripheral blood, i) can be performed routinely, ii) is able to affirm the diagnosis of CT, and iii) may be the first positive examination. Therefore, this unusual practice appears to be a useful alternative diagnostic approach [41].

PCR performed on AF had the highest levels of sensitivity and specificity for the diagnosis of CT. This permits an early diagnosis of most cases and should be recommended [42]. When polymerase chain reaction (PCR) of amniotic fluid, placenta, and/or cord blood is not available, the diagnosis of CT depends only on serologic follow-up after birth. The definite diagnosis is then difficult to assess and delayed [41].

Diagnosis in pregnant women

The infection by *Toxoplasma gondii* during pregnancy may cause fetal harm, such as miscarriages, retarded intrauterine growth, prematurity and neurological and ophthalmic involvement [43, 27]. After infection in pregnant women, the overall risk of fetal infection is 40%. However, this risk varies with the gestational age at which the woman became infected, being lower in the first quarter and highest in the third trimester [44, 27].

The analysis of IgM and IgG antibodies for diagnosis are the most common tests that may be used routinely. Avidity tests should be applied on patients in whom both antibodies are identified as positive [3]. The avidity test is based on the affinity for the antigen having the specific immunoglobulins G according to the time of infection. The IgG antibodies have low avidity for parasite antigens in the early phase of infection. With the maturation of the immune response antibodies acquire greater avidity. It is interpreted as low avidity to values less than 20%, 21-30% intermediate and high avidity higher than 31%. A high avidity result indicates more than 12 to 16 weeks of infection [45, 25]. The interpretation of the results lies in understanding the functional affinity of IgG antibodies to antigens is initially low after primary antigenic response, and increases subsequently after maturation of the immune system [14].

The test avidity IgG anti-T.gondii was performed in 162 patients (92% of sample), lying high avidity (> 30%) in 144 (88.9%) and low avidity (≤ 30%) in 18 (11.1%) of pregnant women studied, being observed variation in the IgG avidity of 19.1% to 74.7%. The association between the results of the IgG avidity tests for toxoplasmosis and fetal/neonatal confirmed infection showed that among 18 women who had low avidity, 4 (22%) resulted in fetal/neonatal and 14 (78%) infection showed no infection congenital. Among the 144 women who had high avidity, 3 (2%) fetuses were infected and 141 (98%) had no congenital infection by the criteria described. There was association (p = 0.003) among the values of high avidity and absence of fetal/neonatal infection *T. gondii* in the studied sample. The prevalence ratio was 13.4 (95% confidence interval: 2.2 to 86.6), i.e., the pregnant women with high avidity were 13.4 times higher chance of your fetus/newborn not be infected [46].

The diagnosis of congenital toxoplasmosis may prove a difficult task, combining clinical characteristics and results from a battery of serologic and molecular tests. From a prospective cohort of 344 women who seroconverted for toxoplasmosis during pregnancy, 344 amniotic fluid, 264 placenta, and 216 cord blood samples were tested for diagnosis of congenital toxoplasmosis using the same PCR assay. The sensitivity and negative predictive value of the PCR assay using amniotic fluid were 86.3% and 97.2%, respectively, and both specificity and positive predictive value were 100%. Using placenta and cord blood, sensitivities were 79.5% and 21.2%, and specificities were 92% and 100%, respectively. Thus, with a molecular diagnosis performing at a high level, and in spite of the persistence of false negatives, posttest risk curves using both negative and positive results prove highly informative, allowing a better assessment of the actual risk of congenital toxoplasmosis and finally an improved decision guide to treatment [10].

Congenital toxoplasmosis occurs only when the woman is infected during pregnancy and transmits the parasite to the fetus through the placenta. The passage is increased with advancing gestation, and is about 15%, 50% or 65% according to the trimester of pregnancy in which the infection occurs [47, 25]. Seventy-three samples were collected from 57 mother-child pairs followed-up for CT, either in Lille 2 University Hospital or in the Cochin-Port Royal-Saint Vincent de Paul University Hospital group. Clinical monitoring for toxoplasmosis in these mother-child pairs showed 12% of termination of pregnancy, 11% of symptomatic CT and 77% of asymptomatic CT. The symptomatic and asymptomatic CT cases were confirmed by toxoplasmosis serological monitoring with an increase of immunoglobulin G (IgG) content in the first 12 months of life or a persistence of IgG after the first year of life or a presence of IgM and/or IgA. CT occurred after maternal infection in the first (7%), second (58%) and third (35%) trimester of pregnancy [35].

There is controversy over the serological tests used in the diagnosis of acute toxoplasmosis in

pregnancy because decisions based on false-positive tests may result in pregnancy interruption and unnecessary treatment [48, 7]. To study the seroprevalence of *Toxoplasma gondii* infection among pregnant women living in Izmir, Turkey, 4,691 pregnant women who were between 15 and 45 years of age and in the first trimester of pregnancy were included in the study. IgG and IgM antibodies were identified as negative (seronegative) in 2.820 (60%). IgG antibody was identified as positive (seropositive) in 1.871 (39.9%) of the pregnant women of which anti *Toxoplasma* antibody results were evaluated. While IgM antibody was identified as negative in 1,823 of 1,871 pregnant women of which IgG was identified as positive, in 48 (2.5%)of them IgM and IgG antibodies were both identified as positive. Intrauterine growth retardation and oligohydramnios developed in fetus in USG follow-up. The gestational age of fetus that was evaluated biometrically was found 31 week rather than 35 weeks and AFI (amniotic fluid index) was measured as 40 in four quadrant measurement [3].

The diagnosis of acute infection in pregnancy is crucial because it is usually during this period that the pregnant woman is at risk of transmitting the disease to the fetus [49, 27]. First trimester infection is associated with 25% transmission with neurologic and ocular sequelae in 75%. Third trimester infection is associated with more than 60% transmission but adverse sequelae in more than 5%.3 Most infants in the latter group will be asymptomatic at birth; some may display sub clinical disease on further evaluation. Without treatment, CT is a recurrent disease that may reactivate and progress at any time [50]. From 1994-2005, in Toulouse University Hospital, France, amniocentesis was performed on 352 pregnant women who were infected during pregnancy. Among the 275 fetuses with follow-up, 66 (24%) were infected. The transmission rates of *Toxoplasma gondii* were 7%, 24% and 59% in the first, second and third trimesters, respectively. The sensitivity and specificity of PCR on amniotic fluid (AF) were 91% and 99.5% and on placentas were 52% and 99%, respectively. PCR performed on AF had the highest levels of sensitivity and specificity for the diagnosis of CT [42].

The prenatal diagnosis of toxoplasmosis is based on routine population screening or on the ultrasonographic recognition of characteristic findings associated with intrauterine infection followed by maternal or fetal laboratory tests [51]. There are additional reports of the imaging findings in CTX, onde pesquisadores describe ventriculomegaly and multiple large periventricular echogenic nodules in a patient diagnosed in the second trimester [52, 51]. Physicians from Prenatal Diagnosis Units in ten Latin American countries were contacted and asked to provide data on fetuses with ultrasound findings suggestive of intrauterine infection and a positive diagnosis of CTX. Intracranial findings suggestive of CTX were identified in eight patients at a median gestational age of 31.5 weeks (range, 24.4–34 weeks). All six survivors have choroidoretinitis and intracranial calcifications, four suffer from developmental delay and three of these four children also suffer from seizures and blindness. Postnatal hydrocephaly was found in five children. Ventriculomegaly associated with multiple echodense nodules is characteristic of severe fetal toxoplasmosis and carries a poor prognosis [51].

We analyzed data from pregnant women diagnosed with acute *Toxoplasma* infection in Lyon (France) from 1987 to 2008 and assessed how the risks of congenital toxoplasmosis and of clinical signs at age 3 years vary depending on GA at the time of maternal infection. Among 2048 mother-infant pairs, 93.2% of mothers received prenatal treatment and 513 (24.7%) fetuses were infected. Probabilities of congenital infection were higher than 10% for maternal infections before 12 weeks of gestation, rose to 20.0% at 19 weeks, and then continued increasing to 52.3% and almost 70%

at 28 and 39 GA weeks, respectively. Because of a significant reduction in risk of clinical signs of congenital toxoplasmosis in infected children born from mothers diagnosed after 1995 when polymerase chain reaction testing on amniotic fluid was initiated (87/794 vs 46/1150; P = .012), probabilities of clinical signs at 3 years were estimated based on 1015 maternal infections diagnosed after 1995 including 207 infected children, with symptoms in 46 (22.2%) [53].

The ideal situation for the diagnosis of infection with *T. gondii* in pregnancy is serologic tests for toxoplasmosis in the early gestational period [54, 27] but often this is not possible, especially in Brazil, where few pregnant women start prenatal care in the first trimester [55, 56, 27]. For the diagnosis of acute *T. gondii* infection in pregnancy the realization of IgG avidity test in the first trimester in pregnant women with positive IgM serology should be considered. The presence of positive IgM serology as a test isolated, has limited value for detecting recent infection and should be used in combination with other complementary tests for the diagnosis of acute infection. The importance of analyzing the avidity index of IgG anti-T.gondii for making treatment decision rests on knowledge of the characteristics of clinical situations of pregnant women at the time of carrying out the serological tests [57, 45, 58, 46]. Such a strategy could reduce the need for treatment of pregnant women and follow-up of children who have ambulatorial suspicion.

Maternal serologic screening at delivery was useful for the early detection of cases of congenital toxoplasmosis that would have otherwise gone undetected in the neonatal period, and allowed for earlier treatment of newborns with retinochoroiditis. The high prevalence of *Toxoplasma gondii* antibodies in pregnant women and of congenital toxoplasmosis justify a prenatal screening program in this population [59].

Diagnosis in neonates

Congenital infection by *Toxoplasma gondii* can lead to severe congenital defects such as hydrocephalus, mental retardation and retinochoroiditis, which can be present at birth or develop later in life [60, 61]. About 10% to 33% of prenatal infections result in abortion, neonatal death, or severe clinical signs at birth [62, 63, 20]. The clinical manifestation of the infant will depend of the gestational week when the mother acquired the infection and is characterized by a broad spectrum of symptoms at birth, including varying degrees of neurologic, ophthalmologic and systemic involvement [60, 64]. More accurate diagnostic tests are needed for newborns identified by prenatal screening [65].

Early diagnosis is essential in order to determine the appropriate antibiotic therapy, but confirmatory tests are needed when specific IgMs or IgAs are not detected or to confirm potential false-positive results originating from maternal IgM or IgA leaks from the placenta in samples taken at birth or during the first 10 days of life [60, 61]. A single IgM and IgG serology test supported the majority of the decisions taken by the doctors regarding therapeutic and prophylactic management. [7]. Poor performance of IgM and IgA tests in the newborn, particularly if the mother seroconverted in early pregnancy, casts doubt on the value of neonatal screening in industrialized countries where the risk of clinical manifestations during childhood is low.

Recent reports indicate that congenital toxoplasmosis is more often symptomatic in South America than in Europe. We collected 15,333 samples from umbilical cord blood between the period of March 2009 to May 2010 in 19 different hospitals and maternal-child health services from seven different cities. We applied an IgM ELISA assay (Vircell, Spain) to determine the frequency of IgM anti Toxoplasma. The results in blood cord samples were confirmed either by western blot and repeated ELISA IgM assay. In a sub-sample of 1,613 children that were negative by the anti-*Toxoplasma* IgM assay,

the frequency of specific anti-*Toxoplasma* IgA by the ISAGA assay was determined. All children with positive samples by IgM, IgA, clinical diagnosis or treatment during pregnancy were recalled for confirmatory tests after day 10th of life [64].

We compared results of the first postnatal IgM or IgA test in infants with infected mothers identified by prenatal screening with the reference standard for congenital infection status of specific IgG status at one year of age. In all, 170 infected and 822 uninfected infants were analysed. The first IgM or IgA test detected just over half the infants with congenital toxoplasmosis. Detection by IgM was lowest for newborns whose mothers seroconverted in the first or second trimesters [65]. More than two-thirds of infants detected by IgM or IgA were born to mothers who seroconverted in the third trimester. For prenatal screening, the poor performance of IgM and IgA tests means that more accurate tests or additional tests and follow up are required to decide on postnatal treatment in the 80% of newborns who have a negative IgM or IgA test result [66, 65]. The ISAGA IgM and IgA and ELISA IgA tests performed best, but positive results need to be quickly confirmed by testing a second sample as the detection rate was optimal up to two weeks of age and declined thereafter.

The children were submitted to clinical examinations to assess macular, neurological and hearing signals. The WB results obtained were compared to the persistence of IgG antibodies at the end of 12 months, which is regarded as the "gold standard" diagnosis of congenital toxoplasmosis. Of the 215 children, 177 had a confirmed congenital toxoplasmosis diagnosis and 38 were uninfected. IgG-WB showed a sensitivity of 73.5% and a specificity of 97.4%. IgM-WB showed a sensitivity of 54.8% and a specificity of 94.7%. The IgG-WB and IgM-WB combination increased the sensitivity to 86.5%. The IgM-WB-positive children had a 1.4-fold greater risk of presenting active macular lesions than did those that were IgM-WB-negative. The WB assay is a useful tool to confirm a diagnosis of congenital toxoplasmosis and that the IgM-WB-positive results can indicate active macular lesions in newborn infants [67].

Eighty five mothers with *Toxoplasma* seroconversion and their offspring were enrolled (among them, 2 spontaneous abortions were documented in the first trimester). Prenatal PCR diagnosis was carried out on 50 patients (60%), with 7 positive cases (14%). Morphological ultrasound scanning revealed anomalies in one fetus. Fourteen (17%) of the infants were infected at one-year serological follow-up. In 69 uninfected infants, anti-*Toxoplasma* IgG immunoblot analysis excluded infection within the 3 months in 18 infants (26%) and in the others within 6 months of life. Western blot analysis may help to evaluate infection within the 6 months of life. The accuracy of ultrasound imaging todetermine the brain damage in the fetus and newborns is doubtful, and should be combined with MR imaging [68].

Approximately 80% of newborns with subclinical toxoplasmosis develop ocular sequelae during their lifetime [69, 70]. Toxoplasmosis seroprevalence was analyzed in 68 712 serum samples from 47 635 pregnant women living in the catchment area of Hospital Miguel Servet during the period of 1992 to 2008. Detection of toxoplasma-specific immunoglobulins (IgM, IgA, IgG) and IgG avidity studies were carried out in the microbiology laboratory. Fifteen thousand two hundred and seven were pregnant with IgG antibodies against *T. gondii*, which means 31.9% immune women, and 32,428 were negative, representing 68.1% of the susceptible women. Among the positive women, 460 cases of women with high titers of IgG were observed, but not IgA or IgM accompanist. IgA was the serological marker more sensible in the detection of congenital infection in children [70].

A retrospective study was conducted with IgM-anti-*Toxoplasma gondii* reagent pregnant women and their children who attended the public health

system in the state of Paraná, Southern Brazil, from January 2001 to December 2003. Information were obtained from clinical, laboratory (ELISA IgM/IgG) and ultrasonographic data and from interviews with the mothers. Two hundred and ninety (1.0%) cases of suspected IgM-reagent infection were documented, with a prevalence of 10.7 IgM-reagent women per 1,000 births. Frequent complaints included headaches, visual disturbance and myalgia. Ultrasonography revealed abnormalities in 13 of 204 pregnancies. Among exposed children, 44/208 were serologically followed up and all were IgG reagent, and three IgM-reagent cases showed clinical symptoms [7].

Real-time PCR analysis significantly improves the detection of *T. gondii* on amniotic fluid. It provides an accurate tool to predict fetal infection and to decide on appropriate treatment and surveillance. However, postnatal follow-up remains necessary in the first year of life to fully exclude infection in children for whom PCR results were negative [71]. Real-time PCR (qPCR) was positive in 72/150 (48%) blood samples of newborns with congenital toxoplasmosis. Among infants with active retinochoroiditis, 68% had positive qPCR results, while positivity was 29% in the absence of ocular involvement. Positive qPCR was associated with the presence of retinochoroidal lesions, with an odds ratio of 2.8. *T. gondii* qPCR positivity is higher in infants with ocular involvement, particularly in those with active retinochoroiditis. The parasite load in peripheral blood of congenitally infected infants within the first 2 months of life is low, with no association between degree of parasitemia and the presence of retinochoroidal lesions [72].

In another study, the confirmatory tests for congenital toxoplasmosis were evaluated in 23 infected and 31 uninfected newborns. Conventional polymerase chain reaction was better than real-time polymerase chain reaction, but did not identify additional cases. Avidity tests added 2 new cases that were not identified by other criteria. Overall sensitivity was 82.6%. Avidity assay, but not polymerase chain reaction, increased the sensitivity of confirmatory assays in congenital toxoplasmosis [61]. Laboratory contamination was discarded with negative controls during DNA extraction and PCR amplification. The presence of inhibitors such as hemoglobin, blood proteins and anticoagulants is a problem in blood-related products [73, 74, 61]. Consequently, there is a need to improve the diagnosis by evaluating alternative confirmatory assays that could increase sensitivity or reduce the need for multiple criteria for diagnoses in newborns [61].

Neonates with congenital toxoplasmosis, even asymptomatic at birth, should be treated early to reduce long-term sequelae. Postnatal diagnosis of congenital toxoplasmosis is essential because prenatal diagnosis fails to detect approximately 15% of cases or cannot be performed when maternal infection is acquired in late pregnancy [8]. Moreover, the sensitivity of CT diagnosis increased to 92.4% when prenatal diagnosis was combined with immune blotting and to 95.8% with the addition of a conventional serological test (specific IgM/IgA) performed during the first month of life [11]. The sensitivity of IgM detection appeared to be related to maternal treatment since IgM was positive in 43% and 75% when mothers were treated or not, respectively (P 0.01). Though 5/7 symptomatic infants had a positive placenta examination, there was no correlation between a positive placenta and the presence of clinical signs during the first year of life [8].

Recombinant antigens (rAg) have several advantages compared with whole parasite extracts. Their costs are lower and they allow standardization procedures for serologic tests. The reactivity values of *Toxoplasma gondii* ROP2, GRA4, and GRA7 recombinant antigens (rAgs) were analyzed by immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) in 23 congenitally infected (I) and 36 noninfected (NI) infants. The results indicate that

rGRA4 and rGRA7 could have a potential to be used in the designing of novel and alternative diagnosis systems of CT. Between 1 and 4 months, the presence of antibodies antirGRA7 suggests a congenital infection, whereas the absence of reactivity against rGRA4 would be suggestive of no infection [20].

Diagnosis in infantis

T. gondii causes more severe ocular disease in congenitally infected children in Brazil than in Europe, with marked differences in frequency, size, and multiplicity of retinochoroidal lesions [75, 72]. There are many difficulties in diagnosing congenital toxoplasmosis; for example, approximately 80% of infected newborns are asymptomatic at birth and may not present any manifestation of this infection for years. However, this does not prevent the late appearance of lesions, mainly in the eyes and brain, which can lead to blindness or mental retardation [76, 12]. In other cases, infected newborns appear to be totally asymptomatic at birth but are at risk of developing retinal diseases during childhood or adolescence [77, 78]. Advances in early diagnosis coupled with early and prolonged treatment during the first year of life appeared to modify the course of CT [32].

Testing of toxoplasma-specific antibodies in infants identifies congenital toxoplasmosis during the first year of life [79]. Immunological markers can vary depending on the trimester of infection, and also maternal and neonatal therapeutic treatment received during pregnancy can block or retard the neonate's immune response [80, 81, 68]. Therefore, even if comparison of maternal and neonatal IgG by immune blot does not show neonatal immune response to any *Toxoplasma gondii* antigens in the first trimester of life, this does not unequivocally exclude congenital infection. In fact, recent studies which have evaluated B-cell subsets and their functional development have shown that memory B-cells are very low at birth and increase significantly at 6 months of life [82, 68]. The presence of maternal immunoglobulin G (IgG) antibodies in the newborn serum obscures and delays the diagnosis during the 1st months of age. Therefore, the infection should be confirmed by persistence of anti-*T. gondii* IgG after 8 months of age [63, 20].

Diagnosis at birth relies mainly on serological tests. Cell-mediated immunity plays the major role in resistance to infection but is not routinely investigated for diagnostic purposes. Here, we describe a simple test based on the gamma interferon (IFN-) response after stimulation of whole blood by crude parasitic antigens. For 62 infants under 1 year of age born to mothers who were infected during pregnancy, the sensitivity and specificity of the test were 94% and 98%, respectively. For a cohort of 124 congenitally infected patients between 1 and 30 years of age, the sensitivity of the assay was 100%. We present a simple test based on IFN- secretion to assess cell-mediated immunity in toxoplasmosis. As only 1 ml of blood is required to investigate humoral and cellular immunity, our assay is well adapted for the study of congenital toxoplasmosis in infants. Using purified antigens or recombinant peptides may improve the test performance [78].

In a retrospective study, serum *Toxoplasma gondii* antibodies were measured in samples from 333 infants, including 212 noninfected infants and 121 infants with congenital toxoplasmosis. A total of 1,157 umbilical cord blood and peripheral serum samples were analyzed. Liaison toxoplasma-specific IgG and IgM antibodies and the IgG avidity index were compared to the infection status of the infant, determined by the Sabin-Feldman dye test and immunosorbent agglutination assay—IgM. All noninfected infants were seronegative by Liaison IgG within the first year of life. In this study cohort, avidity did not show a potential diagnostic benefit for the detection of congenital infection. Despite the significant progress brought about by the analysis of these additional immunoblot bands, postnatal follow-up remains, however, necessary

in the first year of life to fully identify infected children [11]. The Liaison system is a valuable tool to monitor the serologic course of infants at risk. A final serologic confirmatory test is recommended to improve the rate of detection of congenital toxoplasmosis at 1 year of life [79].

Amniotic fluid samples were submitted to DNA extraction and amplification by the following 4 *Toxoplasma* techniques performed with parasite B1 gene primers: conventional PCR, nested-PCR, multiplex-nested-PCR, and real-time PCR. Fifty nine of the 100 infants had toxoplasmosis; 42 (71.2%) had IgM antibodies at birth but were asymptomatic, and the remaining 17 cases had non-detectable IgM antibodies but high IgG antibody titers that were associated with retinochoroiditis in 8 (13.5%) cases, abnormal cranial ultrasound in 5 (8.5%) cases, and signs/symptoms suggestive of infection in 4 (6.8%) cases [5]. PCR had the highest levels of sensitivity and specificity in the diagnosis of (prenatal and postnatal) toxoplasmosis in comparison to serological tests [83, 42, 32]. Besides, PCR does not depend on the immune response of the patient as the serological tests do [84, 32]. Among the 4 proposed methods, the real-time PCR technique detected 58 of the 59 possible cases did not produce any false positive results and was the best-performing test [5].

Maternal serology at delivery and neonatal screening were especially useful in the identification of infants with congenital toxoplasmosis when the mother did not receive regular prenatal serological testing or prenatal care [85]. The presence of positive IgM serology as a test isolated, has limited value for detecting recent infection and should be used in combination with other complementary tests for the diagnosis of acute infection [27]. It will be from 217 newborns initially testing positive for specific IgM in filter paper dried blood spots were tested for specific IgM and IgG by ELFA-VIDAS®. Congenital toxoplasmosis was confirmed in 175 and ruled out in 42 infants. The validity of the ELISA tests was determined using the persistence of IgG antibodies (ELFA-VIDAS® kit) at the end of 12 months, which is considered the reference test for the diagnosis of congenital toxoplasmosis. All of the antigens showed high sensitivity and low specificity in detecting anti-*T. gondii* IgGt and IgG1 and low sensitivity and high specificity in detecting IgG3 and IgG4. The assessment of IgG subclasses using recombinant antigens is a promising complementary tool for CT diagnosis, allowing the identification of a large number of newborns infected with *T. gondii* [1].

The neonatal serologic diagnosis of congenital toxoplasmosis is based on measurement of the IgM and IgA anti-*T. gondii* antibodies. However, 15-30% of the children infected do not present these antibodies at birth and 10-20% of the infants testing positive for these antibodies are false-positives [86, 67]. T lymphocyte proliferation, interferon (IFN) - production and lymphocyte activation antigens expression were evaluated in 23 infected and 65 uninfected neonates at different times, in the first year of life. Evaluation of the specific T cell response allowed identification at 3 months of age or younger, 2 of 23 infected neonates, who had negative serologic tests. Moreover specific T lymphocyte activity increased with age even in neonates undergoing therapy, suggesting that medical treatment does not affect lymphocyte response [9].

The severity of the congenital infection is inversely proportional with the trimester in which the mother is infected with the disease [3]. Maternal prenatal recognition of acute gestational infection and early treatment of infants with congenital infection are important because prenatal and accurate postnatal antibiotic therapy improves the outcomes of infected infants [87].

Conclusion

Toxoplasmosis is usually asymptomatic for mother and the diagnosis accurate and early, and it's essen-

tial in the natural history of the disease. Its diagnosis currently comprises a spectrum of various methods, among which stand out the immunological/serological, PCR and Western Blot

The immunological/serological methods indicate that a significant increase in the levels of specific antibodies or seroconversion during pregnancy is associated with recent exposure to the etiological agent of the disease. Furthermore, the antigen ROP2186-533 is considered effective for detecting Toxo-IgM antibodies and can be used as a specific antigen to capture the IgM and to distinguish between latent and recent infection with good efficiency. The PCR method has the advantage of the ability to detect parasite DNA in the amniotic fluid, placenta or umbilical cord blood not depending on the immune response of those involved. The Western Blot method allows diagnose of congenital toxoplasmosis in cases where the infection is not detectable by serology techniques. Studies also found the frequency of IgM or IgA in cord blood as markers for congenital infections.

The diagnosis beyond the neonatal period may be done by Western blot, which improved the time of diagnosis of toxoplasmosis in postnatal follow-up to about six months. We may associate this method of imaging tests such as ultrasound and MRI. The PCR also showed efficacy for that purpose. We conclude that the current status of health sciences, and technologies in terms of diagnosis of congenital toxoplasmosis are satisfactory. However, we do not rule out the need for developing new and revised methods allowing a specific research percentually more specific, accurate and especially early of the disease, as well as reasonably priced and applicable nationally in pre and postnatal screening programs.

Conflict of interest

Mr. Lima, Ms. Barros, Mr. Teixeira, Mr. Aguiar, Mr. Gonçalves and Drs. Santos, Lima, Bianco, Rolim-Neto, Sevciovic have no conflicts of interest or financial ties to report.

Acknowledgments

We are grateful to the Faculty of Medicine of ABC. We would also like to the Scientific Writing Lab (LABESCI) and the Medical Parasitology League of the Federal University of Cariri (UFCA).

Role of the funding source

We have no fundation source.

References

1. Silva CHS, Andrade GQ, Januário JN, Carneiro ACAV, Carneiro M, Vasconcelos-Santos DV et al (2012) Early diagnosis of congenital toxoplasmosis in newborn infants using igg subclasses against two *Toxoplasma gondii* recombinant proteins. Mem Inst Oswaldo Cruz; 107(3): 342-347
2. Morelle C, Varlet-Marie E, Brenier-Pinchart MP, Cassaing S, Pelloux H, Bastien P et al (2012) Comparative assessment of a commercial kit and two laboratory-developed pcr assays for molecular diagnosis of congenital toxoplasmosis. J Clin Microbiol; 50(12): 3977-82
3. Uysal A, Cüce M, Tañer CE, Uysal F, Atalay S, Göl B et al (2013) Prevalence of congenital toxoplasmosis among a series of turkish women / prevalencia de toxoplasmosis congénita en una serie de mujeres en Turquía. Rev Med Chil; 141(4): 471-476
4. Reis MM, Tessaro MM, D'Azevedo PA (2006) Serologic profile of toxoplasmosis in pregnant women from a public hospital in Porto Alegre. Rev Bras Ginecol Obstet; 28(3): 158-164
5. Teixeira LE, Kanunfre KA, Shimokawa PT, Targa LS, Rodrigues JC, Domingues, W et al (2013) The performance of four molecular methods for the laboratory diagnosis of congenital toxoplasmosis in amniotic fluid samples. Rev Soc Bras Med Trop; 46(5): 584-588
6. Couto JCF, Melo RN, Rodrigues MV, Leite JM (2003) Diagnóstico pré-natal e tratamento da toxoplasmose na gestação. Femina; 31:85-90
7. Castilho-Pelloso MP, Falavigna DLM, Falavigna-Guilherme, AL (2007) Suspected acute toxoplasmosis in pregnant women. Rev Saúde Pública; 41(1): 27-34

8. Robert-Gangneux F, Dupretz P, Yvenou C, Quinio D, Poulain P, Guiguen et al (2010) Clinical relevance of placenta examination for the diagnosis of congenital toxoplasmosis. Pediatr Infect Dis J; 29(1): 33-8
9. Ciardelli L, Meroni V, Avanzini MA, Bollani L, Tinelli C, Garofoli F et al (2008) Early and accurate diagnosis of congenital toxoplasmosis. Pediatr Infect Dis J; 27(2): 125-9
10. Sterkers Y, Pratlong F, Albaba S, Loubersac J, Picot MC, Pretet V et al (2012) Novel interpretation of molecular diagnosis of congenital toxoplasmosis according to gestational age at the time of maternal infection. J Clin Microbiol; 50(12): 3944-51
11. L'Ollivier C, Wallon M, Faucher B, Piarroux R, Peyron F, Franck J (2012) Comparison of mother and child antibodies that target high-molecular-mass *Toxoplasma gondii* antigens by immunoblotting improves neonatal diagnosis of congenital toxoplasmosis. Clin Vaccine Immunol; 19(8): 1326-8
12. Rodrigues IM, Castro AM, Gomes MB, Amaral WN, Avelino MM (2009) Congenital toxoplasmosis: evaluation of serological methods for the detection of anti-*Toxoplasma gondii* igm and iga antibodies. Mem Inst Oswaldo Cruz; 104(3): 434-40
13. Remington JS, Mcleod R, Thulliez P, Desmonts G (2001) Toxoplasmosis. In JS Remington, JO Klein, Infectious diseases of the fetus and newborn infant, 5th ed., WB Saunders, Philadelphia, p. 205-346
14. Carellos EVM, Andrade GMQ, Aguiar RALP (2008) Evaluation of prenatal screening for toxoplasmosis in Belo Horizonte, Minas Gerais state, Brazil: a cross-sectional study of postpartum women in two maternity hospitals. Cad Saúde Pública; 24(2): 391-401
15. Camargo ME (2001) Toxoplasmose. In: Ferreira AW, Ávila SLM, editores. Diagnóstico laboratorial das principais doenças infecciosas e auto-imunes. 2ª ed. Rio de Janeiro: Guanabara-Koogan; p.278-88
16. Isabel TF, Costa PI, Simões MJS (2007) Toxoplasmosis in pregnant women from Araraquara/SP: analysis of toxoplasma-specific igg avidity test utilization in the prenatal care routine. Sci. med; 17(2): 57-62
17. Magi B, Migliorini L (2011) Western blotting for the diagnosis of congenital toxoplasmosis. New Microbiol; 34(1): 93-5
18. Gross U, Lqder CGK, Hendgen V, Heeg C, Sauer I, Weidner A et al (2000) Comparative immunoglobulin G antibody profiles between mother and child (CGMC test) for early diagnosis of congenital toxoplasmosis. J Clin Microbiol 38: 3619– 3622
19. Pinon JM, Dumon H, Chemla C, Franck J, Petersen E, Lebech M et al (2001) Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M, and A antibodies. J Clin Microbiol 39:2267– 2271
20. Altcheh J, Diaz NS, Pepe CM, Martin V, Nigro M, Freilij H et al (2006) Kinetic analysis of the humoral immune response against 3 *Toxoplasma gondii*-recombinant proteins in infants with suspected congenital toxoplasmosis. Diagn Microbiol Infect Dis; 56(2): 161-5
21. Stepick-Biek P, Thulliez P, Araujo FG, Remington JS (1990) IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. J Infect Dis 162: 270-273.
22. Durlach RA, Kaufer F, Carral L et al (2003) Toxoplasmic lym phadenitis-clinical and serologic profile. Clin Microbiol Infect; 9: 625-31.
23. Bessieres MH, Roques C, Berrebi A et al (1992) IgA antibody response during acquired and congenital toxoplasmosis. J Clin Pathol; 45: 605-8
24. Gross U, Keksel O, Darde ML (1997) Value of detecting immunoglobulin E antibodies for the serological diagnosis of *Toxoplasma gondii* infection. Clin Diagn Lab Immunol; 4: 247-51
25. Carral L, Kaufer F, Durlach R, Freuler C, Olejnik P, Nadal M et al (2008) Multicenter study on the prevention of congenital toxoplasmosis in Buenos Aires. Medicina (B Aires); 68(6): 417-22
26. Montoya JG (2002) Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. J Infect Dis;185 (Suppl 1):S73-82
27. Pessanha TM, Carvalho M, Pone MVS, Gomes Júnior SC (2011) Diagnostic and therapeutic management of toxoplasmosis in pregnancy and the effect in the newborn. Rev Paul Pediatr; 29(3): 341-347
28. Remington JS, Mcleod R, Thulliez P, Desmonts G (2006) Toxoplasmosis. In: Remington JS, Klein JO, Baker C, Wilson C, editors. Infectious diseases of the fetus and the newborn infant. 6th ed. Philadelphia: WB Saunders; p. 974-1105.
29. Liu L, LIU T, Yu L, Cai Y, Zhang A, Xu X et al (2012) Rrop2(186-533): a novel peptide antigen for detection of IgM antibodies against *Toxoplasma gondii*. Foodborne Pathog Dis; 9(1): 7-12
30. Liang L, Doskaya M, Juarez S et al (2011) Identification of potential serodiagnostic and subunit vaccine antigens by antibody profiling of toxoplasmosis cases in Turkey. Mol Cell Proteomics;10:M110 006916
31. Kodym P, Machala L, Rohacova H, et al (2007) Evaluation of a commercial IgE ELISA in comparison with IgA and IgM ELISAs, IgG avidity assay and complement fixation for the diagnosis of acute toxoplasmosis. Clin Microbiol Infect;13:40–47
32. Said RN, Zaki MM, Abdelrazik MB (2011) Congenital toxoplasmosis: evaluation of molecular and serological methods for achieving economic and early diagnosis among egyptian preterm infants. J Trop Pediatr; 57(5): 333-9

33. Saathoff M, Seitz HM (1991) Analysis of neonatal and fetal blood for the diagnosis of congenital toxoplasmosis infection. Z Geburtshilfe Perinatol; 195: 262-6.

34. Okay TS, Yamamoto L, Oliveira LC, Manuli ER, Andrade Junior HF et al (2009) Significant performance variation among pcr systems in diagnosing congenital toxoplasmosis in São Paulo, Brazil: analysis of 467 amniotic fluid samples. Clinics (Sao Paulo); 64(3): 171-6

35. Delhaes L, Yera H, Ache S, Tsatsaris V, Houfflin-Debarge V (2013) Contribution of molecular diagnosis to congenital toxoplasmosis. Diagn Microbiol Infect Dis; 76(2): 244-7

36. Filisetti D, Gorcii M, Pernot-Marino E, Villard O, Candolfi E (2003) Diagnosis of congenital toxoplasmosis: comparison of targets for detection of *Toxoplasma gondii* by PCR. J Clin Microbiol 41:4826–4828

37. Faucher B, Miermont F, Ranque S, Franck J, Piarroux R (2012) Optimization of *Toxoplasma gondii* DNA extraction from amniotic fluid using nuclisens easymag and comparison with qiaamp DNA minikit. Eur J Clin Microbiol Infect Dis; 31(6): 1035-9

38. Costa JM, Ernault P, Gautier E, Bretagne S (2001) Prenatal diagnosis of congenital toxoplasmosis by duplex real time PCR using fluorescence resonance energy transfer hybridization probes. Prenat Diagn 21:85–88

39. Romand S, Chosson M, Franck J et al (2004) Usefulness of quantitative polymerase chain reaction in amniotic fluid as early prognostic marker of fetal infection with *Toxoplasma gondii*. Am J Obstet Gynecol.;190:797–802

40. Romand S, Wallon M, Franck J et al (2001) Prenatal diagnosis using polymerase chain reaction on amniotic fluid for congenital toxoplasmosis. Obstet Gynecol.;97:296 –300

41. Sterkers Y, Ribot J, Albaba S, Issert E, Bastien P, Pratlong F (2011) Diagnosis of congenital toxoplasmosis by polymerase chain reaction on neonatal peripheral blood. Diagn Microbiol Infect Dis; 71(2): 174-6

42. Bessières MH, Berrebi A, Cassaing S, Fillaux J, Cambus JP, Berry A et al (2009) Diagnosis of congenital toxoplasmosis: prenatal and neonatal evaluation of methods used in Toulouse University Hospital and incidence of congenital toxoplasmosis. Mem Inst Oswaldo Cruz; 104(2): 389-92

43. Jones J, Lopes A, Wilson M. (2003) Congenital toxoplasmosis. Am Fam Physician;67:2131-8

44. Remington JS, Mcleod R, Thulliez P, Desmonts G. (2006) Toxoplasmosis. In: Remington JS, Klein JO, Baker C, Wilson C, editors. Infectious diseases of the fetus and the newborn infant. 6th ed. Philadelphia: WB Saunders; p. 974-1105

45. Lappalainen M, Hedman K. (2004) Serodiagnosis of toxoplasmosis. The impact of measurement of IgG avidity. Ann Ist Super Sanita; 40: 81-8

46. Souza-Júnior VG, Figueiró-Filho EA, Borges DC, Oliveira VM, Coelho LR (2010) Toxoplasmosis and pregnancy: perinatal results and association of the IgG avidity test with congenital infection in pregnant women with positive anti-*Toxoplasma gondii* IgM. Sci. med; 20(1)

47. Desmonts G, Couvreur J (1979) Congenital toxoplasmosis: a prospective study of the offspring of 542 women who acquired toxoplasmosis during pregnancy: pathophysiology of congenital disease. In: Thalhammer O, Baumgarten K, Pollak A (eds). Perinatal Medicine, Sixth European Congress, Vienna. Stuttgart. Georg Thieme, p 51-60

48. Kravetz JD, Federman DG (2005) Toxoplasmosis in pregnancy. Am J Med.;118:212-6

49. Montoya JG, Rosso F. (2005) Diagnosis and management of toxoplasmosis. Clin Perinatol;32:705-26

50. Ferguson W, Mayne PD, Lennon B, Butler K, Cafferkey M (2008) Susceptibility of pregnant women to *Toxoplasma* infection--potential benefits for newborn screening. Ir Med J; 101(7): 220-1

51. Malinger G, Werner H, Rodriguez Leonel JC, Rebolledo M, Duque M, Mizyrycki S et al (2011) Prenatal brain imaging in congenital toxoplasmosis. Prenat Diagn; 31(9): 881-6

52. Couto JC, Ferreira QT (2006) Fetal toxoplasmosis infection. TheFetus.net.

53. Wallon M, Peyron F, Cornu C, Vinault S, Abrahamowicz M, Kopp CB et al (2013) Congenital *Toxoplasma* infection: monthly prenatal screening decreases transmission rate and improves clinical outcome at age 3 years. Clin Infect Dis; 56(9): 1223-31

54. Jenum PA, Stray-Pedersen B (1998) Development of specific immunoglobulins G, M, and A following primary *Toxoplasma gondii* infection in pregnant women. J Clin Microbiol;36:2907-13

55. Maranhão AQ, Joaquim MM, Siu C (1998) A mortalidade perinatal e neonatal no Brasil. Brasília: Ministério da Saúde, UNICEF

56. Puccini RF, Pedroso GC, Silva EM, Araújo NS, Silva NN (2003) Eqüidade na atenção pré-natal e ao parto em área da região metropolitana de São Paulo, 1996. Cad Saúde Pública;19:35-45

57. Isabel TF, Costa PI, Simões MJ. Toxoplasmose em gestantes de Araraquara/SP: análise da utilização do teste de avidez de IgG anti-*Toxoplasma* na rotina do pré-natal. Sci Med. 2007;17:57-62.

58. Lago EG (2007) Teste de avidez de IgG anti-25. *Toxoplasma gondii* e programa de controle da toxoplasmose congênita [Editorial]. Sci Med;17:54-6

59. Lago EG, Carvalho RL, Jungblut R, Silva VB, Fiori, RM. (2009) Screening for *Toxoplasma gondii* antibodies in 2, 513 consecutive parturient women and evaluation of newborn infants at risk for congenital toxoplasmosis. Sci. med; 19(1): 27-34

60. Gómez-Marin JE (2010) Protozoologia Médica: Protozoos Parásitos en el Contexto Latinoamericano. 1st ed Bogotá Editorial Manual Moderno:65–87

61. Torres E, Rivera R, Cardona N, Sanchez V, Lora F, Gómez-Marín JE (2013) Evaluation of IgG anti-*Toxoplasma* avidity and polymerase chain reaction in the postnatal diagnosis of congenital toxoplasmosis. Pediatr Infect Dis J; 32(6): 693-5

62. Tenter AM, Heckeroth AR, Weiss LM (2000) *Toxoplasma gondii*: from animals to humans. Int J Parasitol 30:1217– 1258

63. Remington JS, McLeod R, Thulliez P, Desmonts G (2001) Toxoplasmosis. In Infectious Diseases of the Fetus and Newborn Infant. 5th ed.Eds, JS Remington and JO Klein. Philadelphia7 The WB Saunders Co.,pp 205– 346

64. Gómez-Marin JE, de-la-Torre A, Angel-Muller E, Rubio J, Arenas J, Osorio E et al (2011) First colombian multicentric newborn screening for congenital toxoplasmosis. PLoS Negl Trop Dis; 5(5): e1195

65. Gilbert RE, Thalib L, Tan HK, Paul M, Wallon M, Petersen E (2007) Screening for congenital toxoplasmosis: accuracy of immunoglobulin m and immunoglobulin a tests after birth. J Med Screen; 14(1): 8-13

66. Binquet C, Wallon M, Metral P, Gadreau M, Quantin C, Peyron F (2004) Toxoplasmosis seroconversion in pregnant women. The differing attitudes in France. Presse Med;33:775–9

67. Machado AS, Andrade GM, Januário JN, Fernandes MD, Carneiro AC, Carneiro M et al (2010) Igg and igm western blot assay for diagnosis of congenital toxoplasmosis. Mem Inst Oswaldo Cruz; 105(6): 757-61

68. di Carlo P, Romano A, Casuccio A, Cillino S, Schimmenti MG, Mancuso G et al (2011) Investigation and management of *Toxoplasma gondii* infection in pregnancy and infancy: a prospective study. Acta Pharmacol Sin; 32(8): 1063-70

69. Meenken C, Assies J, Van Nieuwenhuizen O, Holwerda van der Maat WG, Van Schooneveld MJ, Delleman WJ et al (1995) Long term ocular and neurological involvement in severe congenital toxoplasmosis. Br J Ophthalmol.;79:581–4

70. Roc ML, Palacián MP, Lomba E, Monforte ML, Rebaje V, Revillo Pinilla MJ (2010) Serologic diagnosis of congenital toxoplasmosis. Enferm Infecc Microbiol Clin; 28(8): 517-9

71. Wallon M, Franck J, Thulliez P, Huissoud C, Peyron F, Garcia-Meric P et al (2010) Accuracy of real-time polymerase chain reaction for *Toxoplasma gondii* in amniotic fluid. Obstet Gynecol; 115(4): 727-33

72. Costa JG, Carneiro AC, Tavares AT, Andrade GM, Vasconcelos-Santos DV, Januário JN et al (2013) Real-time pcr as a prognostic tool for human congenital toxoplasmosis. J Clin Microbiol; 51(8): 2766-8

73. Ponce N, Gomez JE (2003) Estandarización y validación clínica de la prueba de reacción en cadena de la polimerasa (PCR) para diagnostico de toxoplasmosis cerebral en pacientes infectados por el VIH. Infectio.;7:8–14

74. Cardona N, Bastos N, Parra B et al (2011) Detection of *Toxoplasma* DNA in the peripheral blood of HIV-positive patients with neuropportunistic infections by a real time PCR assay. J Neuroparasitol. DOI:10.4303/jnp/N110402.

75. Gilbert RE, Freeman K, Lago EG, Bahia-Oliveira LMG, Tan HK, Wallon M et al (2008) Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. PLoS Negl. Trop. Dis. 2:e277. doi:10.1371/journal.pntd.0000277.

76. Caiaffa WT, Chiari CA, Figueiredo AR, Orefice F, Antunes CM (1993) Toxoplasmosis and mental retardation. Report of a case-control study. Mem Inst Oswaldo Cruz 88: 253-261

77. Wallon M, Kodjikian L, Binquet C, Garweg J, Fleury J, Quantin C et al (2004) Long-term ocular prognosis in 327 children with congenital toxoplasmosis. Pediatrics 113:1567–1572.

78. Chapey E, Wallon M, Debize G, Rabilloud M, Peyron F (2010) Diagnosis of congenital toxoplasmosis by using a whole-blood gamma interferon release assay. J Clin Microbiol; 48(1): 41-5

79. Prusa AR, Hayde M, Pollak A, Herkner KR, Kasper DC (2012) Evaluation of the liaison automated testing system for diagnosis of congenital toxoplasmosis. Clin Vaccine Immunol; 19(11): 1859-63

80. Bessières M H, Berrebi A, Rolland M, Bloom MC, Roques C, Cassaing S et al (2001) Neonatal screening for congenital toxoplasmosis in a cohort of 165 women infected during pregnancy and influence of in utero treatment on the results of neonatal tests. Eur J Obstet Gynecol Reprod Biol; 94: 37–45

81. Meroni V, Genco F, Tinelli C, Lanzarini P, Bollani L, Stronati M et al (2009) Spiramycin treatment of *Toxoplasma gondii* infection in pregnant women impairs the production and the avidity maturation of T gondiispecific immunoglobulin G antibodies. Clin Vaccine Immunol; 16: 1517–20

82. Avanzini MA, Maccario R, Belloni C, Carrera G, Bertaina A, Cagliuso M et al (2010) B lymphocyte subsets and their functional activity in the early months of life. Int J Immunopathol Pharmacol; 23: 247–54

83. Romnad S, Walloon M, Franck J et al (2001) Prenatal diagnosis using polymerase chain reaction on amniotic fluid for congenital toxoplasmosis. Obstet Gynecol;97:296–300

84. Montoya JG, Liesenfel O, Kinney S et al (2002) VIDAS test for avidity of toxoplasma-specific immunoglobulin G for confirmatory testing of pregnant women. J Clin Microbiol;40:2504–8

85. Lago EG, Neto EC, Melamed J, Rucks AP, Presotto C, Coelho JC et al (2007) Congenital toxoplasmosis: late pregnancy infections detected by neonatal screening and maternal serological testing at delivery. Paediatr Perinat Epidemiol; 21(6): 525-31

86. Remington JS, Thulliez P, Montoya JG (2004) Recent developments for diagnosis of toxoplasmosis. J Clin Microbiol 42: 941-945

87. Prusa AR, Kasper DC, Olischar M, Husslein P, Pollak A, Hayde M (2013) Evaluation of serological prenatal screening to detect *Toxoplasma gondii* infections in Austria. Neonatology; 103(1): 27-34