Screening for *Toxoplasma gondii* antibodies in 2,513 consecutive parturient women and evaluation of newborn infants at risk for congenital toxoplasmosis

Triagem para anticorpos anti-Toxoplasma gondii em 2.513 parturientes consecutivas e avaliação dos recém-nascidos com risco de toxoplasmose congênita

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ABSTRACT

Aims: *To determine the prevalence of seropositivity for toxoplasmosis in pregnant women, to assess the outcome and the prevalence of congenital toxoplasmosis, and to evaluate the usefulness of routine screening for* ***Toxoplasma gondii*** *antibodies in parturient women.*

Methods: *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.*

Results: *Serologic status for toxoplasmosis were assessed in 2,477 (98.5%) of 2,513 mothers of liveborn infants throughout the study period. Of the 2,477 mothers, 810 (32.7%; 95% CI 30.9-34.6%) were susceptible and 1,667 (67.3%; 95% CI 65.4-69.1%) were immune. Three newborn infants with congenital toxoplasmosis were identified because of the maternal serum tests at delivery, and all of them had*

RESUMO

Objetivos: Avaliar a prevalência de soropositividade para toxoplasmose em gestantes, investigar os desfechos clínicos e a prevalência da toxoplasmose congênita e verificar a utilidade da triagem de rotina para *Toxoplasma gondii* em parturientes.

Métodos: Estudo prospectivo incluindo todas as mães e seus respectivos recém-nascidos vivos, durante os 12 primeiros meses de implantação, em uma maternidade, de uma rotina de triagem para toxoplasmose em parturientes.

Resultados: Foi avaliado o estado imunológico para toxoplasmose em 2.477 (98,5%) entre as 2.513 mães de recém-nascidos vivos durante o período do estudo. Destas, 810 (32,7%; IC 95% 30,9-34,6%) eram suscetíveis e 1.667 (67.3%; IC 95% 65,4-69,1%) eram imunes. Foram diagnosticados três casos de toxoplasmose congênita apenas pela sorologia materna na hora do

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active ocular lesions caused by toxoplasmic retinochoroiditis. The prevalence of congenital toxoplasmosis was 12/10,000 (CI 95%: 6/10,000-21/10,000).

Conclusions: *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.*

KEY WORDS: *CONGENITAL TOXOPLASMOSIS/prevention & control; CONGENITAL TOXOPLASMOSIS/diagnosis; PRENATAL* DIAGNOSIS; PERINATAL CARE; DISEASE TRANSMISSION, VERTICAL; SCREENING.

parto, sendo que os três pacientes apresentavam lesões ativas de retinocoroidite toxoplásmica A prevalência de toxoplasmose congênita foi de 12/10.000 (IC 95%: 6/10.000-21/10.000).

Conclusões: A sorologia materna no momento do parto foi útil na identificação precoce de casos de toxoplasmose congênita que teriam passado despercebidos no período neonatal, permitindo o tratamento precoce de bebês com retinocoroidite. Nesta população, a elevada prevalência encontrada, tanto de anticorpos anti-*Toxoplasma gondii* nas gestantes, quanto de toxoplasmose congênita, justificam um programa de triagem pré-natal.

DESCRITORES: TOXOPLASMOSE CONGÊNITA/prevenção & controle; TOXOPLASMOSE CONGÊNITA/diagnóstico; DIAGNÓSTICO PRÉ-NATAL; ATENÇÃO PERINATAL; TRANSMISSÃO VERTICAL DA DOENÇA; TRIAGEM.

INTRODUCTION

Mother-to-child transmission of toxoplasmosis may occur when maternal infection by *Toxoplasma gondii* (*T. gondii*) is acquired during pregnancy. Even though the involvement of the central nervous system of the fetus is usually more severe in infections that occur during the first and second trimesters of gestation, ocular lesions are not influenced by gestational age and are frequent among fetuses infected during the last weeks of gestation – just when the rate of mother-to-child transmission is higher. In these cases, no abnormal findings are observed on physical examination, but the infants may show clinical abnormalities at complementary tests. On the other hand, the treatment for congenital toxoplasmosis is indicated even in subclinical infections, as the prognosis of this disease improves with early treatment and with its maintenance for at least 12 months.[1-4]

Since toxoplasmosis is asymptomatic in most pregnant women and may go undetected in newborn infants, its early diagnosis depends on a control strategy that is based on serologic testing during the prenatal and/or neonatal period. The choice of a strategy demands some information about the characteristics of the population, such as prevalence of immunity to *T. gondii* among women of childbearing age, and the burden of congenital toxoplasmosis.[5] In populations in which the prevalence of toxoplasmosis is high, it is recommendable to carry out prenatal tests, as in some European countries.[6-8] In populations with a lower prevalence of the disease, alternative strategies (e.g.: neonatal screening) have been successful in the early detection of congenital toxoplasmosis among newborn infants.[9-11]

In the city of Porto Alegre, in southern Brazil, the prenatal care program implemented in 2001 includes tests for *T. gondii*-specific IgG and IgM antibodies when starting prenatal care, which should be repeated in the seronegative pregnant women at the beginning of the third trimester. However, this program did not take into account the vulnerability of the third trimester of pregnancy, and it did not include any test at delivery. Researchers had already identified such a problem even in more rigorous prenatal screening programs. In these cases, fetal infections in the last weeks of gestation may go unnoticed in the first months of extrauterine life, until the infant shows signs of ocular or neurological involvement.[12-14] To get around this problem, routine serum tests for *T. gondii* in previously seronegative parturient women have been adopted in our hospital since May 2002. In addition, serum tests are also requested from mothers whose previous immune status are unknown, since many patients admitted to the obstetric ward of our hospital come from cities or towns where the investigation for *T. gondii*-specific antibodies is not a routine practice in prenatal care.

The major objective of this study was to assess whether the routine investigation of *T. gondii*-specific antibodies in parturient women was able to detect cases of congenital toxoplasmosis which would not be otherwise identified at birth. The study also assessed the prevalence of immunity

to toxoplasmosis in pregnant women and of congenital toxoplasmosis in Porto Alegre, Brazil, identified some problems in the prenatal screening for toxoplasmosis, and described the evaluation of the infants at risk for congenital toxoplasmosis.

METHODS

A cross-sectional study was conducted at the public sector of the maternity ward of Hospital São Lucas, a teaching hospital from Pontifícia Universidade Católica do Rio Grande do Sul, from Porto Alegre, a city from southern Brazil. The study prospectively included all parturient women who gave birth to liveborn infants between May 1, 2002 and April 30, 2003 (first 12 months after the implementation of routine testing for *T. gondii* serology in parturient women).

The investigation of *T. gondii*-specific IgG and IgM antibodies was carried out in three situations: 1) in patients without previous serum tests for toxoplasmosis, or with incomplete serum tests (only IgG or only IgM); 2) in those who were seronegative for toxoplasmosis during pregnancy; and 3) in those with evidence of recent infection (positive *T. gondii*-specific IgM).

Testing at delivery was carried out at the laboratory of Hospital São Lucas using the enzyme-linked fluorescent assay (ELFA) VIDAS® (bioMérieux S.A., Lyon, France). Serum tests during pregnancy, when performed, were carried out at different laboratories, according to the sites of prenatal care, using different commercially available techniques. The *T. gondii*-specific IgG and IgM were considered positive or negative according to the reference values of the technique used. Test results, patient ages and data on the newborn infants were prospectively collected during the study period.

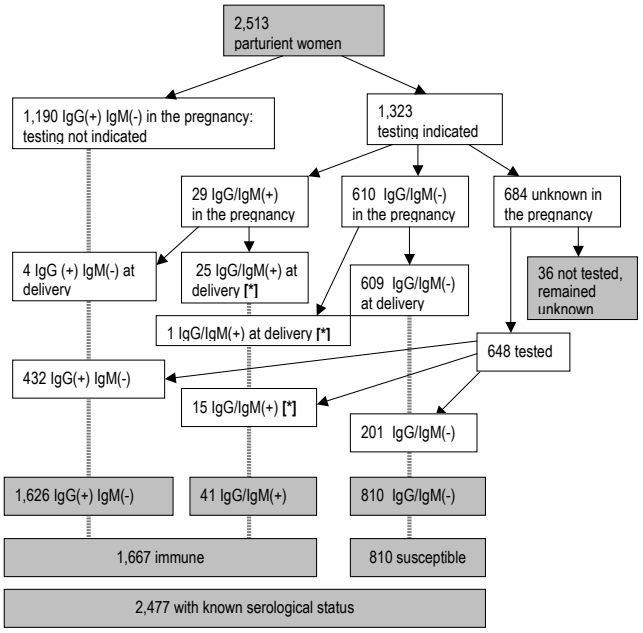
Newborn infants at risk for congenital toxoplasmosis or with confirmed infection were investigated and treated according to current recommendations, and the follow-up was carried out at the Outpatient Clinic of Congenital Infections of Hospital São Lucas. The cases of congenital toxoplasmosis were defined by Lebech's criteria.[15]

The data were analyzed by Epi Info 2002 and SPSS. The statistical tests included frequency distribution and chi-square test for linear trends. The 95% confidence intervals were calculated by binomial distribution.

The study protocol was in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983, and was approved by the Ethics Committee of Pontifícia Universidade Católica do Rio Grande do Sul.

RESULTS

During the study period, 2,513 patients who gave birth to liveborn infants were admitted to the maternity ward. Serum tests for toxoplasmosis were not indicated for 1,190 parturient women, who presented positive *T. gondii*-specific IgG and negative *T. gondii*-specific IgM in the prenatal testing. Testing at delivery was carried out in 29 patients who had positive *T. gondii*-specific IgM results during pregnancy (in four of them, the specific IgM was no longer positive at delivery); in 610 who were susceptible (of whom one patient presented positive IgG and IgM at delivery); and in 648 whose previous immune status was not known (Figure 1).



[*] Groups in which cases of congenital toxoplasmosis were detected

Figure 1 – Serologic status for *T. gondii* infection in 2,513 consecutive

The previous immune status was unknown in these 684 parturient women due to the following reasons: lack of prenatal care in 199 (29%); no testing during prenatal care in 264 (39%); lack of documentation in 179 (26%); and incomplete serology in 42 (6%) – 23 only with IgG and 19 only with IgM antibody tests to *T. gondii* requested during prenatal care. Testing at delivery of these 648 patients revealed that 447 (69%) were IgG-

positive (432 IgM-negative and 15 IgM-positive), and 201 (31%) were susceptible (IgG and IgM-negative).

Tests were inadvertently not requested for 36 parturient women without prenatal testing, whose immune status for toxoplasmosis remained unknown. Therefore, of the 2,513 patients, we were able to determine the serologic status for 2,477 (98.6%), of whom 1,667 (67.3%; 95%CI 65.4-69.1%) had positive results for *T. gondii*-specific IgG (41 with positive IgM at delivery) and 810 (32.7%; 95%CI 30.9-34.6%) were susceptible to toxoplasmosis (Figure 1).

Among the 41 patients with positive IgM at delivery, seven were identified as positive for antibodies to *T. gondii* in a previous pregnancy (residual IgM). Hence, only in the remaining 34 cases, there was a likelihood of sero-conversion during pregnancy. These patients were investigated more closely:

- In one case, fetal infection was detected in the second trimester of gestation, because of hydrocephalus on the fetal ultrasound. The patient was referred to the Division of Fetal Medicine, and fetal toxoplasmosis was diagnosed.
- A woman who was seronegative during pregnancy presented seroconversion in the last trimester of gestation, detected at delivery. The newborn infant had congenital toxoplasmosis.
- Three patients with very high *T. gondii*-specific IgG and IgM concentrations at the first test during pregnancy (two with negative fetal diagnosis and one without fetal diagnosis) had been treated with spiramycin. Their newborn infants showed negative results for specific IgM antibodies and no evidence of congenital infection. Two of these babies were followed up to the complete disappearance of maternal antibodies.
- Two patients without any test for toxoplasmosis during pregnancy showed very high concentrations of *T. gondii*-specific IgG and IgM at delivery, and one of the infants was diagnosed with congenital toxoplasmosis. The other infant showed negative specific IgM and normal physical examination and complementary investigation, but was lost to follow-up.
- In one pregnant woman, only the IgG antibodies had been investigated in the second trimester of gestation, yielding positive result. At delivery, both specific IgG and IgM concentrations were extremely high. The newborn infant was diagnosed with congenital toxoplasmosis.
- In the remaining 26 patients with positive *T. gondii*-specific IgM at delivery, the timing of infection could not be accurately determined. The serologic profile suggested residual IgM levels and indicated that toxoplasmosis had probably been acquired before pregnancy. All newborn infants were IgM-negative and none of them showed clinical evidence of congenital infection. Fourteen infants were followed up to the complete disappearance of *T. gondii*-specific IgG antibodies.

Therefore, four newborn infants were diagnosed with congenital toxoplasmosis. In one case, fetal infection was suspected because of hydrocephalus on the fetal ultrasound. Three cases of congenital toxoplasmosis, however, were identified only through routine maternal serology at delivery. These three infants had normal physical examination at birth, but further investigation revealed positive serology for *T. gondii* and cerebral calcifications in all of them, retinochoroiditis in two, and cerebral ventricular dilatation in one infant (Table 1). In all cases the specific treatment was started in the first days of life. One of them (patient 3) was identified later by neonatal screening, since a trial on neonatal screening for *T. gondii*-specific IgM antibodies was simultaneously being conducted in Porto Alegre with 10,000 newborn infants treated by the public health system, published elsewhere.[16] The other three cases were not detected by that neonatal screening trial because they were born outside that study period, and screening for toxoplasmosis is not included in the public routine for neonatal screening in Porto Alegre.

By excluding the first patient in order to avoid selection bias (as the mother had been referred to the Division of Fetal Medicine), the prevalence of congenital toxoplasmosis in this study population was 3/2,476 liveborn deliveries, or 12/10,000 (95%CI: 6/10,000-21/10,000). Prevalence of congenital toxoplasmosis in live newborns of susceptible mothers was 35/10,000 (IC 95%: 24/10,000-48/10,000).

Maternal ages ranged between 12 and 45 years (median: 22 years). There was a linear trend towards an increase in the prevalence of antibodies to *T. gondii* with age (p=0.015). The increase was more pronounced after the age of 30 years (Figure 2).

TABLE 1 – Outcome of the infants with congenital toxoplasmosis.*

	Patient 1	Patient 2 †	Patient 3 †	Patient 4 †
Sex	female	male	male	male
Birth weight (g)	1,290	2,460	3,055	3,150
Gestational age	32 weeks	36 weeks	40 weeks	39 weeks
Maternal data**	Prenatal care initiated at 28 weeks, with detection of hydrocephalus on fetal ultrasound. After positive IgG and IgM antibodies to toxoplasmosis, treatment was initiated, but not regularly followed.	Prenatal care including more than 10 visits. Negative serology in the first trimester. At delivery, IgG=930 IU/mL IgM=6.87	Prenatal care including 8 visits. No serum tests for *T. gondii* were requested during pregnancy. At delivery, IgG=80 IU/mL IgM=7.28	Only IgG antibodies to *T. gondii* were investigated during prenatal care. Positive results in the second trimester. In the subsequent visit, after 1 month, an IgM test was requested, but the patient gave birth before she could do the test. At delivery, IgG=2,640 IU/mL IgM=4.90
Serum tests of the infant at birth**	IgG=202 IU/mL IgM=0.87	IgG=1,720 IU/mL IgM=8.03	IgG=40 IU/mL IgM=7.69	IgG=2,720 IU/mL IgM=4.61
Physical examination at birth	Microcephaly Microphthalmia Hepatomegaly Splenomegaly	Normal	Normal at birth. Splenomegaly and hepatomegaly in the 3rd week of life.	Normal
Ophthalmologic examination	Microphthalmia (right eye). Extensive active retinochoroiditis in the left eye.	Peripheral retinochoroidal scar in the left eye. Active paramacular retinochoroiditis in the right eye.	Active macular retinochoroiditis in both eyes. Necrotic lesion and vasculitis in the left eye.	Active macular and paramacular retinochoroiditis in both eyes. Temporal papillitis. Vitreous opacity.
Cranial computed tomography	Dilated ventricular system. Cortical cerebral atrophy. Calcifications in the cortical and subependymal regions, and at the basal ganglia.	Multiple cortical and subependymal calcifications. Mildly dilated cerebral ventricles.	Punctiform calcifications in the thalamus and on the floor of the left lateral ventricle. Normal ventricular system.	Disseminated punctiform calcifications. Normal ventricular system.
Follow-up	With ventriculo-peritoneal shunt from the second week of life. Severe mental and neuromotor retardation and nearly total visual impairment at the age of 5 years. Cerebral atrophy, microcephaly. Swallowing impairment, with need for gastrostomy.	No need for ventriculo-peritoneal shunt. Retinochoroidal scars, strabismus, and mild visual impairment at the age of 5 years. No reactivation of retinochoroiditis. Normal mental and neuromotor development. Persistent intracranial calcifications. Normal cerebral ventricles.	Mild visual impairment. Normal mental and neuromotor development at the age of 1 year (was lost to follow-up after the first year).	Retinochoroidal scars at the age of 5 years. Moderate visual impairment. No reactivation of retinochoroiditis. Normal mental and neuromotor development. Persistent intracranial calcifications. Normal cerebral ventricles.

* All infants were treated with pyrimethamine, sulfadiazine and folinic acid for 1 year and were given corticosteroids when active ocular lesions were present (4 to 8 weeks).
** Serum tests of mothers at delivery, and of newborns, carried out by ELFA VIDAS® (IgG positive >8 IU/mL; IgM positive >0.65).
† Patients 2 to 4 identified only through maternal serology at delivery.

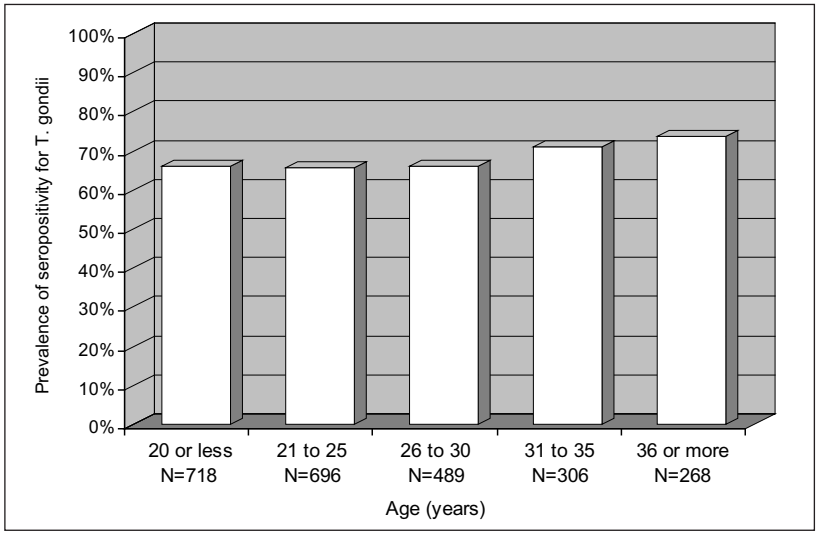


Figure 2 – Rise in the prevalence of seropositivity for *T. gondii* according age of parturient women.

DISCUSSION

The prevalence of 67.3% of antibodies to *T. gondii* observed in this study is considered to be high, as those in countries such as France (54.3%),[17] Belgium (49%)[18] or Cuba (71%),[19] and in other places in Brazil, where the prevalence ranges between 50 and 80%: Araraquara/SP (58%),[20] Fortaleza/CE (71.5%),[21] Cuiabá/MT (70.7%),[22] Londrina/PR (67%),[23] and Erechim/RS (74.5%).[24] Two studies conducted at public maternity wards in Porto Alegre showed similar prevalence rates for antibodies to *T. gondii*: 59.8% (95%CI 57.0%-62.5%)[25] and 61.1% (95%CI 60.2%-62.0%).[26] The high prevalence of toxoplasmosis among women of childbearing age positively affects the cost-benefit ratio of a prenatal screening program, since fewer women are likely to have to repeat serum tests during pregnancy. Moreover, in a contamination-prone environment, susceptible pregnant women, in a smaller but not negligible number, are at higher risk for toxoplasmosis, and prenatal screening is a way to select them for special education regarding primary prevention.[18, 27, 28] However, among the cases of congenital toxoplasmosis observed in this study, only one was diagnosed during pregnancy (due to abnormal findings on fetal ultrasound). It seems that the prenatal screening program with only one or two tests in susceptible patients, as had been used in Porto Alegre, is not enough to detect seroconversion in all patients, although it is important to acknowledge that some patients probably benefited from treatment during pregnancy.[29] Serum tests at delivery were useful as a complement to this strategy. Additionally, tests at delivery detected cases of congenital toxoplasmosis in which the immune status of the mother was unknown. Thus, in regions with a lower prevalence of toxoplasmosis, in which prenatal screening is not a routine procedure, testing of parturient women seems to be a valid alternative for the early detection of congenital toxoplasmosis, and may be complemented by neonatal screening. The advantage of delivery screening is that it allows detecting some cases of congenital toxoplasmosis in which *T. gondii*-specific IgM is already negative in the newborn infant. It has been shown that around 25% of newborn infants with congenital toxoplasmosis have negative specific IgM, and these cases may go undetected if only neonatal screening is performed.[30, 31]

Some cases of congenital toxoplasmosis may have gone unnoticed among mothers with positive IgG and negative IgM results during pregnancy, if the techniques used for prenatal testing were not sensitive enough to detect *T. gondii*-specific IgM antibodies, or in patients infected during pregnancy, but with negative IgM results at delivery, if *T. gondii*-specific IgM antibodies were too short-lived. Therefore, the prevalence of congenital toxoplasmosis may have been underestimated.

The increase in the prevalence of immunity to *T. gondii* with age is consistent with most studies that included this type of analysis.[17, 20-22, 32-34] In our study, as well as in most published studies, the increase is more pronounced after the age of 30 years. However, unlike populations of industrialized countries, in which adolescents show a much smaller prevalence of toxoplasmosis, with a sharper increase as they grow older, prevalence among Brazilian adolescents is already high.[17,18,20,22,32-34] This is probably due to the fact that exposure to soil and water (major risk factors among children and adolescents) is the main route of contamination in the Brazilian population, whereas in other populations the higher risk is posed by food factors, which are more important among adults.[32-37] The increase in seropositivity for toxoplasmosis with age is usually explained by the longer exposure to risk factors, but this can also be due to the cohort effect, that is, to the higher exposure to risk factors in the past, among those aged over 30 years.[34] Another explanation is that the sharp increase in seropositivity for *T. gondii* in women of childbearing age may result from the higher susceptibility of pregnant women to Toxoplasma infection.[38,39] In a Brazilian cohort study, pregnancy enhanced all risk factors for seroconversion to toxoplasmosis.[40]

Among the patients considered as having a recent infection, a significant number may have acquired toxoplasmosis before pregnancy. The duration of positivity of *T. gondii*-specific IgM antibodies in postnatal infection has a quite large individual variability, and IgM may be detected for few weeks or for several years.[41-44] The use of very sensitive techniques for testing for *T. gondii*-specific IgM antibodies during pregnancy has been argued, since the detection of these antibodies over a long period can lead to the incorrect estimation of the timing of infection, especially when the first test during pregnancy shows positive IgM results.[43-45] On the other hand, the low sensitivity can leave very recent

infections (with low antibody titers and high risk of mother-to-child transmission) undetected. The IgG avidity assay can help identify infections that occurred before pregnancy, especially if carried out in the first trimester.[20, 44-46]

Some mothers had already tested positive for toxoplasmosis before pregnancy, but were submitted to tests during prenatal care and at delivery, due to the lack of an information system across health services, and lack of data in their obstetric chart records. The data from previous pregnancies are not usually recorded in the prenatal chart. A similar situation was identified in Italy, where a study showed that 30% of obstetricians and gynecologists did not record and did not ask their patients to keep the results of their tests for future pregnancies, which caused patients to be unnecessarily retested.[47]

We observed that the three cases of congenital toxoplasmosis detected only through routine testing of mothers at delivery had clinical manifestations in the neonatal period. This is not in agreement with the international literature, in which there is a preponderance of subclinical infections.[1, 9] This may be related to the findings of some researchers that *T. gondii* strains found in Brazil have a higher potential for morbidity than in other countries.[48-50]

We concluded that 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 severe retinochoroiditis. Routine screening for *Toxoplasma gondii* in parturient women should be a complement to prenatal screening. In regions with low prevalence of toxoplasmosis, this may be an alternative strategy. However, in our population, the high prevalence of *Toxoplasma gondii* antibodies in pregnant women and of congenital toxoplasmosis justifies a prenatal screening program.

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