

Estimation of the regional distribution of congenital toxoplasmosis in Brazil from the results of neonatal screening

Estimativa da distribuição regional da toxoplasmose congênita no Brasil a partir dos resultados de triagem neonatal

Eurico Camargo Neto¹, Fabiana Amorim², Eleonor Gastal Lago³

¹ Ph.D. in Biochemistry, Universidade Federal do Rio Grande do Sul. Director of the Laboratório Nobel and Technical Manager of the Centro de Triagem Neonatal, Porto Alegre, RS, Brazil.

² Biochemist, Centro de Triagem Neonatal, Porto Alegre, RS, Brazil.

³ Ph.D. in Pediatrics, Pontifícia Universidade Católica do Rio Grande do Sul. Associated Professor, School of Medicine of the Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.

ABSTRACT

Aims: To determine the prevalence of congenital toxoplasmosis in Brazil from samples of dried blood on filter paper from neonates attended by a private program of neonatal screening. **Methods:** Blood samples collected from neonates by puncturing the heel and dried on filter paper, received from all Brazilian states from September 1995 to July 2009, were tested for *Toxoplasma gondii*-specific IgM antibodies. For each positive screening test, confirmatory tests were performed in sera of mothers and newborns, obtained by venipuncture. The infants were monitored and classified as infected according to one of the following criteria: presence of *Toxoplasma gondii*-specific IgM and IgG in the newborn and the mother; *Toxoplasma gondii*-specific IgM and IgG in the newborn only; *Toxoplasma gondii*-specific IgM and IgG in the mother only; or increasing amount of *Toxoplasma gondii*-specific IgG in the infant. **Results:** A total of 800,164 blood samples were tested. The overall prevalence of congenital toxoplasmosis was found to be 1/1,613 (6/10,000) in the country, ranging from 1/5,447 to 1/495 (2/10,000 to 20/10,000) in different states. **Conclusions:** Neonatal screening on a large scale is an important tool for determining the prevalence of congenital toxoplasmosis. The high prevalence of the infection in Brazil and the wide variability of its epidemiology among the states support the need to develop policies on health and education to prevent and control congenital toxoplasmosis across the country, respecting the peculiarities of each state.

Keywords: TOXOPLASMOSIS, CONGENITAL/prevention & control; TOXOPLASMOSIS, CONGENITAL/epidemiology; TOXOPLASMOSIS, CONGENITAL/diagnosis; *Toxoplasma gondii*; EPIDEMIOLOGY; PREVALENCE; CROSS-SECTIONAL STUDIES; NEONATAL SCREENING; INFANT, NEWBORN, DISEASES

RESUMO

Objetivo: estimar a prevalência da toxoplasmose congênita Brasil por meio de amostras de sangue seco em papel filtro, obtidas de neonatos atendidos por um programa privado de triagem neonatal. **Métodos:** amostras de sangue coletadas de neonatos por punção de calcâneo e absorvidas em papel filtro, recebidas de todos os estados brasileiros entre setembro de 1995 e julho de 2009, foram testadas para anticorpos IgM anti-*Toxoplasma gondii*. Para cada teste positivo na triagem, foram realizados exames confirmatórios em soros das mães e dos neonatos, obtidos por punção venosa periférica. Os casos foram considerados confirmados de acordo com um dos seguintes critérios: presença de IgM e IgG anti-*Toxoplasma gondii* no neonato e na mãe; IgM e IgG anti-*Toxoplasma gondii* somente no neonato; IgM e IgG anti-*Toxoplasma gondii* somente na mãe; ou aumento progressivo dos anticorpos IgG anti-*Toxoplasma gondii* no lactente. **Resultados:** foram testadas 800.164 amostras. Observou-se uma prevalência geral de toxoplasmose congênita no país de 1/1.613, variando de 1/1.547 a 1/495 (2/10.000 to 20/10.000) em diferentes estados. **Conclusões:** a triagem neonatal em larga escala é uma ferramenta importante para a determinação da prevalência da toxoplasmose congênita. A alta prevalência dessa infecção no Brasil e a ampla variabilidade de sua epidemiologia entre os estados confirmam a necessidade de políticas de saúde e educação voltadas à prevenção e ao controle da toxoplasmose congênita em todo o país, respeitando as peculiaridades de cada estado.

Descritores: TOXOPLASMOSE CONGÊNITA/prevenção & controle; TOXOPLASMOSE CONGÊNITA/epidemiologia; TOXOPLASMOSE CONGÊNITA/diagnóstico; *Toxoplasma gondii*; EPIDEMIOLOGIA; PREVALÊNCIA; ESTUDOS TRANSVERSAIS; TRIAGEM NEONATAL; DOENÇAS DO RECÉM-NASCIDO

Endereço para correspondência/Corresponding Author:

EURICO CAMARGO NETO
Av. Ipiranga, 5000, cj 201
CEP 90610-000, Porto Alegre, RS, Brazil
Telephone/fax: 55-51-3023-1933
E-mail: eneto.voy@terra.com.br

INTRODUCTION

Toxoplasmosis is caused by *Toxoplasma gondii* (*T. gondii*) and the transmission of the parasite from mother to fetus through the placenta may occur when infection is acquired during pregnancy. Congenital toxoplasmosis (CT) can cause severe eye and central nervous system injuries, seriously compromising the quality of life of untreated individuals.¹ Life cycle of the parasite,² transmission to humans,^{3,4} risk factors for infection,⁵⁻⁹ prenatal and neonatal screening,^{10,11} treatment of pregnant women and neonates^{12,13} and forms of prevention¹⁴ are well described in the literature.

It is recognized the importance of knowing the prevalence of a disease to be able to plan health and educational activities aimed at treatment and prevention. The best way to assess the prevalence of CT is the universal neonatal screening, which in Brazil is restricted to a few clinical laboratories and to private programs for neonatal screening, and are not included in the National Neonatal Screening Program.

The prevalence of CT has been previously established in Brazil¹⁵ by the neonatal screening program of the Center for Neonatal Screening (CTN – *Centro de Triagem Neonatal*), located in Porto Alegre, Rio Grande do Sul (RS) state, which receives samples from all over the country. Brazil has 5.565 municipalities, in an area of over 8 million Km² and an estimated population of 183,987,303 people, according to the Brazilian Institute of Geography and Statistics, from July 2009.¹⁶

The aim of this study is to estimate the regional distribution of congenital toxoplasmosis in Brazil from filter paper blood samples positive for specific IgM antibodies anti-*T. gondii* in the neonatal population served by CTN in each Brazilian state and the Federal District.

METHODS

This observational cross-sectional study used secondary data from a private program of neonatal screening conducted by CTN, which is located in Porto Alegre, capital of Rio Grande do Sul (RS) state, but receives samples from all Brazilian states. Blood samples obtained by puncturing the heel of newborns and applied on filter paper Schleicher & Schuell 903, collected for routine neonatal screening, were analyzed

for *T. gondii*-specific IgM antibodies. All analysis were performed in the Laboratory CTN/Nobel, initially using an indirect enzymatic immunoassay method prepared in-house and, after, with a fluorimetric kit produced by Ani LabSystems (Helsinki, Finland), as previously described.^{10,15} In the last year, the Luminex xMAP platform (Intercientífica, São José dos Campos, SP, Brazil) was also used.

For each test positive at the screening, confirmatory tests in sera of mothers and newborns were performed, initially by indirect immunofluorescence (Biolab-Merieux Diagnostics, Rio de Janeiro, Brazil), and later, owing to the lower sensitivity of the IgM immunofluorescence method, by MEIA IgM kit (Abbott Laboratories, Chicago, IL, USA) and chemiluminescence (Immulite 2000, Siemens, Los Angeles, CA, USA).^{10,15}

When positive for *T. gondii*-specific IgM in the filter paper blood sample, the neonate was classified as infected by meeting one of the following additional criteria: 1) serum *T. gondii*-specific IgM and IgG in the neonate and in the mother, or 2) serum *T. gondii*-specific IgM and IgG in the neonate only, or 3) serum *T. gondii*-specific IgM and IgG in the mother only, or 4) increasing amount of serum *T. gondii*-specific IgG during the follow-up of the infant, excluding maternal origin of the antibodies. Positive cases were followed up as previously reported.^{10,15,17}

All the ethical standards for human experimentation were followed, and the study was approved by the Committee of Research Ethics from Pontifícia Universidade Católica do Rio Grande do Sul.

RESULTS

From September 1995 to July 2009, 800,164 filter paper blood samples of newborns aged from 3 to 15 days of life were analyzed. Of these, 78,350 were tested by the in-house method, 709,807 using the fluorimetric method and 12,007 by the Luminex xMAP platform.

The results are described in Table 1, where it is observed an overall prevalence of CT in Brazil of 1/1,613 (6/10,000). The distribution of prevalence in each state ranged from 1/5,447 to 1/495 (2/10,000 to 20/10,000).

The map of Brazil in Figure 1 groups the states according to the range of the rate of congenital toxoplasmosis. In two Northern states, Amazonas (AM) and Acre (AC), no cases were detected.

Table 1. Prevalence of congenital toxoplasmosis in each Brazilian state in neonates subjected to neonatal screening, from September 1995 to July 2009.

State	Total population of the state	Number of samples	Number of positives	Number of tested neonates for each positive case	Number of positive cases for each 10,000 neonates tested
Pará (PA)	7,065,573	14,835	30	495	20
Rondônia (RO)	1,453,756	2,021	4	505	20
Mato Grosso (MT)	2,854,652	9,875	19	520	19
Maranhão (MA)	6,118,995	4,033	6	672	15
Espírito Santo (ES)	3,351,669	4,158	6	693	14
Sergipe (SE)	1,939,426	829	1	829	12
Santa Catarina (SC)	5,866,252	20,517	21	977	10
Bahia (BA)	14,080,654	22,659	22	1,030	10
Roraima (RR)	395,725	2,124	2	1,062	9
Piauí (PI)	3,032,421	6,409	6	1,068	9
Amapá (AP)	587,311	1,086	1	1,086	9
Tocantins (TO)	1,243,627	1,109	1	1,109	9
Rio Grande do Sul (RS)	10,582,840	280,962	200	1,405	7
Goiás (GO)	5,647,035	14,804	10	1,480	7
Minas Gerais (MG)	19,273,506	61,661	38	1,623	6
Ceará (CE)	8,185,286	20,788	11	1,890	5
Distrito Federal (DF)	2,455,903	11,420	6	1,903	5
Pernambuco (PE)	8,485,386	64,915	28	2,318	4
Paraíba (PB)	3,641,395	26,264	11	2,388	4
Paraná (PR)	10,284,503	32,318	12	2,693	4
Rio de Janeiro (RJ)	15,420,375	65,585	23	2,852	4
Alagoas (AL)	3,037,103	18,105	6	3,018	3
São Paulo (SP)	39,827,570	94,712	29	3,266	3
Rio Grande do Norte (RN)	3,013,740	5,442	1	5,442	2
Mato Grosso do Sul (MS)	2,265,274	10,894	2	5,447	2
Amazonas (AM)	3,221,939	2,402	0	-	-
Acre (AC)	655,387	237	0	-	-
Total	183,987,303	800,164	496	1,613	6

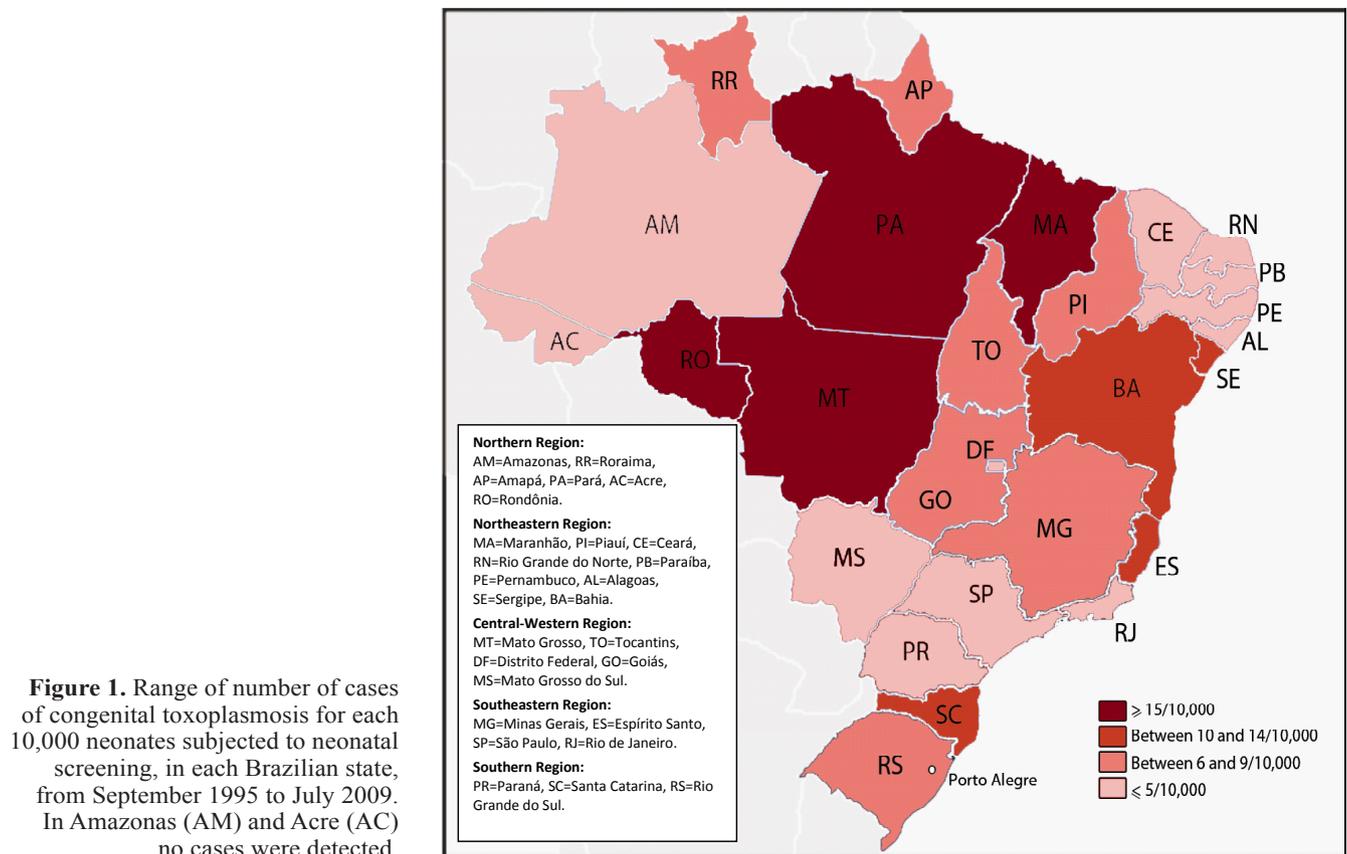


Figure 1. Range of number of cases of congenital toxoplasmosis for each 10,000 neonates subjected to neonatal screening, in each Brazilian state, from September 1995 to July 2009. In Amazonas (AM) and Acre (AC) no cases were detected.

DISCUSSION

Few data in the literature report on the prevalence of CT in Brazil. This study confirms the overall prevalence estimated previously (1/1,867 or 5.3/10,000)¹⁵ and shows the distribution of CT in Brazil estimated by neonatal screening. Considering that in Brazil the strains of *T. gondii* appear to be more virulent,¹⁸ and the clinical presentation of CT is more severe,^{19,20} these findings point to the great burden of the disease in our country.

In interpreting the data on prevalence of CT in each state of the country, we should take into account that some variables, specific to each population, may affect the results. Factors such as level of education and information, maternal age, social background, presence of cats, eating habits, hygiene, indicators of quality of life, clean water and sanitation, among others, should be considered. This study was not designed to evaluate such variables, but some studies already held in Brazil,²¹⁻³⁰ as well as further prospective studies that should be conducted, may aid in the analysis of the causes for the prevalence rates found in each Brazilian state.

Cases of CT were not detected in the state of Amazonas (AM), the largest in size of the country, with an area of more than 1.5 million Km², larger than the areas of France, Spain, Sweden and Greece added, and a population of 3.2 million people, where about 2 million live in the capital, Manaus.¹⁶ A total of 2,344 samples were tested, 97% from Manaus and 3% from the city of Parintins. A study of an indigenous people in Amazonas state has shown a seroprevalence of 77.8% to 92.3% in women between 20 and 39 years age,²¹ but no studies on urban population were found. Perhaps the prevalence in childbearing age women is so high that few cases of CT will occur, as we will discuss below. We should also take into account that the population of neonates born in the state capital and tested by CTN, a private laboratory, may not be fully representative of the general population of neonates from Amazonas (AM) state. Also in Acre (AC) state, with the third smallest population of the country, no record of CT was obtained, but only 205 samples were received from the capital Rio Branco and from the city of Cruzeiro do Sul.

Among the states with a lower prevalence of CT, a frequency of 0.42% for acute infection with *T. gondii* was reported in pregnant women in Mato Grosso do Sul (MS),²² where 92% were previously exposed to infection, and only 8% were susceptible. Pregnant women between 14 and 39 years with positive *T. gondii*-specific IgM antibodies showed a rate of 3.9% of congenital infection.²²

According to Barbosa et al.,²³ little is known about toxoplasmosis in Northeastern Brazil (MA, PI, CE, RN, PB, PE, AL, SE and BA). These authors found a prevalence of toxoplasmosis of 66.3% in 190 pregnant women from Natal, Rio Grande do Norte (RN).²³ In a study conducted in Salvador, Bahia (BA), 73.2% of 1,135 women had positive *T. gondii*-specific IgG antibodies.²⁴ In Sergipe (SE), in a study with 9,550 pregnant women, *T. gondii*-specific IgG antibodies were identified in 6,351 (69.0%).²⁵

The apparently paradoxical findings of high prevalence of CT in states where the prevalence of immunity in pregnant women is relatively low, and low prevalence of CT where the prevalence in pregnant women is very high, results from the balance between the prevalence of immunity due to past infection and the risk of acquiring infection. In a population with a low yearly seroconversion rate, the prevalence of antibodies will be low in young adults. Thus, owing to the higher number of seronegatives, women of childbearing age may have a higher frequency of seroconversion.¹

Naoi and Yano³¹ hypothetically imagined two different scenarios: first, in an area where there is no *T. gondii*, no fetus would suffer from CT. Second, in contrast, in an area where many stray cats roam, excreting huge amounts of oocysts and people who live in that area have contact with soil or water probably contaminated by oocysts, or are highly fond of eating raw meat possibly contaminated with tecdial cysts, seroprevalence could reach 100% before the gestational period, in which case there would be no primary infection among pregnant women. In this case too, we could expect basically no occurrence of CT in that area. These authors comment that it is reasonable to suppose that there should be a peak of risk at a certain level of the yearly primary infection rate, somewhere between 0 and 100%.³¹ Therefore, CT may be higher in regions of medium prevalence (e. g., from 20% to 80%).

In most regions of Brazil the prevalence of *T. gondii*-specific IgG antibodies in pregnant women is between 50 and 80%.^{17,23-29, 32-34} Although there is a smaller (but not negligible) proportion of susceptible pregnant women, these women are prone to infection because they live in an environment with a high risk of contamination. This epidemiologic feature is one of the factors that contribute for the high prevalence of CT in this country.

The infection rate may also vary with the current and past infection rates. For example, in regions in transition from high to low infection rates, it is likely that the influence of decreasing immunity will, at least temporarily, more than outweigh the influence of the

falling infection rates, resulting in a higher number of newly infected pregnant women. Larsen and Lebech³⁵ calculated the expected annual percentage of pregnant women infected with *T. gondii* using models with varying assumptions with regard to the infection rate, assumed to be independent of age but dependent on calendar time. There are no studies about that in Brazil, however, and we can only speculate if this phenomenon may occur in some regions of the country.

Naturally, seroconversion rate depends on many other risk factors, already cited above, which vary among different populations. Also, primary prevention focused on pregnant women may result in fewer seroconversions during pregnancy.³⁶ Furthermore, many variables influence whether congenital transmission will occur. They include the strain and virulence of *T. gondii*, inoculum size, route of infection, time of gestation, and immunocompetence of the pregnant woman.¹

The results obtained in Rio de Janeiro (RJ) state contrast with the limited data available in literature. While we have detected a prevalence of one case of CT for each 2,852 newborns, Bahia-Oliveira et al.³⁷ demonstrated an unusual situation in Campos dos Goytacazes, a city located at North of that state, where enormous social disparities are seen. Four cases of CT were identified among 2,550 neonates aged 3±20 days (a prevalence of one case of CT for each 500 neonates), tested from April 1999 to June 2000 for *T. gondii*-specific IgM antibodies in filter paper blood samples collected for evaluation of phenylketonuria and hypothyroidism in a county-funded program.³⁷ In Campos dos Goytacazes, drinking unfiltered water was found to increase the risk of seropositivity for the lower socioeconomic and middle socioeconomic populations, indicating the potential importance of oocyst transmission in water and the role of socioeconomic status.^{37,38} Although laboratorial analysis for the study of Campos dos Goytacazes were performed in CTN, the 2,550 samples represented a small percentage of the 65,585 neonates tested in Rio de Janeiro (RJ) state throughout the full period of our survey.

In Minas Gerais (MG) we found a prevalence of CT of 1/1,623, similar to that found by a study carried out in Belo Horizonte, the state capital, which detected a prevalence of 1/1,590, or 30 cases of CT in 31,808 neonates screened for *T. gondii*-specific IgM antibodies in filter paper samples.³⁹ Within the state of São Paulo (SP), a study conducted in the city of Ribeirão Preto⁴⁰ showed a prevalence of CT (1/3,032), also identified by neonatal screening, equal to that we identified statewide (1/3,266).

Among the three states of Southern Brazil, published data was not found on the prevalence of

CT in the states of Paraná (PR) and Santa Catarina (SC). In Londrina (PR), it was reported a high rate of recent infection in 4,858 pregnant women, with 2.5% of positive or indeterminate result for *T. gondii*-specific IgM antibodies, of whom 75% were not sent to confirmatory tests.⁴¹ In the same city, a recent study detected 49.2% of seropositivity for *T. gondii* and 1.2% of positive specific IgM in 492 pregnant women.⁴²

In Rio Grande do Sul (RS), we conducted a study in Porto Alegre, the state capital, where 10,000 samples of patients who underwent neonatal screening offered by the National Newborn Screening Program for evaluation of phenylketonuria and congenital hypothyroidism were tested for *T. gondii*-specific IgM. Six cases of CT (confirmed by persistence of specific IgG after 12 months) were identified, showing a prevalence of 6/10,000 (1/1,667),¹⁷ similar to 7/10,000 (1/405) obtained in 280,962 samples analyzed in the whole state. In a recent study, conducted in a large public hospital in the same city, with 41,112 pregnant women and 40,727 live births, CT was detected in 25 neonates (6/10,000) and during follow-up in the first year of life in 12 additional infants, increasing the prevalence of CT to 9/10,000.⁴³ In the Northwestern region of Rio Grande do Sul (RS), where a prevalence of ocular toxoplasmosis of 17.7% was found in a rural population of the city of Erechim,⁴⁴ a study on prenatal screening detected one case of CT among 454 pregnancies, and it was inferred that the prevalence of CT was about 22/10,000.⁴⁵

Among the states with the highest prevalence of CT detected by neonatal screening, there is no literature published in the states of Pará (PA), Maranhão (MA), Rondônia (RO) and Amapá (AP). The same occurred in relation to the states of Tocantins (TO), Goiás (GO) and Brasília (DF).

There is a lack of objective data on the prevalence of CT in Brazil and some studies report the prevalence in isolated populations. Excluding the small number of samples in the states of Sergipe (SE), Amazonas (AM) and Tocantins (TO), and the poor correlation with the information obtained in some localized or specific populations, we can accept the data of this study as very close to the actual prevalence of CT in each state of the country. The long period of accumulation of data, and the large number of subjects, favored a reduction in the effect of transitory conditions, such as outbreaks, which may lead to over or underestimations.

Neonatal screening on a large scale is an important tool for determining the prevalence of CT. Our data indicate the need for more targeted studies on CT in Brazil, aimed at elucidating the nuances of the disease and all the variables involved in its epidemiology.

Notwithstanding, the high prevalence of CT that we found, and the wide variability of its epidemiology among the states, support the need to develop policies on health and education to prevent and control CT across the country, respecting the peculiarities of each state.

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