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Birth prevalence and characteristics of congenital toxoplasmosis in Sergipe, North-east Brazil

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Abstract

OBJECTIVES To estimate, by neonatal screening, the birth prevalence of congenital toxoplasmosis among live-born infants in Sergipe state, Brazil, and to investigate the clinical features of affected infants.

METHODS Dried blood spot specimens obtained from 15 204 neonates were assayed for the presence of anti-*T. gondii* IgM antibodies. Duplicate retesting was done in infants with positive and borderline results. Confirmatory testing in peripheral blood samples consisted of testing for anti-*T. gondii* IgG and IgM in infants and mothers. Those with possible congenital toxoplasmosis were evaluated and followed up to a median age of 20 months. Congenital infection was confirmed in the presence of persisting anti-*T. gondii* IgG antibodies beyond 12 months of age. All infants with confirmed infection were treated with pyrimethamine, sulfadiazine and folinic acid for 1 year.

RESULTS Fifty-three infants had detectable IgM in dried blood spot specimens. Confirmatory testing was reactive in 39/50, of which, 38 completed follow-up. Six of 15 204 newborns were diagnosed with congenital toxoplasmosis, resulting in an estimated birth prevalence of four per 10 000 [CI 95% 1.4–8.0]. Four infants (67%) showed signs of congenital toxoplasmosis in their first year of life; three (75%) had retinochoroidal scars, and one had cerebral calcifications. Two infants remained asymptomatic until 20 months of age.

CONCLUSIONS The birth prevalence of congenital toxoplasmosis is high in the Brazilian state of Sergipe, with most of the infants showing ocular lesions. Preventive measures are strongly warranted.

keywords Congenital toxoplasmosis, *Toxoplasma gondii*, perinatal infections, neonatal screening, heel prick test

Introduction

The chance of exposure to infecting forms of *Toxoplasma gondii (T. gondii)* is determined by socio-economic and cultural conditions, including food production practices, water treatment, hygiene and dietary habits, soil exposure and climate (Jones *et al.* 2007). In Brazil, the seroprevalence of *T. gondii* in pregnant women has been reported to range from 31% to over 90% in different regions (Detanico & Basso 2006; Figueiró-Filho *et al.* 2007; Inagaki *et al.* 2009), suggesting that this population is still highly susceptible to toxoplasma infection.

Neonatal screening has been used to estimate the prevalence of congenital toxoplasmosis (CT) in Brazilian newborns. However, previous studies have focused mainly on the south and south-east regions (Carvalheiro *et al.* 2005; Lago *et al.* 2007; Vasconcelos-Santos *et al.* 2009) of Brazil. In Sergipe, a hot and humid state located in the north-east, the only study evaluating the prevalence of CT (Neto *et al.* 2010) so far was not based on a representative sample of the population. Also, the gold standard to diagnose CT, that is, persistent positive IgG titres after the first year of life (Montoya & Rosso 2005), was not employed in that study.

The present study used neonatal screening to estimate the prevalence of CT in a representative population sample in the state of Sergipe, Brazil. The clinical features of affected infants were also investigated.

Methods

About 35 000 infants are born each year in the state of Sergipe. Most receive public healthcare assistance (Gurgel *et al.* 2009). All infants born from April to October 2009 who had dried blood spot samples (DBS) collected as part of the mandatory neonatal screening programme for phenylketonuria and hypothyroidism were enrolled in the study.

Dried blood spot on Guthrie cards are routinely obtained by a standard heel puncture technique in primary healthcare centres. DBS samples were collected by trained technicians, stored at 4–9 °C and transported weekly, in a cooler, to the Central Laboratory at the Hospital of the Federal University of Sergipe. After routine tests were performed, an additional ELISA immunocapture technique was used to detect anti-*T. gondii* IgM in DBS, according to manufacturer's instructions (Q-Preven Toxo IgM – DBS®; Symbiosis Diagnóstica Laboratories, São Paulo, Brazil). Positive and borderline results were repeated in two aliquots from the same DBS. Infants with negative results in the repeated DBS tests were considered not infected. If any of the aliquots remained positive or borderline, the infant was invited to return for diagnostic confirmation.

For confirmation, peripheral blood was collected from infants and their mothers and submitted to quantitative anti-*T. gondii* IgG and qualitative IgM tests, using a commercial microparticle enzyme immunoassay performed according to manufacturer's instructions (AxSYM® System – MEIA; Abbott Laboratories, Wiesbaden-Delkenheim, Germany). For IgG, results ≥3.0 IU/ml were interpreted as positive, and results <2.0 IU/ml as negative. For IgM, ≥0.6 IU/ml was interpreted as positive and <0.5 IU/ml as negative. Infants were considered not infected when peripheral blood tests did not detect IgG and IgM in either the mother or the infant. If infants and/or mothers had positive or borderline IgG and/or IgM test results, infants were considered to have possible CT.

The research protocol was approved by the Research Ethics Committee at the Federal University of Sergipe. Prior to the study, professionals in charge of sample collection in the 75 towns received information about the study and its purpose and were instructed to provide information to the families. Written informed consent for additional evaluation and follow-up was obtained from the mothers or legal guardian of infants with positive or borderline results.

Assuming a prevalence of CT of 1:1000 in this population, 15 119 newborns would be required to estimate the prevalence of infection with a confidence level of 95% and a precision of 0.0005. All calculations were performed using the Epi-Info 6 software (CDC, Atlanta, GA, USA).

Clinical assessment, treatment and follow-up of infants

Infants with possible CT were submitted to complete clinical assessment, including ophthalmological examination, cranial ultrasonography, blood cell count, liver enzymes and cerebrospinal fluid examination. Indirect ophthalmoscopy was performed without sedation, after topical anaesthesia and placement of a lid speculum. Anti-*T. gondii* IgG and IgM tests were repeated every 3 months. Confirmation of seroreversion with two negative tests at least 30 days apart was the criterion to rule out CT. A definitive CT diagnosis was established when specific IgG was detected beyond 12 months of age and/or when there was an increase ≥3-fold in IgG titres after suspension of antiparasitic treatment, regardless of the presence of signs of CT.

Infants with positive IgM tests in peripheral blood and/or who had IgG titres ≥3 times maternal titres and/or who had any signs suggestive of CT after exclusion of other congenital infections and/or whose mothers had confirmed gestational seroconversion were submitted to 1-year treatment with sulphadiazine, pyrimethamine and folinic acid, according to current recommendations (McAuley *et al.* 1994). Asymptomatic infants who had negative specific IgM in confirmatory tests and whose mothers did not have proven gestational seroconversion were not treated. When available, maternal serological tests performed during the pregnancy were obtained.

Results

From April to October 2009, 21 046 babies were born in the state of Sergipe: 5872 were born in the capital, Aracaju, and 15 174 in the remaining 74 state cities. A total of 15 204 (72.2%) infants had DBS collected by the public healthcare system's neonatal screening programme and were enrolled in this study – 3325 (21.9%) born in the capital and 11 879 (79.1%) in the remaining cities.

Babies underwent a heel prick test at a median age of 7 days (range 3–21). Initially, 233/15 204 (1.5%) infants had positive IgM tests. On subsequent duplicate testing, only 53/233 (22.7% or 0.3% of the total) remained positive in at least one of the duplicate tests. Confirmatory peripheral blood testing was performed in 50 (94.3%) of these 53 infants and their mothers. After the confirmatory test, one mother dropped out of the study. Eleven (22.4%) of 49 infant–mother pairs had negative IgG and IgM confirmatory tests. The remaining 38 (77.6%) mothers were positive for IgG. IgM was detected in five of these mothers and in only two infants. Follow-up was performed for the 38 infants with possible toxoplasmosis.

Estimation of the birth prevalence of congenital toxoplasmosis

As summarised in Figure 1, CT was ruled out in 30 of 38 (78.9%) infants. In seven of these infants, the presence of toxoplasma antibodies was not confirmed in peripheral blood, but IgG was detected in the mother. These seven infants were tested 2–3 times and had two negative IgG results at a median interval of 2 months. The remaining 23 infants with positive results for *T. gondii* antibodies were tested at a median interval of 79 days or three times (range: 2–6) during a median period of 9 months (range: 5–14) of follow-up. Seroreversion from positive to negative anti-*T. gondii* IgG tests was found at a median age of 183 days (range: 102–344).

Of the eight infants with a possible CT diagnosis, two were lost to follow-up. None had clinical abnormalities or detectable IgG and IgM in the only test, performed at 6 months of age. CT was confirmed in the six remaining infants, corresponding to a birth prevalence of four per 10 000 [CI 95% 1.4–8.0] or one per 2500 live newborns. The prevalence found for infants born to mothers living in the capital or in the other cities was similar: six per 10 000 [CI 95% 0.0–20.0] and three per 10 000 [CI 95% 0.0–8.0], respectively, as shown by a prevalence ratio of 1.8 [CI 95% 0.3–9.8] calculated using the Poisson model.

Characteristics of infants with congenital toxoplasmosis and their mothers

Characteristics of mothers and infants with CT are shown in Table 1. None of the mothers had been diagnosed with toxoplasmosis during pregnancy. Although four mothers had negative prenatal tests, they were not retested while

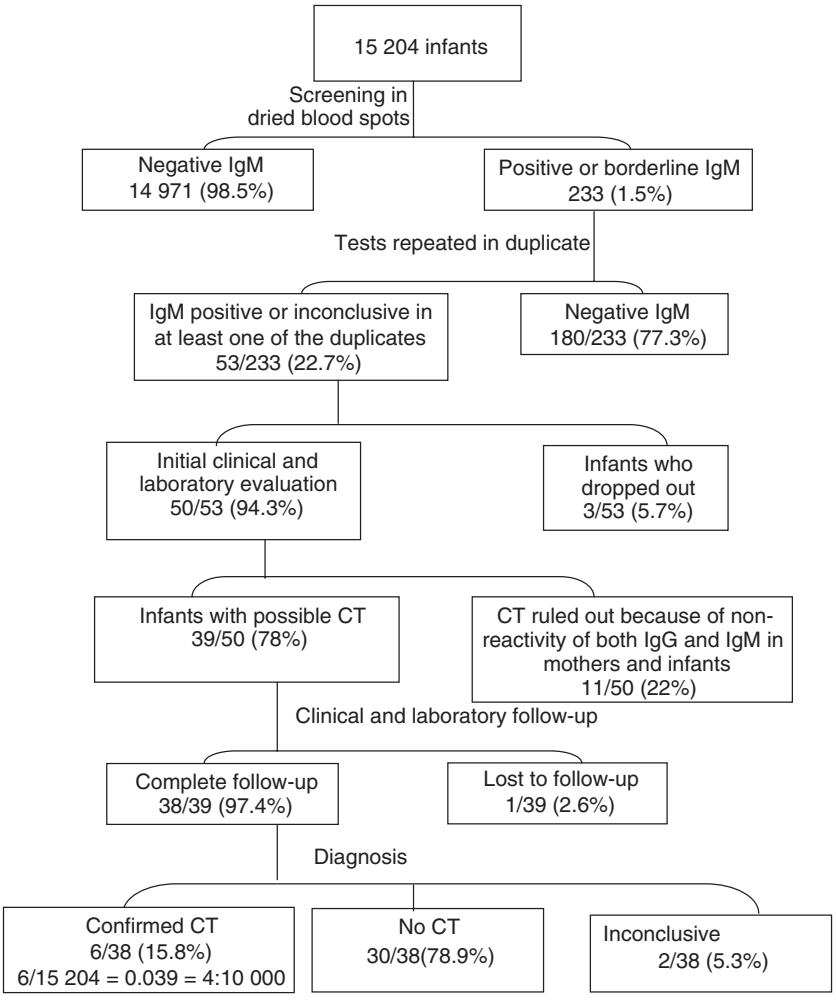

Figure 1 Neonatal screening for congenital toxoplasmosis in the Brazilian state of Sergipe, April–October 2009, and follow-up of infants until final diagnosis.

Table 1 Characteristics of six mothers and infants with confirmed congenital toxoplasmosis

Case number	Prenatal serology	Child age (days) at first clinical evaluation	Child's first serology (peripheral blood) (MEIA*)	Maternal serology (MEIA*)	Child's findings at first evaluation	Child's serology after treatment interruption (MEIA*)	Follow-up period (months)	Final clinical findings
1	IgG (–) IgM (–)	49	IgG 7965.0 IgM (+)	IgG 5742.0 IgM (+)	Cerebral calcifications	IgG 1331.8 IgM (–)	20	Normal neurological exam
2	IgG (–) IgM (–)	91	IgG 1956.5 IgM (–)	IgG 1449.0 IgM (+)	Unilateral retinochoroidal scar involving the fovea	IgG 1000.4 IgM (–)	21	Unchanged ocular lesion
3	Not performed	105	IgG 6237.0 IgM (–)	IgG 2125.6 IgM (+)	Asymptomatic	IgG 15370.0 IgM (–)	20	Asymptomatic
4	IgG (–) IgM (–)	91	IgG 5252.0 IgM (–)	IgG 1587.1 IgM (+)	Asymptomatic	IgG 334.0 IgM (–)	20	Unilateral peripheral retinochoroidal scar
5	IgG (–) IgM (–)	109	IgG 7000.0 IgM (–)	IgG >300.0 IgM (+)	Asymptomatic	IgG 231.0 IgM (–)	20	Asymptomatic
6	IgG (+) IgM (–)	58	IgG 916.0 IgM (+)	IgG >300.0 IgM (–)	Hepatosplomegaly	IgG 961.7 IgM (–)	25	Bilateral peripheral retinochoroidal scars

+, Positive; –, Negative; MEIA, Microparticle enzyme immunoassay.
*Results in IU (International Units)/ml.

pregnant. It is likely that five mothers seroconverted during gestation, as their IgM testing resulted positive after delivery. The last mother (#6) had chronic myeloid leukaemia; she had one serological test performed at 32–34 weeks of gestation, showing an IgG titre of 28.23 IU/ml. When the infant was evaluated by the research team, at 58 days of age, maternal IgG titres were >300 IU/ml, suggesting the possibility of reactivation of chronic toxoplasmosis or reinfection during pregnancy.

While confirmatory DBS screening results were inconclusive in (87.5%) of the 32 uninfected infants, all six infants with CT had positive results in both duplicates. However, when peripheral blood was analysed for IgM in these six infants, only the two blood samples drawn at ages 49 and 58 days tested positive for IgM. The other four infants, tested at 91–109 days, had negative IgM results on peripheral blood. These 6 infants were tested between six and 10 times in their first 2 years of life. Although IgG titres decreased during the first months and reached a nadir around 6–12 months (data not shown), IgG titre rebounds were detected after treatment interruption and remained positive until the conclusion of the follow-up, at a median age of 20 months.

With respect to the findings at initial physical examination (median age of 3 months), only one infant had a typical feature of congenital infection, that is, hepatosplenomegaly. Two other babies had subclinical signs, identified after fundoscopy (retinochoroidal scar) and cranial ultrasound examinations (cerebral calcifications). None had abnormalities in blood cell count, liver enzymes or cerebrospinal fluid examinations. During follow-up, additional retinochoroidal scars were identified in one infant who was previously asymptomatic and in the infant with hepatosplenomegaly. Neuromotor development was adequate in all six infants at the end of the follow-up. Infected infants were treated for 1 year (Table 1).

Discussion

The present results indicate that the prevalence of CT in the state of Sergipe [four per 10 000 live newborns (95% CI 1.4–8.0)] is within the range reported for other places in Brazil, varying from three to 19 per 10 000 (Carvalheiro *et al.* 2005; Lago *et al.* 2007; Vasconcelos-Santos *et al.* 2009; Neto *et al.* 2010). Assuming that the four infants who did not complete follow-up were infected (an unlikely hypothesis), the estimated prevalence of CT in this population would be twice as high, about one per 1000. However, it would still be within the expected range for the country.

Even though we enrolled 72% of the eligible live-born infants, the fact that foetal losses were not included could indicate a higher incidence of CT in the state of Sergipe. Conversely, because we selected only infants born in public maternities and did not study those of higher socio-economic status, the birth prevalence we found might be overestimated. A high prevalence of CT in the state was expected, considering the elevated seroprevalence of toxoplasmosis in pregnant women (69.3%) and the high proportion (30.7%) of susceptible women who were at risk of infection during pregnancy (Inagaki *et al.* 2009).

The prevalence of CT in Sergipe is similar to the 3.0 per 10 000 (95% CI 2.0–4.4) rate that has been reported in Denmark (Lebech *et al.* 1999) and the 2.9 per 10 000 (95% CI 2.5–3.2) reported in France (Villena *et al.* 2010). In the United States (Guerina *et al.* 1994) and Sweden (Evengård *et al.* 2001), the prevalence of CT tends to be lower, below one per 10 000.

The use of anti-*T. gondii* IgM tests as a marker of CT has some limitations. The sensitivity of neonatal DBS has been reported to range from 41% to 86% (Robert-Gangneux 2001; Montoya & Liesenfeld 2004; Rodrigues *et al.* 2009). IgM may not be detected at birth in infected children, and the sensitivity is even lower when gestational treatment for toxoplasmosis is used or when the infection occurs in the first half of pregnancy (Gilbert *et al.* 2007; Petersen 2007). Furthermore, when DBS for neonatal screening are not collected in the first days of life, as occurs in some Brazilian regions, IgM may no longer be detected (Lebech *et al.* 1999; Lago *et al.* 2007). In our study, only 2 of the 6 infected infants tested positive for IgM on peripheral blood, probably because they were tested at <60 days of age. Another disadvantage is the necessity to retest and the high frequency of false-positive results. In this study, the proportion of false-positive results was 97.4% (6/233), even higher than the 14–78.5% range described in other studies (Evengård *et al.* 2001; Carvalheiro *et al.* 2005; Gómez-Marin *et al.* 2011). A high proportion of false-positive results is generally accepted for screening tests, but may produce unnecessary emotional stress and increase costs. Despite these limitations, anti-*T. gondii* IgM tests are still widely used in the clinical investigation of CT and in epidemiological studies, owing to their availability and applicability in a large number of infants.

One advantage of our study is that the definitive diagnosis of CT was not based solely on the initial results of screening tests but was rather confirmed by detection of positive anti-*T. gondii* IgG results beyond the first year of life and/or after suspension of specific treatment, both of which are markers of infection (Lebech *et al.* 1996). However, because the sensitivity of IgM tests is below 100%, the estimated prevalence of CT in Sergipe might have been underestimated.

As the women receiving care from the private sector were not included in this study, the results can be only applied to the population assisted by the public healthcare system, in which screening for gestational toxoplasmosis is mandatory as part of the routine prenatal care. That corresponds to approximately two-thirds of the population in Sergipe. However, serological tests were not performed in the first prenatal visit and repeated in the third trimester for all susceptible women, as recommended; gestational toxoplasmosis was not identified in some women, who were consequently not treated. Similarly, in the south-eastern state of Minas Gerais, only 5.8% of the mothers of infants with CT were treated while pregnant (Vasconcelos-Santos *et al.* 2009). Therefore, we believe that the investment made by the public healthcare system of Sergipe would best be spent on primary prevention programmes focused on providing information about hygiene or dietary measures to prevent acquisition of toxoplasmosis during pregnancy.

When it comes to the clinical presentation of the infants with CT, in the first assessment, 3 (50%) had signs of toxoplasmosis; one had hepatosplenomegaly, one had retinochoroidal scars and the third infant had brain calcifications. Additionally, it should be noted that three of six infants with CT in our cohort had ophthalmological lesions that developed along their first year of life. These problems would not have been identified and treated if the infants had not been submitted to neonatal screening.

Other Brazilian studies performed in the south and south-east regions of the country have reported clinical problems in 80–100% of congenitally infected infants (Carvalheiro *et al.* 2005; Lago *et al.* 2007; Vasconcelos-Santos *et al.* 2009). All five infants identified by Carvalheiro *et al.* (2005) presented retinochoroiditis and 80% had neurological disorders in the first assessment. Similarly, Lago *et al.* (2007) describe 83% of symptomatic infants, 67% of whom with ophthalmological problems. In the study performed by Vasconcelos-Santos *et al.* (2009), the frequency of retinochoroiditis was 79% among 178 infants. Only one Brazilian study (Neto *et al.* 2010) found a lower frequency of clinical problems (17%), similar to that described in a study performed in Colombia (37.5%) (Gómez-Marin *et al.* 2011). Nevertheless, in these studies, ophthalmological and neurological assessments were not performed in all children. In the United States and Europe, large studies describe lower frequencies of clinical manifestations. In Sweden, two-thirds of the infants with CT diagnosed by neonatal screening had clinical abnormalities (Evengård *et al.* 2001); in the USA (Guerina *et al.* 1994) and Denmark (Lebech *et al.* 1999; Schmidt *et al.* 2006),

40% and 25% of newborns submitted to neonatal screening, respectively, were symptomatic among 50 infected infants. In France, where universal prenatal treatment and pregnancy termination are performed, 12% of 234 live-born infants had clinical manifestations (Villena *et al.* 2010). Such differences in the presentation of CT have been attributed to the highest virulence of *T. gondii* genotypes found in South America, as well as differences in genetic characteristics of the population and in the modes of infection (Gilbert *et al.* 2008; Vasconcelos-Santos *et al.* 2009).

In conclusion, this first large study performed in the state of Sergipe shows a worrisome prevalence of CT and provides a contribution to the discussion about the preventive measures that are appropriate to the reality of the state and other regions of the world.

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