Communication/Comunicação

Incidence of congenital toxoplasmosis in the City of Belém, State of Pará, Northern Brazil, determined by a neonatal screening program: preliminary results

Incidência de toxoplasmose congênita na Cidade de Belém, Estado do Pará, norte do Brasil, através de um programa de triagem neonatal: resultados preliminares

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ABSTRACT

Introduction: The aim of this study was to determinate the incidence of congenital toxoplasmosis among a group of newborns (NBs) from *Belém* using neonatal screening. **Methods:** Among the 6,000 newborns referred for investigation of genetic and metabolic diseases, 1,000 were selected for screening for congenital toxoplasmosis by determining the amount of IgM in the eluates of blood collected on filter paper. Positive tests were confirmed using paired serology of the NB and his mother. **Results:** Out of the 1,000 NBs assessed, one had a positive screening result that was confirmed by paired serology. **Conclusions:** The incidence of congenital toxoplasmosis in Belém was 10/10,000 live NBs.

Keywords: Congenital toxoplasmosis. Neonatal screening. Incidence.

RESUMO

Introdução: O objetivo do estudo foi determinar a incidência da toxoplasmose congênita em um grupo de recém-nascidos (RNs) de Belém, pela triagem neonatal. **Métodos:** Entre 6.000 RNs, encaminhados para investigação de doenças genéticas e metabólicas, foram selecionados 1.000 para triagem de toxoplasmose congênita, através da pesquisa de anticorpos IgM em eluatos de sangue colhido em papel de filtro. Os testes positivos foram confirmados, através da sorologia pareada do RN e respectiva genitora. **Resultados:** Dos 1.000 RNs investigados, um apresentou triagem positiva confirmada pela sorologia pareada. **Conclusões:** A incidência de toxoplasmose congênita em Belém foi de 10/10.000 RNs vivos.

Palavras-chaves: Toxoplasmose congênita. Triagem neonatal. Incidência.

Toxoplasmosis is an important protozoan zoonosis found worldwide that is caused by *Toxoplasma gondii*. This infection is more common in areas with tropical and very humid climates, which are favorable conditions for the maintenance and dissemination of the oocysts, the infecting parasite form[1].

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In different areas of Brazil, the prevalence of toxoplasmosis in the human population is as high as 80% in the Northern region[2]. A study performed in the metropolitan area of the City of Belém involving 1,600 individuals showed that the seroprevalence in the general population was 78%, with an incidence of 5.1%[3].

The clinical evolution of toxoplasmosis is usually asymptomatic, but some factors, such as the genetic characteristics of the *T. gondii* strain and the immune status of the host, can result in serious disease, especially in immunocompromised individuals or those with congenital infection[4].

The congenital form of toxoplasmosis is characterized by primary infection of a pregnant woman with consequent fetal infection via the placenta. The risk of transmission increases as the pregnancy progresses, but the disease is more serious when transmission occurs during the first trimester. In this case, infection can cause abortion, fetal death, prematurity or serious fetal disease. After this period, toxoplasmosis tends to manifest itself with late side effects that mainly affect the eye system tissues[4].

The diagnosis of toxoplasmosis in pregnant woman is based mainly on serological methods to detect specific antibodies[5]. However, as in most other countries, Brazil does not have a free national program to investigate toxoplasmosis during pregnancy, in spite of the high prevalence of the infection. Some Brazilian states provide serological evaluation during prenatal care, but regardless, in most cases, these exams are performed too late to make a helpful diagnosis; furthermore, when the first exam is negative, there is no follow up in the subsequent months. Thus, there is no possibility of the early diagnosis of serological conversion and specific treatment during pregnancy[6].

In addition, these states are performing toxoplasmosis diagnosis in newborns by measuring the levels of anti-*T. gondii* IgM or IgA antibodies in dry blood samples on filter paper obtained as part of the neonatal screening program for metabolic disorders[7,8].

Based on the toxoplasmosis situation in the Amazon region and the severity of the clinical picture observed in the newborns, the aim of this study was to determinate the incidence of congenital toxoplasmosis in a group of newborns in the City of Belém, by neonatal screening using blood samples on filter paper.

From October to December 2009, a random sample (n = 1,000) was selected from a population of 6,000 newborns evaluated by the

neonatal screening program of the *Laboratório de Pesquisa e Apoio Diagnóstico da Universidade do Estado do Pará* (LAPAD-UEPA). These selected newborns were screened for phenylketonuria, sickle cell anemia, congenital hypothyroidism and toxoplasmosis. Four drops of total blood (200μL each drop) from each newborn were placed on filter paper cards (S&S 903) using the standard heel puncture procedure. Each card was individually identified, dried at room temperature and refrigerated until the tests were performed.

Screening for toxoplasmosis consisted of measuring the levels of IgM anti-*T. gondii* antibodies in the eluates of the dried blood samples by the capture immunoenzymatic assay (cELISA). For this method, commercial kits (Imunoscreen Mbiolog *Diagnósticos*) were used, and the procedures were performed according to the manufacturer's instructions. Briefly, using a paper perforator, a 3mm disk was obtained from one of the blood samples on the filter paper of each patient. The total blood volume in each disk was approximately 3μL. The disks were placed on microplates coated with anti-IgM and eluted with the elution buffer solution in the approximate ratio of 1:50. After an incubation period, the disks were removed, and the microplates were washed. In the following steps, the conjugate was added along with the soluble *T. gondii* antigen, the chromogen substrate and the blocking solution, and the absorbances were read in a microplate analyzing apparatus (*BIORAD CODA*®). Negative and positive controls supplied in the kit were used for each microplate, and the cutoff was calculated according to the manufacturer's instructions.

As has been done previously, when a newborn (NB) was positive in the screen, the mother was requested to go to the LAPAD-UEPA so that venous blood could be collected from both the newborn and his/her mother for use in the confirmation tests. The presence of anti-*T. gondii* IgG and IgM in the sera was determined using an immunofluorometric assay using commercial kits and the automated VIDAS® system (*bioMérieux*). This method, which is completely automated, was performed according to the manufacturer's instructions and consisted of performing two immunoenzymatic methods, an indirect method for IgG and an immunocapture method for IgM, both with final detection by fluorescence. Positive and negative calibration controls were included for each group of sample tests supplied by the kit, and the cutoff was calculated according to the manufacturer's instructions.

IgM was detected in one of the 1,000 NBs investigated by the screen using an enzyme-linked immunosorbent assay (ELISA). The joint analysis of the samples of the NB's serum and his mother's serum by enzyme-linked fluorescence assay (ELFA) confirmed the diagnosis because anti-*T. gondii* IgG and IgM were detected in both sera.

Clinical assessment of this NB showed that he was born at full term and presented good general conditions (*Apgar index* = 9, weight: 2,800g, length: 40cm, cephalic perimeter: 33cm). However, during the neonatal period, signs and symptoms consistent with chorioretinitis, hydrocephalus and diffuse hypodensity with multiple dispersed calcifications were observed by computerized tomography. The mother started prenatal care in the second month of pregnancy with seven consultations, but the first serology for toxoplasmosis was not performed until the seventh month. This testing revealed that she was positive for IgM and IgG. Treatment was carried out for 40 days with pyrimethamine, sulfadiazine and folinic acid.

The preliminary results of this study demonstrate that the incidence of congenital toxoplasmosis was 0.1% (1:1,000), or 10 positive cases are expected for every 10,000 newborns tested (CI 95% 0.0-0.2%). This prevalence is considered high in comparison to that observed in other countries where neonatal screening programs for congenital toxoplasmosis have been implemented; these countries include Poland (4.7/10,000)[9], Sweden (0.73/10,000)[10], Italy (1.38/10,000)[11] and Denmark (1.6/10,000)[12]. However, the prevalence observed herein is lower than that observed in México (20/10,000)[13].

Neto et al.[14] published data about the incidence of congenital toxoplasmosis obtained by the neonatal screening of newborns assisted by private health care in different Brazilian states over a period of 14 years (1995 to 2009). In this study, the rates ranged from 12 to 20/10,000 live NBs in the States of Rondonia, Mato Grosso, Maranhão, Espírito Santo, Sergipe and Pará, indicating the importance of this disease in the population enrolled in this study.

The data from the present study indicate that there is a high incidence of congenital toxoplasmosis in Belém, which is in accordance with the reality observed in the Brazilian Amazon, especially in the State of Pará, where the prevalence of this infection is known to be high as a result of the suitable environmental conditions for the survival of the parasite, the population's occupational and food habits and the probable involvement of atypical, highly virulent strains.

Toxoplasmosis serology has been shown to be an important tool in neonatal screening programs to prevent sequelae and to estimate the prevalence of congenital infections[14,15]. The high incidence of congenital toxoplasmosis observed in Belém in the present investigation justifies the inclusion of the infection in the municipal neonatal screening program that is currently limited to the diagnosis of metabolic and genetic disorders.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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