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DETECTION OF NEWBORN INFANTS AT RISK FOR CONGENITAL TOXOPLASMOSIS IN RIO DE JANEIRO, BRAZIL

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SUMMARY

Within the third to fifth day after birth, blood samples for indirect immunefluorescence tests (IF) for toxoplasmosis were taken from 1032 neonates. 377 (36.5%) IF IgG and IF-IgM seronegative cases and 655 (63.5%) IF-IgG seropositive cases reacting from 1:16 to 1:1024 were found. In 15 such cases (1.4%), sera were reactive in both the IgG and IgM classes, 12 cases being IF-IgM = 1:16, and 3 cases IF-IgM = 1:64. These cases were considered at potential risks for congenital infection by **T. gondii.** Of these 15 cases, a morphological study of 13 placentas showed data suggestive of prolonged fetal injuries in 11 such cases. All these 13 placentas showed signs of hematogenous inflammatory processes. Four placentas revealed structures with morphological characteristics similar to cysts of **Toxoplasma gondii** at the microscopical examination. Of these four neonates where the parasite was found in the placentas, one infant was underweight and showed discrete microcephaly. Two children had hepatosplenomegaly, one of them with unilateral retinochoroiditis. The fourth case was clinically normal. In the other 11 cases, selected due to possible risk of congenital infection, one infant was premature, eight were normal, and two showed symptomatology non-suggestive of congenital toxoplasmosis.

INTRODUCTION

Toxoplasmosis is an infection caused by **Toxoplasma gondii**, which may infect human beings as well as a great number of other animal species.

Serological examinations of urban or indigenous populations in Brazil have shown a prevalence of positive sera, generally between 40% and 80% [3,6,18]. COUTINHO et al.[9] have found, in Rio de Janeiro, 78.7% of positive sera in 6079 ambulatory patients or pregnant women, of which 5.1% with titres ≧ 1:4096.

In almost all cases of congenital toxoplasmosis, intrauterine transmission happens when acute maternal infection occurs for the first time during pregnancy [11,12,20,24]. In such cases, serious consequences may arise, such as abortions, premature births or other diseases, such as: retinochoroiditis, cerebral calcification, oligophrenia, macro or microcephaly [2,10,13].

Such very serious occurrences, although infrequent, clearly reveal the importance of toxoplasmosis in its congenital form which, when poorly diagnosed, may cause damages that may be of irreversible nature.

Various Authors [1,11,12,23,24] have recently mentioned that the congenital transmission of toxoplasmosis may be responsible not only for severe cases, with neurological lesions, to neonates,

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but also for a discrete or unapparent symptomatology during the neonatal period, which may lead to retardation in development, retinochoroiditis which may develop into blindness, etc. The clinical aspect of neonatal congenital toxoplasmosis may be so varied that, according to EICHENWALD [13], it should be taken into consideration during the differential diagnosis of practically all forms of obscure diseases occurring during the first months of life. Specific treatment, immediately after birth, may possibly prevent worsening of the clinical picture [1,11, 23,26].

Thus, laboratory diagnoses may render valuable aid, not only for an early diagnosis of individual cases, but also for epidemiological studies on the prevalence, or incidence, of congenital transmission.

The most widely utilized method of serological diagnosis has been the indirect immunefluorescence test (IF) [6,22], which permits detection of IgG and IgM anti **Toxoplasma** antibodies. The ratios of occurrence of maternal acute toxoplasmosis during pregnancy have been estimated at between 2 and 4 per 1000, in Scandinavia [25,27]; 5 to 6 per 1000, in the USA [4,24]; 10 per 1000, in France [11]. However, the ratios of congenital toxoplasmosis in the USA and Europe vary from 1 to 2 births per 1000 [1,4,25,26]. CASTILHO [8] using an indirect method has estimated this ratio at 16 infected births per 1000 in the city of São Paulo, Brazil. Several studies, such as by REMINGTON & DESMONTS [20], DESMONTS & COUVREUR [11], and STRAY-PEDERSEN [25], indicate that only approximately 39% to 46% of women, who acquired toxoplasmosis during pregnancy, gave birth to infected children. However, even when infection does occur, more frequently a discrete, or subclinical picture, rather than severe disease is the result, which may later cause mental retardation or ocular lesions [26].

As far as we know, this is the first study that has been published in Brazil, to evaluate the birthrate of neonates infected by **T. gondii**, with or without clinical symptomatology at a routine neonatal examination.

The placentas of newborn infants, serologically suspected of congenital toxoplasmosis, were microscopically examined in an attempt to detect the parasite.

MATERIAL AND METHODS

During the period from August, 1978, to February, 1980, 1,032 newborn infants were examined, who had been selected, at random, from a total of 1,131 births in the Fernandes Figueira Institute, of FIOCRUZ, in the city of Rio de Janeiro. The majority of the mothers pertained to the lower socioeconomic classes.

In all 1,032 cases, the blood for serological tests was taken between the third and fifth day of life, by means of a micro hematocrit tube. The newborn infant's heel was picked with a disposable sterile lancet, and the resultant drop of blood was collected in a micro hematocrit tube (Kimble 73811), with an internal diameter of 1.1 to 1.2 mm and a total capacity of approximately 85 microliters. After being filled with blood, one of the extremities of the tube was sealed with wax. Using the formula to determine the cilinder volume, it was found that 20 microliters were contained in 19 mm lenght of the tube. After centrifugation, the tubes were cut at a height corresponding to 19 mm (=20 microliters), free of cells, both ends of the tube being open. The cut tube was emptied into a recipient containing 300 microliters of PBS (pH 7.2), thus rendering an initial dilution at 1:16. Thereafter, successive four-fold dilutions were made. The immunefluorescence tests were done with anti-IgG and anti-IgM conjugates, from Hyland, Travenol Laboratories, USA.

Such cases where sera were positive in both the IF-IgG and IF-IgM at 1:16 or higher, or were reactive exclusively in IF-IgG at 1:4096, or higher, were serologically considered as being high risks for the congenital transmission of toxoplasmosis. Thirteen placentas of the fifteen cases thus diagnosed were submitted to histopathological examination after fixation in 10% formoline. Histological sections were Eosin-Hematoxylin stained.

These newborn infants were clinically examined and had ambulatorial follow up.

RESULTS

The immunefluorescence tests of sera, taken between the third and fifth day of life, showed 377 (36.5%) IF-IgG and IF-IgM serone-

gative cases and 655 (63.5%) IF-IgG seropositive cases within titers of 1:16 to 1:1024. In 15 (1.4%) of the latter cases, sera were positive in both the IF-IgG and IF-IgM tests. Clinical and serological data on these 15 neonates are resumed in Table I.

TABLE I

Neonates at risk for congenital toxoplasmosis, presenting positive indirect immunefluorescent test for toxoplasmosis (IF-IgG and IF-IgM). Clinical and laboratorial data

NEONATES							
Number	Sexe	Weeks of pregnancy	Birth weight (grams)	Antibody anti-Toxoplasma IF-IgG	IF-IgM	Clinical data	Placenta Histo-pathology
1	F	39	2600	1:256	1:64	Hepato-spleno-megaly	PFI, HIP *T.gondii* like forms
2	M	39	3400	1:1024	1:64	Hepato-spleno-megaly, chorio-retinitis	PFI, HIP *T.gondii* like forms
3	F	38	2300	1:64	1:64	Low weight micro-cephaly	PFI, HIP *T.gondii* like forms
4	M	38	3650	1:256	1:16	Normal	PFI, HIP *T.gondii* like forms
5	M	39	3600	1:1024	1:16	Normal	HIP
6	M	39	3675	1:64	1:16	Uni-lateral congenital catarata	PFI, HIP
7	M	38	3070	1:256	1:16	Normal	PFI, HIP
8	F	39	3300	1:64	1:16	Normal	PFI, HIP
9	M	38	2775	1:64	1:16	Normal	PFI, HIP
10	M	38	2800	1:256	1:16	Normal	PFI, HIP
11	M	39	2900	1:64	1:16	Down's syndrome	PFI, HIP
12	M	39	3300	1:256	1:16	Normal	PFI, HIP
13	F	39	2500	1:256	1:16	Normal	HIP
14	M	39	3500	1:64	1:16	Normal	ND
15	F	36	2120	1:256	1:16	Pre-term	ND

F= female M= male ND= not done PFI= prolonged fetal injury
HIP= haematogenous inflammatory process

The results of a detailed morphological study of placentas of cases No. 1 to No. 13 will be published elsewhere. Summing up, data suggesting prolonged fetal injuries were observed in eleven such cases.

All thirteen placentas examined showed signs of hematogenous inflammatory processes: affection of the villus disc, with villusitis and chronic vascularitis, funiculitis, in addition to other data.

In cases No. 1 to No. 4 (Table I), microscopic examinations of placentas showed structures similar to cysts of **Toxoplasma gondii.**

DISCUSSION

According to serological criteria, considered in this paper as representing a high risk of congenital transmission of toxoplasmosis, fifteen suspected cases of congenital toxoplasmosis (15 cases of IF-IgG and IF-IgM $\geq$ 1:16 and no cases of only IF-IgG $\geq$ 1:4096) were diagnosed. Such criteria was based on the fact that congenital transmission of toxoplasmosis occurs almost exclusively when the pregnant woman acquires an acute infection during pregnancy [11,12,20,24]. In such cases, titers in the IgG classes are usually high and pass passively through the placenta to the foetus. In the present investigation, 63.5% of positive IF-IgG were found in low or medium titers, probably in relationship to the passive passing of antitoxoplasma antibodies of the IgG class through the placenta. In previous investigations, carried out among pregnant women and their newborns in the city of São Paulo, JAMRA et al. [15] found 38.7% of newborns sera reactive to the dye test, and HYAKUTAKE et al. [14] determined 47.4% by IF-IgG antitoxoplasma.

Titers in the IgM class are usually early present, although they become negative within a short period. Their presence in the newborn infant leads to suspicion of congenital transmission of the parasites, since they rarely passively traverse the placenta. This possibly signifies that they were produced by the neonate itself, due to the presence of the parasite antigen [1,5,21]. To minimize the possibility of false negative IF-IgM, which occasionally occurs in the case of blood samples taken from the umbilical cord [1], sera taken between the third and fifth day of life was preferred. In addition to false negative results [19,26] mention has been made of cases of false positive IF-IgM for toxoplasmosis in newborn infants [1,5]. Thus, IF-IgM antitoxoplasma may lead to suspicion of congenital transmission of toxoplasmosis, without however definitely signifying this to have been the case.

Preliminary examinations showed that the titers observed in blood samples taken with capillary tubes, according to the technique described herein, did not significantly vary from titers of blood samples simultaneously taken by venipuncture. Thus, this method permits the obtention of quantitative serological results in small samples of blood taken with disposable lancets.

All of the fifteen newborn infants, serologically suspected of having congenital toxoplasmosis (1.4%) had shown acceptable clinical conditions. The clinical examinations of nine infants appeared to be normal. Of the four cases (0.4%) where forms similar to cysts of **T. gondii** were found in the placenta, only one case was clinically normal (case No. 4), whilst the other three cases (No. 1, 2 and 3) presented clinical symptoms compatible with congenital toxoplasmosis (Table I).

However, in all these cases, clinical data was discrete and the ophthalmological examination, that permitted diagnosis of retinochoroiditis in case No. 2, was only performed on this infant due to serological finding compatible with congenital toxoplasmosis. Possibly the therapeutic treatment (sulfa and pyrimethamine) will arrest progress of the disease [1,11,23,26].

Case No. 15 was pre term, an occurrence compatible with **T. gondii** [1] congenital infection, but unfortunately the placenta was not examined to attempt to find evidence of the parasite.

Two other cases (No. 6 and No. 11) showed respectively congenital cataract and genetic anomaly diagnosed as Down's syndrome, not related to the clinical aspect of congenital toxoplasmosis.

In the thirteen cases that were examined, histopathological examination of placentas revealed changes compatible with hematogenous inflammatory processes. In all thirteen cases, samples were exhaustively examined by microscope in an attempt to find evidence of forms similar to **T. gondii.** No attempt was made to isolated the parasite by inoculation of placenta material in mice, because the placentas have been conserved in formoline during four to six days after date of birth, when they were then selected for histopathological examination, in accordance with results obtained by IF-IgG and IF-IgM tests.

STRAY-PEDERSEN [25,26] was able to detect the parasite in the placenta or amniotic fluid of three births, amongst the thirteen cases that

were examined and, from a serological viewpoint, considered as presenting high risks of infection.

DESMONTS & COUVREUR [11] detected the parasite in 25% of placentas of pregnant women, who contracted toxoplasmosis during pregnancy. KIMBALL et al. [17], CARDOSO et al. [7], also detected **T. gondii** in placentas, the latter having referred to a prevalence of 0.5% cases of congenital toxoplasmosis amongst 1,200 necropsies of stillborns or newborn infants, in the city of Rio de Janeiro.

The present study found serological suspicion of congenital toxoplasmosis in 1.4% of newborn infants, of which at least four cases (4 per 1000) were confirmed through detection of forms similar to the parasite in placentas, in addition to clinical symptoms compatible with congenital toxoplasmosis in three of the cases. The remaining newborn infants had ambulatory follow up, but congenital infection by **T. gondii** cannot be inferred as being exclusively due to positive IF-IgM, since false positive results in such tests have been mentioned [5,19].

It should be stressed that none of the fifteen cases, including the four cases where **T. gondii** was detected in the placentas, showed signs of hydrocephalus or cerebral calcification, which are classic and very evident signs of congenital toxoplasmosis [13,29].

Similar results of oligosymptomatic or subclinical cases of congenital toxoplasmosis with possibility of worsening after birth, have been identified by various Authors and are considered to be more frequent than severe cases with evident symptomatology [1,11,23,24,26].

Thus, early diagnosis of congenital toxoplasmosis, including subclinical cases, is very important [1,11,23,26]. Serological data, principally the IF-IgG, IF-IgM and the dye test for toxoplasmosis, may be helpful, without however being decisive, due to the possible occurrence of cases of non-reactive IF-IgM in newborn infants with congenital infection [5,16,19,26]. REMINGTON & DESMONTS [19], ALFORD et al. [1], KARIN & LUDLAN [16], state that certain human anti IgM fluorescent conjugates may show false cases of negative IF-IgM. Recent methods to measure cell mediated immunity, such as the in vitro lymphocyte stimulation test (LST) have been tested with good results [28], but unfortunately only became positive at a much later time [26].

In view of the relatively high incidence of congenital transmission of toxoplasmosis in our circles, the serological follow up during pregnancy appears to be indicated as the best measure for its detection.

RESUMO

Detecção de recém-nascidos com risco de toxoplasmose congênita no Rio de Janeiro, Brasil

Foi coletado sangue entre o 3.° e o 5.° dias após o nascimento de 1032 recém-nascidos para a realização do teste de imunofluorescência indireta (IF) para toxoplasmose. Verificou-se 377 (36,5%) soro-negativos nas classes IgG e IgM e 655 (63,5%) soro-reagentes entre 1:16 e 1:1024 na classe IgG. Em 15 destes (1,4%) os soros eram reagentes tanto nas classes IgG como IgM, 12 deles IF-IgM = 1:16 e 3 deles IF-IgM = 1:64, sendo considerados com risco potencial de infecção congênita pelo **T. gondii.** Um estudo morfológico foi realizado em 13 das 15 placentas demonstrando, em 11 delas, dados sugestivos de sofrimento fetal prolongado. Todas as 13 placentas examinadas apresentavam sinais de processo inflamatório hematogênico. Em 4 placentas, o exame microscópico ainda evidenciou estruturas com características morfológicas semelhantes a cistos do **Toxoplasma gondii.** Destes 4 recém-nascidos, em que se evidenciou o parasito na placenta, um deles era de baixo peso e com discreta microcefalia. Dois outros apresentavam hepatoesplenomegalia, tendo um destes retinocoroidite unilateral. O quarto caso era clinicamente normal. Dos outros 11 casos selecionados por apresentarem risco de infecção congênita pelo critério sorológico adotado, um deles, era pré-termo, oito dentro da normalidade, e dois outros com sintomatologia não sugestiva de toxoplasmose congênita.

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