

Incidence of Congenital Chagas' Disease in Bahia, Brazil

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The first proven case of congenital Chagas' disease was reported in 1911 by Chagas¹ two years after he described the disease.² Since then there have been about 160 cases of congenital Chagas' disease reported in the literature. However, the incidence of this form of transmission of *Trypanosoma cruzi* in endemic areas remains to be established.³

The few studies concerning the incidence of congenital chagasic infection in South America have varied greatly in their criteria for selection of participants.⁴⁻¹⁰ Some of these studies included only premature newborns of chagasic and nonchagasic mothers,^{7,8} while others described the frequency of congenital transmission of *T. cruzi* among newborns of chagasic women.^{4,5,6,9} In addition, different methods were used to detect parasitemia.⁴⁻⁶ Bittencourt *et al.*¹ working with livebirths and stillbirths weighing less than 2000 g, performed xenodiagnosis, examination of placenta and adnexae as well as complete autopsy of both stillbirths and neonates who died. Using this methodology they found an overall frequency of congenital transmission of 10.5 per cent in Salvador, Bahia, Brazil.¹⁰ The incidence of transmission was two times greater among mothers who

delivered conceptuses weighing 401 to 1000 g than in the group whose fetuses weighed between 1001 to 2000 g. Among conceptuses weighing less than 400 g the frequency of transplacental transmission of *T. cruzi* was 6.2 per cent.¹¹

In order to evaluate the frequency of congenital transmission of Chagas' disease, stillbirths and newborn infants of all intra-uterine ages of infected mothers must be examined. Therefore, the present investigation was carried out to determine the incidence of transmission among conceptuses weighing more than 2000 g in the same area where conceptuses weighing less than 2000 g had been studied. The clinicopathological aspects of the congenital cases identified in this study are presented.

Materials and Methods

Study population

We examined a non-selected sample of women admitted to the obstetric unit of the Hospital Professor Roberto Santos (Department of Health, State of Bahia) and Maternity Hospital Climério de Oliveira (Federal University of Bahia) who gave birth to conceptuses weighing more than 2000 g during the period from January, 1981 to August, 1982. Both hospitals admit women of low socio-economic status, many of them (58 per cent) originally from rural areas of the state of Bahia but currently living in the city of Salvador.

Study protocol

Women were examined within 24 hours of delivery while still in the hospital. Ten ml of venous blood were obtained for serological examination (see below). Seropositive women were further submitted

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to clinical examination, xenodiagnosis and blood culture for *T. cruzi*. Epidemiological history of contact with triatomines was also sought. Newborns of seropositive women were submitted to clinical examination, xenodiagnosis and direct blood examination (DBE) of venous blood to detect the presence of trypomastigotes.

We considered as a case of congenital transmission of Chagas' disease, every newborn of a seropositive woman who had either a positive xenodiagnosis or bloodstream forms of *T. cruzi* on direct examination. In cases of stillbirths we diagnosed congenital transmission when we found inflammation and amastigotes in the fetal organs.

Clinical management of cases of congenital transmission

Newborn infants with congenital Chagas' disease identified in this study underwent chest X-ray, electrocardiography (ECG), and direct examination of the cerebral spinal fluid for *T. cruzi*. Serum was tested for IgM antibodies to *T. cruzi* by enzyme-linked immunosorbent assay (ELISA).

Detection of Parasitemia

Ten non-infected laboratory-raised 5th instar nymphs of *Triatoma infestans* were used for xenodiagnosis. The rectal contents of individual bugs were collected 30 days after feeding on the patients, mixed with saline, and examined for the presence of *T. cruzi* at 400 \times magnification. To detect parasitemia by blood culture, 3 ml of sterile heparinized blood was processed on the day of collection. The blood was separated over Ficoll-hypaque and the mononuclear cell layer (where trypomastigotes concentrate) was suspended in 2.0 ml of F-29 medium, and overlaid on NNN medium using methods previously described.^{12,13} Venous blood from babies of seropositive mothers was collected for direct examination by heel puncture using a heparinized microhematocrit tube.¹⁴ After centrifugation, the leucocyte layer was examined under a coverslip for the presence of *T. cruzi* at 400 \times phase contrast magnification.

Serology

Serum from mothers was tested for IgG antibodies to *T. cruzi* using indirect immunofluorescence (IFAT) and ELISA. Bloodstream trypomastigotes were used as antigens for immunofluorescence and the test was performed as previously described, except that sera were tested at a 1:40 dilution instead of 1:16.¹³ A modification of Voller's method¹⁵ was utilized in the ELISA test. Ninety-six well ELISA-plates (Dynatech, Alexandria, Virginia) were sensitized with 200 μ g/ml soluble epimastigote proteins. Serum samples were tested at a dilution of 1:200 with alkaline phosphatase conjugated anti-human IgG and para-nitro phenyl phosphate (both from SIGMA, St. Louis, Missouri) as assay reagents. Optical density was measured with

a Titertek Multiskan (Flow, McLean, Virginia). Optical densities greater than 0.1 were considered positive. Positive and negative control sera routinely gave optical densities of ≥ 0.25 and ≤ 0.02 , respectively. Concordance between the two serological methods was 100 per cent. Serum from the infected newborns were submitted to IgM-ELISA using a similar procedure.

Histology

The placentas and adnexae of seropositive mothers were fixed in 10 per cent neutral formalin and examined macroscopically according to Benirschke's method.¹⁶ Eight sections of the whole thickness of the placenta were taken from different areas, including the umbilical cord insertion area, for microscopic examination. Two sections of the umbilical cord and one section of the roll of extraplacental membranes were also examined. Paraffin sections were stained by hematoxylin and eosin. A complete autopsy was performed in all stillbirths including macerated fetuses.

Results

Of 2651 pregnant women who underwent serologic examination, 226 (8.5 per cent) were seropositive. The prevalence rate of seropositivity varied according to the mother's birthplace. For a sample of 472 women who were born in Salvador, 1.7 per cent were seropositive, and for a sample of 1127 of those born in rural areas of Bahia 10.9 per cent were seropositive. Forty mother-infant pairs were excluded because the newborns were not examined. Sixty-two cases were included in the study even though their protocol was not complete; details are summarized in Table 1A.

All the seropositive mothers studied had no previous diagnosis of Chagas' disease.

A group of seropositive mothers was examined by xenodiagnosis and/or blood culture to assess parasitemia. A total of 28.3 per cent (26/92) were positive; 35 per cent (17/48) were positive by xenodiagnosis and 16 per cent (15/92) by blood culture; two mothers who transmitted their infection to the conceptuses had positive parasitemia. Other details of the seropositive mothers and of their newborns are summarized in Table 1B.

Three of the 186 conceptuses studies were stillbirths. In two of them the autopsy and placental examination did not show any evidence of chagasic infection. The results of xenodiagnosis and of DBE were concordant in all the newborns.

Three cases of conceptuses with Chagas' disease were identified: one stillbirth plus two living infants. The overall incidence of transmission was 1.6 per cent (3 cases from 186 seropositive women who delivered conceptuses weighing more than 2000 g). The mothers of the three congenital cases were born in rural areas of Bahia. The clinicopathological aspects of the congenital cases are summarized in Table 2.

TABLE I
A. Summary of 62 cases included in the study despite an incomplete protocol

	Examination performed on child		
	Only DBE	Only xenodiagnosis	DBE and xenodiagnosis
Placentas not examined	2	3	43
Placentas examined	10	4	—

B. Demographic details of seropositive mothers and their children

	Mean \pm S.D.	n
Age of mothers	28.0 \pm 6.2 yrs	183
Parity of mothers	4.9 \pm 3.4 births	184
Weight of infants	3110 \pm 460 g	174
Infants < 2500 g	9.2 per cent (16/174)	
'Symptomatic'* mothers	52.1 per cent (49/94)	

* 'Symptomatic' refers to complaints which might be related to chronic Chagas' disease e.g., palpitations, dyspnea, dizziness, dysphagia, constipation, thoracic pain.

From the 138 placentas examined, four showed specific hematogenous placentitis; three of them belonged to infected conceptuses and one to a newborn free of infection (DBE and xeno negative). The latter one represents a case of congenital transmission at placental level. In all sixteen sections of placenta examined only two parasitized macrophages and two foci of villus inflammation were seen.

Discussion

Our results show that congenital transmission of *T. cruzi* to conceptuses weighing more than 2000 g is much lower than that previously found among fetuses weighing less than 2000 g.¹⁰ In Santa Cruz, Bolivia, Azogue has found incidence rates of transmission of 4 per cent and 14.8 per cent among fetuses weighing more than and less than 2000 g respectively (Personal communication). These rates are higher than those observed in Salvador, Bahia (1.6 per cent in conceptuses > 2000 g, 10.5 per cent in conceptuses \leq 2000 g) particularly as Azogue did not include stillbirths and did not eliminate seronegative mothers. Besides, the rate of congenital chagasic infection is lower in Bahia than in some areas of Argentina where rates of transmission up to 10 per cent are found among newborns of chagasic mothers.⁹ These data indicate that there are regional differences in congenital chagasic transmission. Various authors, elsewhere in Brazil, have not identified cases of congenital Chagas' disease in prevalence studies, but they examined few cases and used different methods for detection.¹⁷⁻²² However, Dias²³ working in a previously endemic area (Bam-

bui, Minas Gerais) where vector-mediated transmission had been interrupted for several years, found no cases of infection in more than 300 children of chagasic mothers. His study did not exclude the possibility of congenital transmission as it is possible that most or all of the infected conceptuses died *in utero*.

The frequency of parasitemia among seropositive mothers studied was comparable to the rate of parasitemia in seropositive individuals of the same age group in an endemic area of Bahia.¹³ Due to a small number of positive cases of congenital chagasic infection we were unable to demonstrate relationship between parasitemia and transmission.

Histological studies limited to the placenta cannot exclude congenital Chagas' disease in the newborn because parasites can be absent in chagasic placentitis as demonstrated in cases I and II (Table 2). On the other hand, the determination of specific IgM may give negative results in positive cases of transmission as occurred in some cases of the literature²⁴⁻²⁶ and in our cases I and II (Table 2). Thus, the parasitologic examination remains the first choice for detecting congenital chagasic infection. However, the DBE without concentration, a simple method generally used in the diagnosis of congenital Chagas' disease, may fail in detecting parasites.²⁷⁻²⁸ Otherwise, the xenodiagnosis and the methods of DBE with previous blood concentration are much more sensitive procedures in the diagnosis of acute Chagas' disease.²⁹ The xenodiagnosis gives 100 per cent of positivity in non-congenital cases of acute Chagas' disease,³⁰ but it is a time-consuming method. In congenital chagasic infection it is generally used in incidence studies.¹⁰ In

TABLE 2
Clinico-pathological aspects of the cases of congenital Chagas' disease

IUA-weight	Clinical and laboratorial data	Specific pathological aspects
Case I 38 w 2700 g	Male clinically normal. DBE (+). Xeno (+). ELISA for IgM (-). Chest x-ray and ECG normal.	Focal granulomatous villitis and perivillitis. Acute choriovasculitis and omphalitis. Parasites in the umbilical vessels (Fig. 1A).
Case II 32 w 2160 g	Female, at birth: Severe anemia, jaundice, hyporeflexia, dyspnea and hepatosplenomegaly. DBE (+). Xeno (+). ELISA for IgM (-). Association with perinatal hemolytic disease. After an exchange transfusion symptomatology improved, but disappeared only with specific treatment.	Focal granulomatous villitis and perivillitis. Acute omphalitis. Parasites in the vessels and amniotic epithelium of the umbilical cord (Fig. 1B).
Case III 35 w 2160 g	Female, macerated stillborn.	Diffuse granulomatous villitis and perivillitis. Chorioamnionitis. Myocarditis. Inflammatory foci in esophagus and intestines (muscular layer) and in the tongue. Meningo-encephalitis (Fig. 2A). Parasites on placental villi, chorionic plate and brain (Figs. 2B and 3).

IUA = Intrauterine age
 W = weeks

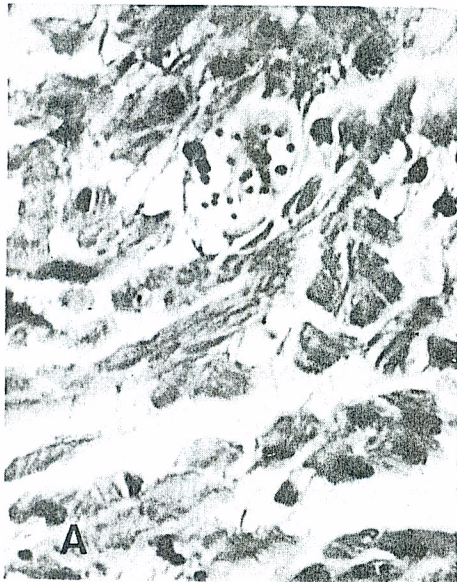


FIG. 1. A. Case I. Parasitized cell in the umbilical vein wall. H and E, $\times 400$. B. Case II. Amastigotes within the amniotic epithelium, umbilical cord. H and E, $\times 1,000$.

the present study, the results of xenodiagnosis and of DBE with previous concentration using microhematocrit tubes were concordant in all the newborns. This technique was used for detection of congenital Chagas' disease for the first time in the present study. It is a simple, sensitive and cheap method which gives instant results without requiring veni-puncture, highly recommended for the diagnosis of Chagas' dis-

ease in the newborn and which can be used even in newborns with very low birth-weight.

In our congenital cases of Chagas' disease the severity of placental involvement was proportional to the severity of fetal involvement as previously observed.³¹ Case I was a normal-appearing newborn and the placenta was only slightly involved. In contrast, the placenta of case III was severely and dif-

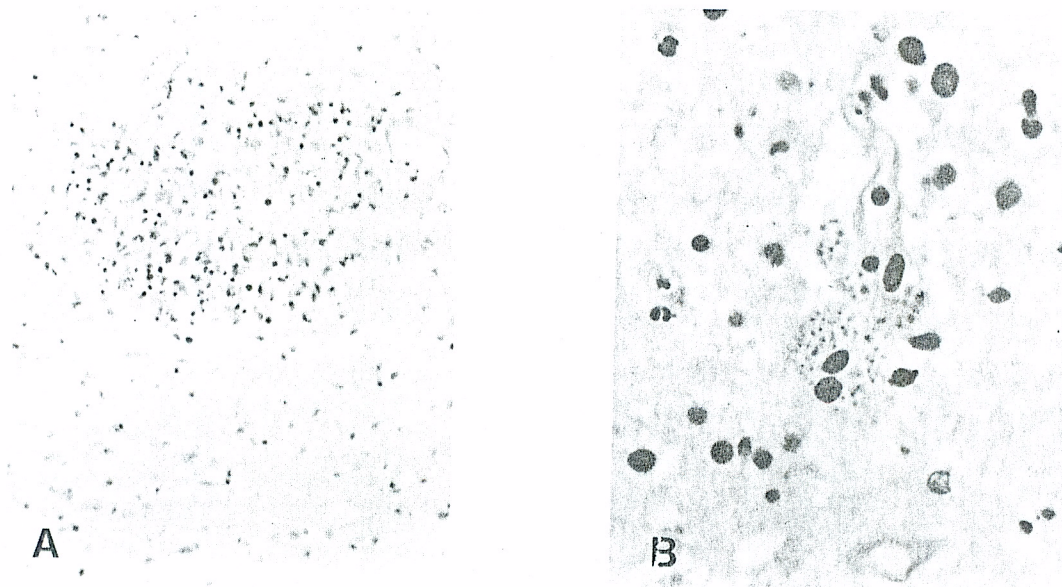


FIG. 2. Case III. Brain. A. Granuloma. H and E, $\times 100$. B. Macrophages filled with amastigotes near a blood vessel. H and E, $\times 400$.

fusely involved, had a marked parasitism (Fig. 3) and was associated with a macerated fetus with severe myocarditis, meningoencephalitis and inflammatory foci in the muscular coat of the digestive tract. In this case, despite the severe grade of maceration, inflammatory foci and parasites were observed in the brain (Figs 2A and 2B). These findings show the importance of the study of macerated fetuses in the diagnosis of congenital chagasic infection.

Case I corresponded to an inapparent acute form of congenital Chagas' disease. In the naturally acquired disease, this form is at least two times more frequent than the symptomatic one.³² Case II had an association with perinatal hemolytic disease. It is known that Chagas' disease can also mimic this disease,⁷ but we believe that the child's jaundice and hepatosplenomegaly were partially due to Chagas' disease, because complete recovery occurred only after specific treatment.

The presence of specific placentitis in the absence of fetal infection has already been referred to in the literature⁹ even in cases of acute maternal disease when parasitemia is high and persistent. In the case here observed placental parasitism was scarce and only two foci of inflammation were demonstrated in all the sections examined.

This study indicates that, in Salvador, the prevalence of Chagas' disease is high (10.9 per cent) amongst mothers originally from the rural areas of the State of Bahia. Therefore a serologic examination for Chagas' disease in these mothers would be advisable during the prenatal period. Although the inci-

dence of congenital transmission of Chagas' disease in conceptuses weighing more than 2000 g in Salvador, Bahia is not markedly elevated, a DBE with the microhematocrit technique of such newborns is justified so that cases of transmission can be identified and treated.

Summary

The incidence of transmission of *Trypanozoma cruzi* from infected mothers to offspring was determined in a hospital-based study of conceptuses weighing more than 2000 g. Out of 186 mother-conceptus pairs examined, three cases of congenital Chagas' disease (two livebirths and one stillbirth) were identified, an incidence of 1.6 per cent. Direct microscopic examination of the child's blood by the microhematocrit technique was found to be as sensitive as xenodiagnosis in detecting infection. The clinicopathological data of the congenital cases are presented.

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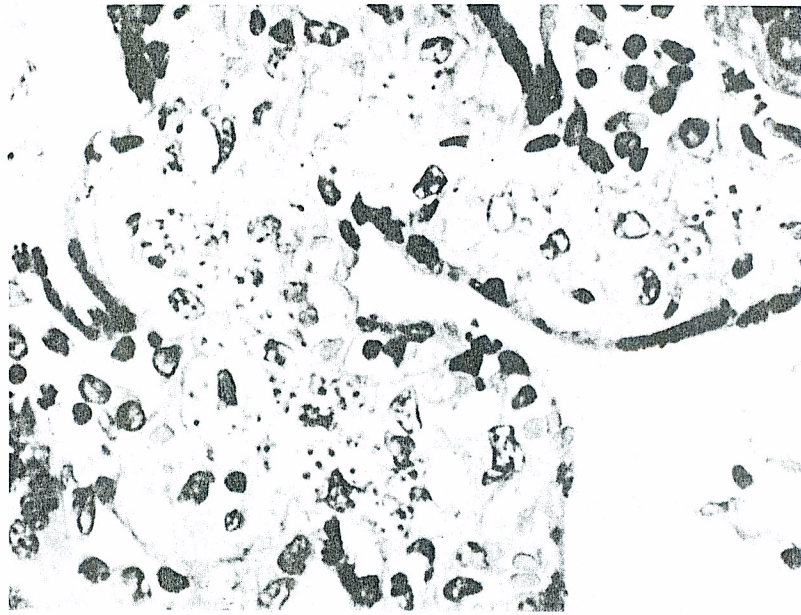


FIG. 3. Case III. Placenta. Villus with many macrophages filled with amastigotes. H and E, $\times 400$.

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