

## EVALUATION OF CHAGAS' DISEASE TRANSMISSION THROUGH BREAST-FEEDING

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*One hundred milk or colostrum samples from 78 mothers with chronic Chagas' disease were parasitologically studied for Trypanosoma cruzi infection by means of direct examination and inoculation of mice. The mice were submitted to direct blood examination three times a week. At the end of 45 days, xenodiagnosis and indirect immunofluorescent test (IFAT) for T. cruzi antibodies were carried out in the animals. No parasitized sample was observed even though five mothers had parasitemia at milk collection. In addition, 97 breast-fed children of chronic chagasic mothers, born free of infection, were tested for IgG antibodies to T. cruzi using IFAT. No case of T. cruzi infection was detected. The authors conclude that breast-feeding should not be avoided for children of chronic chagasic women. However, as these mothers had intermittent parasitemia, they should avoid nursing when there is nipple bleeding.*

Key words: Chagas' disease transmission – milk parasitism – lactogenic transmission

Chagas' disease is a major public health problem in South America. The World Health Organization (1986) estimates that 15 to 18 million South Americans are infected. The infection is generally transmitted by insects (triatomines) but transfusional and transplacental infections have also been reported (Bittencourt et al., 1985; Dias, 1979). Lactation is also considered a possible means of transmission (Mazza et al., 1936; Medina-Lopes, 1983). Mazza et al. (1936) and Medina-Lopes (1983) found trypomastigotes in the milk of two mothers in the acute phase of the disease. In one of these cases there was transmission through lactation (Mazza et al., 1936).

Until now, there was no evidence of milk infection during the chronic phase of Chagas' disease. Recently, Medina-Lopes (1983) reported a two-month-old infant with acute Chagas' disease who acquired the infection during lactation. Congenital and vector transmission were excluded. The mother was in the chronic phase of the disease and presented nipple

bleeding. The search for parasites in the milk was negative; thus, infected blood could have been the source of infection.

The prevalence of Chagas' disease among pregnant women in South America ranges from 2 to 51% in urban centers and from 23 to 81% in rural areas (Bittencourt, 1987). Acute cases caused by vector transmission are not as frequent as some years ago because of insecticide control programs (Silva, 1979). Otherwise, acute Chagas' disease occurs mainly in children and is rarely observed in pregnant women (Bittencourt, 1987). However, the real significance of transmission through lactation among chronic chagasic mothers has not been adequately evaluated, which is our intention in the present study.

### MATERIAL AND METHODS

Serum from mothers admitted to prenatal care of Maternity Hospital Climério de Oliveira (Federal University of Bahia) was tested for IgG antibodies to *Trypanosoma cruzi* using indirect immunofluorescence test (IFAT) and enzyme-linked immunosorbent assay (ELISA), or by the complement-fixation reaction (Guerreiro-Machado). Mothers who had at least two positive serological tests were considered chagasic.

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Most of the mothers were also submitted to one or more xenodiagnoses (xeno). Ten laboratory-raised 5th instar nymphs of *Triatoma infestans* were used; their rectal contents were collected individually 30 days after feeding, mixed with saline, and examined for the presence of *T. cruzi*. One sample of colostrum and one of milk, or two samples of milk taken at intervals of at least two weeks, were collected from 22 mothers; from the other 56 mothers, only one sample of milk or colostrum was collected. The total of 100 samples was examined, corresponding to 66 samples of milk and 34 of colostrum. The samples were usually collected in the first month postpartum but a few were taken up to five months postpartum. Soon after collection, samples were submitted to direct microscopic examination and inoculated in mice.

*Inoculation* — 0.2 ml of the sample was administered intraperitoneally to two or three young outbred mice soon after weaning. Study of the animals was done by direct blood examination (DBE), by xeno, and by serological test. The DBE was done three times a week from the 8th to the 45th day after inoculation. The xeno and the serological tests were performed 45 days after inoculation. Five non-infected laboratory-raised 5th instar nymphs of *Rhodnius prolixus* were used for xeno, and the bugs were examined 30 days after feeding on the mice. Serum from mice was tested for IgG antibodies to *T. cruzi* using indirect immunofluorescence test. Promastigote forms of *T. cruzi* São Felipe strain, cultivated in LIT-R9 medium, were used as antigens (Sadigursky & Brodskyn, 1986). They were washed three times in phosphate-buffered saline (PBS), pH 7.3, and suspended to  $1 \times 10^6$  parasites per ml. Ten  $\mu$ l of the parasite suspension were added to each well of glass slides. Before usage, the slides were fixed 5 minutes in acetone. Mice sera were two-fold diluted in PBS, from 1:5 to 1:2.560. Twenty  $\mu$ l of diluted serum were added to each well, and after incubation for 1 h at room temperature the slides were washed three times in PBS. Twenty  $\mu$ l of goat anti-mouse IgG, FITC conjugate (Sigma F 0257) diluted to 1:20, were added to each well. After incubation of 1 h at room temperature, the slides were washed three times in PBS, mounted, and observed at a fluorescent microscope.

During the collection of milk or colostrum, the mothers were submitted to another xeno.

*Serological study of children of chronic chagasic mothers* — Ninety-seven breast-fed children, in whom congenital infection was excluded soon after birth by DBE (microhematocrit technique) and xeno (Bittencourt et al., 1985), were serologically examined at ages ranging from six months to two years. The serum was tested for IgG antibodies for *T. cruzi* using IFAT as described above; the anti-serum was a goat anti-human IgG, FITC conjugate. Average time of breast-feeding was 7 months. To prevent possible transmission by blood contamination of milk, all the mothers were recommended to avoid breast-feeding if nipple bleeding occurred.

#### RESULTS

Five of the 76 xenos performed on the mothers simultaneously with milk collection were positive. Otherwise, 38 of 77 mothers submitted to one to five xenos during gestation had parasitemia on at least one examination; ten of them had three or more positive xenos.

*Parasitological examination* — Of the 100 milk or colostrum samples, two were not submitted to the complete protocol: the serological examination or the xeno of the inoculated mice was missing. Two hundred and seventy-two mice were examined, being all negative for *T. cruzi*.

*Serological examination of children of chagasic mothers* — All serological tests were negative.

#### COMMENTS

Our results did not show milk parasitism by *T. cruzi*. The 97 children of chagasic mothers did not become infected through breast-feeding. It is important to emphasize that five mothers had parasitemia at sample collection, proving that blood parasitemia did not correspond to milk infection as was observed in the experimental acute phase (Disko & Krampitz, 1971). In the case described by Medina-Lopes (1983), infection of the newborn certainly occurred through nipple bleeding. Miles (1972) demonstrated experimentally that even in the acute phase of the disease, transmission through breast-feeding rarely occurs unless parasites have been frequently found in the milk of mice (Miles, 1972; Disko & Krampitz, 1971; Nattan-Larrier, 1913). Other authors could not experimentally confirm transmission through breast-feeding during the acute phase

of the disease (Disko & Krampitz, 1971; Fine, 1933; Werner, 1954). It has been suggested that the rarity of *T. cruzi* transmission through breast-feeding in acute Chagas' disease may be due to the protection given by maternal antibodies transferred to the young through the milk (Miles, 1972; Disko & Krampitz, 1969; Kolodny, 1939; Siqueira, 1975). Moreover, *T. cruzi* infection by the digestive route is difficult to establish (Miles, 1972).

Considering that breast-feeding is highly beneficial in providing protection against infection and that chagasic women have a low socioeconomic status, the prohibition of breast-feeding could represent a major problem to these women. It is essential to recommend these mothers to avoid breast-feeding when nipple bleeding occurs. However, we believe that chagasic mothers should not be accepted as donors in milk bank programs.

#### RESUMO

**Avaliação da transmissão da doença de Chagas através da amamentação** — Foram estudadas, parasitologicamente, 100 amostras de leite ou colostro de 78 mães chagásicas crônicas, através do exame direto e inoculação em camundongos. Os animais foram submetidos a exame direto, três vezes por semana e, ao fim de 45 dias, também, a xenodiagnóstico e a exame sorológico para pesquisa de anticorpos anti-*Trypanosoma cruzi*. Não houve evidência de parasitismo pelo *T. cruzi* nas amostras estudadas, muito embora em cinco mães tivesse sido documentada parasitemia no momento da colheita do material.

Foram, também, examinados, sorologicamente, através da pesquisa de anticorpos IgG anti-*T. cruzi*, filhos de chagásicas crônicas, nos quais excluiu-se, ao nascer, infecção pelo *T. cruzi*. Nestas crianças não se comprovou infecção chagásica. Os autores concluem que não se deve proibir a amamentação nessas mães, mas como apresentam parasitemia intermitente devem ser recomendadas a não amamentar quando houver sangramento do mamilo.

Palavras-chave: transmissão da doença de Chagas — parasitismo do leite — transmissão lactogênica

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