

# Vertical transmission of *Trypanosoma cruzi* in the Province of Choapa, IV Region, Chile. Preliminary Report (2005-2008)

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## DEDICATION

*The group of doctors who finished their basic studies in 1962 in the Facultad de Medicina of the Universidad de Chile will never forget the official reception we received when we began the program in March, 1955. It was our first day in the Faculty, and we were received in the Anatomy Auditorium by Dr. Gustavo Hoecker. We soon had him as a professor in the first year Biology course, and in meetings on Saturday afternoons in Borgoño 1470, the site of the former Parasitology Hall of Dr. Amador Neghme. Years later some of us joined forces with Dr. Hoecker in research projects on Chagas disease in which he led the group. We took many field trips to the different endemic areas of the IV Region; Tulahuén, Río Hurtado, El Palqui and Huatulame, among others. We left early in the morning and usually returned late at night. In these interactions I got to know the real Dr. Hoecker; intelligent, lively, discerning and with a great group spirit. When my class of 1962 celebrated our 40<sup>th</sup> year as doctors in June, 2002, we did so with a solemn ceremony in the Facultad de Medicina, in the same auditorium where he received us on our first day of classes. In this ceremony we gave recognition to two professors, Gustavo Hoecker and Carlos Oberti (Histology Professor), which faithfully reflected the opinion of his former students. Dr. Hoecker is no longer with us, but his spirit is. In modest homage to him, we dedicate our most recent research on congenital Chagas disease, a subject which united us on more than one occasion.*

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## ABSTRACT

Congenital Chagas disease acquired special importance in Chile after the certification of the control of *Triatoma infestans* and transmission by blood donors affected with *Trypanosoma cruzi*. In order to establish adequate protocols for intervention and control in infected mother-neonate pairs in endemic zones of Chagas disease, we present partial results (2005-2008) of a pilot project which is being carried out in the Province of Choapa, IV Region, Chile, whose objectives are: determine the current prevalence of the disease in pregnant women, estimate the incidence of vertical transmission of *T. cruzi* to newborns, determine the lineages of the parasite present in mothers who do and do not transmit the disease, determine the prevalence of Chagas disease in maternal grandmothers of neonates and study placental histopathology. Preliminary results indicated that in this study period, 3.7% of the women who gave birth in the Province have Chagas disease and 2.5% of their newborns were infected. The most frequent *T. cruzi* genotypes found in mothers studied during pregnancy were TCI and TCII, either alone or in mixed infections. A high percentage (74.3%) of the grandmothers studied was infected with the parasite. In 29 placentas from mothers with Chagas disease we observed edema, necrosis, fibrinoid deposits and slight lymphoplasmocyte infiltration. In three placentas we found erythroblastosis and in one of them amastigote forms of *T. cruzi*; this was one of the cases of congenital infection. The evaluation of the diagnostic and control protocols generated will allow us to determine if it has been possible to modify the natural history of vertical transmission of *T. cruzi* in Chile.

**Key terms:** Congenital Chagas disease, Province of Choapa, multidisciplinary study.

## INTRODUCTION

The IV Region of Coquimbo is located between 29° 02' and 32° 16' S in the area of transverse valleys of Chile; it is divided into the Provinces of Elqui, Limarí and Choapa. It has three principal geographical features; the Andes Range, the complex formed by the Coast Range and Andes foothills, and flat coastal areas. Its climate is semi-arid or steppe, with a shrubby vegetation dominated by *Acacia* spp. (Benítez,

1994). It has an area of 40,580 km<sup>2</sup> and a population of 603,210, of which 21.9% are rural (INE, 2003).

Some rural areas of the IV Region are still considered as zones of high Chagas endemism due to the high indices of domestic infestation (66.9%) (Correa et al., 1984), and infection of humans (41.2%) (Apt and Reyes, 1986), animals (41.2%) (Rios et al., 1986) and triatomines (37.8%) (Apt and Reyes, 1986) reported in past decades. Although in 1999 Chile was certified to have interrupted the mechanisms of

vector transmission of *Trypanosoma cruzi* by *Triatoma infestans* and by blood bank transfusions, vertical transmission is still a public health problem (Schenone et al., 2003).

In 2005 we began a multidisciplinary study of congenital Chagas disease in the Province of Choapa, whose preliminary results we present here. Our original objectives included determining the current serological frequency of Chagas infection in pregnant women, the incidence of congenital transmission and the lineages of parasites in mothers who do and do not transmit the disease, as well as determining the percentage of infection in the maternal grandmothers of the neonates as epidemiological information and studying the characteristics of placental histopathology.

## MATERIAL AND METHODS

### *Implementation of Control Pilot Plan*

We began to apply the protocols designed for the vertical study of *T. cruzi* in the province of Choapa in 2006, with the collaboration of professional teams from the urban and rural health centers of the Ministry of Health and the Municipal Departments of Health, after formal training. The protocols were designed to find Chagas infection in pregnant women, study the mother-neonate pair, confirm or discount vertical transmission in newborns and/or long-term follow up, treat infected neonates and incorporate Chagasic mothers to the regular controls of chronic Chagas disease post partum for specific treatment.

### *Study population*

The study included pregnant women from the four communities of the Province of Choapa; Los Vilos, Canela, Salamanca and Illapel, who gave birth in one of the medical centers in the period 2006-2008. Mothers whose children were born in 2005 were retrospectively tested serologically for parasites in 2006. According to the protocol, local centers requested a screening serological exam (ELISA IgG) during the first or second third of pregnancy. The confirmation of positive cases and detection of false positive reactions were performed in our laboratory or the regional laboratory (La Serena Hospital, Coquimbo Health Service), by Indirect Immunofluorescence (IIF IgG). It was recommended that this information be included on the health card with which the mother is admitted to the maternity ward to give birth, thus alerting personnel to perform the study of the mother and newborn.

### *Conventional quantitative serological tests*

IIF tests were performed according to Zulantay et al (1998). *T. cruzi* epimastigote forms of the Tulahuén strain were used as antigens. The diagnosis titer of IIF IgG was 1/20. The ELISA test of neonates was performed in the Laboratorio de Referencia de Parasitología, Instituto de Salud Pública de Chile, ISP (Apt et al., 2008).

### *Study of the mother-neonate pair*

Before birth, 4 cc of venous blood was extracted from the mother; 2 cc in a tube without anticoagulant for IIF IgG and 2

cc in a tube with guanidine-EDTA for the parasitological study with PCR. In the delivery room, 5 cc of blood was taken from the umbilical cord of the neonate, which was divided into 1 cc for the direct parasitological study searching for mobile trypomastigotes of *T. cruzi*, 2cc for the parasitological study with PCR to detect kinetoplastic DNA of *T. cruzi* and 2 cc for the conventional serological study (IIF and ELISA IgG).

### *Blood PCR*

This test was performed under conditions already described (Solari et al., 2001b, Zulantay et al., 2004). The blood sample for the PCR test was boiled for 15 min at 97° C to decatenate mini-circles from the kDNA network and stored at 4° C. DNA extraction was performed from 200 µl of the mixture and chromatographic purifications (Favorgen, Biotech Corp). The PCR components included a buffer, oligonucleotides 121 and 122, dNTP, Mg, Taq polymerase and a DNA source of template from each patient in 50 µl total volume. The PCR conditions were: an initial cycle of 98° C for 2 min, a second cycle of 98° C for 1 min and 72° C for 2 min; 33 cycles of 94° C for 1 min, 64° C for 1 min and 72° C for 2 min and a final cycle of 72° C for 10 min. Later 10 µl of the final product was electrophoresed in a 2% agarose gel for 90 min at 113 volts and stained with ethidium bromide. All PCR tests were performed three times, each time using negative and positive PCR controls. The presence of a 330 bp DNA band was considered a positive test. Finally, a digital photo was taken of the gel.

### *Genotyping of T. cruzi infecting chagasic patients*

DNA samples amplified by PCR were characterized with hybridization tests and Southern analysis using a panel of *T. cruzi* genotype-specific probes (Solari et al., 2001a). Identical membranes containing DNA samples were prepared to be hybridized with each of the six available DNA probes belonging to the subgroups TcI, TcIIb, TcIIId and TcIIe circulating in Chile. The construction of the probes and their validation as a typing method is available (Veas et al., 1991; Galupo et al., 2009).

### *Study of Chagas infection in maternal grandmothers of newborns*

After the delivery, the maternal grandmothers of babies born between 2005 and 2008 were studied serologically, either by citation to the medical centers or by a house visit.

### *Anatomic and pathological study of the placenta*

Twenty-nine placentas of mothers with Chagas were submitted to macroscopic analysis (dimensions, weight, conformation, alterations), routine optical microscopy with hematoxyline and eosin and an immunohistochemical study with the S-ABC method using monoclonal antibodies against actin. Each placenta was photographed and then a complete serial and systematic section was performed; sections were fixed in 10% formalin buffer (10:20:1). For the routine optical microscope study we took sections from the four quadrants, while the immunohistological study used syncytiotrophoblast cells from selected sections stained with hematoxyline and eosin.

## RESULTS

### *Serological prevalence of Chagas infection in pregnant women*

There were 3778 live births in the Province of Choapa in the period 2005-2008. Serological information of the mothers was previously available for 87.6%, 88.3% and 77.6% of the births in the centers of Illapel, Salamanca and Los Vilos, respectively (except for the year 2005 for the Los Vilos Hospital, in which serological studies were not performed). Infection by *T. cruzi* was confirmed in 123 mothers, which represents 3.7% of the births in this period. The mean age of mothers was 32 years. Nine cases (7.3%) were from Los Vilos, 26 (21.1%) from Canela, 42 (34.1%) from Illapel and 46 (37.4%) from Salamanca.

### *Study of the mother-neonate pairs*

We performed genotyping studies of all mothers in which *T. cruzi* was found when they delivered (Solari et al., 2001a). Sixty percent of the mothers had been examined regularly as chronic Chagas cases, including clinical, serological, electrocardiographic and parasitological examinations. In addition, the evaluation of hepatic function, hematology and other exclusion factors such as pregnancy or nursing were a pre-therapy condition for specific treatment. We were able to perform a study at birth and serological and parasitological follow-up on 80 of the 123 children of mothers with Chagas disease who gave birth in the years 2005-2008. Thirty three of the 43 chagasic mothers not included in this investigation correspond to mother-child pairs of 2005 on whom we have only performed a retrospective study. Congenital transmission was confirmed in two cases, which gives a preliminary transmission index of 2.5%, with 95% confidence limits of 0.3% and 8.7%. The other cases not examined included babies who are still less than a year old, deceased neonates, mothers who did not agree to participate in the study and families who moved out of the area or gave their child in adoption.

### *Study of maternal grandmothers of newborns*

We investigated infection of *T. cruzi* in 70 of the mothers of the 123 women in the study who were found to have Chagas disease (maternal grandmothers of neonates). We found that 74.3% (95% confidence limits 66.4% and 84%) were infected with the parasite, and 53% of those did not know they were infected (95% confidence limits 39.5% and 67.8%). The age range of the grandmothers was from 40 to 82 years, with a mean of 62 years.

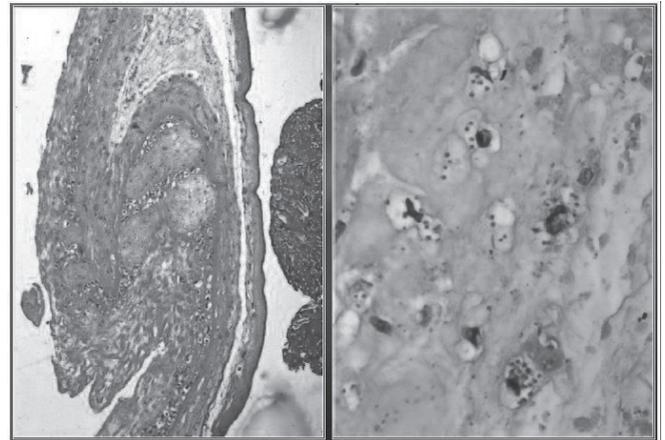
### *Genotyping of mothers infected with *T. cruzi**

The preliminary evaluation of *T. cruzi* genotypes infecting chronic mothers provided epidemiological data from 20 persons. The most frequent *T. cruzi* genotypes were TCI and TCII alone or combined as mixed infections. Other *T. cruzi* genotypes (TCIb and TCIIe) have not yet been detected.

### *Anatomical and pathological study of the placentas*

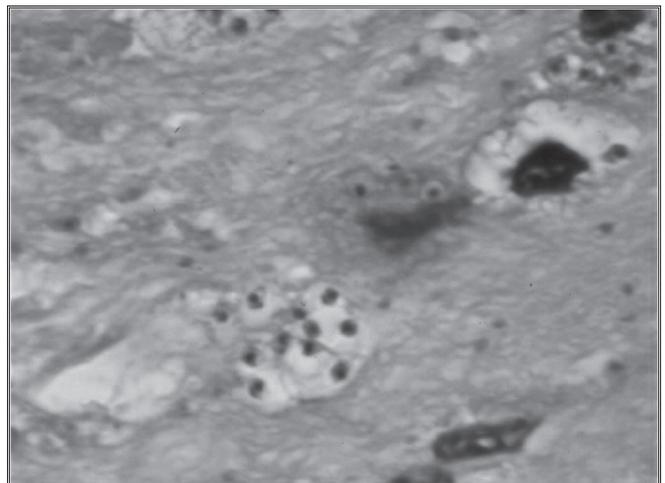
The membranes were complete in 22 cases; the umbilical cord measured an average of 31.2 cm with a range from 8.5

to 58 cm; mean diameter of the cord was 1.4 cm (range 0.5-1.8 cm). The insertion of the cord was paracentral in 21 cases, marginal in 4, central in three and had an eccentric location in one case. Three of the cases had membranes with scarred lesions, two had edema and one had a lesion with imprecise border. Microscopic examination demonstrated edema, necrosis, fibrinoid deposit and slight lymphoplasmatic infiltration in 29 placentas. We found erythroblastosis in three placentas, in one of which we found amastigotes of *T. cruzi* (one of the neonates with congenital infection). In three cases the immunohistological study demonstrated a decrease in actine expression in the trophoblast cells (Fig. 1). In the placentas of the non-Chagasic mothers there were no differences with the placentas of the Chagasic mothers, with the exception of erythroblastosis and amastigotes forms that were present only in the latter group where the lymphoplasmatic infiltration was more intense than in the normal placentas.



**Fig. 1a:** Placenta membranes with inflammation and parasites in the cytoplasm of macrophages. Hematoxiline & Eosine 40x.

**Fig. 1b:** Placenta membranes with inflammation and parasites in the cytoplasm of macrophages. Hematoxiline & Eosine 200x.



**Fig. 2:** Macrophages with parasites in the cytoplasm Hematoxiline & Eosine 1000x.

## DISCUSSION

The prevalence of Chagas infection in the general population of the endemic zones of Chile has decreased notably in the last two decades, due principally to the success of the Program of Vector Control (Moncayo, 2003). This reduction in seropositive cases of the general public has also been favored by the housing policy developed in rural areas of Chile, which has produced important advances in groups of people who had grave housing deficiencies (materials or health conditions) (Mideplan, 1998). Rural subsidies have reduced this percentage from 16.7% in 1990 to 9.9% in 1998 (Mideplan, 2000). Between 1992 and 2002 the percentage of rural houses with electricity increased from 54% to 79% (CNE, 2003). Finally, the level of education has played an important role in the decrease in the incidence of Chagas disease. In 1904, 49.7% of the population of Chile was analphabetic, while the 2002 census indicated only 4.3%. In the IV Region, of a total of 68,277 inhabitants 10 years or older, there are about 5,582 analphabetic persons; in other words, 91.82% of the inhabitants know how to read and write (INE, 2003). However, these advances are not indispensable to avoid the congenital transmission of *T. cruzi*, which is a pressing public health problem in countries of the Southern Cone (Carlier and Torrico, 2003). Since there is not yet a ministerial norm for the study of congenital transmission in Chile, in order to establish appropriate actions of intervention and control it is indispensable to know the current prevalence of Chagas infection in pregnant women, especially among those who live in endemic areas and are known to be at risk because they have been in contact with the domestic vector (vinchuca) or report having been bitten by this insect. Previous studies of infected women have shown the prevalence in urban and rural areas of Latin American countries to vary between 2% and 51% (WHO, 1991); yearly cases of congenital infection by *T. cruzi* have been estimated at 1500 (Carlier, 2007), with rates from 1-12% (Virreira et al., 2006).

Preliminary studies of the prevalence of infection of pregnant women in the IV Region of Chile found 33.1% (Lorca et al., 1987), 15.6% (Aguilera et al., 1988) and 7.8% (García et al., 2001). Our study found a prevalence of 3.7% in the Province of Choapa, IV Region, for the period 2005-2008. Since pregnancy is considered as a physiological state, these percentages of the prevalence of Chagas disease in pregnant women, considering the range of fertility to be from 15 to 49 years (INE, 2003), are similar to that of the general population in this age range. The preliminary estimation of congenital transmission of *T. cruzi* (2.5%) is similar to estimates in the international literature (Howard, 1962; Muñoz et al., 1992; Carlier and Torrico, 2003; Gürtier et al., 2003; Torrico et al., 2004). The current information on maternal infection, congenital transmission and fertility in the Province of Choapa will allow prediction of the cases expected for the region, and thus establishment of adequate protocols of diagnosis and treatment, which is the purpose of this pilot project.

The information gathered up to now about *T. cruzi* genotypes found in the mothers indicates that the TCI is one of the most represented, suggesting that this lineage is probably transmitted to newborns, in contrast to Bolivia and

Argentina where most vertical transmissions are only TCIId genotypes (Virreyra et al., 2006; Corrales et al., 2009).

A number of authors have suggested that congenital Chagas disease requires a multidisciplinary approach within the family group. The prevalence of congenital transmission in siblings of infected newborns in Salta, Argentina was 31.4% (32 of 102 children), while no infected siblings were found in families without congenital cases. In four of these cases two generations of transmission were documented (grandmother to mother and mother to newborn), thus it was recommended to test siblings of infected neonates even if they are asymptomatic (Sánchez et al., 2005). Our study confirmed the importance of a family epidemiological study. As expected, the maternal grandmothers had a high percentage of infection (74.3%), since they acquired the disease in an epoch when there was not an adequate control of parasitosis. We are currently studying *T. cruzi* infection in siblings of mothers with Chagas disease whose offspring were born in the period 2005-2008.

The study of placentas allowed us to demonstrate that the infection by *T. cruzi* induces the depletion of microfilaments in the syncytiotrophoblast of the human placenta and is associated with premature birth, spontaneous abortion and placentitis. The parasite has been found especially in the corionic fibroblasts and in the subamniotic mesenchyme of the marginal cavity where the membranes attach to the corionic plate. Since we did not observe signs of villitis or lesions of the intervillitis, we suggest that the passage of parasites from the mother is by the corionic route, without direct invasion of the trophoblast (Sartori et al., 2003). Mjihdi et al. (2002) observed that placentas infected with *T. cruzi* contained inflammatory infiltrations and developed ischemic necrosis, deposits of fibrin and vascular thrombosis. Morphological alterations due to chronic and acute infection have been described principally in extraplacental areas, both at the level of optical microscopy and ultrastructure (Oddo et al., 1992; Labarca et al., 1992; Solari et al., 1993). In our observations, the macroscopic microscopic alterations observed without congenital transmission of *T. cruzi* were notable. In one placenta with erythroblastosis, we demonstrated the presence of amastigote forms of the parasite, which coincided with the congenital infection of the newborn. The decrease of the actine expression in the trophoblast cells has been interpreted as a diminishing and reorganization of the microfilaments of the cytoskeleton by the earlier action of *T. cruzi* in the vertical transmission. The evaluation of the diagnostic and control protocols generated will allow in the future evaluating whether it has been possible to modify the natural history of the vertical transmission of *T. cruzi* in Chile according to the international recommendations for our endemic zones (OPS, 2004).

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