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ORIGINAL ARTICLE

Congenital infection by *Trypanosoma cruzi* in an endemic area of Chile: a multidisciplinary study

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Background: This study investigated the prevalence of Chagas disease (ChD) in pregnant women in Choapa Province (IV Region, Chile) and the vertical transmission of *Trypanosoma cruzi*.

Method: ELISA and IFI IgG for ChD was performed for the pregnant women. PCR for *T. cruzi* was done for all chagasic mothers and their newborns. The congenital infection was confirmed by serial positive PCR and/or ELISA or IFI IgG after age of nine months. The placentas of mothers, with and without ChD, were submitted for histopathology and immunohistochemical study.

Results: From 4831 deliveries in 2005–2009 with a serological coverage of 88.6%, it was established that 147 cases (3.4%) had ChD. More than 80% of the pregnancies had a physiological evolution and 90% of the newborn were term. Congenital transmission was demonstrated in six children (4.7%) of the 127 newborn studied by serial PCR (at birth and/or between 3–18 months) and/or ELISA or IIF IgG after age nine months. Most of congenital cases were asymptomatic (67%). The histopathology shows edema, necrosis, fibrinoid deposit in the placentas of 28 of 29 chagasic mothers. In three cases the immnunochemistry demonstrated a decrease in actin expression in trophoblast cells. In one congenital case amastigote nests was observed.

Conclusions: These results indicate that *T. cruzi* infection in pregnant women and vertical transmission in Chile are still prevalent. For this reason it is important to propose control measures in endemic areas of the country.

Keywords: Congenital Chagas disease, Newborn, Maternal infection, *Trypanosoma cruzi*

Introduction

Chagas disease (ChD) is an important public health problem in Latin America, affecting 8–12 million people of endemic countries.[1,2] It is one of the neglected parasitic diseases, along with others, including sleeping sickness and leishmaniasis. The infection is acquired mainly by triatomine insect vector, blood transfusion, transplacental, oral and transplant routes. In Chile and other South American countries the control of the domestic vectors (*Triatoma infestans*) by insecticides, education programs, improvement of dwellings and the screening of blood donors in blood banks dramatically decreased the incidence of the disease,[3] increasing the relative importance of vertical transmission in the development of new cases.[4] Congenital and oral transmission represent important active routes of *Trypanosoma cruzi* infection for which no kind of control exists in many endemic countries.[2,5,6] Maternal–foetal transmission has become partially responsible for the urbanization of ChD and its spread into non-endemic countries.[1,5] The congenital transmission rate varies between different countries, distinct geographical areas, rural and urban localities, ranging between 2.4 and 18.2%. [7–10] It has been postulated that the main factors involved in vertical infection are the intensity of the parasite load (parasitemia), the specific immune response of the mother/newborn and possibly the genotype of the infecting *T. cruzi* (TcI-TcVI).[9–12]

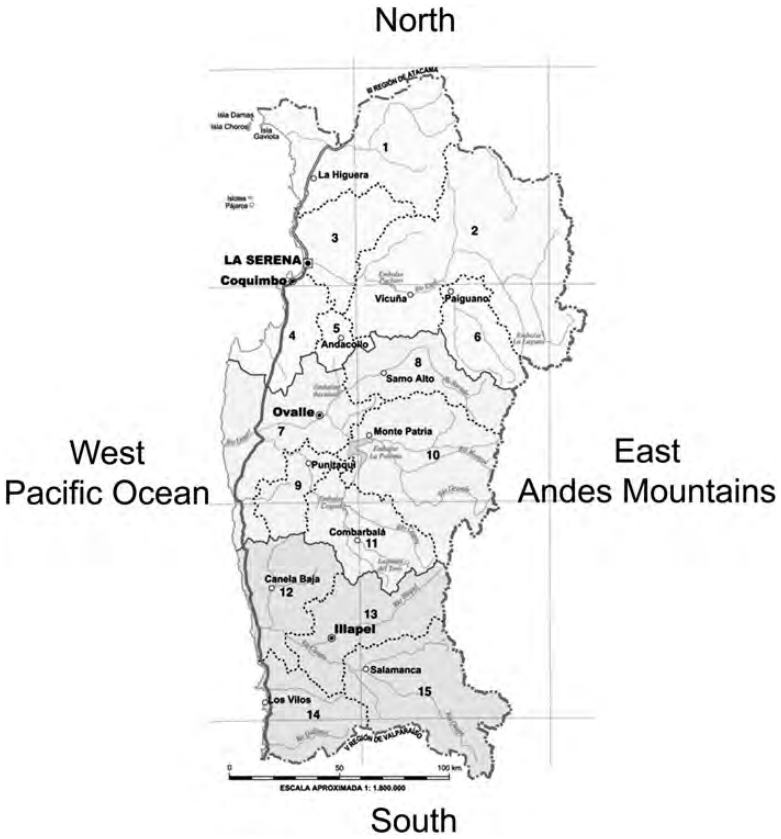

Figure 1. IV Region, Chile, Province of Choapa. (Longitude 71°40min and -70°10min West. Latitude 31°15min and 32°10min South). Localities of Salamanca, Illapel, Los Vilos and Canela.

Choapa Province belongs to the transverse valleys of Chile, IV Region, and is divided by four localities, Salamanca, Illapel, Los Vilos and Canela (Figure 1). It has three principal geographical features: the Andes Range; the complex formed by the Coast Range and Andes foothills; and coastal valleys. Its climate is semi-arid or steppe, with a shrubby vegetation.[13] The rural area of the province is still considered a high endemic Chagas region.[13,14] Most infected people acquired the parasitosis 10–20 years ago. In 2005, we began a prospective multidisciplinary study of congenital ChD in this province to determine the frequency of the disease in pregnant women, the incidence of congenital transmission and the characteristics of the placental histopathology. For this purpose, we designed three protocols to study congenital ChD.[15] Here we present our final results for 2005–2009.

Material and methods

Design of protocols

The protocols were designed jointly with professionals of the local hospitals to detect ChD in pregnant women, study the mother–neonate pair, confirm or discard vertical transmission in newborns and/or in follow-up, treat infected neonates and incorporate mothers with ChD to the regular controls of chronic ChD for specific treatment post lactation.[16]

Population studied

Pregnant women and their newborns from the IV Region, Province of Choapa which is located between 29° 02′ and 32° 16′ South, area of transversal valleys of Chile, localities of Los Vilos, Canela, Salamanca and Illapel with children born in 2006–2009 were included. Mothers whose children were born in 2005 were retrospectively studied in 2006. The informed consent for this study was approved by the Ethics Committee of the Faculty of Medicine of the University of Chile. All the infected mothers were submitted to an epidemiological survey considering her proceeding (rural or urban), education level, obstetrical gynaecological history and co-morbidity.

Serological study

In the health centres of the province serological screening for *T. cruzi* was performed by ELISA IgG during the first or second third of pregnancy with an ELISA CHAGAS III kit (Grupo-Bios, SA, Chile). All the positive results of the screening were confirmed by ELISA (cut-off value 0.2 optical density) and indirect immunofluorescence IgG (IIF) (diagnosis titre 1/20), using as antigens *T. cruzi* epimastigote forms of the Tulahuén strain DTU TcVI obtained from axenic culture (Dr J. Diego Maya, Farmacology Laboratory, Faculty of Medicine, University of Chile). Positive results were included in the health card by which the mother is

admitted to the maternity ward to give birth, thus alerting health personnel to perform the study of the mother and newborn. The same serological techniques were applied in the follow-up of the newborn.

Educational program

Mothers with positive serology for *T. cruzi* were invited to educational meetings performed by the staff of investigators of the study. The education program included biological, epidemiological, clinical, diagnostic, therapeutic and preventive aspects of ChD, a diagnostic test and a test of acquired knowledge, oral presentations with audio-visual support and delivery of printed material. The educational actions had special reference to congenital transmission and the agreement of the mother to the follow-up of their newborn and eventually treatment if congenital infection was confirmed.

Study of pre-delivery mothers and their newborn

Before birth, 4mL of venous blood was extracted from the mother; 2mL were received in a tube without anticoagulant for ELISA and IIF IgG and 2mL in a tube with guanidine-EDTA for the parasitological study with PCR. In the delivery room, 4mL of blood was taken from the umbilical cord of the neonate, 2mL for the parasitological study with PCR to detect kinetoplastic DNA of *T. cruzi* and 2mL for the conventional serological study (ELISA and IIF IgG).

PCR

This test was performed under conditions described previously.[17] Briefly, the blood sample for the PCR test was boiled for 15 min at 97 °C to break mini-circles from the kDNA network, and stored at 4 °C. DNA extraction was performed with 200 μL of the mixture and chromatographic purification (Favorgen, Biotech Corp, Vienna, Austria). The reactions were performed in triplicate with oligonucleotides 121 and 122, which anneal to the four conserved regions present in the minicircles of *T. cruzi*, dNTP, Mg, Taq polymerase and 5 μL of DNA source of template in 20 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 extension of 72 °C for 10 min. The 330-base pair PCR product was separated by electrophoresis in a 2% agarose gel and visualized by staining with ethidium bromide. We conducted a random sampling of specimens with negative PCR to perform a control test inhibition with ß globin. Each experiment included 5 μl of BenchTop 100 bp DNA ladder (Promega, Madison, Wisconsin, USA); a PCR control that contained water instead of DNA; DNA of non chagasic patients and a positive control of the purified DNA *T. cruzi* Tulahuen strain.

Clinical aspects of the newborn

The following features were recorded: gender, weight, size, Apgar test and gestational age; premature or term was calculated based on the last menstrual period to which was added ten days (date of pregnancy) and nine months (date of birth), and by ultrasound. Physical examination of all cases was performed.

Confirmation of congenital cases

The newborns with positive PCR in umbilical cord blood were confirmed by serial positive PCR in peripheral blood and/or ELISA or IIF IgG after age nine months. The cases with negative PCR at birth were followed by conventional serology after age nine months.[4]

Study of the placenta

The placentas of mothers with and without ChD were submitted to macroscopic analysis (dimensions, weight, conformation and alterations), routine histopathology analysis (hematoxiline and eosin staining [HE]) and 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 histopathology study we took sections from the four quadrants. The immunochemistry analysing specifically the syncyotrophoblast was performed in previous selected areas from de HE staining. Electron microscopy was performed in samples fixed in glutaraldehyde buffer and included in epon. The semi and fine cuts were stained with uranyl acetate.

Treatment of congenital cases

Five of the six confirmed congenital cases were treated with nifurtimox 15mg/Kg/day for 60 days under medical supervision. Biochemical profiles and haemogram were performed before treatment, once a month during treatment and up to two months post-therapy, according to established treatment protocols.[15]

Statistical analysis

The data were entered into SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The data are nominal, thus descriptive statistics used tables and especially graphs to summarize and illustrate.

Results

The Province of Choapa is an endemic zone of ChD. For this reason it was important to know the current situation of congenital infection. In this province during 2005–2009 there were 4831 deliveries. With a serological coverage of 88.6% (4280 deliveries) we found that 3.4% (147 cases) of the women presented anti-*T. cruzi* antibodies by IFI IgG with titres from 1/40–1280 and ELISA with optical density between 0.2 and 2.20. The prevalence decreased from 6.2% to 1.9% in this period (Table 1).

The maternal average age was 30.1 years (range 15–44 years). Of the population studied 59.7% was rural (86 cases) and 40.3% urban (58 cases). Most (71.7%; 104 cases) of the mothers were from the communities of Salamanca and Illapel, where the majority of the inhabitants of the Province live, 37.9% (55) and 33.8% (49) cases respectively. Of the other mothers 18.6% (27) were from Canela and 9.7% (14 cases) from Los Vilos; 71.4% (10) of the mothers from this last locality were from rural zones, as were most mothers of Salamanca and Canela (67.3% (37 cases) and 59.3% (16 cases), respectively).

Table 1. Prevalence of *Trypanosoma cruzi* infection in pregnant women, IV Region, Chile, Province of Choapa, localities of Salamanca, Illapel, Los Vilos and Canela, 2005–2009

Year	Deliveries	Deliveries with serology		Prevalence of *T. cruzi* infection	
	n	n	%	n	%
2005	766	550	71.8	34	6.2
2006	926	834	90.1	29	3.5
2007	1041	935	89.8	32	3.4
2008	1045	966	92.4	33	3.4
2009	1053	995	94.5	19	1.9
TOTAL	4831	4280	88.6	147	3.4

However, this relationship was reversed in Illapel where only 47.9% (23 cases) were rural. In relation to maternal education 97.9% (137 cases) had primary school education, yet only 27.1% (38) had secondary education and 5.0% (7 cases) higher education 2.1% (3 cases) complete and 2.9% (4 cases) incomplete. The majority of the mothers are housewives (80.7% (113)) and only 12.9% (18) have some work activity.

The obstetrical gynaecological history revealed that 22.7% (32) of the mothers were primiparous, 51.1% (72) had a history of one or two previous pregnancies and 26.2% (37) had three or more. 13.6% (20) of the mothers had any comorbidity during pregnancy; two pathologies were more frequent, hypertensive syndrome and gestational diabetes.

More than 81.2% (108) of the pregnancies had a physiological evolution; 68.4% (91) of the deliveries were vaginal and 94.7% (125) in cephalic presentation. 90.2% (120 cases) of the newborns were term.

Each child of chagasic mothers was assisted at birth by a paediatrician; an 'ad hoc' file was designed to record all the data of the physical examinations. Because the majority of the children did not have a full record, it was necessary to review the clinical medical records of both mothers and newborn. Despite this, it was possible to rescue 90% of the records.

In relation to clinical parameters of the newborns, 55% were female with an average weight of 3.28 kg, average height 49.4 cm and head circumference 34 cm. Over 90% of the newborns had a normal physical examination at birth. The Apgar average was 8.5 at one minute and 8.9 at 5 minutes. Of the 18 newborns (14%) who presented alterations in the physical examination at birth or in the first 24 hours, 39% were pre-term newborns, with an average weight less than 3 kg; four of them weighed less than 2.5kg (low birth weight).

Of the six confirmed congenital cases, five were term newborns and only one was premature (33 weeks). The average weight was 3.11 kg and average size was 49.2 cm. The average head circumference was 34.4 cm. Apgar scores averaged 8.8 the first minute and 9.2 after 5 minutes. Three cases were apparently healthy at birth and were considered as asymptomatic; one of the remaining three cases presented a transient hypotonia and the other two had respiratory symptomatology cyanosis and grunting (costal retraction and respiratory difficulty). One of these developed bronchopneumonia and the other respiratory distress. Both had a good evolution after treatment.

Of the 147 mothers with ChD it was possible to complete the study in 113 of their newborns at birth; 92 of them had serological study (81.4%) of which 93.5% (86 cases) were IIF and ELISA positive. Six cases had negative serology at birth and were followed up and checked again for anti-*T. cruzi* antibodies at one year old with the same results. In relation to the parasitological study from 89 newborns, only 9 (10%) had positive PCR. From them, the serological and/or parasitological follow-up confirmed the congenital infection in three cases and in four cases it was discarded by serial PCR and serological study. The other two cases were lost by transfer to other regions.

Serological and parasitological follow-up during 2005–2009 was performed in 86.4% of the cases (127 children). The first follow-up was performed at an average of 11.3 months (range 2–40 months) of which 10.2% were serologically positive (13 cases) and 4.9% PCR positive. The principal reason of late first follow-up was migration of the mothers to other non-endemic localities. The follow-up allowed confirmation of congenital transmission in six children (4.7%). Two cases researched in 2006 were confirmed before one year of age (8 and 11 months). In the first case no tests were conducted at the time of delivery. At three months of age serology and PCR were performed; these tests were repeated at eight months and persisted as positive and the infection was confirmed. The second case was confirmed by positive conventional serology and PCR at birth. A second follow-up performed at 11 months of age gave the same result. One case in 2008 was confirmed by serial conventional serology. The other three cases were confirmed in 2009 by serial PCR and conventional serology.

From the newborns of the 147 mothers with ChD, 20 were not studied (13.6%) for the following reasons: transfer to other regions (6), abortions (2), stillbirths (4) and rejection of the mothers (8).

In relation to histopathology, 29 placentas of chagasic mothers and 10 of non chagasic mothers were studied. In the placentas of the chagasic cases, the membranes were complete in 22 cases; the umbilical cord measured an average of 31.2 cm with a mean diameter of 1.4 cm. Its insertion was paracentral in 21 cases, marginal in four, central in three and had an eccentric location in one case. Microscopic examination demonstrated edema, necrosis, fibrinoid deposit and slight lymphoplasmatic infiltration in 28 placentas. In three cases the immunochemical study demonstrated a decrease in actine expression in the trophoblast cells and intense erytroblastocis. In the placenta of one congenital case amastigotes forms, erythroblastosis and intense lymphoplasmatic infiltration was observed.

Due to administrative problems (late receipt of nifurtimox) therapy was initiated at 14 months of age in the first congenital case of the 2006 newborns. The treatment was carried out for two months, with clinical and laboratory monitoring with blood counts and liver function tests at baseline, monthly during treatment, until 15 days after therapy. An acute diarrhoea syndrome with dehydration was observed during the first month of treatment, and a slight rash on the back during the second month. After two years the serology was borderline positive and PCR negative. In the second case, the mother rejected the therapy.

A second interview to raise awareness was unsuccessful: she did not accept the treatment. The four congenital cases confirmed in 2008 and 2009 were treated after one year of age. All of them are under control.

Discussion

The prevalence of *T. cruzi* infection in the general population of endemic zones in Chile has declined in the last two decades, mainly due to vector control, but also due to the improvement in the living conditions of the population, especially in rural zones.[4,14,19] These advances have allowed certification of the interruption of vector transmission of *T. cruzi* and control of transmission by blood transfusions, but they have not managed to avoid vertical (mother–foetal) transmission, another route which constitutes a major public health problem in endemic countries.[10]

While Chile has recently updated clinical ministerial guidelines for ChD and published recommendations for screening for congenital transmission in pregnant women and their newborns,[14] routine and systematic screening is not yet performed for all pregnant women in endemic areas, as in most of the endemic countries.[6,20] Therefore, the magnitude of the congenital transmission of *T. cruzi* as a public health problem has not yet been established, which is essential for developing strategies and programs of control, detection, and early treatment of this infection.

Previous studies in diverse Latin American countries have demonstrated an infection prevalence in pregnant women in rural and urban zones which varies between 2% and 51%.[9] In the IV Region of Chile, studies performed in the 1980s demonstrated a prevalence of 15.6–33.1% in pregnant women.[4,21] Subsequent studies found lower prevalence, 7.8%, in highly endemic areas and 1.4% in low endemic zones.[22]

The application of the protocols designed in this study demonstrated a prevalence of 3.4% infection by *T. cruzi* in pregnant women of the Choapa Province, IV Region, for 2005–2009. It is important to note that before 2006 no systematic serological screening for ChD was performed in hospitals or rural or urban outpatient clinics of the Choapa Province. This investigation allowed us to start training and education of health professionals in this endemic zone, to create awareness of the importance of a serological study of the pregnant women and their newborns in the different maternities of the province.

In relation to maternal epidemiological antecedents, the high percentage of rurality and low percentage of higher education and maternal activity are notable, characteristics which are generally associated with poverty conditions and poor quality of housing, factors that have shown to be an increased risk of infection.

In this study we obtained a congenital transmission rate of 4.7%, similar to that reported in international scientific literature.[10,23–28] For the studied period, taking as reference the 3.4% infection rate obtained in pregnant women of the Choapa Province and considering a total of 18 081 births in highly endemic areas (excluding La Serena and Coquimbo due to their low rurality) it is estimated that there may be 615 infected pregnant women in the region. According to this investigation vertical transmission occurs in 4.1%, therefore 25 congenitally infected children are expected, information which is currently unknown.

The serological and parasitological study of the newborns was performed at birth in 80% of the cases, with a high percentage of positive cases (94%). However, antibody detection in newborns does not distinguish whether they are produced by the child or by the infected mother; in the study we observed six newborn cases from mothers with ChD with negative serology at birth. This observation has been published in the scientific literature.[11] Only 10% of the newborns had positive PCR at birth; in these cases it was necessary to perform serial PCR or serological tests to discount infection, since this result may be due to the presence of nonviable DNA that crosses the placenta.

In relation to the clinical manifestations of infected newborns at birth, most congenital cases were asymptomatic (67%), as described in the literature (40–100%).[10] The two symptomatic cases had respiratory manifestations that could be attributable to the congenital infection. This study revealed that in endemic areas where there is no congenital transmission study, newborns with no symptoms or signs at birth might be infected, but not diagnosed or treated.

It is important to know that the hospitals of the IV region are subdivided according to the complexity of its structure and medical specialities. All the pregnancies of chagasic mothers with risk of premature delivery were considered high risk and for that reason were transferred to hospitals of high complexity with neonatology services.

Histopathology showed edema, necrosis, fibrinoid deposit and slight lymphoplasmatic infiltration in 28 of 29 placentas. In three cases the immunohistochemistry study demonstrated a decrease in actin expression in the cells of the trophoblast and intense erythroblastosis. Studies about the correlation between the histopathological findings and the congenital transmission are on going. The decrease of actin in the trophoblast has been shown previously in ex vivo infection of human placental chorionic villi explants[29] and is indicative of cellular and molecular damage of the trophoblast. The trophoblast is the first tissue of the placental barrier in contact with the maternal blood and therefore with the parasite. However, the trophoblast as an epithelia suffers a continuous turnover and the presence of the parasite may accelerate the trophoblast differentiation inducing detachment of the parasite from the placental tissue. This is consistent with the fact that only one placenta of the congenital cases shows amastigote nests in the tissue. If the low congenital transmission rate (4.7%) is also considered, it could be proposed that some local placental antiparasitic factors may exist. The pathological differences observed in the placentas of the mothers with ChD in relation to the controls, were: higher lymphoplasmatic infiltration, erythroblastosis in three cases and the presence of amastigotes forms of *T. cruzi* in one case.

Although the infection was confirmed before the age of one year in the case who received delayed treatment due to problems of availability of nifurtimox, this delay motivated us to conduct training of medical and pharmacy staff of the four hospitals of the province. This has enabled the drug to be currently available to treat the congenital cases properly and early.

The rejection of therapy by the mother of a congenital case reflects the lack of education about the disease and its long-term consequences for the population. It has been shown that all children treated before the age of one year are cured and

that adverse effects are minor compared with adults.[16] Another important element that we have observed in relation to the treatment of congenital ChD is the lack of adherence to therapy in adults and the high rate of side effects. This could explain the reluctance of mothers to treat their children with this drug.

The 4.7% prevalence of congenital ChD in the Province of Choapa is the most important result of this investigation and could be useful to the other endemic regions of Chile to perform control protocols of this transmission mechanism.

Conclusions

The prevalence of ChD in pregnant women is 3.4% and vertical transmission of *T. cruzi* 4.7% in the Choapa Province. These data allow us to estimate the current prevalence of infection at the regional and national levels.

Despite progress in controlling ChD, congenital transmission remains a significant public health problem in time (pool of infected women) and in space (migration). It is thus essential to maintain epidemiological surveillance of this zoonosis, since the early treatment of congenital cases allows 100% cure, thereby preventing sequelae in children with ChD and decreasing the infected population in Chile.

Authors' contributions: WA and IZ designed the study, supervised the data collection, JR analysed the data, MA, AS, SO, DO, SG, JR, UK, CT, YC participated in the developing of the investigation and all authors participated in drafting and revising the manuscript critically for intellectual content. All authors read and approved the final manuscript. WA is guarantor of the paper.

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Competing interests: None declared.

Ethical approval: The informed consent was approved by the Ethical Committee of the Faculty of Medicine of the University of Chile.

References

1 Organización Panamericana de la Salud. Quantitative estimation of Chagas disease in the Americas [in Spanish]. Montevideo, Uruguay: OPS; 2006.

2 Rodriguez Coura J, Pinto Dias JC. Epidemiology, control and surveillance of Chagas disease-100 years after discovery. Mem Inst Oswaldo Cruz 2009;104:31–40.

3 WHO. Chagaśdisease interrupted in Chile. TDR News 2000;61:10.

4 Apt W, Zulantay I, Solari A et al. Vertical transmission of *Trypanosoma cruzi* in the Province of Choapa, IV Region, Chile. Preliminary Report (2005-2008). Biol Res 2010;43:269–74.

5 Briceño-León R. Chagas disease in the Americas: an ecohealth perspective [in Spanish]. Cad Saude Pública 2009;25:71–82.

6 Yadon Z, Schmunis G. Congenital Chagas disease: estimating the potential risk in the United States. Am J Trop Med Hyg 2009;81:927–33.

7 Andrade AQ, Gontijo E. Neonatal screening for congenital Chagas infection: application of latent class analysis for diagnostic test evaluation. Rev Soc Bras Med Trop 2008;41:615–20.

8 Burgos JM, Altcheh J, Petrucelli N et al. Molecular diagnosis and treatment monitoring of congenital transmission of *Trypanosoma cruzi* to twins of a triplet delivery. Diagn Microbiol Infect Dis 2009;65:58–61.

9 Carlier Y, Truyens C, Deloron P, Peyron F. Congenital parasitic infections: A review. Acta Tropica 2011;121:55–70.

10 Carlier Y, Torrico F, Sosa-Estani S et al. Congenital Chagas disease: recommendations for diagnosis, treatment and control of newborns, siblings and pregnant women. PLoS Negl Trop Dis 2011;5:e1250.

11 Freilij H, Biancardi M, Lapeña A et al. Contributions to the topic congenital Chagas. In: PAHO Consultation on congenital Chagas disease, its epidemiology and management [in Spanish]. Montevideo, Uruguay;2004.

12 Klein JO, Baker C, Remington JS, Wilson C. Current concepts of infections of the fetus newborn infant. In: Remington JS, Klein JO, Wilson C, Bake C. (Editors). Infectious Diseases of the Fetus, Newborn Infant. Philadelphia: Elsevier Saunders; 2006; p. 13–25.

13 Ministry of Health. Diagnostic Situation of Health, 4th Region: Coquimbo [in Spanish]. Santiago: Government of Chile; 2005.

14 Ministry of Health. Clinical Guide. Guide to Diagnosis, Treatment and Prevention of Chagas disease [in Spanish]. Santiago: Ministry of Health; 2011.

15 Zulantay I, Corral G, Guzmán MC et al. The investigation of congenital infection by *Trypanosoma cruzi* in a endemic area of Chile: three protocols explored in a pilot project. Ann Trop Med Parasitol 2011;105:123–8.

16 Apt W. Current and developing therapeutic agents in treatment of Chagas disease. Drug Des Devel Ther 2010;4:243–53.

17 Zulantay I, Honores P, Solari A et al. Use of polymerase chain reaction (PCR) and hybridization assays to detect *Trypanosoma cruzi* in chronic chagasic patients treated with itraconazole or allopurinol. Diagn Microbiol Infect Dis 2004;48:253–7.

18 Veas F, Cuny G, Breniere S, Tibayrenc M. Subspecific kDNA probes for major clones of *Trypanosoma cruzi*. Acta Tropica 1994;48: 79–82.

19 Dias JCP. Elimination of Chagas disease transmission: perspectives. Mem Inst Oswaldo Cruz 2009;104:41–5.

20 OPS/MSF. Organization and structure of health care for the sick or infected by *Trypanosoma cruzi* (Chagas Disease) [in Spanish]. Montevideo, Uruguay: OPS; 2005.

21 Schenone H, Contreras M, Borgoña J et al. Epidemiology of Chagas disease in Chile. Frequency of human *Trypanosoma cruzi* infection by age groups and regions [in Spanish]. Bol Chil Parasitol 1989;1:84–6.

22 Garcia A, Bahamonde M, Verdugo S et al. *Trypanosoma cruzi* transplacental infection: situation in Chile. Rev Méd Chile 2001;129:330–3.

23 Luquetti AO, Dias JC, Prata A. Diagnosis and treatment of congenital infection caused by *Trypanosoma cruzi* in Brazil. Rev Soc Bras Med Trop 2005;38:27–8.

24 Russomando G, Almiron M, Candia N et al. Implementation and evaluation of a locally sustainable system of prenatal screening that allows the detection of cases of congenital transmission of Chagas disease in endemic zone of Paraguay. Rev Soc Bras Med Trop 2005;38:49–54.

25 Sánchez O, Mora MC, Basombrio MA. High prevalence of congenital *Trypanosoma cruzi* infection and family clustering in Salta, Argentina. Pediatrics 2005;115:668–72.

26 Schijman A, Altcheh J, Burgos JM et al. Aetiological treatment of congenital Chagas disease diagnosed and monitored by the polymerase chain reaction. J Antimicrob Chemother 2003;52:441–9.

27 Sosa-Estani S, Gamboa-Leon MR, del Cid-Lemus J et al. Working Group. Use of a rapid test on umbilical cord blood to screen for *Trypanosoma cruzi* infection in pregnant women in Argentina, Bolivia, Honduras and Mexico. Am J Trop Med Hyg 2008;79:755–9.

28 Torrico F, Alonso-Vega C, Suarez E et al. Maternal *Trypanosoma cruzi* infection, pregnancy outcome,morbidity, and mortality of congenitally infected and noninfected newborns in Bolivia. Am J Trop Med Hyg 2004;70:201–9.

29 Sartori MJ., Pons P, Mezzano L et al. Trypanosoma cruzi infection induces microfilament depletion in human placenta syncyotrophoblast. Placenta 2003;24:767–71.