The investigation of congenital infection by *Trypanosoma cruzi* in an endemic area of Chile: three protocols explored in a pilot project

I. ZULANTAY*, G. CORRAL†, M. C. GUZMAN*, F. ALDUNATE*, W. GUERRA‡, I. CRUZ‡, A. ARAYA‡, V. TAPIA§, F. MARQUEZ¶, C. MUÑOZ* and W. APT*

*Laboratorio de Parasitología Básico–Clínico, Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Independencia 1027, P.O. Box 427, Santiago 3, Chile

†Hospital de Illapel, Servicio de Salud Coquimbo, IV Región, Avenida Independencia 0512, Illapel, Chile

‡Hospital de Salamanca, Servicio de Salud Coquimbo, IV Región, Matilde Salamanca 0113, P.O. Box 053, Salamanca, Chile

§Consultorio de Canela, Departamento de Salud, Municipalidad de Canela, IV Región, Joel Muñoz 495, Canela Baja, Chile

¶Hospital de Los Vilos, Servicio de Salud Coquimbo, IV Región, Calle Arauco 400, Los Vilos, Chile

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Given the increasing travel of pregnant women from areas where *Trypanosoma cruzi* is endemic, the congenital transmission of the parasite has become a global public-health problem. In a recent pilot study, which ran in Chile from 2006 to 2010, three strategies for exploring and managing *T. cruzi*-infected mothers and their infected or uninfected neonates were investigated. Any protocols applied to the investigation of such mother-and-child pairs need to include the detection of infection in pregnant women, the detection of infection, if any, in the children born to the women, the appropriate treatment of the infected neonates, and the serological–parasitological follow-up of all of the neonates until their medical discharge.

The success of attempts to prevent and control human American trypanosomiasis (Chagas disease) via reductions in vector numbers and the transfusion-related transmission of the causative parasite, *Trypanosoma cruzi*, has increased the relative public-health importance of congenital infection. Congenital transmission represents the only active route of transmission for which no kind of control exists in many endemic countries, and, since pregnant women infected with *Try. cruzi* are increasingly likely to travel or

emigrate to non-endemic regions, it is a form of transmission that may occur anywhere in the world (WHO, 2002; Gascón *et al.*, 2007). Although, if detected, the infections in neonates that result from congenital transmission can usually be treated effectively, without any serious adverse events (Altcheh, 2010), it has been estimated that such transmission will remain a problem for at least the next 30 years — that is, until the number of infected women of fertile age becomes negligible (Dias, 2009). In Latin America there are currently about two million women of fertile age who are infected with *Try. cruzi* (Anon., 2006), seroprevalence among women of this age

Reply to: W. Apt.
E-mail: wapt@med.uchile.cl.

ranging from 5%–40% (Altcheh, 2010). Between 1% and 12% of mothers with American trypanosomiasis will each transmit *Try. cruzi* to their foetus (Carlier and Torrico, 2003) but most of the infected foetuses will be asymptomatic at birth (Yadon and Schmunis, 2009).

In Chile, about 130,000 women of fertile age (15–49 years) live in rural areas where *Try. cruzi* is endemic. Although it has been estimated that about 122,000 females aged >5 years migrated from such areas between 1997 and 2002, mostly to urban sites within Chile (Anon., 2007a), the importance of such migration to the epidemiology of American trypanosomiasis remains unclear. About 160,000 people in Chile are thought to be infected with *Try. cruzi*, of whom about 34,000 are women of fertile age (Anon., 2006). These numbers, together with estimates of birth rates and the incidence of congenital transmission, indicate that about 445 new cases of congenital American trypanosomiasis should be expected each year in Chile (Anon., 2006). In their recent study in the Chilean province of Choapa (in region IV), Apt *et al.* (2010b) estimated that there were about 20,130 women of fertile age in the province and found that 3.7% of the women who had their babies while in the care of the provincial maternity services had American trypanosomiasis and that, at parturition, about 4% of the pregnant women with the disease had transmitted *Try. cruzi* to their foetuses. If these values are similar across region IV (i.e. the provinces of Elqui, Limari and Choapa), there should be about 10 congenital cases in the region each year.

Between 2006 and 2010, in a pilot study based in region IV, three different protocols were used, in parallel, to investigate and manage the *Try. cruzi*-infected mothers and their children (i.e. those who were *in utero* when their mothers were infected), in the hope that the results would lead to recommendations that would complement other, relevant, national (Apt *et al.*, 2010a) and international guidelines (Carlier and Torrico, 2003; Gascón *et al.*, 2007).

PROTOCOLS

Protocol 1. Serological Examination of Pregnant Women from the Endemic Zone and Interaction with the Women and their Families

All pregnant women who live in a known endemic area or have migrated from such an area should be asked to submit to a serological examination to see if they are infected with *Try. cruzi* (Carlier and Torrico, 2003). Such serology should preferably be performed during the first prenatal examination, at the same time as routine serological tests for several other infectious agents (such as HIV and *Treponema pallidum*). Unfortunately, 1.6%–2.5% of individuals infected with *Try. cruzi* appear false-negative in the serological tests that are frequently used (WHO, 2002). Adequate quality control should be established in the laboratories in which the diagnostic tests for Chagas disease are performed (WHO, 2002) and, according to international consensus, all positive results should be confirmed using at least two different serological techniques (Luquetti *et al.*, 2005), such as indirect haemagglutination (IHA), indirect immunofluorescence (IIF) and/or ELISA (Luquetti *et al.*, 2005; Apt *et al.*, 2010a). Rapid detection tests, based on serology, are being developed, primarily for testing mothers with chronic infections (Gomes and Virginia, 2009).

Serological testing should be repeated in each pregnancy (Dias, 2005) and the results of such investigation should be recorded on the mother's hospital card and in her general clinical record and pregnancy record, so that they can be seen by any maternity service that cares for the woman. Under current Chilean law, Chagas disease is notifiable, with any new cases of human infection with *Try. cruzi* recorded on a standard form — the *Formulario de Notificación de Enfermedades de Declaración Obligatoria* — and reported daily to the regional health service and weekly to the national Ministry of Health. It is, however, generally assumed that only a proportion of the cases detected, and perhaps only a

small proportion, appears in any national database (Apt *et al.*, 2010a).

A strategy of information, education and communication (IEC) should be used to enhance levels of knowledge about congenital Chagas disease among all pregnant women (and their partners), to improve the detection and management of the disease and social attitudes towards it (Guillén and Cuentas, 2003; Anon., 2007b). As part of this IEC strategy, women of fertile age should be told the place where, and the manner in which, they and their neonates will be tested (assuming consent), the actions that should be taken if mother and/or child is found seropositive (including chemotherapy of the child), the danger of re-infection from contact with the faeces of infected *vinchucas* (i.e. the triatomine vectors), and the wisdom of having their older children, especially those aged <5 years, checked for *Try. cruzi* and, if found positive, immediately started on a course of treatment. If such advice and information can be given, in part, by health staff seeing women (individually or in small groups) at their prenatal checkups, this tends to improve maternal/parental compliance in the serological investigation of the women and their neonates and the follow-up of both the seropositive neonates who are treated and their seronegative counterparts (who should also be followed-up until their discharge). Currently, the serological testing of neonates born to women who are known (or, at least, know themselves) to be seropositive for *Try. cruzi*, so that infected neonates can be identified and treated early, is the greatest challenge in the control of congenital Chagas disease in non-endemic countries (Gascón and Pinazo, 2008).

Protocol 2. Serological and Parasitological Examination of the *Try. cruzi*-infected Mothers and their Neonates, at Parturition and over a Prolonged Follow-up

The serological and parasitological investigation of infected mothers (in the pre-partum

room) and their neonates (in the delivery room), using peripheral and umbilical-cord blood, respectively, is recommended. In a child aged <9 months, it is currently impossible to distinguish anti-*Try. cruzi* antibodies produced by the child from those produced by his or her infected mother. Some neonates born to infected mothers are seronegative (Szarfman *et al.*, 1975; Freilij *et al.*, 2004; Apt *et al.*, 2010a) but all children born to infected mothers should be carefully followed-up and checked again for anti-*Try. cruzi* antibodies at an age (e.g. 1 year) when maternal antibodies will have cleared from their bloodstream. Compared with other infected mothers, those found positive for *Try. cruzi* by blood culture are particularly likely to give birth to infected neonates (Hermann *et al.*, 2004). Although transmission may occur at any stage of the pregnancy (Freilij *et al.*, 2004), there is some evidence (elevations in parasitaemia, as detected by xenodiagnosis, and the detection of specific IgM) indicating that the risk of congenital transmission may be highest during the final stages of gestation (Szarfman *et al.*, 1975; Storni and Bolsi, 1979). Congenital transmission may occur in one or more pregnancies of an infected woman (Freilij *et al.*, 2004). The identification of mobile trypomastigote forms in umbilical-cord blood at birth or, later, in the peripheral blood of the neonate unequivocally confirms congenital infection and indicates the need for immediate treatment (Carlier and Torrico, 2003; Freilij *et al.*, 2004; Rodríguez Coura and Pinto Dias, 2009; Apt *et al.*, 2010a).

The direct parasitological technique that is most frequently recommended for the detection of *Try. cruzi* in blood is the microhaematocrit. In the detection of congenital infection (which is often associated with high parasitaemia), this method can offer a sensitivity of at least 90% (Freilij *et al.*, 2004). Although, in the absence of adequate training, the sensitivity of the microhaematocrit method may be much reduced (as seen in the recent study in region IV; unpubl. obs.), the method

remains useful for the early diagnosis of congenital infection. It is recommended that blood from the umbilical cord of the neonate is collected in a heparinized tube, with microhaematocrit capillaries only filled with this blood in the health-unit's laboratory (to reduce the exposure of delivery-room personnel to blood that may be infected with *Try. cruzi*). If the neonate's blood cannot be tested at birth, a sample of peripheral blood should be taken at an age of 15–30 days and similarly investigated (Azogue and Darras, 1995).

Another parasitological technique whose utility has already been demonstrated in several studies of congenital American trypanosomiasis is the PCR-based assay, in which specific segments of the DNA of *Try. cruzi* are amplified (Schijman *et al.*, 2003; Virreira *et al.*, 2003). The serial parasitological study of neonates with strong clinical indications of congenital infection by *Try. cruzi* (e.g. prolonged fever, hepato-splenomegaly, acute myocarditis and/or premature birth) is recommended (Rodríguez Coura and Pinto Dias, 2009). In such serial investigation, Diez *et al.* (2008) found a PCR-based assay to be more sensitive than direct parasitological tests such as the microhaematocrit. If such an assay is used to test a neonate at birth, it should be repeated after 15 and 30 days (irrespective of any earlier results), especially when a neonate has been found PCR-positive but negative in direct parasitological tests (since PCR positivity may be caused by cell-free *Try. cruzi* DNA from the infected mother, that has passed the placental barrier).

Rapid diagnostic tests for neonates, such as the *T. cruzi* OligoC-Test (Deborggraeve *et al.*, 2009), could be very useful when they become available commercially and, also in the future, real-time PCR may replace conventional PCR (Duffy *et al.*, 2009).

Based on experience gained in the recent study in region IV, it seems reasonable to set three laboratory criteria, each of which can be considered to confirm a congenital infection:

- (1) at least four positive results in direct parasitological tests of umbilical-cord blood of the neonate and/or peripheral blood of the neonate collected 1–2, 15 and 30 days after the birth (irrespective of the results of any PCR-based assay);
- (2) consistently positive results in serial PCR-based assays performed until the child is aged at least 15 days (irrespective of the results of any direct parasitological tests); and/or
- (3) **if** the post-partum infection of the child can be discounted (e.g. because the child has always lived in areas where the triatomine vectors do not occur), the presence in a child of 9–12 months of anti-*Try. cruzi* antibodies, confirmed by two conventional serological techniques (e.g. IIF and ELISA).

Protocol 3. Treatment and Prolonged Post-treatment Follow-up of Children with Confirmed Congenital Infection

Once congenital infection by *Try. cruzi* has been confirmed, the prevention of cardiac and digestive damage and neurological lesions depends upon adequate specific treatment (Abad-Franch *et al.*, 2010). Such treatment should be obligatory for all confirmed cases (Carlier and Torrico, 2003; Apt *et al.*, 2010a; Altcheh, 2010), since frequencies of cure approaching 100% can be obtained — when nifurtimox or benznidazole are given early (WHO, 2002; Carlier and Torrico, 2003; Schijman *et al.*, 2003; Yadon and Schmunis, 2009). The recommended treatment, as agreed upon by the relevant experts, is to give either benznidazole (at 5–10 mg/kg.day) or nifurtimox (at 5–15 mg/kg.day) for 30–60 days. Nifurtimox should be given at 5 mg/kg on each of the first 3 days of treatment and only increased to 10 mg/kg.day if no adverse events have then been observed (Apt, 2010; Apt *et al.*, 2010a). As paediatric preparations of benznidazole and nifurtimox are not currently available, a pharmacist will have to prepare the prescribed doses.

It is recommended that any drug be given under the direct supervision of a responsible family member or health worker, to assure compliance, avoid over-dosage and allow any adverse effects to be monitored (Carlier and Torrico, 2003). There should be clinical follow-up (with weekly clinical examinations and measurements of weight and height during treatment), general laboratory examinations (haemograms, liver-function tests and two different serological tests for anti-*Try. cruzi* antibodies during and at the end of treatment) and parasitological examinations (PCR recommended; every 6 months for 2 years after the therapy). Only after at least 2 years of negative results in serological and serial parasitological tests should the patient be declared cured (Molinedo *et al.*, 2005). Persistent seropositivity indicates therapeutic failure, when a second course of treatment with prolonged follow-up should be considered (Rodríguez Coura and Pinto Dias, 2009).

CONCLUSIONS

All neonates with *Try. cruzi*-infected mothers should be studied until their congenital infection can be confirmed or discounted (Anon., 2007b). The first step in this process is the identification of the infected mothers (Yadon and Schmunis, 2009). Most congenital infections are asymptomatic in infancy, and most infected babies have normal birthweights. Premature infants infected with *Try. cruzi* may have a variety of clinical manifestations, such as low birthweights for their gestational age, jaundice, anaemia, hepatomegaly, splenomegaly, perinatal hypoxia, meningo-encephalitis and/or myocarditis, and may soon die (Freilij *et al.*, 2004; Rodríguez-Coura and Borges-Pereira, 2010). The mortality and morbidity still associated with Chagas disease, and the relative ease with which the disease can be cured in most young children, justify the establishment of

protocols for the multidisciplinary care of infected-mother/neonate pairs in Chile. The training of health teams is fundamental, especially in endemic areas without control programmes and in non-endemic areas with female migrants from endemic areas (Buekens *et al.*, 2008; Gascón *et al.*, 2009; Yadon and Schmunis, 2009). American trypanosomiasis is currently a global parasitosis and congenital transmission is one of its manifestations. The protocols presented here are intended to be a contribution to the strategies for the control of this disease.

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