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## Prevalence of *Trypanosoma cruzi* infection in pregnant Latin American women and congenital transmission rate in a non-endemic area: the experience of the Valencian Health Programme (Spain)

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C. BARONA-VILAR<sup>1,2,3\*</sup>, M. J. GIMÉNEZ-MARTÍ<sup>4</sup>, T. FRAILE<sup>5</sup>,  
C. GONZÁLEZ-STEINBAUER<sup>1</sup>, C. PARADA<sup>6</sup>, A. GIL-BRUSOLA<sup>4</sup>,  
D. BRAVO<sup>7</sup>, M. D. GÓMEZ<sup>4</sup>, D. NAVARRO<sup>7,8</sup>, A. PEREZ-TAMARIT<sup>9</sup>,  
L. FERNANDEZ-SILVEIRA<sup>9</sup>, A. FULLANA-MONTORO<sup>1,2</sup> AND R. BORRÁS<sup>7,8</sup>

<sup>1</sup> Department of Health, General Directorate of Public Health, Generalitat Valenciana, Spain

<sup>2</sup> Centre for Public Health Research (CSISP), Valencia, Spain

<sup>3</sup> Spanish Consortium for Research on Epidemiology and Public Health, CIBERESP, Spain

<sup>4</sup> Department of Microbiology, Hospital Universitario La Fe, Valencia, Spain

<sup>5</sup> Department of Microbiology, Consorcio Hospital General, Valencia, Spain

<sup>6</sup> Centre for Blood Transfusions, Valencia, Spain

<sup>7</sup> Department of Microbiology, Hospital Clínico Universitario, Valencia, Spain

<sup>8</sup> University of Valencia, Valencia, Spain

<sup>9</sup> Department of Paediatrics, Hospital Universitario La Fe, Valencia, Spain

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### SUMMARY

This study describes the results of the health programme implemented in the Valencian Community (Spain) to achieve an early diagnosis of Chagas disease in pregnant Latin American women and their newborns. During 2009 and 2010, 1975 women living in the health districts of three university hospitals were enrolled via midwives or at the time of delivery. Diagnosis of disease was performed using two serological tests with different antigens. Congenital infection was diagnosed by parasitological, molecular or serological methods from blood samples obtained at birth or in subsequent controls. The overall seroprevalence of Chagas infection in pregnant women from 16 different endemic countries was 11·4%. Infection was higher in those from countries in the Gran Chaco Region (Bolivia, 34·1%; Paraguay, 7·4%; Argentina, 5·3%). Eight newborn infants from Bolivian mothers had congenital Chagas which represents a vertical transmission rate of 3·7%. In conclusion, this work supports the benefits of offering an early diagnosis to pregnant women and newborns during routine prenatal healthcare.

**Key words:** Chagas disease, congenital Chagas, pregnant women, prevalence, *Trypanosoma cruzi*.

### INTRODUCTION

During the last decade, Chagas disease has become an emergent public health problem in Spain [1] due to the high number of immigrants from endemic countries.

\* Author for correspondence: Dr C. Barona-Vilar, Perinatal Health Unit, General Directorate of Public Health, Conselleria de Sanitat, Avda Cataluña, 21, 46020 València (Spain), Fn. 34-96-192 57 39.  
(Email: barona\_car@gva.es)

Spain is the European country with the greatest number of people arriving from Latin America, with more than one million immigrants in 2008. It has been estimated that 25/1000 of these people could be infected by *Trypanosoma cruzi* [1] and that, since the disease has a slow evolution, most would be in the chronic asymptomatic stage [2].

In 2005 Spanish legislation stipulated the need to perform universal screening of blood and organ

donors from endemic countries [Act 1088/2005 published in the Official Bulletin of the State, BOE number 225]. Another possible mode of transmission in Spain is from mother to child during pregnancy or at delivery. In this sense, the potentially exposed population is high, since there were 31 522 births in women from endemic countries during 2009 in Spain, representing 31% of newborns from foreign mothers [3].

Epidemiological studies conducted in Spain to evaluate the prevalence of the infection in pregnant women from endemic countries have been confined to specific areas. The estimated prevalence in Catalonia and the Valencian Community ranges from 1.75% to 9.7% [4–9] and this variability has been related to the origin of the women included in each study. Nevertheless, all the studies agree in highlighting that women from Bolivia have the highest risk of infection. With respect to mother-to-child transmission, epidemiological studies have not been performed extensively on the susceptible population in Spain, even though some cases of congenital infection have been reported [10–12].

As mother-to-child transmission cannot be prevented, an early diagnosis of the infection in newborns is essential. Successful aetiological treatment has been reported in children, with a lower incidence of side-effects than in adults [13–15]. Therefore, it is especially recommended that health programmes be implemented for early diagnosis in pregnant women, together with protocols aimed at following up their newborns [10]. In 2009 the Public Health Authorities in the Valencian Community decided to design and perform a strategy for dealing with the early diagnosis of Chagas disease in pregnant women from endemic countries [16]. The aims of this study were to evaluate the level of implementation of the protocol for early diagnosis of Chagas disease in pregnant women in the city of Valencia, to determine the prevalence of infection depending on the geographical origin of the women and to discover the status of the congenital disease.

## METHODS

### Design and setting

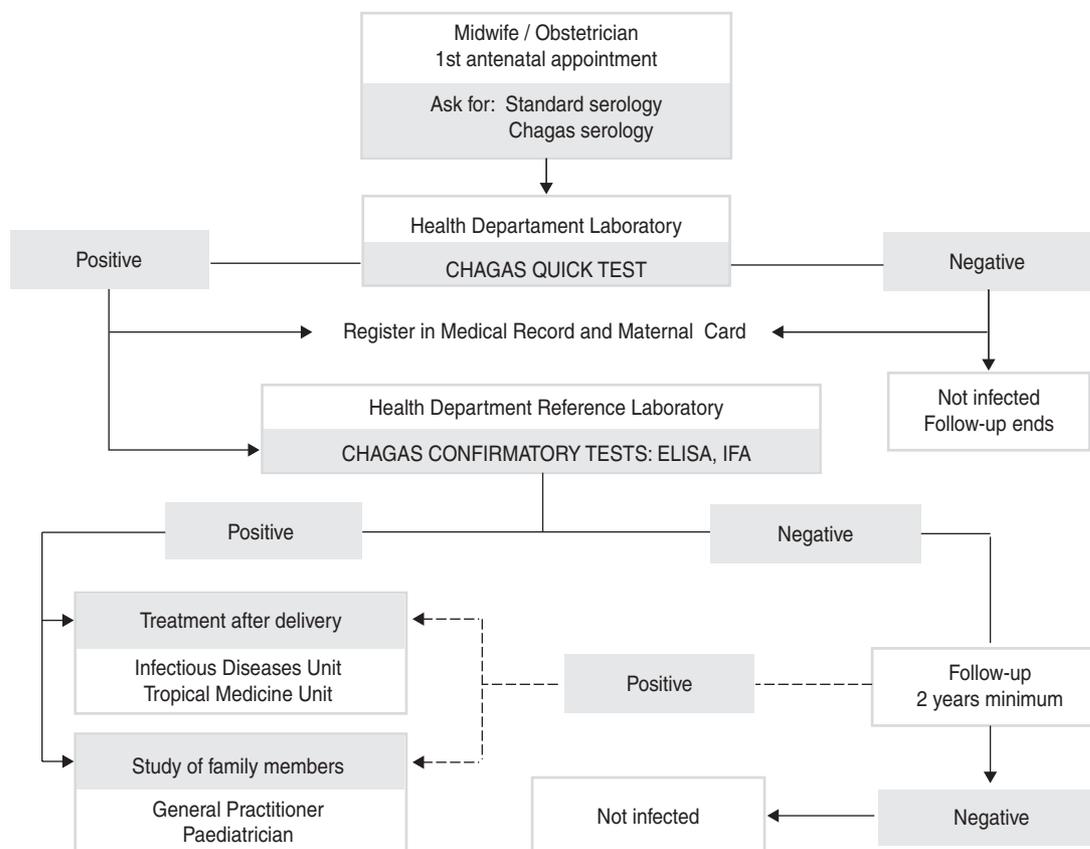
A cross-sectional study was performed during 2009 and 2010 in the city of Valencia (Spain). The sources used were the records obtained from the microbiology laboratories' information systems of the university

hospitals with the largest number of births in the city of Valencia: Hospital Universitario y Politécnico La Fe, Hospital Clínico Universitario and Consorcio Hospital General Universitario. Additional information on births was obtained from the regional Neonatal Metabolic Screening Registry, with thorough coverage and high quality on the main perinatal variables, including the mother's country of origin. This source allowed us to calculate the percentages of mothers from the 16 endemic countries who gave birth in the period who had been screened (targeted women).

### Participants and laboratory techniques

The populations studied were pregnant women from endemic countries and their newborns, recruited mainly via midwives from primary health centres. In the Valencian Community, midwives are the primary caregivers of uncomplicated pregnancies and deliveries, providing continuous care with the support of obstetricians when necessary. Free healthcare is offered during pregnancy and up to 40 days after delivery to every woman resident in our region. Women are encouraged to arrange an appointment with the midwife early in the first trimester of pregnancy in order to initiate routine care [17], which includes, among other studies, a blood test in order to determine their immunological status to some infectious diseases (rubella, toxoplasmosis, syphilis, hepatitis B, HIV). In the case of non-serologically controlled women, these analyses are performed at the time of delivery in the hospital. The detection of antibodies to *T. cruzi* in pregnant women from countries with endemic Chagas disease was reported in 2007 and formalized in 2009 by the Public Health Authorities [16, 18]. The protocol for serological studies that the midwife should offer pregnant women and the algorithm to be followed up is shown in Figure 1. The women were required to give oral consent to screening after being informed by midwives.

The detection of antibodies against *T. cruzi*, following the WHO recommendations [19], was performed using different antigenic substrates that, depending on the policy of the laboratories, were used either as a combination of two ELISA tests, one whole cell lysate antigen test (Ortho<sup>®</sup> *T. cruzi* ELISA Test System, Johnson & Johnson, USA, or Chagatek ELISA, bioMérieux, France) and the other with recombinant antigens (*T. Cruzii* Ab, DIAPRO, Diagnostic BioProbes, Italy), or by the combination of an



**Fig. 1.** Diagnostic algorithm of Chagas disease in pregnant Latin American women.

ELISA test (Chagatek ELISA) with a rapid test of recombinant antigens (agglutination assay, ID-PaGIA Chagas, DiaMed AG, Switzerland, or immunochromatographic assay; Stick Chagas, Operon SA, Spain). The reactive sera were titrated by indirect immunofluorescence (IFA Kit Trypanosomiasis, MarDx Diagnostics, USA, Innogenetics Ibérica, Spain, or Immunofluor Chagas, Biocientífica, Inverness Medical Ibérica, Spain). Agreement between the results of both tests was considered to be evidence of Chagas disease. Any discrepancies in the results obtained by each laboratory, following the established protocol, were analysed by the other two laboratories participating in the study.

In newborns from mothers with serological evidence of *T. cruzi* infection, a blood sample from the umbilical cord or peripheral blood was collected and tested immediately after birth for the: (i) microscopic observation of parasites (microhaematocrit); (ii) detection of IgM anti-*T. cruzi* by IFA, in serum samples previously treated with an anti-rheumatoid factor, and when negative for the detection and titration of IgG antibodies, with the above procedures; (iii) detection of DNA by a home-made nested PCR

(target: minicircle kDNA, primer 121/122) performed at the Instituto de Salud Carlos III (Majadahonda, Spain) or real time-PCR (RealCycler<sup>®</sup> CHAG, Progenie Molecular, Spain; Ingenie Molecular<sup>®</sup>, Progenie Molecular, Spain). Vertical transmission was considered positive when at least one test was positive: (i) the microscopic visualization of the parasite; (ii) the presence of IgM; (iii) the persistence or increase of IgG antibody titres to time; (iv) PCR positive on two separate blood samples for at least one month. Serological controls were performed from 1 to 12 months of age, as shown in Figure 2.

### Statistical analysis

The outcome variable of study was the mother's antibodies against *T. cruzi*. Demographic and obstetric variables were considered as covariables. A descriptive analysis was used to calculate absolute and relative frequencies of the main demographical and epidemiological variables. Seroprevalence and 95% confidence intervals (CI) by country of origin were calculated to obtain the epidemiological profile of the women at higher risk of disease. We compared the

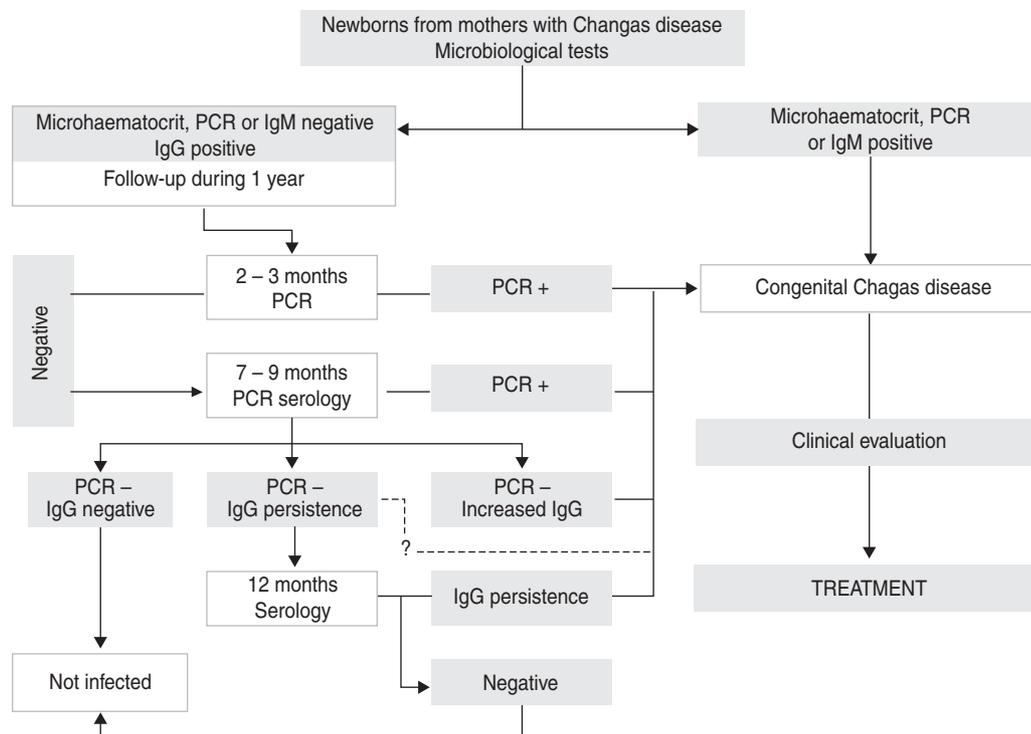


Fig. 2. Diagnostic algorithm of Chagas disease in newborns.

screening coverage and prevalence between countries of origin using  $\chi^2$  and Fisher's tests. A statistically significant level of  $P < 0.05$  was assumed. The statistical analysis was performed with Epi Info version 3 (CDC, USA) and SPSS version 14 (SPSS Inc., USA) statistical packages.

## RESULTS

In absolute figures, 7346 births from Latin American women occurred between 2009 and 2010 in the Valencian Community, representing about 30% of births from all immigrant mothers. This population was mostly settled in the urban area of the city of Valencia, where 2147 Latin American women gave birth at the three university hospitals included in the study. Of those women, 2070 came from endemic regions: 1132 (54.7%) from the Andean Region, 854 (41.3%) from the South Cone and 84 (4%) from Mexico and Central America. Ecuador with 30% of the mothers, Bolivia with 21.7%, Colombia with 17.8% and Argentina with 6.4% were the most representative countries, as shown in Figure 3.

The mean age of the mothers studied was 29 years (s.d. = 6, range 14–48 years). Vaginal delivery was predominant (75%). Male newborns represented 53.5% and females 46.5%; they reached 3335 g

(s.d. = 583) of mean birth weight and a preterm rate ( $< 37$  weeks gestation) of 8.3%.

A total of 1975 pregnant women were recruited for screening, all being asymptomatic. This total represents coverage of 95.4% of the target population and there was no significant difference between regions of origin. The prevalence of Chagas disease in pregnant women from endemic countries as a whole was 11.4%. There were no discrepancies between the different serological tests, being in all cases the antibody titres against *T. cruzi*  $\geq 128$ . Nevertheless, there were many differences depending on the region of origin. Women from South American countries had significantly higher rates than those from Central American countries (11.8% vs. 2.5%,  $P < 0.01$ ). Of the first group, the prevalence of the disease was higher in women born in countries from the Southern Cone than from the Andean Region (24.8% vs. 0.1%,  $P < 0.0001$ ). Mothers from the Gran Chaco Region, especially those from Bolivia, obtained the highest prevalence (34.1%) in Southern Cone countries and there were statistical differences ( $P < 0.0001$ ) with the other countries in that region: Paraguay (7.4%) and Argentina (5.3%). It should be highlighted that 94.7% (214/226) of the detected cases in this study corresponded to Bolivian mothers (Table 1).

Table 1. Prevalence of *T. cruzi* infection in pregnant Latin American women resident in the city of Valencia by geographical area and country of origin (2009–2010)

| Pregnant women             |               | Women with <i>T. cruzi</i> infection |            |               |
|----------------------------|---------------|--------------------------------------|------------|---------------|
| Region/country of origin   | Screened      | Number                               | Prevalence | 95 % CI       |
| Mexico and Central America | 80 (95.2 %)   | 2                                    | 2.5        | 0.31 to 8.7   |
| El Salvador                | 2             |                                      |            |               |
| Guatemala                  | 1             |                                      |            |               |
| Honduras                   | 55            | 2                                    | 3.6        | 0.44 to 12.5  |
| Mexico                     | 8             |                                      |            |               |
| Nicaragua                  | 7             |                                      |            |               |
| Panama                     | 7             |                                      |            |               |
| South America              | 1895 (95.4 %) | 224                                  | 11.8       | 10.3 to 13.3  |
| Andean Region              | 994 (87.8 %)  | 1                                    | 0.1        | –0.15 to 0.35 |
| Colombia                   | 312           |                                      |            |               |
| Ecuador                    | 571           | 1                                    | 0.2        | –0.25 to 0.61 |
| Peru                       | 59            |                                      |            |               |
| Venezuela                  | 52            |                                      |            |               |
| South Cone                 | 901 (100 %)   | 223                                  | 24.8       | 21.9 to 27.6  |
| Argentina*                 | 75            | 4                                    | 5.3        | 1.5 to 13.1   |
| Bolivia*                   | 628           | 214                                  | 34.1       | 30.3 to 37.9  |
| Brazil*                    | 83            |                                      |            |               |
| Chile                      | 13            |                                      |            |               |
| Paraguay*                  | 68            | 5                                    | 7.4        | 2.4 to 16.3   |
| Uruguay                    | 4             |                                      |            |               |
| Total                      | 1975 (95.4 %) | 226                                  | 11.4       | 10.0 to 12.9  |

CI, Confidence interval.

Values in parentheses are percentage of target mothers.

\* Countries with territories included in the Gran Chaco Region.

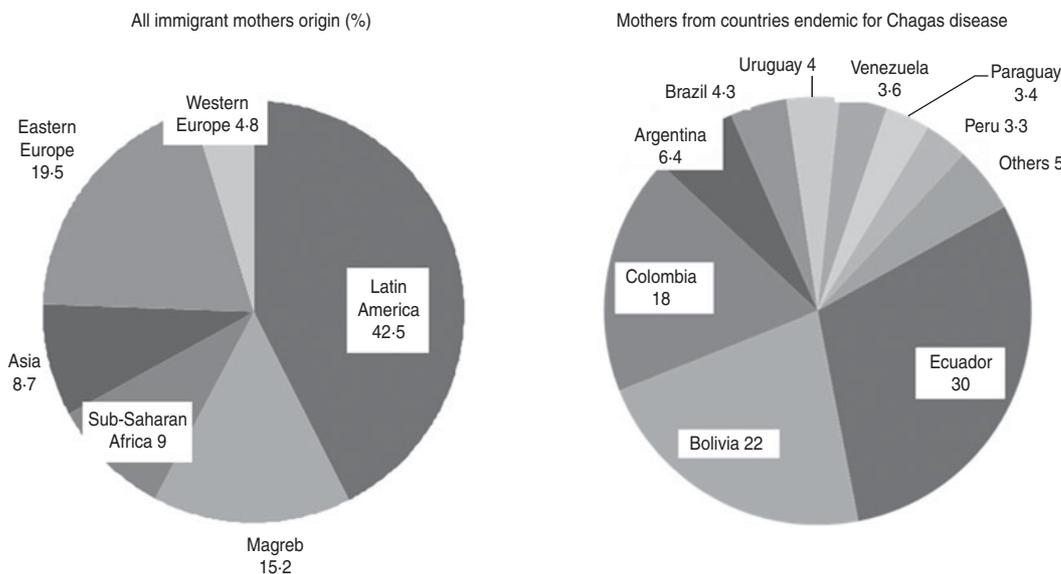


Fig. 3. Geographical origin of immigrant mothers in the city of Valencia, Spain (2009–2010).

Eight newborn infants from Bolivian mothers were diagnosed with congenital Chagas disease, which represents a vertical transmission rate of 3.7%.

Only one of the newborns showed hepatosplenomegaly at birth, a sign associated with congenital Chagas disease. The PCR test was the most efficient

Table 2. *Diagnostic methods of congenital Chagas cases*

| Positive diagnostic methods             | No. of cases |
|---|--------------|
| PCR                                     | 4            |
| PCR and microhaematocrit                | 2            |
| PCR and presence of IgM antibodies      | 1            |
| Persistence of IgG antibodies >6 months | 1            |
| Total                                   | 8            |

PCR, Polymerase chain reaction.

diagnostic procedure for this cohort since it was positive in 7/8 cases, four of which were exclusively diagnosed by it (Table 2). All cases were followed up and successfully treated with benznidazole (7 mg/kg per day for 60 days) without adverse pharmacological effects.

## DISCUSSION

Our findings reveal that the incorporation of serological screening for Chagas disease into the prenatal care programme conducted by midwives is a good strategy for dealing with mother-to-child transmission in our setting. This was reflected by the fact that 95.4% of pregnant women from 16 different countries where the disease is endemic were screened during the study period. The need to implement public health policies in the host country where immigrant women from these different countries have settled has been highlighted, ensuring detection and treatment of acute and chronic cases, including Chagas congenital infection [20, 21]. This has become a priority in the Valencian Community, taking into account that it has the third largest concentration of immigrant women from developing countries in Spain and that Latin American mothers contribute the highest proportion of births [3].

The overall seroprevalence of *T. cruzi* infection in pregnant women from 16 endemic countries in this study was 11.4%. This rate is similar to that described by Jackson *et al.* [22] in Latin American women living in Geneva (12.8%) and higher than those previously described in the Valencian Community, with rates between 1.75% and 10% in pregnant women [4, 6, 9, 23], and in Catalonia with rates from 3.4% to 4.3% in pregnant women and women of child-bearing age, respectively [8, 24].

These differences may be related to sample size, indicating that particular countries of origin of the population under study play an important role in prevalence studies [21]. This fact has been demonstrated in our study, where the prevalence of Chagas disease was lower in pregnant women born in Mexico or Central America than in those from South America (2.5% *vs.* 11.8%). In our cohort, infection is more prevalent in women coming from Southern Cone countries in the Gran Chaco Region, than in those born in countries from the Andean Region (24.7% *vs.* 0.1%).

In Latin America, infection rates in mothers range from 2% to 51% [25], being higher in rural areas of Bolivia [26], where the proportion of infected women of childbearing age varies between 20% and 60%, depending on area of residence [27]. The infection rate in pregnant Bolivian women (34.1%) found in this study is higher than that reported in Madrid (18%) in pregnant women [28] and our data suggests that these women could have migrated from rural areas.

In our study there have been eight diagnosed cases of congenital Chagas disease in the newborns from Bolivian mothers, which represents a vertical transmission rate of 3.7% for this cohort. Other researchers have reported variable rates of mother-to-child transmission (1–10%) [8, 25, 27]. In our setting, the number of cases detected is within the range expected according to transmission rates of between 1.3% and 5% estimated by Schmunis & Yadon [21] for the Bolivian cohorts, which would indicate between three and 11 cases, respectively. In newborns, the most effective diagnostic tool was the PCR, since it allowed diagnosis of seven cases, four of them in isolation, compared to the four diagnoses that could have been made with other tests if PCR had not been performed. These findings support the recent data that PCR is more sensitive and allows an earlier diagnosis of congenital infection than conventional techniques [29].

This study identified that women from the Southern Cone and especially those from Bolivia are the most vulnerable to Chagas disease in immigrant mothers in the Valencian Community. We have demonstrated the benefits of offering early diagnosis to pregnant women and newborns as part of routine primary healthcare given by midwives. In order to achieve good results in a health programme applied to a newly imported or emerging infectious disease, it is important that health professionals are adequately

trained and are made aware of the specific health needs of the group in question.

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### DECLARATION OF INTEREST

None.

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