Trypanosoma cruzi infection in Elche (Spain): comparison of the seroprevalence in immigrants from Paraguay and Bolivia

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Chagas disease is a global public health problem due to the recent emigration of people from Latin America to other regions, including Europe. The aim of this study is to determine the prevalence of *Trypanosoma cruzi* infection among Paraguayans and Bolivians living in Elche (Spain), a city located in the Mediterranean Coast of Spain. A cross-sectional study was conducted. Capillary blood samples were obtained through a finger prick, and collected on filter paper. An enzyme-linked immunosorbent assay and indirect immunofluorescence tests were performed to search for anti-*T. cruzi* IgG antibodies in the filter papers. Thirteen out of 201 participants were infected with *T. cruzi* in this study, seven out of 73 Bolivians and six out of 128 Paraguayans, corresponding to seroprevalences of 9.59% (95%CI, 4.72–18.5%) and 4.69% (95%CI, 2.17–9.85%), respectively. Palpitation, chest pain, and migration from rural endemic areas were the most common clinical and epidemiological risk factors associated with *T. cruzi* infection detected in the Paraguayan group. This study highlights that Chagas disease is no longer limited to the Bolivian population living in Spain. It is important to note this wider prevalence and, therefore, not discount Paraguayans in the screening for Chagas disease in Spain. Indeed, this should be considered for all immigrants from Latin America.

Keywords: Chagas disease, *Trypanosoma cruzi* infection, Paraguay, Bolivia, Immigrants

Introduction

Chagas disease, or American trypanosomiasis, is a systemic, chronic, parasitic infection caused by *Trypanosoma cruzi*. In recent years, cases have increased in non-endemic countries due to the migration of Latin Americans from endemic areas.^{1–4}

Spain has received the largest number of immigrants in its history within the last 10 years. The number of foreigners registered in Spain from 1998 to 2009 grew by approximately eight to nine times, from 637 058 to 5 708 940. In 2010, 12.2% of the total Spanish population were immigrants, with 1.72 million from Latin America.⁵ The greatest number of Latin American immigrants in Spain are from Ecuador (23.0%) followed by Colombia (16.9%) and Bolivia (12.3%).

In 2006, the Pan American Health Organization (PAHO) estimated the morbidity, prevalence, and burden of *T. cruzi* infection in Latin America. Bolivia was the country with the highest rate of *T. cruzi* infection, followed by Argentina, El Salvador, Honduras,

and Paraguay.⁶ In a study done in Barcelona, Spain,² it was found that 194 out of 202 *T. cruzi*-infected individuals were from the Southern Hemisphere of South America, mainly from Bolivia. Prenatal screenings performed in several hospitals in Spain also showed that a high proportion of *T. cruzi*-infected pregnant women were from Bolivia.^{7,8} Although several Spanish studies have investigated the seroprevalence of *T. cruzi* infection in pregnant women, adults, and children who had attended primary healthcare centres or international health centres,^{2,7–9} there is no information on the prevalence of Chagas disease in each of the immigrant communities. In a previous study involving serological screening on 210 pregnant women living in Elche (Spain), we detected two positive women from Bolivia and two from Paraguay.¹⁰ Following on from these findings, we designed a population-based study to investigate the prevalence of *T. cruzi* infection among Paraguayans and Bolivians living in Elche.

Materials and Methods

Design and setting

A cross-sectional study was conducted on Paraguayans and Bolivians living in Elche. Out of a total

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population of 230 112, 10 837 (4.8%) inhabitants came from Latin America and the Caribbean. The origins of the Latin Americans were 2785 from Colombia, 2372 from Ecuador, 1700 from Argentina, 1224 from Paraguay, and 470 from Bolivia.⁵

Recruitment and participants

In order to test 10% of the Paraguayans and 10% of the Bolivians living in the city, a sampling of 122 and 47 individuals, respectively, was calculated. The enrolment of the participants was conducted over 1 year from November 2009 to November 2010.

We established strong, positive relationships with members of the Paraguayan and Bolivian communities in Elche through a variety of avenues, including joining them in social activities, visiting them in their homes, and attending the Paraguayan community association. This presented opportunities to provide information about Chagas disease, and then to recruit residents to participate in the study. From those who agreed to participate, we obtained written informed consent.

Epidemiological data were obtained via interview and included: age; sex; country; place of residence, rural or urban, in the country of origin; dwelling type (construction materials for floors, walls, etc.) in the country of origin; date of arrival in Spain; any history of blood transfusion; knowledge of Chagas disease; relatives with Chagas disease; and, any previous diagnosis of Chagas disease in their home country. Clinical symptoms associated with *T. cruzi* infection were also evaluated such as chest pain, palpitation, constipation, and dysphagia.

The ethics committee of the Hospital General Universitario de Elche approved this study, and all the participants gave their written informed consent.

Screening test procedures

Capillary blood samples obtained by finger prick were taken from each participant. Several drops of whole blood were put on filter paper, Whatman protein saver 903 card (Whatman GmbH, Dassel, Germany) for detection of IgG anti-*T. cruzi* antibodies. Two serological tests were used, enzyme-linked immunosorbent assay (ELISA), and indirect immunofluorescent antibody test (IFAT). The ELISA was prepared in-house using an antigen prepared from a proportional mix of epimastigotes obtained from a culture of the stationary phase of two strains of *T. cruzi*, (MC and T) and from another *T. cruzi* I (Dm28). The IFAT was also prepared in-house, and was done according to the protocol adapted from Camargo;¹¹ the antigens were prepared from cultures of epimastigotes in the stationary phase of the same strains of *T. cruzi* used in ELISA.^{12,13} The ELISA test was considered positive when the optical density was >0.40 and for the IFAT test, titers $\geq 1/40$ were considered positive. When a

positive result from ELISA and/or IFAT was obtained, the person was contacted for further tests to confirm the result. Whole blood was drawn via venous puncture (4 ml) and the same tests were repeated. An individual was considered infected if the results of the two serological tests from serum samples were positive. Polymerase chain reaction (PCR) on blood was performed for people who had two positive serological tests. The PCR was done in-house using oligonucleotides 121–122 and Tcz1–Tcz2, which amplify the variable region of the kinetoplast DNA minicircle (330 bp), and a repetitive sequence of satellite DNA (195 bp), respectively.¹⁴ *T. cruzi*-infected patients were studied using a protocol that included a 12-lead electrocardiogram, chest-X-ray, and hematology, biochemistry, and HIV serology.

Statistical analysis

A descriptive analysis was performed by setting the rates. We obtained estimates of the prevalence with 95% confidence intervals (95% CI) using the Wilson procedure. We used the Z-ratio for the significance of the differences between prevalence in this study and that in the country of origin. We analysed the variables associated with *T. cruzi* infection using chi-square or Fisher's exact test for the categorical variables, and the Wilcoxon rank sum test for the continuous variables. Odds ratio with 95% CI was calculated. Statistical tests were performed using a two-sided test at a significance level of 0.05. Data for each study participant were entered into Microsoft Excel and analysed with SPSS 12.0 (SPSS, Inc., Chicago, IL, USA).

Results

Participants in the study included 128 Paraguayans and 73 Bolivians, and of these 10.5 and 21.4%, respectively, were residents of Elche, Spain. The demographic, clinical, and epidemiological data from the participants are shown in Table 1. The median age was 30 years and 114 (56.7%) were female. Of the total study cohort, 65.8% of Bolivians and 35.9% of Paraguayans said that they lived in a house mainly constructed of mud ($P<0.001$). The percentages who had personally experienced or had known of relatives with Chagas disease were 34.2% Bolivians and 8.6% Paraguayans ($P<0.001$).

There were 13 study participants infected with *T. cruzi*, seven Bolivians and six Paraguayans. The seroprevalences in the Bolivian group were 9.59% (95% CI: 4.72–18.5%) and 4.69% (95% CI: 2.17–9.85%) in the Paraguayans. PCR was positive for *T. cruzi* in eight of the 13 (61.5%) cases.

Table 2 shows the epidemiologic characteristics according to infection status. Variables significantly associated with *T. cruzi* infection for all participants were age ($P=0.03$), palpitations ($P=0.02$), and chest

pain ($P=0.02$). In Paraguayans, the variables associated were palpitations ($P=0.03$), chest pain ($P=0.01$), and living in the departments of Central, Itapúa, Paraguari, and Presidente Hayes ($P=0.02$). In the study population of Bolivians, no clinical variables associated with *T. cruzi* infection were detected, and six out of seven positive cases lived in the higher endemic areas of Bolivia, Cochabamba, and Santa Cruz. No independent variables associated with *T. cruzi* infection were identified using multivariate logistic regression analysis.

Discussion

This study on Chagas disease is complementary to other studies carried out in Spain and focused on hospital^{2,10,15} and primary health care centres.^{9,16} *T. cruzi* infection in Paraguayan immigrants in a European country. The rate of *T. cruzi* infection among all the study participants is correlated with the estimated prevalence of the disease in the population living in the same region in their home country and within the same age range.¹⁷

Chagas disease is a major public health problem in Bolivia.¹⁸ It is as much of a health problem in rural areas and small villages, as it is in urban areas.¹⁹ Fifty-five percent of the national territory of Bolivia ($\sim 600\,000\text{ km}^2$) is endemic for Chagas disease, with approximately 3.5 million people at risk of infection.²⁰ Seven out of the nine Bolivian departments are endemic for Chagas disease, regardless of the altitude.²¹ The seroprevalence rates are higher in communities from the departments of Cochabamba, Chuquisaca, Tarija, and Santa Cruz.^{19,22} According to the PAHO, the overall seroprevalence estimate in Bolivia is 6.75%.⁶ However, the prevalence in a rural area in the southern Bolivian Chaco ranged from 40 to 80%.²²

Moreover, in the peripheral urban districts of Cochabamba, between 15 and 25% of children under 14 years were infected.¹⁹ Our results, however, show a prevalence of 9.59% of *T. cruzi* infection among Bolivians, which is lower than that reported in other studies performed in pregnant women living in Spain (13.3–26.5%).^{7,9,10,23}

Paraguay is one of the countries from the Southern Cone, bordering Bolivia. Paraguay, Bolivia, and Argentina share a wide area named the Gran Chaco territory, where the prevalence of *T. cruzi* infection is high. Paraguayans in this territory are only 1.5% of the total population. According to PAHO, the seroprevalence of *T. cruzi* infection of the total population in Paraguay is 2.54%,⁶ but in pregnant women from endemic areas, it is 6%.²⁴ Our results show 4.69% prevalence among Paraguayans living in Elche, Spain, which is considerably higher than the prevalence for the total population living in Paraguay.⁶ Immigration from Paraguay to Spain is more recent than from Bolivia, hence the little understood prevalence of Chagas in the Paraguayan immigrant community. References in the medical literature regarding *T. cruzi* infection in people, other than those coming from Bolivia, are rare.²⁵ and only a few cases of Chagas disease have been reported in Paraguayans living in Spain.^{7,10,26} In addition, a literature search about seroprevalence of *T. cruzi* in Paraguay also shows few reports.^{24,27} The prevalence of *T. cruzi* infection seems to be higher in populations inhabiting the Gran Chaco territory of Paraguay than in those living elsewhere in the country. The study by Ferrer *et al.*²⁷ shows that the prevalence of *T. cruzi* infection was 43.5% in Paleoamerindians but only 2.5% in non-Indians (Mennonites and Paraguayans) in the Department of Boqueron (Gran Chaco). Chagas disease is also

Table 1 Demographic and epidemiological characteristics of the participants in the study

Variable	Paraguayans (<i>n</i> =128)	Bolivians (<i>n</i> =73)	Total (<i>n</i> =201)	<i>P</i> value
Gender				0.04
Female	80 (62.5%)	34 (46.6%)	114 (56.7%)	
Male	48 (37.5%)	39 (53.4%)	87 (43.3%)	
Age				0.07
Median years (range)	30 (5–78)	29 (2–60)	30 (2–78)	
<30 years	58 (45.3%)	39 (53.4%)	97 (48.3%)	
≥30 years	70 (54.7%)	34 (46.6%)	104 (51.7%)	
Time in Spain				0.1
Median months (range)	54 (1–170)	55 (24–176)	54 (1–176)	
Knowledge about Chagas disease				0.9
No	47 (36.7%)	27 (37.0%)	74 (36.8%)	
Yes	81 (63.3%)	46 (63.0%)	127 (63.2%)	
Relatives with Chagas disease				<0.001
No	117 (91.4%)	48 (65.8%)	165 (82.9%)	
Yes	11 (8.6%)	25 (34.2%)	36 (17.7%)	
Living in mud houses				<0.001
No	82 (64.1%)	25 (34.2%)	107 (53.2%)	
Yes	46 (35.9%)	48 (65.8%)	94 (46.8%)	
History of blood transfusion				0.2
No	126 (98.4%)	69 (94.5%)	195 (97.0%)	
Yes	2 (1.6%)	4 (5.5%)	6 (3.0%)	

prevalent in the departments of Cordillera and Paraguari (~7.7% of Paraguay population). Data from surveys conducted in 2000 on pregnant women living in these two departments showed prevalences of 15 and 12%, respectively. The prevalence declined to 6% in both departments in 2009.^{24,28} The decrease was attributed to improvements in living conditions, and vector control activities.^{24,28} In this study, the cases of Chagas disease were from the departments of Central, Itapúa, Paraguari, and Presidente Hayes. Among 32 participants from the department of Cordillera (not in Gran Chaco), no cases of Chagas disease were detected.

Demographic and epidemiological data obtained from Latin American immigrants may help to establish a way of predicting the risk of infection.⁹

Muñoz *et al.*⁸ identified independent variables associated with *T. cruzi* infection, such as female gender, aged over 25 years, coming from countries below the equator and living in mud houses. Our study suggests that auto-referred symptoms (chest pain and palpitations) may also be useful in identifying those patients with *T. cruzi* infection in immigrants from Paraguay.

One limitation of this type of study is that not all the participants come from endemic regions of their countries. This may account for the discrepancies observed in the seroprevalence described for Latin American immigrants residing in different parts of Europe when attributing prevalence data to a specific country. This observation may be applicable to countries such as Argentina and Paraguay, because

Table 2 Epidemiological characteristics in all patients, Paraguayans, and Bolivians *Trypanosoma cruzi*-infected and non-*T. cruzi*-infected

Variable	All		Paraguayans		Bolivians	
	<i>T. cruzi</i> -infected (n=13)	Non- <i>T. cruzi</i> -infected (n=174)	<i>T. cruzi</i> -infected (n=6)	Non- <i>T. cruzi</i> -infected (n=122)	<i>T. cruzi</i> -infected (n=7)	Non- <i>T. cruzi</i> -infected (n=64)
Gender						
Female	7 (53.8%)	107 (56.9%)	4 (66.7%)	76 (62.3%)	3 (42.9%)	31 (47.0%)
Male	6 (46.2%)	81 (43.1%)	2 (33.3%)	46 (37.7%)	4 (57.1%)	35 (53.0%)
Age						
Median years (range)	35 (22–51) [†]	29.5 (2–78)	45.5 (22–51) [§]	30 (5–78)	34 (27–48)**	28 (2–60)
<30 years	3 (23.1%)	94 (50%)	1 (16.7%)	57 (46.7%)	2 (28.6%)	29 (43.9%)
≥30 years	10 (76.9%)	94 (50%)	5 (83.3%)	65 (53.3%)	5 (71.4%)	29 (43.9%)
Mud houses						
Yes	8 (61.5%)	86 (45.7%)	3 (50%)	43 (35.2%)	5 (71.4%)	43 (65.2%)
No	5 (38.5%)	102 (54.3%)	3 (50%)	79 (64.8%)	2 (28.6%)	23 (34.8%)
Knowledge about Chagas diseases						
Yes	9 (69.2%)	118 (62.8%)	4 (66.7%)	77 (63.1%)	5 (71.4%)	41 (62.1%)
No	4 (30.8%)	70 (37.2%)	2 (33.3%)	45 (36.9%)	2 (28.6%)	25 (37.9%)
History of relatives with Chagas diseases						
Yes	3 (23.1%)	33 (17.6%)	0	11 (9.0%)	3 (42.9%)	22 (33.3%)
No	10 (76.9%)	155 (82.4%)	6 (100%)	111 (91.0%)	4 (57.1%)	44 (66.7%)
History of transfusion						
Yes	1 (7.7%)	5 (2.7%)	0	2 (1.6%)	1 (14.3%)	3 (4.5%)
No	12 (92.3%)	183 (97.3%)	6 (100)	120 (98.4%)	6 (85.7%)	63 (95.5%)
Departments*						
A			6 (100%) [‡]	56 (45.9%)	6 (85.7%)	51 (77.3%)
B			0	66 (54.1%)	1 (14.3%)	15 (22.7%)
Palpitations						
Yes	7 (53.8%) [‡]	42 (22.3%)	4 (66.7%) [†]	27 (22.1%)	3 (42.9%)	15 (22.7%)
No	6 (46.2%)	146 (77.7%)	2 (33.3%)	95 (77.9%)	4 (57.1%)	51 (77.3%)
Chest pain						
Yes	5 (38.5%) [‡]	23 (12.2%)	3 (50%) [¶]	10 (8.2%)	2 (28.6%)	13 (19.7%)
No	8 (61.5%)	165 (87.8%)	3 (50%)	112 (91.8%)	5 (71.4%)	53 (80.3%)
Constipation						
Yes	5 (38.5%)	48 (25.5%)	2 (33.3%)	31 (25.4%)	3 (42.9%)	17 (25.8%)
No	8 (61.5%)	140 (74.5%)	4 (66.7%)	91 (74.6%)	4 (57.1%)	49 (74.2%)
Dysphagia						
Yes	0	6 (3.2%)	0	1 (0.8)	0	5 (7.6%)
No	13 (100)	182 (96.8%)	6 (100)	121 (99.2%)	7 (100)	761 (92.4%)

Notes: *Paraguayans (*T. cruzi*-infected/non-*T. cruzi*-infected) Departments A: Central (3/42) Itapúa (1/8), Paraguari (1/6), and Presidente Hayes (1/1); Departments B: Caaguazú (0/2), Alto Paraná (0/3), Caazapá (0/3), Concepción (0/3), San Pedro (0/3), Ñeembucú (0/5), Distrito Capital (0/12), Cordillera (0/32), and born in Spain (0/2). Bolivians (*T. cruzi*-infected/non-*T. cruzi*-infected) Departments A: Cochabamba (4/36) and Santa Cruz (2/15); Departments B: Oruro (0/6); La Paz (0/3), Potosí (1/1), Beni (0/1), and born in Spain (0/3).

The numbers printed in bold letters indicate a significant difference of the corresponding patient characteristic with respect to test.

[†]*P*=0.03; [‡]*P*=0.02; [§]*P*=0.06; [¶]*P*=0.01; ^{**}*P*=0.08.

they have marked regional differences in the seroprevalence. Among the 126 participants in this study that were identified as coming from Paraguay, 73 (58%) reported that they lived in six of the non-endemic departments (Central, Itapúa, Alto Paraná, Caazapá, Ñeembucú, and Distrito Capital) but their prevalence for *T. cruzi* infection was relatively high at 5.5% (4/73) showing a high risk of transfusional or vertical transmission even in non-endemic areas of Paraguay. Therefore, the distinction in Spain between non-endemic and endemic regions of *T. cruzi* infection in Paraguay is not pertinent. Another limitation of this study is the relatively small sample size, even though it represents more than 10% of local immigrants from those countries living in Elche.

This study highlights the importance of Chagas disease in the Paraguayan population living in Spain. The prevalence observed in this population is rather high, even though the disease occurs more commonly in Bolivians. These findings show the necessity of implementing diagnostic strategies and health resource planning. More epidemiological data are necessary to estimate the magnitude of this public health problem in Spain.

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