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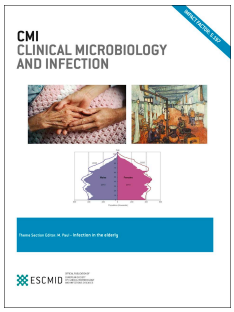


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# CHAGAS DISEASE KNOCKS ON OUR DOOR: A CROSS-SECTIONAL STUDY AMONG LATIN AMERICAN IMMIGRANTS IN MILAN, ITALY

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**RUNNING TITLE**: Chagas disease in Italy

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**ABSTRACT**

**Objectives:** We aimed to assess the prevalence and risk factors for Chagas Disease (CD) in Latin American immigrants and to evaluate the accuracy of diagnostic tests. Moreover, we offered to all positive subjects a complete free-of-charge clinical/instrumental evaluation as well as benznidazole treatment in order to stage the disease and verify drug tolerability.

**Methods:** A cross-sectional survey of CD among Latin Americans living in Milan and its metropolitan area was conducted between July 2013 and July 2014. Blood samples were tested for serologic evidence of CD together with a questionnaire covering demographic and clinical-epidemiological information.

**Results:** Forty-eight (9.6%) of the 501 tested subjects were conclusively diagnosed as having CD. The highest prevalence of CD was among those from Bolivia (43/169, 25.4%) and El Salvador (4/68, 5.9%). Older age (adjusted odds ratio [aOR] 1.05, $p$=0.004), a Bolivian origin (aOR 8.80; $p$=0.003), being born in the department of Santa Cruz (aOR 3.72, $p$=0.047 ), having lived in mud houses (aOR 2.68; $p$=0.019), and having an affected relative (aOR 12.77, $p$=0.001) were independently associated with CD. The ARCHITECT Chagas test showed the highest sensitivity (100%) and specificity (99.8%). Twenty-nine of the subjects with CD (60.4%) underwent disease staging, ten of whom (35.7%) showed cardiac and/or digestive involvement. Benznidazole treatment was associated with high frequency of adverse reactions (19/27, 70.4%) and permanent discontinuation (8/27, 29.6%)

**Conclusions** CD is highly prevalent among Bolivians and Salvadorans living in Milan. Regions with a large Latin American immigrant population should implement programmes of active detection and treatment.

**KEY WORDS**: *Trypanosoma cruzi*; screening; Chagas disease; Latin American immigrants; Italy

## INTRODUCTION

Chagas disease (CD) or American trypanosomiasis, which is caused by *Trypanosoma cruzi*, is a predominantly vector-borne parasitic zoonosis that, under ecological conditions is endemic in Central and South America, Mexico and Texas [1,2]. However, it can also be transmitted by oral route, blood transfusions, organ transplantations, and from mother to child [3], with the last three modes of transmission being a matter of concern in non-endemic countries. The World Health Organisation (WHO) estimates that about six million people are infected by *T. cruzi* throughout the world, 62% of whom live in countries of the Southern Cone initiative against CD, particularly Argentina, Brazil, Bolivia and Mexico [4].

Over the last 10-15 years, massive migration from Latin America to Europe and the USA has led to the urbanisation of CD in non-endemic countries, and a recent meta-analysis of studies of CD among Latin Americans living in the European Union (EU) has calculated a pooled prevalence of 4.2% [5]. Like Spain, Italy hosts many people who have immigrated to the EU but, despite this, the disease is largely unknown to Italian physicians, which has led to a call to "break this epidemiological silence" [6-9].

The aims of this study were: 1) to assess the prevalence and risk factors for *T. cruzi* infection in a population of Latin American living in Milan and its metropolitan area; 2) to compare the overall accuracy of serological methods to detect *T. cruzi* infection; 3) to define the clinical stage of the disease among infected patients; and 4) to evaluate benznidazole tolerability among treated patients.

## METHODS

### Study design and setting

This cross-sectional survey of CD was conducted in a population of Latin American (LA) subjects living in Milan and its metropolitan area (which hosts the largest LA community in Italy) [10] between 30 July 2013 and 30 July 2104. The top 5 countries of origin for this population are Brazil, Bolivia, Ecuador, El Salvador and Peru (Fig.1). The participants were recruited by means of an active outreach to the local Latino communities with the support of Médecins Sans Frontières

(MSF): three MSF health promoters of LA origin participated in 20 health fairs in 13 sites including local churches (n= 8) cultural community organizations (n=5) and the Bolivian consolate (n=1). Screening sessions took place 2-3 times monthly in a dedicated outpatient clinic at our Hospital and at "Opera San Francesco per i Poveri", an outpatient care facility for indigent subjects and undocumented immigrants.

Demographic and clinic-epidemiological information was collected at the time of blood sampling using a standardised questionnaire. The study protocol also included serological testing for *Strongyloides*. Free clinical investigations and treatment were offered to all of the subjects diagnosed as having CD.

The study was approved by the Ethics Committee of AO-Polo Universitario Luigi Sacco, Milan, Italy (Protocol No. 480/2013/61/AP) and the Médecins San Frontières Ethics Review Board (Geneva, Switzerland). Written informed consent was obtained for subjects before their enrolment (in the case of children, this was given by their parents/guardians).

**Laboratory methods**

CD screening was carried using two serological tests in parallel: an enzyme-linked immunosorbent assay (ELISA) based on a lysate antigen of *T. cruzi* (BioELISA Chagas III, BiosChile, Santiago, Chile) and a chemiluminescence immunoassay (CLIA) based on recombinant antigens of distinct regions of the parasite (ARCHITECT Chagas, Abbott, Chicago, IL, USA), which was performed using an automated ARCHITECT $i2000_{SR}$ system (Abbott).

A subset of blood samples were also tested by means of a CLIA directed against three distinct antigenic regions (LIASON® XL MUREX Chagas, DiaSorin, Saluggia, Italy) and an immunochromatographic test (*Trypanosoma cruzi* IgG Rapid Test, ImmunoSpark, SD, Rome, Italy) in order to investigate their performance in screening CD. Serological testing for anti-*Strongyloides stercoralis* IgG was carried out using the *Strongyloides rattii* ELISA (Bordier Affinity Products, Effegiemme Diagnostici, Milan, Italy).

**CD diagnosis and treatment**

CD was diagnosed on the basis of the concordant positive results of two independent serological tests in accordance with the WHO guidelines [11].

Cardiac involvement was investigated by means of electrocardiography (ECG), chest X-rays, and echocardiography. The Brazilian Consensus Classification was used to classify cardiac CD [12].

Digestive involvement was assessed by means of a colonic barium enema and a barium X-ray of the esophagus and considered pathological as previously reported [13,14].

All of the CD-positive participants were offered treatment with benznidazole 5 mg/kg/day (maximum 300 mg/day) for 60 days.

**Statistical analysis**

The continuous variables were compared using the Mann-Whitney non-parametric test; the categorical variables were compared using the chi-squared test, with Fisher's corrections when necessary. Univariate and multivariate logistic regression models were used to identify the factors associated with CD. The variables in the multivariate analysis were selected using a backward procedure based on a cut-off *p*-value of 0.05.

The diagnostic accuracy of serological test was calculated as sensitivity, specificity, validity index, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratio and the Youden index, which is a measure of the overall discriminative power of a diagnostic procedure and Cohen's kappa coefficient which describes the level of concordance among tests.

**RESULTS**

A total of 501 Latin Americans (63% female) were screened for CD: 471 adults with a median age of 39 years (interquartile range [IQR] 32.0-48.7), and 30 children with a median age of 10.5 years (IQR 8.2-14.0), of whom seven were born in Latin America and had been adopted by Italians, and five were born in Italy of Latin American parents. The median time from their arrival in Italy was eight years (IQR 3.5-11.0). Ninety-one percent of the subjects came from four countries (Bolivia, Peru , El Salvador, Ecuador); the others came from Brazil (16), Argentina (6), Venezuela (4), Chile

(3), Colombia (3), Guatemala (2), Paraguay (2) and Uruguay (1), or had no recorded country of origin (8).

**Serology diagnosis**

Initially 46 subjects (9.2%) were diagnosed with *T. cruzi* infection on the basis of two concordant serology results; 451 subjects (90.0%) had negative serology with both tests and 4 (0.8%) had initially discordant results: three positive with ARCHITECT (2.87, 4.69 and 1.21 S/CO, respectively) and negative with BioELISA (0.03, 0.7 and 0.22 index value, respectively) and one negative with ARCHITECT (0.61 S/CO) and positive with BioELISA (1.2 index value). Repeated serology of discordant cases (using also the other available tests) allowed us to resolve the discrepancies with two confirmed cases (both with the highest S/CO on ARCHITECT and one of whom had a subsequent diagnosis of megacolon) and two negative cases.

In table 1 are summarised the diagnostic results and performance of serological and immunochromatographic assays used for the diagnosis of *T. cruzi* infection. A high proportion of patients were correctly classified by ARCHITECT and BioELISA (validity index 99.80 and 99.40, respectively) and both tests showed a high level of agreement (k index 0.955) although the former performed better for all measures. The Liaison Chagas assay has a high sensitivity and specificity but was slightly less efficient than the counterpart assay employing the chemiluminescence detection system. The median S/CO values were 13.8 (IQR 10.6-14.9) for the ARCHITECT assay and 11 (IQR 7.6-12) for the Liaison assay. With reference to true positive serum values, 43/48 samples (89.6%) showed results of > 6S/CO with the ARCHITECT and 25/31 samples (80.6%) with the Liaison.

The performance of the rapid diagnostic test was characterised by low sensitivity (89% and a validity index of 91.6%

**Prevalence of CD and risk factors for the disease**

CD was diagnosed in 48 subjects (overall prevalence: 9.6%) of whom 43 coming from Bolivia (25.4% of the 169 enrolled Bolivians), four from El Salvador (5.9% of the 68 enrolled

Salvadorans), and one from Argentina. Bolivians accounted for 89.6% (43/48) of the subjects with CD and only 27.8% (126/453) of those without CD ($p$<0.0001). Subjects with CD came more frequently from the departments of Santa Cruz (24/48, 50% *vs* 47/453, 10.4%) and Cochabamba (15/48, 31.3% *vs* 43/453, 9.5%; $p$<0.0001); had more frequently lived in rural areas (39/48, 81.3% *vs* 233/453, 51.4%; $p$=0.0001) and mud houses (38/48, 79.1% *vs* 201/453, 44.3%; $p$<0.0001); were more frequently related to people affected by CD (11/48, 22.9% *vs* 5/453, 1.1%; $p$<0.0001 ); and were more frequently infected with *S. stercoralis* (7/48, 14.6% *vs* 18/453, 4.%; $p$=0.001) (Tab. 2). Serology for *S. stercoralis* was positive in 5% of screened individuals (25/501), seventeen of whom from Bolivia, 3 from El Salvador, 2 from Peru and 1 from Brazil. Median serology titres were 1.47 (IQR 1.15-1.95). All patients with CD and strongyloidiasis were from Bolivia. Four out of 14 patients who underwent culture tested positive for *S. stercoralis*.

The results of the multivariate analysis (Tab.3) showed that the factors independently associated with an increased risk of developing CD were an older age (aOR: 1.05 for every year older, 95% CI 1.02-1.09; p=0.004), Bolivian nationality (aOR 8.80, 95% CI 2.10-36.87; p=0.003), being born in the department of Santa Cruz (OR 3.72, 95% CI 1.02-13.64; p=0.047), having lived in a mud house (OR 2.68, 95% CI 1.17-6.13; p=0.019), and having a relative with a diagnosis of *T. cruzi* infection (OR 12.77, 95% CI 2.96-55.06; p=0.001).

**Staging and treatment of CD cases**

Twenty-nine (60.4%) of the subjects diagnosed with CD underwent clinical evaluations. On the basis of the findings of instrumental examinations, 17 subjects (58.6%) were classified as having indeterminate CD, four (13.8%) Chagas cardiomiopathy (Cc), five (17.2%) digestive Chagas disease, and one Cc + digestive disease (Tab. 4). Only two of these patients were symptomatic: one complained of palpitations and one of constipation. Twenty-seven of the 29 patients accepted benznidazole treatment. Nineteen (70.4%) experienced adverse reactions, which led to permanent treatment discontinuation in eight cases (29.6%): 4 women (4/19, 21%) and 4 men (4/8, 50%). The most frequent side-effects were skin rash (37%), pruritus (18.5%) and fever (14.8%). Two patients

had severe polyarthritis (7.4%) requiring hospitalisation, and two complained of severe arthralgias. Nervous system disturbances (headache, anxiety, dysgeusia and paresthesia) were recorded in five patients (18.5%).

## DISCUSSION

This is the first survey of CD among Latin Americans living in the metropolitan area of Milan which hosts the largest community of LA immigrants in Italy. Previous retrospective studies carried in Italy have reported CD prevalence rates ranging from 4.2% to 17% [7,9], and one cross-sectional study recorded a prevalence of 7.9% [15]. In line with these data, the prevalence of *T. cruzi* infection in our population as a whole was 9.6%, being much higher (25.6%) among Bolivians, a finding that is similar to previous studies carried out in Italy, Spain and Switzerland [7-9,15-17]. In a meta-analysis by Requena-Méndez *et al*., the pooled prevalence of *T. cruzi* infection among Bolivians living in Europe was 18.1% [5], which is three times higher than that estimated in Bolivia by the WHO (6.1%) [4].

We found that Bolivian nationality was associated with a 8.8 higher risk of testing positive for *T. cruzi*, which may reflect the fact that many of these people came from areas in which *T. cruzi* is hyperendemic [8,9,16,17]. Immigrants from Santa Cruz accounted for 42% of our Bolivian population, and being born there was associated with a three times higher risk of developing CD in comparison with other Latin American localities outside Bolivia.

It should also be noted that the prevalence of CD among immigrants from El Salvador was as high as 5.9%. According to WHO estimates, El Salvador is fifth most affected Latin American country, with a prevalence of 1.3% [4]. However, two recent studies of pregnant women and blood donors living in the departments of Sonsonate and Ahuachapàn found prevalence rates of respectively 3.8% and 2.5% [18,19], and it has been reported that the prevalence of CD among Salvadoran immigrants living in Los Angeles is 3.4% [20]. There is little information about the prevalence of CD among

Salvadorans living in Europe: the above mentioned meta-analysis [5] that included 67 Salvadoran migrants found a pooled prevalence of 3.7%: three times that estimated in El Salvador by the WHO [4].

Our findings also show that, regardless of nationality and department of origin, an older age is associated with an increased risk for CD, which probably reflects longer and more intense exposure to vector transmission in the country of origin [17] as none of the children in our study had CD. As expected, having lived in a mud house and having a relative with CD were also independently associated with an increased risk of CD [8,21,22]. The significant higher prevalence of strongyloidiasis observed among patients with CD confirms the results of a recent retrospective Spanish study highlighting possible shared epidemiologic and socioeconomical risk factors and the need of a combined screening for both infections [23].

CD diagnosis based on simultaneous use of two serological tests is costly and time consuming. Moreover, even with this expedient a discordance between tests can be observed. In our study, a discordance was observed in the 0.8% of cases but this rate was much higher (3.3%) in a recent Spanish-Italian study [24]. Repeating the tests on a new serum sample can usually solve much of discordant cases but for those with still inconclusive results it is indicated to employ a western blotting directed toward trypomastigote excreted-secreted antigens (TESA). On the other hand there is increasing need to make serology screening for CD simpler, faster and cheaper possibly using a single test. In this regard Abras *et al*. proposed the use of ARCHITECT Chagas as a single technique for routine screening based on its high sensitivity and specificity [25]. We observed a high level of agreement between the two different serological tests employed in our study but we confirm that ARCHITECT Chagas had the best performance (100% sensitivity , 99.8% specificity) and we concur with the strategy suggested by Abras *et al*. to reserve a second test only for positive sera with a result < 6 S/CO that in our study were about 10% [25].

Previous studies have shown that the sensitivity of rapid diagnostic tests is unacceptable for screening purposes [26,27], and our own experience with the ImmunoSpark assay resulted unsatisfactory (validity index 91.6%).

More than one-third of our patients with CD showed organ involvement, with digestive disease being the most frequent (17.2%). This is consistent with the findings of two Italian studies [8,9], and suggests that digestive CD may be more frequent in Italy than in Spain or Switzerland [17,21,22,28]. This may be due to differences in the origins of the study populations and the disease classification criteria (inclusion of dolichocolon) [8], which may lead to the over-diagnosis of digestive CD [29]. However, even excluding people with dolichocolon, the 10.3% frequency of megacolon in almost all of our asymptomatic subjects is higher than that reported in other studies of patients complaining of digestive symptoms [17,21], thus supporting the importance of radiographic evaluation of all patients testing positive for CD. Conversely, we did not detect any abnormalities in the esophagus X-ray in our asymptomatic subjects, which argues against the usefulness of systematic esophageal assessments suggested by some authors [8,21].

The efficacy of benznidazole in the treatment of chronic CD is still debated, particularly its ability to slow the progression of Chagas cardiomyopathy [30,31]. A considerable proportion (70%) of our patients experienced adverse drug reactions (mainly skin rash) and about one-third permanently discontinued treatment a rate similar to that observed in studies carried out in non-endemic countries, but higher than that reported in endemic countries [31-34]. It remains to be established whether this reflects European physicians' lack of confidence and experience in treating CD, or other reasons such as the gender composition of the study population [33].

We acknowledge that our study has several limitations. Firstly, individuals who came to make the screening may be not representative of the whole population of LA immigrants living in our metropolitan area and might have been more motivated by perceving themselves at risk of CD and thus our results can overestimate the true prevalence of the infection, a selection bias observed also

in other studies conducted in Europe [5,35]. Secondly, our results may not be generalizable to other European countries or Italian regions.

In conclusion, CD is highly prevalent among Bolivians and Salvadorans and therefore regions with large LA immigrant communities, such as ours, should implement programmes aimed at actively detecting and treating CD with particular attention to special population such as pregnant women and immunocompromised patients.

**Figure legend**

Figure 1. Countries of origin and number of people living in Milan and its metropolitan area (overall) in 2014 for the five more represented communities of Latin American immigrants (Ref.10)

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**Trasparency declaration**

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**Table 1.** Performance of four serological tests for the diagnosis of *T.cruzi* infection [a].

| Measure | ARCHITECT Chagas [b] (n=501) | | BioELISA Chagas III [c] (n=501) | | Chagas Liaison XL [d] (n=263) | | Chagas Rapid ImmunoSpark [e] (n=95) | |
|---|---|---|---|---|---|---|---|---|
| | Number/total | 95% CI | Number/total | 95% CI | Number/total | 95% CI | Number/total | 95% CI |
| Sensitivity (%) | 100 (49/49) | 91.01-100 | 95.92 (47/49) | 85.34-99.56 | 96.77 (30/31) | 82.15-100 | 89.29 (25/28) | 71.79-96.96 |
| Specificity (%) | 99.78 (451/452) | 98.60-100 | 99.78 (451/452) | 98.60-100 | 99.14 (230/232) | 96.66-99.95 | 92.54 (62/67) | 83.21-97.08 |
| Validity index (%) | 99.80 (500/501) | 99.41-100 | 99.40 (498/501) | 98.73-100 | 98.86 (260/263) | 97.58-100 | 91.58 (87/95) | 85.99-97.16 |
| PPV (%) | 98.00 (49/50) | 94.12-100 | 97.92 (47/48) | 93.88-100 | 93.75 (30/32) | 85.36-100 | 83.33 (25/30) | 70.00-96.67 |
| NPV (%) | 100 (451/451) | 100-100 | 99.56 (451/453) | 98.95-100 | 99.57 (230/231) | 98.72-100 | 95.38 (62/65) | 90.28-100 |
| LR+ | 452.00 | 63.81-3201.83 | 433.55 | 61.15-3073.76 | 112.26 | 28.20-446.85 | 11.96 | 5.10-28.07 |
| LR- | 0.00 | - | 0.04 | 0.01-0.16 | 0.03 | 0.00-0.22 | 0.12 | 0.04-0.34 |
| Youden's index | 1.00 | 1.00-1.00 | 0.96 | 0.86-1.00 | 0.96 | 0.83-1.00 | 0.82 | 0.65-0.92 |

a- In case of discordant results serology was repeated using a new sample after 2-4 months.
b- The chemiluminescent reaction is measured in relative light units (RLUs). The results are expressed as sample RLUs/cutoff value (S/CO) with ratio < 0.8 considered negative, inconclusive from ≥ 0.8 to < 1 and ≥ 1 positive.
c- For the ELISA test results were expressed as the index between the absorbance of the test serum and the cutoff value. Tests were considered negative if the index was < 0.9, equivocal if ≥ 0.9 and < 1, and positive if ≥1.
d- The chemiluminescent reaction is measured in relative light units (RLUs). The results are expressed as sample RLUs/cutoff value (S/CO) with ratio < 0.9 considered negative, inconclusive from ≥ 0.9 < 1 and ≥ 1 positive.
e - 5 µL of serum were added in the sample port following the transfer of 2 full drops of conjugate into the reagent port. The results were read within 5 minutes after the sample application. The test was considered positive if a pink/purple line was seen in the test and control areas. The test was considered negative if a pink/purple line was seen only in the control area. The test was invalid (and was repeated) if the control line failed to appear.

PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR-: negative likelihood ratio.95%CI, 95% confidence interval.

**Table 2. Characteristics of the participants who were positive and negative to *T. cruzii* screening.**

| Variable | Total screened N=501 | Negative for CD (n=453) | Positive for CD (n=48) | *P* value |
|---|---|---|---|---|
| Age in years, median (IQR) | 39 (31-48) | 39 (31-48) | 41 (17-66) | 0.405 |
| Females | 315 (62.9) | 283 (62.5) | 16 (33.3) | 0.448 |
| Males | 186 (37.1) | 170 (37.5) | 32 (66.7) | |
| Country of origin, n (%) | | | | |
| Bolivia | 169 (33.7) | 126 (27.8) | 43 (89.6) | <0.0001 |
| Peru | 159 (31.8) | 159 (35.1) | 0 (0.0) | |
| El Salvador | 68 (13.6) | 64 (14.1) | 4 ( 8.3) | |
| Ecuador | 60 (12.0) | 60 (13.2) | 0 (0.0) | |
| Other | 45 (9.0) | 44 ( 9.7) | 1 (2.1) | |
| Department of origin, n (%) | | | | |
| Santa Cruz | 71 (14.2) | 47 (10.4) | 24 (50.0) | <0.0001 |
| Cochabamba | 58 (11.6) | 43 (9.5) | 15 (31.3) | |
| La Paz | 18 ( 3.6) | 18 (4.0) | 0 (0.0) | |
| Other | 354 (70.7) | 345 (76.2) | 9 ( 18.7) | |
| Had lived in rural areas, n (%) | | | | |
| Yes | 272 (54.2) | 233 (51.4) | 39 (81.2) | 0.0001 |
| No | 229 (45.7) | 220 (48.6) | 9 (18.7) | |
| Had lived in mud houses, n (%) | | | | |
| Yes | 239 (47.7) | 201 (44.3) | 38(79.2) | <0.0001 |
| No | 262 (52.3) | 252 (55.7) | 10 (20.8) | |
| Had received transfusion, n (%) | | | | |
| Yes | 43 (8.6) | 3 (6.3) | 40 (8.8) | 0.569 |
| No | 458 (91.4) | 45 (93.7) | 413 (91.2) | |
| Had a relative with CD, n (%) | | | | |
| Yes | 16 (3.2) | 5 (1.1) | 11 (22.9) | <0.0001 |
| No | 485 (96.8) | 448 (98.9) | 37 (77.1) | |
| Positive to *Strongyloides* serology, n (%) | | | | 0.001 |
| Yes | 25 (5.0) | 18 (4.0) | 7 (14.6) | |
| No | 476 (95.0) | 435 (96.0) | 41 (85.4) | |

**Table 3- Univariate and multivariate logistic regression analysis of the risk factors for Chagas disease**

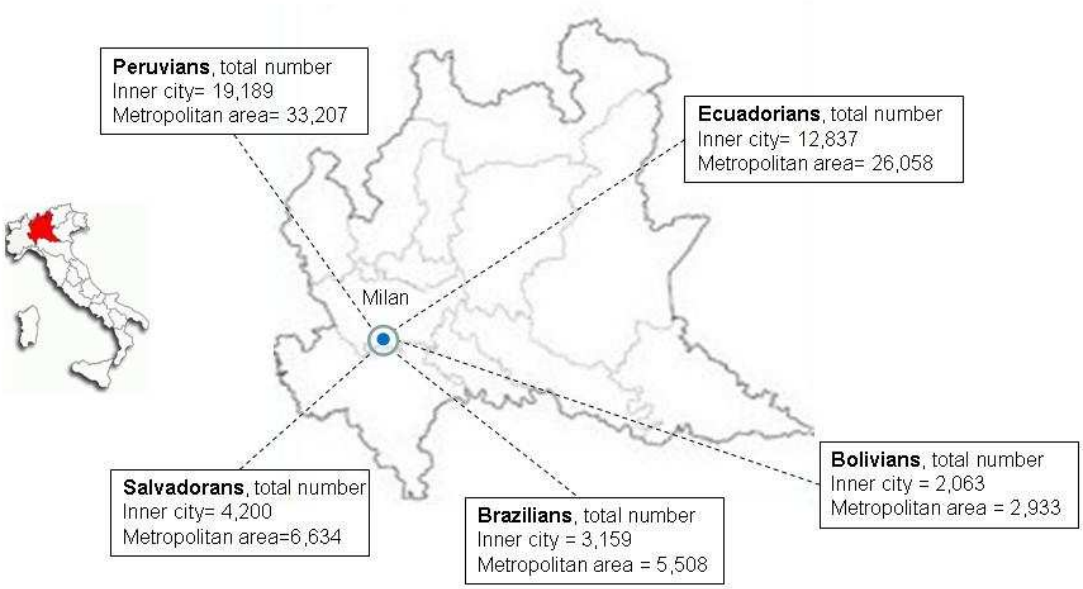
| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *p* value | aOR | 95% CI | P value |
| Age, for 1 year more | 1.01 | 0.99-1.04 | 0.314 | 1.05 | 1.02-1.09 | 0.004 |
| Gender | | | | | | |
| Males | 1 | | | | | |
| Females | 1.28 | 0.67-2.44 | 0.449 | - | - | - |
| Country of origin | | | | | | |
| All but Bolivia | 1 | | | 1 | | |
| Bolivia | 21.80 | 8.43-56.35 | <0.0001 | 8.80 | 2.10-36.87 | 0.003 |
| Department of origin | | | | | | |
| Provinces outside Bolivia | 1 | | | 1 | | |
| Santa Cruz | 17.99 | 7.96-40.66 | <0.0001 | 3.72 | 1.02-13.64 | 0.047 |
| Cochabamba | 12.96 | 5.42-30.96 | <0.0001 | 2.09 | 0.56-7.78 | 0.272 |
| La Paz | 0.98 | 0.05-18.92 | 0.991 | 0.18 | 0.01-4.06 | 0.278 |
| Having lived in rural areas | | | | | | |
| No | 1 | | | | | |
| Yes | 3.99 | 1.88-8.44 | <0.001 | - | - | - |
| Having lived in mud houses | | | | | | |
| No | 1 | | | 1 | | |
| Yes | 4.64 | 2.25-9.56 | <0.0001 | 2.68 | 1.17-6.13 | 0.019 |
| Previous transfusions | | | | | | |
| No | 1 | | | | | |
| Yes | 0.70 | 0.21-2.37 | 0.571 | - | - | - |
| Having a relative with CD | | | | | | |
| No | 1 | | | 1 | | |
| Yes | 24.22 | 7.86-74.57 | <0.0001 | 12.77 | 2.96-55.06 | 0.001 |
| Serological positivity for *Strongyloides* | | | | | | |
| No | 1 | | | 1 | - | - |
| Yes | 4.23 | 1.67-10.73 | 0.002 | 12.77 | | |

The strength of the associations was measured by means of odds ratios (ORs) and adjusted ORs (aORs) with their 95% confidence intervals (95% CI). All of the data were statistically analysed using SAS version 9.4, and differences with *p*-values of <0.05 were considered statistically significant.

**Table 4- Clinical characteristics of 29 subjects with Chagas disease who underwent clinical staging.**

| Characteristic | N (%) |
|---|---|
| Country of origin | |
| Bolivia | 26 (89.7) |
| El Salvador | 2 (6.9) |
| Argentina | 1 (3.4) |
| Median age (IQR) | 37.5 (17-67) |
| Gender | |
| Male | 7 (24.1) |
| Female | 22 (75.9) |
| Clinical stage, | |
| Indeterminate | 18 (62.1) |
| Chagas cardiomyopathy | 4 (14.3) |
| Digestive Chagas | 5 (17.8) |
| Chagas cardiomyopathy + digestive disease | 1 (3.6) |
| ECG, | |
| Normal | 22 (75.9) |
| RBBB* | 2 (6.9) |
| Right focal BBB | 1 (3.4) |
| Sinus bradycardia (< 50 bpm) | 2 (6.9) |
| Sinus bradycardia+RBBB | 1 (3.4) |
| IRBB§ | 1 (3.4) |
| Chest radiography | |
| Normal | 27 (93.1) |
| Enlarged heart | 1 (3.5) |
| Hylar lymphadenopathy‡ | 1 (3.5) |
| Echocardiography | |
| Normal ejection fraction (>50%) | 29 (100) |
| Normal findings | 12 (41.4) |
| Mild tricuspid regurgitation | 7 (20.7) |
| Mild mitral regurgitation | 5 (17.2) |
| Mild mitral and aortic regurgitation | 1 ( 3.4) |
| Tricuspid insufficiency | 2 (6.9) |
| Esophageal barium | |
| Normal | 29 (100) |
| Barium enema | |
| Normal | 22 (75.0) |
| Dolichocolon | 4 (14.3) |
| Megacolon | 3 (10.7) |

*RBBB: right bundle branch block; §IRBB: incomplete right bundle-branch block; ‡ Confirmed to be sarcoidosis


**Peruvians**, total number
Inner city= 19,189
Metropolitan area= 33,207
**Ecuadorians**, total number
Inner city= 12,837
Metropolitan area= 26,058
Milan
**Salvadorans**, total number
Inner city= 4,200
Metropolitan area=6,634
**Brazilians**, total number
Inner city = 3,159
Metropolitan area = 5,508
**Bolivians**, total number
Inner city = 2,063
Metropolitan area = 2,933