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Age-related anomalies of electrocardiograms in patients from areas with differential Seroprevalence of Chagas disease in Southern Bolivia

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ABSTRACT

Introduction: Chagas disease currently affects some 6 million people around the world. At the chronic stage, cardiomyopathy occurs in about 20–30% of infested people. Most prevalence studies have focused on young to adult people due to the drastic consequences of acquiring the pathogen and the possibility to cure the disease at this age; the prevalence of this disease, the effect of patients' sex and the consequences to senescent people have been largely neglected. This study looks to characterize the seroprevalence of Chagas disease and its relation with occurrence of electrocardiographic anomalies associated with sex and age, and to compare rural and urban populations in Bolivia.

Methodology: Seroprevalence of Chagas disease was determined in blood samples and electrocardiograms were performed on seropositive individuals.

Results: The rural population showed higher seroprevalence than the urban population (92% and 40%, respectively). The proportion of Chagasic cardiac anomalies in seropositive persons was highest in patients of the 50–59 age group (36%) as compared with the 40–49 (8%) and the ≥60 (17%) age groups.

Conclusions: Higher seroprevalence in rural population was attributable to a higher probability to encounter the vector in rural areas. Increased exposure to infection and to development of the disease symptoms together with increased lethality of the disease as patients age explains the age-related Chagasic electrocardiographic anomalies. Since rural and urban populations showed different reactions under Chagas disease and the rural population was mainly of guaraní stock, the genetic and environmental determinants of the results should be further explored.

1. Introduction

American trypanosomiasis or Chagas disease was discovered and described by Carlos Chagas in 1909. The disease is caused by the protozoan parasite, *Trypanosoma cruzi*, which is transmitted by some 150 species of triatomine insects (Hemiptera: Reduviidae) and more than 100 mammal species, including humans, are reservoirs of *T. cruzi*. *T. cruzi* is transmitted mainly

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through the feces of triatomines, but it can also be transmitted vertically from infected mothers to their children during pregnancy, through blood transfusions, organ transplants, laboratory accidents and by ingestion of contaminated food (Rassi Jr. and Rassi, 2012; Bern, 2015).

Chagas disease has been detected worldwide although vector transmission is limited to Latin America, where it also shows the highest prevalence (Méndez et al., 2019; Olivera et al., 2019; Serra et al., 2019; Lidani et al., 2019). Bolivia is the country with the highest prevalence and incidence of the disease: more than 600,000 people are infected with an annual average of 8000 new infections (World Health Organization, 2015). Although the Bolivian government has designed policies against the disease such as, inter alia, the promulgation of law N° 3374 declaring Chagas disease a national priority, and the improvement of housing conditions in rural areas, such strategies have not achieved an adequate coverage in relation with the size of the population at risk of infection (Bosch, 2017).

Precarious living conditions in rural areas are the main risk factors in acquiring the disease because the most epidemiologically important vectors live in the cracks of mud walls and thatched roofs of rustic rural houses (Bern, 2015). Considerable effort has been made to reduce the rate and the effect of *T. cruzi* infestation in the human population: promotion of vector control (Rassi et al., 2003), better housing, early diagnosis of the disease to avoid contagion, and increased availability of advanced cardiac care. In infected people, treatment exists which can eliminate the disease even if it is at an acute stage or attenuate its effects if it is at a chronic phase. Notably, most newly infected patients experience only a small discomfort, and develop disease symptoms 20 or 30 years later; thus, Chagas is most often an insidious and silent disease (Punukollu et al., 2007).

Cardiac disorders are one of the most important consequences of infection by *T. cruzi (*Oliveira de Figueiredo and Pinho Ribeiro, 2018*)*. The main cardiopathic symptoms related with Chagas disease are an increase in the size of the heart, specific anomalies in cardiac rhythms and electrocardiographic alterations such as left ventricular enlargement with global or segmental systolic function impairment, that can be diagnosed by a specialist via a cardiological evaluation. Main causes of death are malignant arrhythmias (leading to sudden death) and cardiac failure (Rassi et al., 2017; Fernández et al., 2018).

The large number of studies carried out with people up to 50 years of age contrasts with the poor knowledge concerning older people. Chagasic elderly people frequently show advanced heart disease which, in some cases, is attenuated through the effects of pacemaker implantation (Arce et al., 2012). However, the prevalence of Chagas disease in elderly people is poorly known in comparison with other age groups. The objective of this study was to determine the seroprevalence of Chagas disease and electrocardiographic anomalies in relation to sex and age in rural and urban populations of the department of Chuquisaca, Bolivia. This is an area which is not yet free of vector transmission in spite of various governmental control programs which have been applied in this area (Ministerio de Salud y Deportes, 2020). The study used identical protocols in both populations, and analyzed the frequency of seropositive subjects and the electrocardiographic anomalies related with Chagas disease as a function of locality, sex and age.

2. Materials and methods

2.1. Populations

The municipality of Macharetí, with a population of less than 1% in urban areas (Mitelman and Giménez, 2007), was chosen as source of the rural population in the Chaco region of Chuquisaca department, while the municipality of Sucre, the capital of Chuquisaca department with over 91% urban population, was chosen as the source of the urban population (Fig. 1). In both cases, people of both sexes and over 40-years old were incorporated in a cross-sectional study performed between July and October 2018. Age was categorized in three groups: 40–49, 50–59 and ≥ 60 years. The rural population consisted of 91 individuals who volunteered to participate in the study. Blood samples and electrocardiograms were obtained by a mobile work team composed by a medical officer, three biochemists, three students and a Guaraní chief, since the rural population was mainly of guaraní stock. For the urban population, 95 persons born in Sucre were selected in coordination with the health centers of nine districts within the municipality. The urban participants were cited at a medical service office for blood sampling and electrocardiographic study.

2.2. Seroprevalence

The seroprevalence of Chagas disease was determined using the blood sample of each subject. Two types of tests were performed: i) the immunography test Chagas Ab Rapid kit from SD BIOLINE from Abbott, and ii) the immunoenzymatic test Chagatest (ELISA recombinant v.4.0) from Wiener Lab. A true seropositive individual was considered when both tests were positive. In all samples, both tests showed 100% of concordance.

2.3. Electrocardiograms

A 6-channel ECG (RT10405, RESTOMED) was used to perform electrocardiograms (ECGs) on seropositive individuals of the sampled population. ECGs were reported and analyzed following standardized protocols related with Chagas disease (World Health Organization, 2015; Mitelman and Giménez, 2007; Miorillo et al., 2015), using conventional 12-lead ECG (6 frontal plane and 6 horizontal plane sections) with extended D2 derivation for rhythm control. In those cases when alterations were found, the record was extended in order to determine aspects such as permanence or transience.

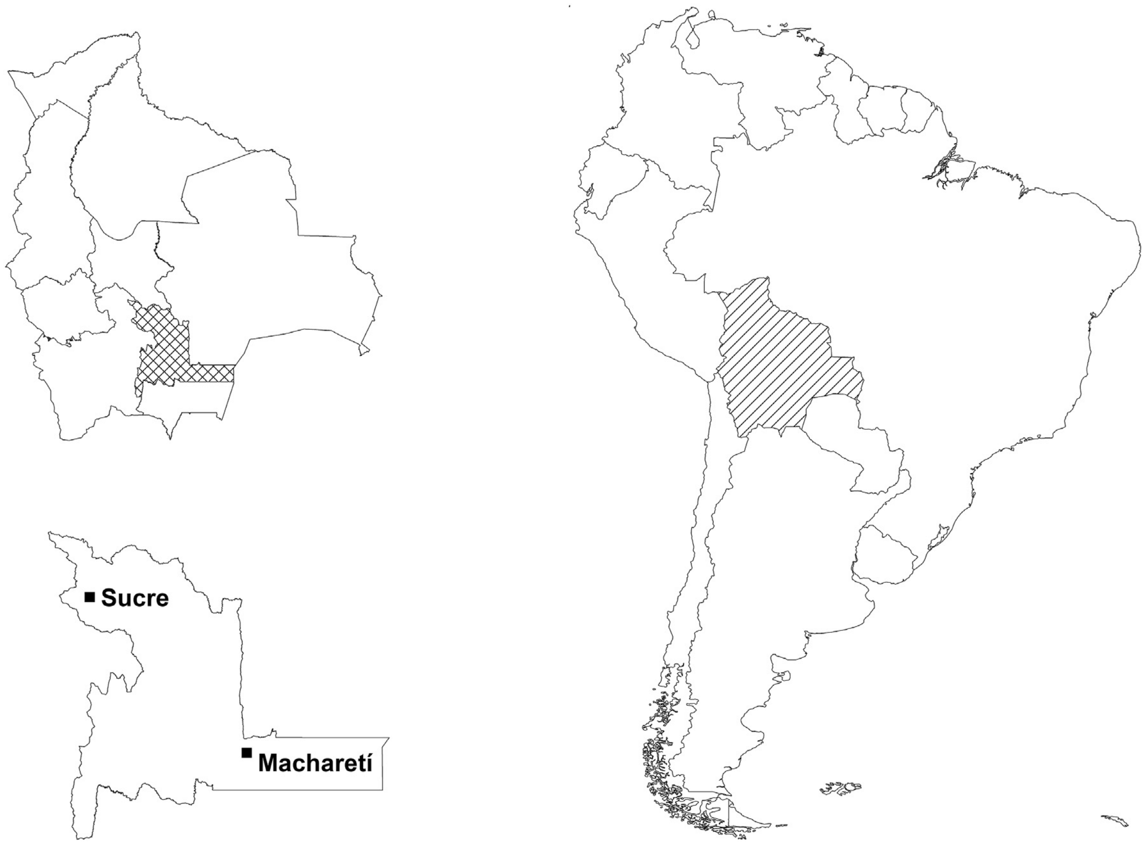

Fig. 1. Sampling sites. Ninety-five individuals were studied from the urban population of Sucre and 91 from five communities in the rural Municipality of Macharetí. Maps were drawn using ArcGIS.

2.4. Statistical analysis

Calculations on descriptive data were performed in Excel. The pwr library implemented in the R software was first used to verify that the sample size was adequate. Using a Coehn value = 0.3 (moderate value) with 196 patients and alpha = 0.05, the resulting statistical power was 0.972, thus showing adequacy of sample size used. Then, a Generalized Linear Model (GLM) using a binomial distribution of error and a logit link function was performed to assess simultaneously which explanatory variables and/or their interactions better explained differences in Chagas seroprevalence. The effect of the explanatory variables, locality (rural and urban), sex (women and men) and age (40–49, 50–59 and > 60) were tested. A forward analysis was used to search for the best model with the lowest AIC (Akaike information criterion) value. In order to explore differences in the proportion of individuals with Chagasic cardiac anomalies within rural and urban populations, a similar GLM using a binomial distribution was performed using locality, sex and age as factors. A forward analysis was also used to search for the model with the lowest AIC value. The GLM analysis was conducted using the R software v3.4.2 (R Core Team R, 2017). Finally, the odds ratio and the CI of 95% was estimated using the odds.ratio function implemented in the R software.

2.5. Ethical considerations

A written informed consent was obtained from all patients. This study was reviewed and approved by the Universidad San Francisco Xavier de Chuquisaca's bioethical committee.

3. Results

3.1. Seroprevalence

Of the 186 individuals screened, 122 (65.5%) were seropositive. Seroprevalence was 92.3% in the rural population and 40.0% in the urban population (Table 1). Men represented 46.2% of the individuals screened (34.1% of the rural population and 57.9% of the urban population) and women represented 53.8% of the individuals screened (65.9% of the rural population and 42.1% of the

Table 1
Study of Chagas disease at the Chuquisaca region of Bolivia. Immunographical and immunoenzymatic tests were performed. Seropositive individuals gave both tests positive and seronegative individuals both tests negative.

Locality	Age	Sex	Tested individuals	Seropositive individuals	Seronegative individuals	Chagas seroprevalence (%)
Rural	40–49	Male	9	8	1	88.9
Rural	50–59	Male	11	11	0	100.0
Rural	>60	Male	11	11	0	100.0
Rural	40–49	Female	27	25	2	92.6
Rural	50–59	Female	14	11	3	78.6
Rural	>60	Female	19	18	1	94.7
		Rural subtotals	91	84	7	92.3
Urban	40–49	Male	26	11	15	42.3
Urban	50–59	Male	21	12	9	57.1
Urban	>60	Male	8	4	4	50.0
Urban	40–49	Female	14	4	10	28.6
Urban	50–59	Female	19	5	14	26.3
Urban	>60	Female	7	2	5	28.6
		Urban subtotals	95	38	57	40.0
		Totals	186	122	64	

urban population). The average age of participating men was 53.3 years (55.5 years in the rural population and 52.0 years in the urban population) and that of women 54.5 years (55.6 years in the rural population and 53.0 years in the urban population).

The model containing locality and sex showed the lowest AIC value (39.35) compared to other models examined containing all possible comparisons of factors and their interactions. Hence, the forward analysis performed for the GLM exhibited a significant effect only of locality and sex as main factors (Table 2A). The proportion of seropositive individuals was significantly higher in the rural population than in the urban population ($p < 0.0001$, Fig. 2A). The odds ratio indicated that a person infected with Chagas disease was 25.3 (95% CI: 10.38–71.27, $p < 0.0001$) times more likely to be found among the rural than among the urban population. Furthermore, men showed a higher proportion of seropositive subjects than women (66% vs. 64%; $p = 0.018$, Fig. 2B). The odds ratio indicated that a person infected with Chagas disease was 2.7 (95% CI: 1.21–6.09) times more likely to be a man than a woman.

3.2. Electrocardiograms

Of the 122 patients subjected to an ECG, 98 (80.3%) showed normal results and 24 (19.7%) showed anomalies. Forty anomalies were found (27 in rural patients and 13 in urban patients): sinus bradycardia (1 rural, 5 urban; 15%), left anterosuperior hemiblockage (9 rural, 3 urban; 30%), complete blockage of right branch (8 rural, 4 urban; 30%), diffuse disorders of ventricular repolarization (8 rural, 1 urban; 22.5%), and overload of the right ventricle (1 rural; 2.5%).

The GLM model containing age as factor showed the lowest AIC values (34.67) compared to other models containing all possible comparisons of factors and their interactions. This model with the lowest AIC value for Chagas related anomalies in the electrocardiograms showed an effect of age as main factor (Table 2B). Eight percent of subjects showed abnormal electrocardiograms in the 40–49 age group, 36% the 50–59 group and 17% the ≥60 group (Table 3). The statistical analysis suggests that among seropositive individuals, those of the 40–49 age group showed a lower proportion of abnormal electrocardiograms than those of the 50–59 age group ($p = 0.042$, Fig. 2C) and no difference with the ≥60 years age group ($p = 0.223$). Considering the significant p-value obtained in the GLM, we estimated the odds ratio for the 40–49 vs 50–59 age group using the 40–49 age group as comparator. This analysis showed that persons with abnormal electrocardiogram were 6.2 (95% CI: 1.97–23.61, $p = 0.003$) times more likely to be found in the 50–59 age group than in the 40–49 age group.

Table 2
Results for the Generalized Linear Model for prevalence of Chagas disease (A) and for cardiopathic anomaly/normality ratio of seropositive subjects (B). In both analyses, the explanatory variables tested were locality (rural and urban), sex (women and men) and age (40–49, 50–59 and ≥ 60).

A: Prevalence of Chagas disease				
Subject classes	Estimate	Standard error	z value	Pr(>|z|)
Urban vs Rural	3.231	0.487	6.631	< 0.0001
Women vs Men	0.974	0.410	2.376	0.018
B: Cardiopathic anomaly/normality ratio of seropositive subjects				
Subject age groups	Estimate	Standard error	z value	Pr(>|z|)
40–49 vs 50–59	1.818	0.620	2.933	0.0034
40–49 vs > 60	0.822	0.688	1.195	0.232

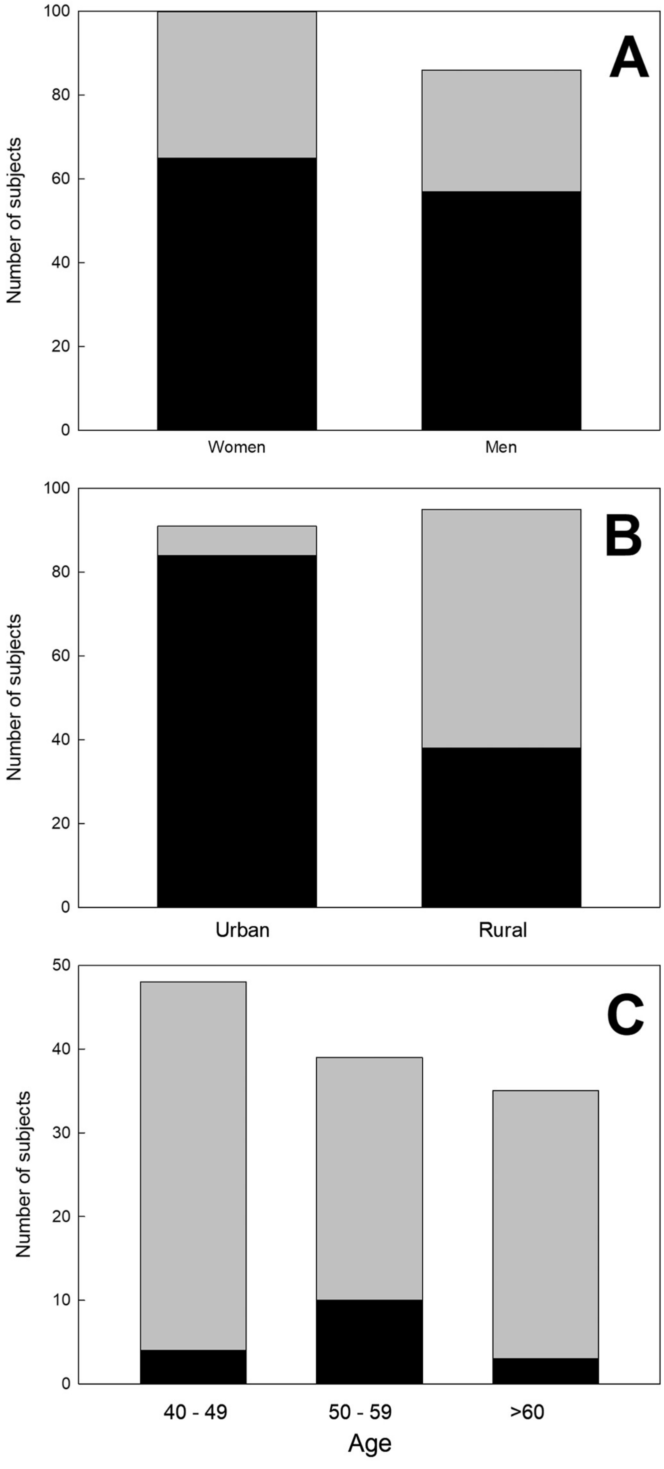

Fig. 2. A) Number of seropositive (black bars) and seronegative subjects per locality (gray bars). The analysis revealed a higher proportion of seropositive individuals in the rural than in the urban population. B) Number of seropositive (black bars) and seronegative (gray bars) subjects per sex. The analysis revealed that both men and women showed more seropositive than seronegative subjects and that the proportion of seropositivity was higher in men than in women. C) Number of subjects with normal (gray bars) and abnormal (black bars) electrocardiograms as a function of age group. The analysis revealed a higher proportion of abnormal subjects in the age group 50–59 compared to the other age groups.

Table 3
Number of seropositive individuals with or without Chagas related electrocardiographic (ECG) abnormalities.

Locality	Age (y)	Sex	Tested individuals	Individuals with abnormal ECG	Individuals with normal ECG
Rural	40–49	Male	8	1	7
Rural	50–59	Male	11	4	7
Rural	>60	Male	11	1	10
Rural	40–49	Female	25	2	23
Rural	50–59	Female	11	2	9
Rural	>60	Female	18	4	14
Urban	40–49	Male	11	1	10
Urban	50–59	Male	12	6	6
Urban	>60	Male	4	1	3
Urban	40–49	Female	4	0	4
Urban	50–59	Female	5	2	3
Urban	>60	Female	2	0	2

4. Discussion

Results showed a clear contrasting pattern in the seroprevalence of Chagas disease which was higher in the rural than in the urban population in the study area. However, seropositive persons of both areas showed similar patterns of seroprevalence per age group, with the highest percentage of patients with electrocardiogram abnormalities occurring in the 50–59 age group.

In the present century, several studies have reported prevalence of Chagas disease in rural populations in the Chaco ecoregion of South America, which comprises areas within Bolivia, Argentina and Paraguay. For example, in the Chaco region of the Santa Cruz province in Bolivia, studies have shown prevalence of Chagas disease of 91% in people up to 30 years old (Samuels et al., 2013), 71.3% for people in the 21–49 age group and 87.9% for people above 50-years old (Chippaux et al., 2008). In the Chaco region of Argentina, prevalence figures reported are 76.0% in rural areas of Misión Nueva Pompeya in subjects within a wide age range (<1 to ≥60-years old) (Moretti et al., 2010), 27.8% in a population ranging from 1 to >20-years old and inhabiting Chacabuco and 12 de Octubre counties (Diosque et al., 2004), between 26 and 34% in subjects less than 70-years old from rural localities of San Roque (Bar et al., 2005), and 53% in 17 rural populations of all ages from El Monte Impenetrable (Biancardi et al., 2003). In the Chaco region of Paraguay, prevalences of 29.1% in Guazu-Cuá and 20% in Pozo Hondo were reported (Arias and Rojas, 1984). These data, taken together with the data presented in this paper (92% seroprevalence) show that the highest prevalence of Chagas disease are found in the Chaco ecosystem of Bolivia. This is the area where *T. infestans* shows its highest genetic diversity and has been described as the region of origin and the epicenter of the spread of *T. infestans* (Giordano et al., 2005).

On the other hand, prevalence of Chagas disease in urban populations of the Chaco ecoregion have also been reported: for example, 23.4% in Santa Cruz, Bolivia (Chippaux et al., 2009) and 58.4% in Misión Nueva Pompeya, Argentina[17] These data show that seroprevalence in urban populations tends to be lower than in rural populations possibly on account of a high level of education about prevention of the disease, better quality of food storage, better housing, accessibility to modern health systems for early diagnosis and treatment, and possibilities to control the vector.

Information is still scarce in relation to seroprevalence in women relative to men. Our results showed a higher proportion of seropositive cases in men compared with women, a pattern not previously described. Other studies have not detected differences between sexes, for example in a population from Veracruz, Mexico (Segura and Escobar-Mesa, 2005) and in pre-Hispanic human mummies from the coast and lower valleys of northern Chile and southern Peru (Aufderheide et al., 2004). Further studies enquiring on potential differences between sexes and their relationship with the vertical transmission of Chagas disease would be desirable.

Our study showed higher prevalence of Chagasic related abnormalities in the ECG of patients in the 50–59 age group (36% of subjects) than in the preceding (40–49: 8%) and the following (≥ 60: 17%) age groups. This increase-followed-by-decrease age pattern in the proportion of Chagasic cardiopathies has also shown up in other studies. For example, a higher proportion of cardiopathies related with Chagas disease in the 40–59 age group (33.4% of patients) when compared with preceding (20–39: 30.2%) and the following (13.3%, ≥60) age groups in people inhabiting rural areas of Santa Cruz, Bolivia, was reported (Fernandez et al., 2015), and a higher proportion of Chagasic cardiopathies in the 30–39 age group (ca. 50% of patients) than in the preceding (20–29: ca. 30%) and following (50–59: ca 35%) age groups in patients mainly from different areas of Brazil was found (Coura and Borges-Pereira, 2010). The age-related raise in the proportion of Chagasic cardiopathies may be caused by an increased exposure to infection and increased development of the disease symptoms as patients age, while the decrease in the proportion of Chagasic cardiopathies may be related to a higher mortality among Chagasic patients in the older age group. Consistent with this proposal, a higher proportion of mortality in patients with advanced abnormal ECG in patients in the 55 ± 11 age group has been reported (Espinosa et al., 1987), and only half of Chagasic people survived after 60 years in a Brazilian rural community showing 42% of prevalence of Chagas disease (Maguire et al., 1987). Moreover, the age group of maximal incidence of cardiopathies was found to shift from 30 to 39 in Brazil in data collected since 1960 (Coura and Borges-Pereira, 2010; Coura et al., 1983) to 40–59 in data collected in Bolivia between 2011 and 2012 (Fernandez et al., 2015) and to 50–59 in data collected in Bolivia in 2017 (this work). This age group shift is consistent with an increase in life expectancy over time, which has been 21 years in

Brazil and 27 years in Bolivia from 1960 to 2015 (World Bank, 2017). Age-related studies on Chagasic patients should be implemented covering a larger range of geographical areas, including urban and rural populations and examining eventual differences between men and women, within the countries in Latin America with highest incidence of Chagas disease.

5. Conclusion

Seroprevalence of Chagas disease was higher in the rural than in the urban population; however, seropositive persons of both populations showed similar patterns of seroprevalence per age group, and the highest prevalence of electrocardiogram abnormalities occurring in the 50–59 age group. Since the rural population was mainly of guaraní stock, the results suggest a genetic component to the differences observed. The genetic and environmental determinants of these differences results should be further explored.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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