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Original Article

Trypanosoma cruzi infection in the human population of the Bolivian Chaco: four serosurveys over a 26-year period (1987-2013)

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Abstract
Introduction: Chagas disease (CD) remains a public health concern in several Latin American countries. At global level, Bolivia has the highest CD burden and the Chaco region, in the southeast of the country, is the most affected area. We report the results of four serosurveys for *Trypanosoma cruzi* antibodies, carried out approximately ten years apart from each other, during the lapse 1987-2013, in different localities of the Bolivian Chaco.
Methodology: Four cross-sectional surveys were conducted in various localities, mostly rural, of the Bolivian Chaco, during the period 1987-2013.
Results: Although a reliable analysis of CD epidemiological trend is challenging, a partial reduction of anti-*T. cruzi* seroprevalence over the past four decades in the Bolivian Chaco may be assumed. In particular, in 1987 the exposure to *T. cruzi* in rural setting was universal since the first years of life, while it resulted gradually lower and age-dependent thereafter. Moreover, *T. cruzi* seroprevalence among women of reproductive age (15-45 years) has been persistently high in rural areas.
Conclusions: *T. cruzi* transmission is still active and CD remains a concern throughout the Bolivian Chaco. More efforts are needed in order to achieve a sustainable interruption of vector-borne CD transmission in this area.

Key words: Chagas disease; seroprevalence; Bolivia; Chaco; *Trypanosoma cruzi*.

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Introduction
Chagas disease (CD) or American trypanosomiasis, caused by the infection with the protozoan parasite *Trypanosoma cruzi*, is a public health concern in several Latin American countries. An estimated 6-7 million people worldwide are infected by *T. cruzi*, and the most part remains unsuspected (it is named "the invisible disease"), undiagnosed or untreated [1]. Thanks to regional intergovernmental control programs, launched during the 1990s, *e.g.* the Southern Cone Initiative (1991), the Andean Pact Initiative, and the Central America Initiative (1997), vector-borne transmission due the main vector, *Triatoma infestans* (Reduviidae, Triatominae), has been interrupted in Uruguay, Chile, Brazil, Eastern Paraguay, and few provinces in Argentina [2]. By contrast, Bolivia has still the highest prevalence over the planet (6.1%), with an estimated 607,186 prevalent infections and an endemic area covering 60% of the country [3,4]. For this reason, Bolivian Health Authorities declared CD prevention

and control a national priority in Law 3374 dated March 23, 2006 [5]. Control campaigns are promoted and managed by the National Program for Chagas Disease Control (NPCDC), with the main goal of reducing the proportion of infested houses to less than 3%. A considerable reduction was achieved in the last decades at national level, from an estimated 47.7% infestation rate (IR) in 1999 to 1.9% in 2016 [6]. However, control interventions obtained a limited success in the Bolivian Chaco, southeast of the Plurinational State of Bolivia, where the risk for *T. infestans* transmission continues to be high, and the house IR is still above 3% in the great part of the municipalities of the three Departments (Santa Cruz, Chuquisaca, and Tarija) [2,6].

Among the research activities, realized since the late 1980s within the longstanding collaboration between the Infectious and Tropical Diseases Unit, University of Florence, Italy and the Bolivian Ministry of Health, four seroprevalence studies for *T. cruzi* antibodies have been carried out, in different rural and urban/semi-urban localities of the Bolivian Chaco. In this paper, we report the results of those *T. cruzi* surveys, conducted approximately ten years apart from each other during the lapse 1987-2013, in order to set out CD prevalence trend and to strengthen the awareness on the current CD burden in the extremely affected Bolivian Chaco region.

Methodology

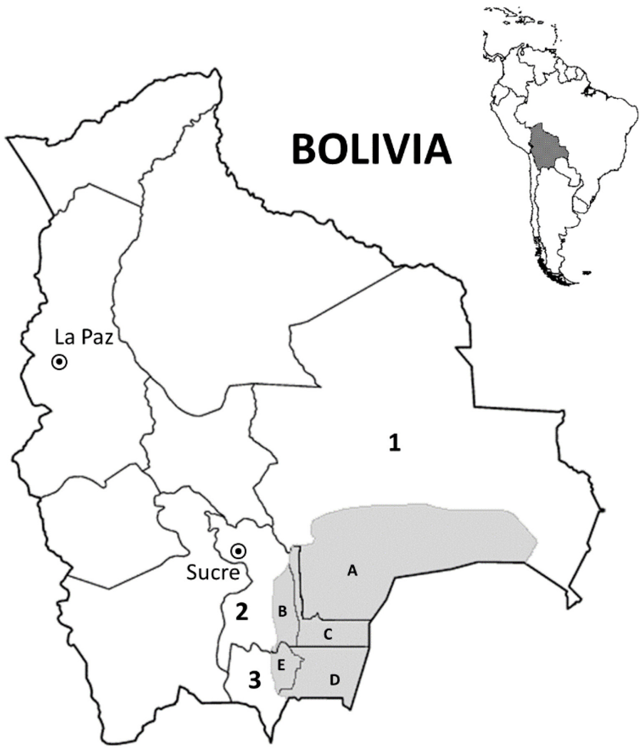
Study area

The Bolivian Chaco region is a semi-arid, homogeneous ecological zone, situated between the latitudes 17°59’ - 22°21’ South and the longitudes 64°31’ - 58°51’ West, and is approximately 127 755 km² in size. The region is sparsely-populated and consists of three Departments and five provinces (Cordillera, Luis Calvo, Hernando Siles, Gran Chaco, and O’Connor) (Figure 1). The inhabitants live in few small urban centres, and, for the most part, in rural communities, where households are poor dwellings, with walls of sticks, straw and clay and thatched roofs. People are mainly of the Guaraní ethnic group. Local economy is based on agriculture and animal farming [7].

Study populations

We carried out four cross-sectional surveys throughout the territories of the Bolivian Chaco region, in 1987, 1997, 2006, and 2013, respectively. In all cases, prevalence of anti-*T. cruzi* antibodies was obtained as part of broader epidemiological surveys in human population of the Chaco region.

Figure 1. Map of the Bolivian Chaco (grey area).



The area of the Bolivian Chaco lies in the south-east of the country, and includes 3 Departments (numbers on the map: Santa Cruz = 1; Chuquisaca = 2; Tarija = 3) and 5 Provinces (letters on the map: Cordillera = A;, Hernando Siles = B; Luis Calvo = C; Gran Chaco = D; O’Connor = E).

Each study was firstly explained during a preparatory meeting, involving local health care providers and community leaders. With their collaboration, the invitation to participate, directed to all individuals of the communities, was disseminated during public meetings. Therefore, the surveyed populations consisted of healthy volunteers, consecutively enrolled among those who consented to participate (with the exception of the studies carried out in Camiri and Boyuibe in 1987, which included a number of hospitalized patients).

In 1987, the study population included individuals from three different settings of the Cordillera Province (Department of Santa Cruz): Camiri, (a little urban centre of approximately 30,000 inhabitants); Boyuibe, (a semi-urban municipality of about 2,500 inhabitants), and Javillo (an extremely isolated rural community of about 110 people). In Camiri and Boyuibe, the study population consisted of primary and secondary school children and in- and out-patients of the local hospitals (178 people in Camiri, 184 in Boyuibe, age range 1-75 years), whereas in Javillo almost the entire population was enrolled (84 people, age range 4-70 years).

In the following studies, we surveyed only populations living in rural areas. In 1997, the survey concerned rural communities in the neighborhood of Camiri and Villa Montes (Gran Chaco Province, Department of Tarija), each having approximately 3,300 inhabitants. The sample size was adequate for an expected anti-*T. cruzi* antibodies prevalence of 80%, with a worst acceptable error of 5% and a confidence interval of 95%. The study populations consisted of 238 individuals from Camiri and 250 individuals from Villa Montes (age range 1-85 years).

In 2006, we surveyed the rural community of Bartolo (Hernando Siles Province, Department of Chuquisaca). Blood samples were taken from ≈80% of the community populations (161 persons, age range 1–77 years).

In 2013, 90 individuals (≈45% of the community populations; age range 1–83 years) were enrolled in the rural community of Bartolo, and 126 people (≈25% of the community populations, age range 1–83 years) were enrolled in Ivamirapinta (Cordillera Province, Department of Santa Cruz).

Serologic assays

For all subjects, demographic data (sex, age) were recorded, and a sample of 5-10 ml venous blood was collected, stored at -20°C in Bolivia, then transported to Italy and stored at -70°C until tested. Serological tests were performed by using an indirect hemagglutination assay (IHA) in 1987 (Cellognost-Chagas, Behring Institute, Germany) and 1997 (Chagas Polychaco kit, Lemos Laboratories, Argentina), fixing the cutoff at 1:8 dilution, according to WHO recommendations [8]. In the surveys conducted in 2006 and 2013, two conventional tests based on different principles and detecting different antigens were used. In the case of ambiguous or discordant results, a third test has been used. In details, in 2006 samples were tested by a high-performing rapid diagnostic test (RDT) (Chagas Quick test, Cypress Diagnostic, Belgium), a based on a recombinant antigen, composed of nine different epitopes and by an immunoenzymatic assay (EIA) (Chagas *Trypanosoma cruzi* IgG, DRG Diagnostic, Germany), which used as antigens the *Trypanosoma cruzi* lysate.

In 2013, we used two enzyme-linked immunosorbent assays (ELISA) (NovaLisa Chagas *Trypanosoma cruzi* IgG, NovaTec Immundiagnostica GmbH, Germany; ORTHO *T. cruzi* ELISA Test System, Ortho-Clinical Diagnostics, Inc) based on *T. cruzi* recombinant and whole cell lysate antigens, respectively. A third test (BioELISA Chagas, Biokit S.A, Spain) based on recombinant antigens, was used to disclose discordance. Assays were performed and cut-off determined according to the respective manufacturer's instructions. Negative and positive controls provided in the kit were included in each serological run.

Ethics

The studies were programmed and conducted in agreement with the Ministry of Health of the Plurinational State of Bolivia (within the Convenio Ministerio de Salud y Deportes, Estado Plurinacional de Bolivia/Cátedra de Enfermedades Infecciosas, Universidad de Florencia, Italia), and with the support of the Guaraní political organization (Asamblea del Pueblo Guaraní). The study was approved by a local Ethics Committee (Colegio Médico de Santa Cruz, TDEM CITE No. 028/2017).

Statistical analysis

Statistical analysis of data was performed with STATA 11.0 (StataCorp, College Statio, TX, USA). Frequencies and percentages with 95% confidence intervals (CI) for categorical variables, medians, and interquartile ranges (IQR) for continuous variables were calculated. Chi-square test and logistic regression were used to investigate the association of positive tests with sex and age, respectively. Results were considered significant when the p-value was ≤ 0.05.

Results

Demographic details and age-stratified *T. cruzi* seroreactivity of the populations studied in our cross-sectional surveys are shown in detail in Table 1. In the Cordillera Province (Department of Santa Cruz), in 1987 we found a seroprevalence for *T. cruzi* of 73% (129/178) and 64% (117/184) in urban and semi-urban areas, respectively. At the same time, a significantly higher rate was found in the rural community of Javillo, where 98% (82/84) of the inhabitants presented anti-*T. cruzi* antibodies ($p < 0.001$, age-stratified *Cochran-Mantel-Haenszel* test). In the subsequent decades, we conducted two further surveys in similar rural settings of the Cordillera Province. In 1997, seroprevalence was 81% (193/238) in some rural communities near Camiri, while in 2013, we found 60% of positivity (76/126) in Ivamirapinta (municipality of Gutierrez).

As far as the Chaco territories belonging to the Department of Chuquisaca are concerned, in the two consecutive surveys we conducted in the rural community of Bartolo (municipality of Monteagudo, Hernando Siles Province) in 2006 and 2013, a not significant decline in *T. cruzi* seroprevalence was observed (70% and 63%, respectively, p = 0.106). Similarly, seropositivity rate among 1-10 year-old children showed a slight, not significant decline from 2006 to 2013 (25% and 20%, respectively, p = 0.666).

In the Department of Tarija, we found a seroprevalence of 74% (184/250) in rural communities around the city of Villa Montes (Gran Chaco Province) in 1997.

In all the surveyed populations, distribution of CD did not differ by sex, while it increased according to age (in all cases excluding Javillo – 1987, where exposure resulted universal since the first years of life). Moreover, *T. cruzi* seroprevalence among women of reproductive age (15-45 years) has been persistently high in rural areas, ranging from 100% in 1987 to 74-79% of the most recent determinations in 2013 (Table 1).

Discussion

Relatively few studies reported data on CD epidemiology in the Bolivian Chaco, although it is acknowledged as one of the most affected areas in the planet. In Table 2, we report a summary of the main available studies concerning anti-*T. cruzi* antibodies seroprevalence in the human population of the Bolivian Chaco [9–13]. A detailed and accurate analysis of the epidemiological evolution is challenging, being hindered by several limitations. Above all, various serological techniques have been used in the different periods (IHA, EIA, ELISA, RDT), basically due to the sequential development and availability of newer assays along the years. However, as for the older IHA, whereas sensitivity widely varied from different manufacturers, specificity was considered very high (98-99%), suggesting that the high prevalence found in the first studies was not overestimated [14,15]. On the other hand, results from more recent studies are generally based on more than one serological test, being a definitive diagnosis of *T. cruzi* infection defined by at

Table 1. Seroprevalence for IgG anti *Trypanosoma cruzi* in the human population of the surveyed localities in 1987, 1997, 2006, 2013.

	Age	**Seroprevalence for IgG anti *Trypanosoma cruzi***								
	Median (range)	**Male**	**Female**	**1-5 years**	**6-10 years**	**11-20 years**	**21-40 years**	**> 40 years**	**Women of reproductive age (15-45 years)**	**Overall prevalence** (95%CI)
1987										
Camiri[1]	17 (1-75)	24/30 80.0%	105/148 71.0%	3/15 20.0%	10/12 83.3%	58/88 66.0%	44/49 89.8%	14/14 100%	75/102 73.5%	129/178 **72.5%** (66.0-79.0)
Boyuibe[1]	13 (4-75)	46/75 61.3%	71/109 65.1%	1/3 33.3%	37/51 72.5%	66/108 61.1%	12/21 57.1%	1/1 100%	21/36 58.3%	117/184 **63.6%** (56.6-70.5)
Javillo[1]	16 (4-70)	35/36 97.2%	47/48 98.0%	5/5 100%	21/23 91.3%	23/23 100%	21/21 100%	12/12 100%	22/22 100%	82/84 **97.6%** (94.4-100)
1997										
Camiri[1] **(rural communities)**	20 (2-80)	84/104 80.8%	109/134 81.3%	14/24 58.3%	35/54 64.8%	29/43 67.4%	53/55 96.4%	62/62 100%	48/51 94.1%	193/238 **81.1%** (76.1-86.1)
Villa Montes[2] **(rural communities)**	20.5 (1-85)	87/118 73.7%	97/132 73.5%	12/27 44.4%	32/51 62.7%	34/47 72.3%	51/64 79.7%	55/61 90.2%	39/47 83.0%	184/250 **73.6%** (68.1-79.1)
2006										
Bartolo[3]	24 (1-77)	65/92 70.7%	47/69 68.1%	3/16 18.8%	7/24 29.2%	18/33 54.5%	38/39 97.4%	46/49 93.9%	21/22 95.5%	112/161 **69.6%** (62.5-76.7)
2013										
Ivamirapinta[1]	25 (2-75)	28/57 49.1 %	48/69 69.6%	0/7 0.0%	1/19 5.3%	13/32 40.6%	26/30 86.7%	36/38 94.7%	26/33 78.8%	76/126 **60.3%** (51.8-68.9)
Bartolo[3]	30 (1-83)	31/49 63.2%	26/41 63.4%	2/9 22.2%	2/11 18.2%	5/11 45.5%	22/30 73.3%	27/29 93.1%	14/19 73.7%	57/90 **63.3%** (53.4-73.2)

[1]Cordillera Province, Department of Santa Cruz; [2]Gran Chaco Province, Department of Tarija; [3]Hernando Siles Province, Department of Chuquisaca.

least two positive results, as recommended by the WHO [16]. Commercial ELISA used in our studies, have shown a pooled sensitivity of 99.3% (97.9%–99.9%) and a pooled specificity of 97.5% (88.5%–99.5%) in a meta-analysis [17]. Moreover, the surveys took place in different communities, and comprehensive information on entomological situation and insecticide-based interventions are sparse or not available for the great part of the surveyed communities. The Bolivian Chaco is a homogeneous ecological zone and the rural populations share the same living and health conditions. Here, the maintenance of active transmission is fostered by the reinvasion of the poor human dwellings by residual triatomine populations from areas not well treated in the domicile or in the peridomicile, or from neighboring untreated areas. This problem is enhanced by the recent emergence of foci of resistance to pyrethroid insecticides [10,18]. In the area of Gutierrez (Cordillera Province), systematic spray campaigns against domestic *T. infestans*, through blanket spraying

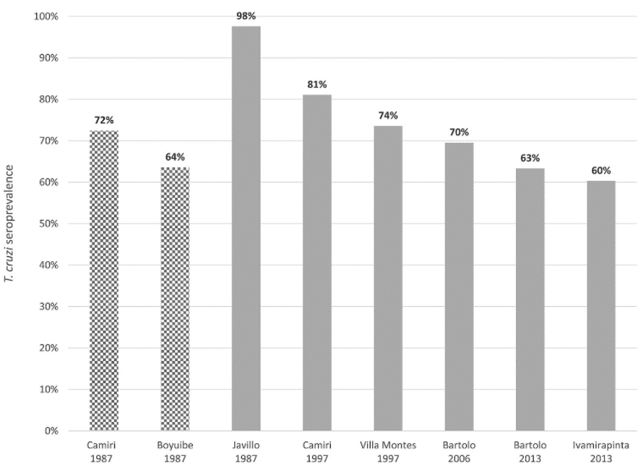
Table 2. Available studies reporting anti-*Trypanosoma cruzi* antibodies seroprevalence in the human population of the Bolivian Chaco.

References	Year	Setting	Municipality	Population	Age median (range)	anti-*T. cruzi* seroprevalence	Test
Santa Cruz Department (Cordillera Province)							
Romero, *et al*. [8]	1977	Gutierrez - Ipitá (semi-urban area)	Gutierrez	General population	NA (1-70)	70.6% (381/540)	IHA
This study	1987	Camiri (urban area)	Camiri	School children & in- / out-patients	17 (1-75)	72.5% (129/178)	IHA
		Boyuibe (Semi-urban area)	Boyuibe	School children & in- / out-patients	13 (4-75)	63.6% (117/184)	IHA
		Javillo (rural community)	Gutierrez	General population	16 (4-70)	97.6% (82/84)	IHA
This study	1997	Vicinity of Camiri (rural communities)	Camiri	General population	20 (2-80)	81.1% (193/238)	IHA
Samuels, *et al*. [9]	2011-12	Eity health sector (rural communities)	Gutierrez	General population	15 (IQR: 8-33)	51.7% (799/1545)	IHA, EIA, ELISA*
Shah, *et al*. [10]	2011-12	Vicinity of Gutierrez (rural communities)	Gutierrez	Children	9.5 (2-17)	39.5% (79/200)	IHA, IFA, EIA*
This study	2013	Ivamirapinta (rural community)	Gutierrez	General population	25 (2-75)	60.3% (76/126)	ELISA (3 assays)*
Chuquisaca Department (Hernando Siles Province)							
This study	2006	Bartolo (rural community)	Monteagudo	General population	24 (1-77)	69.6% (112/161)	ICT, EIA, ELISA*
This study	2013	Bartolo (rural community)	Monteagudo	General population	30 (1-83)	63.3% (57/90)	ELISA (3 assays)*
Tarija Department (Gran Chaco and O'Connor Province)							
This study	1997	Vicinity of Villa Montes (rural communities)	Villa Montes	General population	20.5 (1-85)	73.6% (184/250)	IHA
Chippaux, *et al*. [11]	2007	Vicinity of Caraparí (rural communities)	Caraparí	General population	25.4 (NA)	48.4% (482/995)	ELISA
Yun, *et al*. [12]	2002-06	Vicinity of Entre Rios (rural communities)	Entre Ríos	Children	< 15 years	19.4% (1475/7613)	ELISA (2 test), IHA*

IHA: indirect hemagglutination assay, IFA: immunofluorescent-antibody test, EIA: immunoenzymatic assay; ELISA: enzyme-linked immunosorbent assay. NA: not available; IQR: interquartile range; Data are presented in chronological order, after grouping by Department; *Final status was defined as positive if results were positive by at least two of the three tests and negative if results were positive by none or one of the assays.

with alphacypermethrin 20%, were conducted in 2000 and 2003. Moreover, in early 2000, housing improvements such as whitewash, wall plastering and/or substitution of tin roofs for those of thatch, were provided within an intervention program by Caritas, a nongovernmental organization [10]. In the following years (2006-2013), periodical assessments for household vector IR and focal spraying of infested houses were performed in the municipalities of the Cordillera Province, as part of the NPCDC. Focal interventions achieved a partial, temporary control of the IR, which sharply fluctuated along the years. In the municipality of Gutierrez, household (*vivienda*) IR had a dramatic decline after the 2000-2003 systematic interventions (from 81.5% in 2000 to 0.8% in 2004), then rose to 44.8% in 2008, and decreased again to 10% in 2012. In Ivamirapinta, the most recent intervention was carried out few months before our survey in 2013, and detected a household IR of 16% (31/189 *viviendas*) (personal communication, R. Vargas, 2018). Given the limited efficacy of the traditional programs, based on indoor and outdoor residual insecticide spraying, alternative experimental strategies have been proposed. In 2006, a vector control intervention, based on microencapsulated organophosphate formulations, instead of the traditional suspension concentrate formulation applied with spraying pumps, showed promising results in the municipalities of Cuevo and Lagunillas (Cordillera Province, Santa Cruz Department) [19]. The study included an entomological evaluation of the house infestation by *T. infestans* before the intervention, which showed an overall infestation of either intra and/or peridomestic infestation of 30.5%. Furthermore, an ecosystem approach for the control of CD in the Bolivia Chaco, based on the identification of the ecological, biological and social determinants of CD transmission was proposed in rural villages of the Cordillera Province to reduce the risks of house infestation [20]. Knowledge, attitudes and practices on the vector, CD and its prevention were deficient. Living conditions, which encompass wall conditions and cleanliness of the room, and the presence of domestic animals in rooms, in particular poultry, resulted the main risk factor for house infestation. Education activities and an integrated vector control approach, based on simple and low cost interventions on the domicile environment were realized through community participation, leading to a significant decrease *T. infestans* densities in the domiciles. Recently, the presence of multiple animal reservoirs, including canine population, was confirmed as a further challenge for disease control and prevention strategies in this area [21].

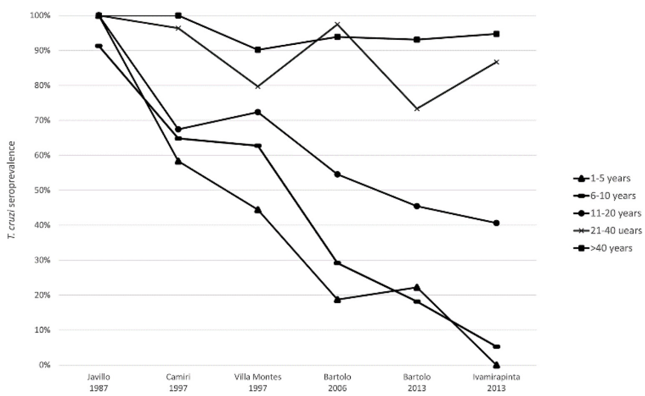
Figure 2. *Trypanosoma cruzi* seroprevalence in the human population from urban, semi-urban and rural areas of the Bolivian Chaco, in the period 1987-2013.



Checkered columns = urban/semi-urban area; full columns = rural area.

In fact, whilst burdened by some limitations, available data confirmed that *T. cruzi* transmission is still active throughout this area, both via vectorial and vertical route, albeit a partial reduction over the past 40 years may be assumed (Figure 2). This trend is especially accounted for by the youngest generations, reflecting a contained CD transmission in recent years, likely due to an improvement in housing conditions over time and the successful vector control programs. On the other hand, positivity rates in people >40 years old are still over 90%, reflecting the intense vector-borne *T. cruzi* exposure over the past decades, which resulted in a chronic, lifelong infection (Figure 3). Subjects with chronic asymptomatic infection (indeterminate phase) had 30% to 40% of likelihood to

Figure 3. Age-specific trends of T. cruzi seroreactivity rates, in the human population living in rural communities of the Bolivian Chaco, in the period 1987-2013.



develop the chronic phase complications, with a cardiac, digestive, neurological, or mixed involvement, decades after the initial infection [22]. Cardiac disease could lead to progressive heart failure and sudden death, which are the main causes of death in patients with chronic Chagas heart disease [23]. In 2011, a high prevalence of ECG abnormalities and substantial evidence of Chagas cardiomyopathy (55/398, 13.8%), including bundle branch and/or atrioventricular blocks and rhythm disturbances associated with impaired left ventricular end-diastolic dimensions or ejection fraction, were found in a population-based survey among *T. cruzi* infected inhabitants living in a rural area of the Bolivian Chaco [24]. As expected, the prevalence of any abnormality rose proportionally with the age. The authors called for improving the access to basic cardiac care, such as annual ECG, antiarrhythmics, pacemakers, which could have an immediate impact on CD morbidity and mortality. By merging these findings, and the CD prevalence reported in the few available studies, the Working Group on Chagas Disease in Bolivia and Peru estimated that between 3,000 and 4,000 people in the Bolivian Chaco are in need of pacemakers [25].

The screening of pregnant women and the early treatment of infected newborns are priorities for the Bolivian NPCDC started in 2004. The program was based on serological testing during pregnancy, follow-up of children born from positive mothers until one year old, and treatment of positive children [26]. At national level, in 2016 the infection rate among pregnant women was 16.4%, and maternal transmission in Bolivia was estimated between 1.3% and 2.3% [6]. In the Bolivian Chaco both these rates are likely higher than the average of the country, especially in rural areas, where maternal seroprevalence stands at 60-70% and congenital transmission rate at 4-6%. Maternal risk factors for vertical transmission included higher circulating parasite loads, alterations in maternal immune response, younger age and twin births [27,28]. Of note, sustained domestic vector exposure, occurring in women living in infested houses, has been associated with decreased parasitemia and lower congenital transmission risk [28]. Furthermore, available data are still too scarce to elucidate whether maternal *T. cruzi* infection has an impact on fetal outcome. In the absence of effective vertical transmission, chronic maternal *T. cruzi* infection did not show effect on gestation outcome and fetal development [29]. However, early detection of congenital infection remains crucial. Benznidazole showed good efficacy and tolerance in the treatment of congenital Chagas disease [30]. In addition, the impact of treatment of women of childbearing age in preventing the vertical CD transmission have been recently highlighted [31].

Conclusion

Although positive steps have been taken in the last decades, CD remains a major public health concern throughout the Bolivian Chaco. Here, as well as in the neighbouring areas of Northwest Argentina, and Paraguay (known as Gran Chaco region in its entirety), an effective vector control is still challenging. The reduction to 0 of the high risk municipalities and the strengthening of the Program (Atención Integral de la Enfermedad de Chagas) are a specific target of the Developing Sectorial Plan 2016-2020 [6]. Long-term repeated insecticide-based campaigns need to be integrated with housing improvements and surveillance system in order to achieve a sustainable interruption of vector-borne CD transmission in this area.

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