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Seroprevalence of *Trypanosoma cruzi* infection and vector control activities in rural communities of the southern Gran Chaco (Argentina)

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ABSTRACT

We compared age-related seroprevalence of *Trypanosoma cruzi* infection with history of vector control interventions and social and ecological changes in three historically endemic departments of Cordoba province, Argentina, covering an area of 42,600 km^2 of the Gran Chaco region. Using a cross sectional design, blood samples of 5240 people between 6 months and 40 years of age, living in 192 rural communities were analyzed to detect *T. cruzi* infection using ELISA tests, and confirmed with indirect immunofluorescent antibody test and indirect haemoagglutination. Overall seroprevalence was 5.4%, 7.9% and 7.5% in the north, northwest and west studied areas (average for all areas 6.95%). Seroprevalence for *T cruzi* increased with population age, especially in age classes older than 15 years of age. Communities of the north and west areas showed 0.59% seroprevalence for *T. cruzi* in children below 15 years of age, whereas children of the same age in the northwest region showed a seroprevalence of 3.08%. Comparative analyses indicate that vector control activities and land use changes during the last decades are the most likely causes of the overall reduction of *T. cruzi* prevalence. Results suggest that the vectorial transmission of *T. cruzi* has been strongly reduced and probably interrupted in the north and west areas, but it is still active in the northwestern rural settlements of Córdoba province.

1. Introduction

Chagas disease (American trypanosomiasis) is one of the main endemic diseases of Latin America, produced by *Trypanosoma cruzi* and mainly transmitted by triatomine bugs (Hemiptera, Reduviidae, Triatominae). Following successful vector control campaigns that started in the 1980s and 1990s, together with improvement of living conditions of many people in rural areas, the affected population in Latin America decreased from around 20 millions in the 1980s to less than 10 millions during the first years of 2000 (Remme et al., 2006). With the successful elimination of domestic infestations of *Triatoma infestans* (the main vector species) in most of the affected areas of the southern cone of South America, the epidemiological situation of Chagas disease is at present a heterogeneous mosaic in the area (Schofield et al., 2006).

Vectorial transmission of *T. cruzi* by *T. infestans* in the Southern Cone countries of South America has been interrupted in Chile (1997), Uruguay (1999), Brazil (2006), and some regions of Argentina and Paraguay (OPS, 2006). Within the areas where vectorial control was successful, the detection of infected children allows for elimination of the infection through available parasitological treatments. In areas where the vectorial transmission of *T. cruzi* is interrupted, the infection can be found only in adults that were not treated as children or did not respond positively to the parasitological treatment (OPS, 2001, 2002a,b, 2003; Dias et al., 2002; Gürtler et al., 2003).

But vectorial transmission of *T. cruzi* is still apparent in several areas of the Gran Chaco region of Argentina, Bolivia and Paraguay. In this area, that covers around 1 million km^2, people of all ages can be found infected by *T. cruzi*, especially in those areas where the vectorial control interventions has been sporadic and not sustained over time (Gürtler et al., 2005).

Towards the southern part of the Gran Chaco, the northern departments of Córdoba province (Argentina) were early identified as highly endemic. By the mid 1960s, rural houses of these departments showed rates of infestation by *T. infestans* averaging 50% (Segura, 2002). As in many endemic areas of Argentina, political and economic instability affected the activities of the vector control program in Córdoba, with sporadic pulses of activity followed by years of stagnation (Sosa Estani et al., 2006). Yet even with poorly sustained vector control activities, house infestation rates

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decreased to 20% between 1988 and 1992. By 1992, all activities for the control of vectorial and non-vectorial transmission adhered to the protocol approved by the Southern Cone Initiative, and house infestation was reduced to less than 5% by the year 2000 through selective spraying of infested houses reported by the community (Chüit et al., 1992; Segura et al., 1994; Segura, 2002). Because of the Argentinean economic crisis that started in 1999, vector control activities were reduced and house infestation increased to 6.5% in the intradomestic structures and 10.7% in the peridomestic structures (OPS, 2001; Segura, 2002; Dirección de Zoonosis, Córdoba Ministry of Health, unpublished data).

The recrudescence of the domestic infestation was reflected by the detection of 7 acute cases in the north and west of the province, between 1998 and 2005 (Coordinación Nacional Control Vectores Argentina, unpublished data). From 1994, newborns of infected mothers were being monitored for infection, and serological screening in blood banks was well established in the province of Córdoba (Segura et al., 1994, 2000; OPS, 2002a,b; Segura, 2002).

This investigation is part of a larger project that studies the association between environmental changes and the epidemiology of Chagas disease in the southern part of the Gran Chaco. We report here on the age-specific seroprevalence of *T. cruzi* infection in human populations up to 40 years of age, and assess this against vector control activities during the last decade in rural communities in the north, northwest and west of Córdoba province (Argentina).

2. Material and methods

2.1. Study area

The study area is located towards the south of the Gran Chaco of Argentina, in the north and west of the province of Córdoba (Fig. 1). The region covers around 42,600 km^2 and has a Mediterranean climate, with annual average temperature of 19 °C with hot summers and cold winters (extreme temperatures over 35 °C and below 5 °C are frequent). Rainfall (especially between November and March) decreases from an average annual total of 700 mm in the SE to less than 550 mm in the NW of the study area (Zak and Cabido, 2002; Zak et al., 2004). The selected area covers the departments historically endemic for Chagas disease, with high seroprevalence and high domestic infestation by *T. infestans* in rural houses, reported in a number of studies (Chüit, 1989; Esteco, 1984; Giojalas et al., 1990; Martínez, 1996; Crocco and Sanmartino, 2000). Within the selected region, three areas were identified, according to history of land use and land cover changes during the last 30 years. A north area includes parts of the departments of Río Seco and Tulumba, a northwest area includes parts of the departments Cruz del Eje and Ischilín, and the west area includes parts of Pocho and San Alberto (Fig. 1). During the last 40 years, the north area showed a strong change towards intensive soya production, the northwest area showed a land use change towards an increase of cattle production, and the west area developed low-impact tourism and showed few land cover changes. Rural communities of each area were cartographically located using a Garmin GPS.

2.2. Study design

The seroprevalence of *T. cruzi* infection was estimated through a cross sectional sampling design (Nelson et al., 2001). Individuals included in the study were those that lived their entire life in the rural community (or spent a maximum of 2 years outside it) and had between 6 months and 40 years of age. The age limit of 40 and residence time was imposed by the period of interest of the study that seeks to understand the epidemiology of Chagas disease during the last 40 years in the north and west of Córdoba province.

Minimum sample size was estimated in two steps. In the first step, a preliminary minimum sample size (n_0) was calculated as pq/EE^2, where p is the expected prevalence of Chagas disease in the

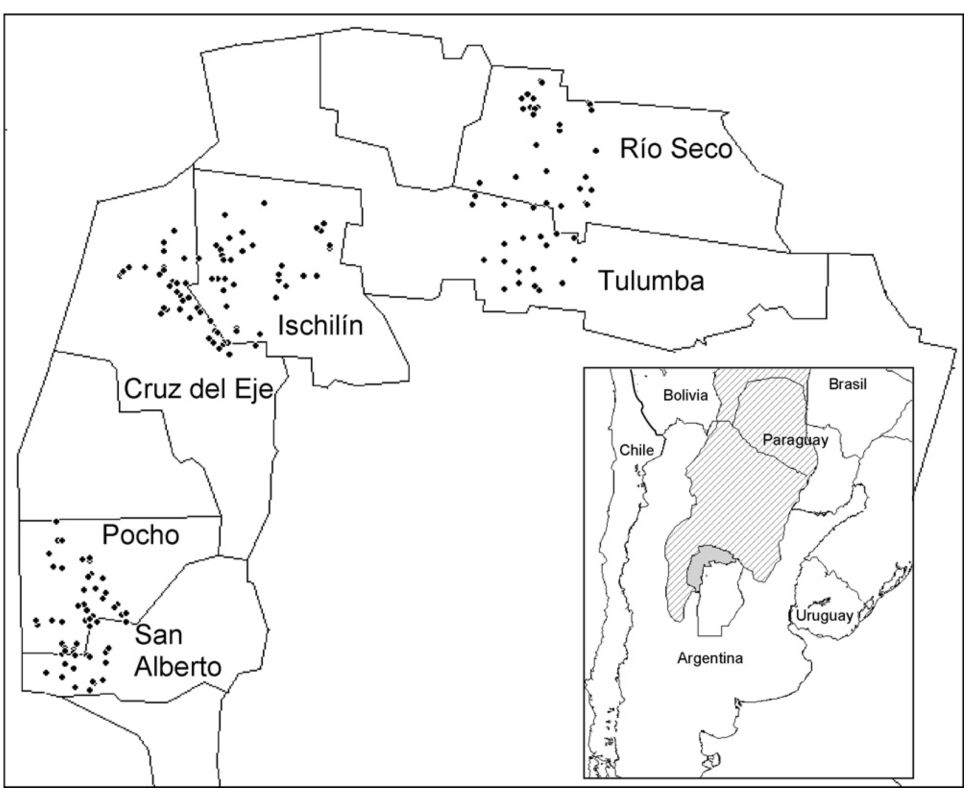

Fig. 1. Study area north, northwest and west of Córdoba province (in grey), showing the localities included for the collection of serology samples. The Gran Chaco area is shown by the diagonal fill in the inset.

study area ($p = 0.02$) [based on reports by OPS, 2002a,b], $q = 1 - p$ and *EE* the accepted relative error in the estimation ($EE = 0.15$) (Cochran, 1982). On a second step, the adjustment for finite populations was estimated as $n = n_0/[1 + (n_0/N)]$, where n is the minimum sample size and N the total population size. Considering the estimated rural populations aged 40 years or younger reported by the 2001 national census in the three areas (3975, 2999 and 3142), the minimum sample sizes were 1407, 1262 and 1286 for the north, northwest and west areas, respectively. Most (90%) of children below 15 years of age were included, using a census carried out between 2004–2006 in rural schools, by the Ministry of Health of the province of Córdoba.

The local population of each area was invited to participate in this study through communications sent to schools, primary health centers, local FM radios or through person-to-person contacts. On a particular date, one of the researchers (MLM), a technician of the Coordinación Nacional de Control de Vectores (CNCV) together with a primary health care agent of the area, went to a rural school or primary health center where the community attended for the study. Where community response was low, the research team carried out a house-by-house visit.

For each child, 300 μL samples of fingertip blood were collected into one of the 96 vials of a commercial kit (Serokit®; Polychaco, Buenos Aires, Argentina). Each vial contains 500 μl of preservative solution that can stabilize antibodies and antigens in the whole blood for 2 months at temperatures of up to 37 °C. The blood samples collected with Serokit were analyzed by a recombinant ELISA essay (Chagastests recombinant Elisa, Wiener Lab) in the laboratory of the CNCV (Córdoba), and processed according to the protocol of the national Chagas program of Argentina (www.msal.gov.ar/chagas). Reactive samples were confirmed using a second blood sample, taken by venepunction, and analyzed using indirect immunofluorescent antibody test (IFAT) and indirect haemoagglutination (IHA). A case was confirmed when at least two of the tests were positive. Cut off point for IHA was 1:16 and 1:32 for IFAT.

Blood samples of persons older than 15 years of age were taken directly by venepuncture and stored with cooling bags to keep the sample at low temperature; these samples were centrifuged (2500 rpm) for 10 min at the end of the day in the nearest hospital. The serum was recovered and stored in a fridge (at about 8 °C) of the hospital until the research team returned to Córdoba city. Sera were then processed by the laboratory of the CNCV, as described above. Age-specific seroprevalence was calculated as the number of seropositives for *T. cruzi* divided by the number of studied in 4-year wide age classes.

All members of the communities participating in this study received a detailed explanation of the objective of the study and signed an informed consent document, previously approved by the Córdoba Ministry of Health. According to the signed document, the name and serological result was confidential and individually informed. Seropositive individuals younger than 15 years of age received the parasitological treatment according to the protocol of the federal Ministry of Health. Seropositive persons older than 15 years of age were offered access to the nearest hospital for clinical studies according to the protocol of the federal Ministry of Health of Argentina (http://www.msal.gov.ar/chagas/secciones/prod/normas.htm).

Although a special effort was made to obtain maximum coverage of human populations of the selected rural areas, the realized sample size in some age classes was relatively small, because there was not enough population in a particular region, mainly because of migration to cities. The number of individuals included in this study represented 42%, 79% and 28% of the rural population below 40 years of age reported by the national census of 2001 in the north, northwest and west areas, respectively. The number of localities included in this study was 77%, 71% and 87% of the total number of localities existing in the north, northwest and west areas, respectively. In order to reveal possible congenital transmission, 49% of the mothers of seropositive children were included in the study. Although we tried to study all mothers of seropositive children younger than 15, many were not studied because they were older than 40, were not available and/or did not want to participate in the study.

People older than 40 years of age were not initially planned to be included. However, when people above 40 years of age knew that the study team was in the region, many asked for the blood analysis. In all these cases, the procedure was carried out as in the younger persons. In this article, we report seroprevalence of this age class as the group of older than 40 years, bearing in mind that sample selection differed from that applied to the younger age groups.

Data on acute cases of Chagas disease in children younger than 15 years of age during the last 10 years were compiled by the Córdoba Ministry of Health and from the Coordinación Nacional de Control de Vectores (CNCV).

2.3. Vector control activities

Historical data on vector control interventions using insecticide application in the study area was provided by the Dirección de Zoonosis of the Health Ministry of the province of Córdoba. Vector control data was used in analysis of the association between vector control and the observed age-specific serology. Although control interventions against *T. infestans* using pyrethroid insecticides in the study region started during the 1970s, no systematic, detailed and reliable historical data could be found before 1997. From 1997 to 2007, house infestation by *T. infestans* and frequency of insecticide application and/or house evaluation in our study area was recorded. These records were used to estimate the annual coverage of house spraying/evaluation, as the number of sprayed and/or evaluated houses per year divided by the number of rural houses in the area. A locality was considered sprayed or evaluated when at least one house of the locality was sprayed and/or evaluated. Annual coverage of the intervention was estimated as the number of localities sprayed (or evaluated) per year divided by the total number of localities in the area. The north area has 2585 houses distributed in 48 localities; the northwest area has 2766 houses in 81 localities; the west area has 2720 houses in 63 localities.

2.3.1. Data analysis

A maximum likelihood multiple logistic regression (multiplicative risk type), odds ratio (OR) calculation and corresponding 95% confidence intervals (CI95) were carried out using EGRET (1999) software, to analyze the *T. cruzi* prevalence across age classes and study areas, including their interaction. The association of house and locality coverage was measured by Spearman's rank correlation and CI95 for prevalence values were calculated with Statxact 8.0 (Cytel Inc., 2007).

3. Results

3.1. Seroprevalence

Overall infection by *T. cruzi* in the study region was 6.95% (out of 5240 studied). The average infection by *T. cruzi* was 5.4%, 7.9% and 7.5% in the north, northwest and west areas, respectively. Concordance between IHA and ELISA was 92%. Of the positive samples, 91% had reactive IHA and ELISA, whereas among the negatives, 92.7% showed non-reactive IHA and ELISA. Table 1 shows the age-specific seroprevalence of *T. cruzi* in the study area.

Seroprevalence for *T. cruzi* in the studied population increased with age in all areas ($p < 0.001$, OR = 1.13, CI95 = 1.10–1.15). Infec-

Table 1
Age-specific seroprevalence for *T. cruzi* infection in the study region. Overall prevalence refers to prevalence in the whole region studied.

Age class (years of age)	Region						
	North Studied	Sero+ (%)	Northwest Studied	Sero+ (%)	West Studied	Sero (%)	Overall prevalence
0–4	97	0 (0.0)	105	3 (2.9)	59	0 (0.0)	1.15
5–9	273	0 (0.0)	528	12 (2.3)	190	1 (0.5)	1.31
10–14	501	6 (1.2)	626	22 (3.5)	226	1 (0.4)	2.14
15–19	247	5 (2.0)	441	32 (7.3)	138	8 (5.8)	5.45
20–24	139	9 (6.5)	164	12 (7.3)	58	5 (8.6)	7.2
25–29	159	8 (5.0)	181	35 (19.3)	71	14 (19.7)	13.87
30–34	149	15 (10.1)	164	27 (16.5)	72	16 (22.2)	15.06
35–39	123	14 (11.4)	164	21 (12.8)	59	14 (23.7)	14.16
>40	139	42 (30.2)	128	33 (25.8)	39	9 (23.1)	27.45
Total	1827	99 (5.4)	2501	197 (7.9)	912	68 (7.5)	6.95

tion risk in the NW area was higher than in the other two areas (OR = 8.855, CI95 = 3.72–21.05). Interaction between area and age was significant only for the NW area, with a marginally significant OR (Table 2). When data for the group older than 15 years of age was analyzed by logistic regression, seroprevalence showed a significant increase with age ($p < 0.001$, OR = 1.115, CI95 = 1.07–1.16), and risk in the NW and W areas showed significantly higher compared with the *N* area, but not between them (OR = 15.51 and 18.35, respectively). The group of younger than 15 years of age showed a seroprevalence of 0.59% (CI95 = 0.26–1.17) in the north and west areas (aggregated data), whereas it was 3.08% (CI95 = 2.08–4.03) in the NW area (OR = 4.88, CI95 = 1.88–10.68). The age group older than 40 years of age showed a seroprevalence of 27.4% (CI95 = 22.5–32.8).

3.2. Vector control activities

The coverage of insecticide application at the house and locality levels during the vector control interventions against *T. infestans* during the period 1997–2007 was heterogeneous in time and space within the studied region. Intradomestic infestation (i.e. houses with *T. infestans* within bedrooms) was relatively low in the north area during 2005–2007 (below 3%) and very high in the west area in 2004 (63%)

If all houses were visited at least once, the frequency of visit (for spraying and/or evaluation purposes) per house ranged between 1.2 and 1.9 times during the period 1997–2007 (west and northwest, respectively). The frequency of visit per locality during the same period ranged from 1.8 to 2.8 (west and northwest, respectively). Houses and localities of the northwest area were the most frequently visited (1.9 and 2.8, respectively), whereas the west area were the lowest (1.2 and 1.8, respectively) (Table 3). There was significant positive association (Spearman correlation = 0.65 $p < 0.03$) between coverage (either house or locality) of the northwest and west areas, suggesting the existence of periods with more vector control activities than others that temporally affected both areas in the same fashion. Vector control activities of the north area were not associated with vector control activities of the other areas.

Table 2
Multiple logistic regression result for *T. cruzi* infection by age and study area.

Terms	Coefficient	Odds ratio (95% conf. int.)
Constant	−6.221**	0.002 (0.001–0.004)
Northwest area	2.181**	8.855 (3.724–21.053)
West area	1.010 NS	2.746 (0.902–8.361)
Age	0.119**	1.126 (1.102–1.151)
NW area × age	−0.051**	0.95 (0.927–0.975)
W area × age	−0.012 NS	0.988 (0.957–1.021)

NS, non-significant.
** $p < 0.001$.

During the reported period, active ingredients used for house spraying were always pyrethroids (deltamethrin, cypermethrin, α-cypermethrin), applied at the recommended concentrations (OPS, 2002a,b). There were no reports of control failures and house spraying was always focal (i.e. insecticide was only applied to houses with reported infestations).

4. Discussion

Vectorial transmission of *T. cruzi* is the main route of infection in many rural communities of the Gran Chaco of Argentina, where domestic infestation by *T. infestans* is still present. When the domestic populations of *T. infestans* are eliminated, the transmission rate of *T. cruzi* drops strongly (Moya et al., 2005; Gürtler et al., 2003). Considering this effect, seroprevalence of younger age classes was considered a reliable indicator of the impact of vector control interventions (OPS, 2001, 2002a,b, 2003; Dias et al., 2002).

The collected data suggest that vectorial transmission in the north and west areas has been strongly decreased and probably interrupted about 15 years ago, as *T. cruzi* prevalence in children below 15 years of age is less than 1%, a figure that is well below the expected rate due to congenital transmission in this region of Argentina (Moya et al., 2005; Gürtler et al., 2003). However, between 1998 and 2005, 7 acute cases of Chagas disease have been reported from Cordoba, and of these, 6 occurred within the study area of the present report. Of the 6 cases, 3 occurred within the northwest area, 2 within the west area and 1 within the north area of this study (Ministry of Health, Córdoba province and Coordinación Nacional Control Vectores, unpublished data). In the northwest area, prevalence in younger age classes is over 2%, increasing with age. Although we cannot discard the possibility that some of these may have been congenital, the figure is high enough to consider the vectorial route as a likely contributor.

The realized sampling may impose some limitations on the present study. A sample selection bias could be present among the studied persons that, knowing they were infected, were more easily motivated to participate in the study than people that did not know their infection status. However, only 31 persons (all older than 16 years of age) mentioned they knew they were infected by *T. cruzi*. This figure represents 9.7% of the positive cases older than 16 years of age.

The overall average seroprevalence found in the study region was 6.95%, several times less than the figures found in the province of Chaco (northern Argentina) of 27.8% (Chacabuco and 12 de Octubre departments) (Biancardi et al., 2003) and 53.2% (Guemes department) (Diosque et al., 2004). The same is true for the children below 15 years of age. In this study, the overall observed value was 1.7%, contrasting with 24.1% (Chacabuco and 12 de Octubre departments) and 45.8% (Guemes department) (Diosque et al., 2004 and Biancardi et al., 2003, respectively) in the Chaco province. The

Table 3
House and locality coverage of vector control interventions (house evaluation and/or insecticide application) in the studied regions between 1997 and 2007. ID infestation is the number of houses with intradomestic infestation by *T. infestans*. Inspected/sprayed localities is the number of localities visited by a field team to evaluate and/or spray houses. House and locality coverage in %, out of the respective total.

Study area	Year	ID infestation	Inspected houses	House coverage	Inspected or sprayed localities	Localities coverage
North	1997	0	22	0.9	3	6.3
	1998	70	1096	42.4	29	60.4
	1999	31	755	29.2	25	52.1
	2000	9	187	7.2	3	6.3
	2001	21	520	20.1	13	31.3
	2002	13	151	5.8	7	14.6
	2003	*	0	0.0	0	0.0
	2004	0	3	0.1	2	4.2
	2005	7	325	12.6	9	18.8
	2006	8	260	10.1	4	8.3
	2007	1	172	6.7	4	8.3
Northwest	1997		0	0.0	0	0.0
	1998	104*	990	35.8	37	45.7
	1999	35	915	33.1	48	59.3
	2000	10	339	12.3	33	40.7
	2001	28	817	29.5	25	30.9
	2002	17	278	10.1	13	16.0
	2003		0	0.0	0	0.0
	2004	143*	1189	43.0	13	16.0
	2005	30*	358	12.9	28	34.6
	2006	4	153	5.5	17	21.0
	2007	9	163	5.9	10	12.3
West	1997		0	0.0	0	0.0
	1998	170	697	25.6	22	34.9
	1999	50	520	19.1	19	30.2
	2000	**	0	0.0	0	0.0
	2001	67	360	13.2	18	28.6
	2002		0.0	0.0	0.0	0.0
	2003		0.0	0.0	0.0	0.0
	2004	313	499	18.3	20	31.7
	2005	17	368	13.5	10	15.9
	2006	27	329	12.1	16	25.4
	2007	6	590	21.7	11	17.5

* Occurrence of an acute case of Chagas disease.
** Two acute cases in the year 2000 in the West area.

difference in seroprevalence between the findings in the present study and the ones carried out in the Chaco province confirms the high geographical heterogeneity of epidemiological scenarios of the Gran Chaco region in Argentina, suggested by Diosque et al. (2004) among others. The geographical heterogeneity is also present at lower scales, as shown by the result of this study in the different areas. The heterogeneity in seroprevalence could be related to differences in the history of vector control interventions, land use change and/or changes in the socioeconomic status of the affected populations. All areas within the study region had been heavily infested with domestic populations of *T. infestans* until the 1980s, reflected in the 27% prevalence of *T. cruzi* in people older than 40 years of age (Chüit, 1989; Segura, 2002; this study).

The historical data on vector control interventions during the last decade showed that localities in the west area received lower coverage than localities in the north and northwest areas. The frequency of vector control interventions was highest in the northwest area and similar between the north and west areas. Higher intervention coverage and frequencies in the northwest area did not produce lower prevalence in children below 15 years of age, suggesting that other factors affecting *T. cruzi* transmission were also operating during the same period and/or that the differences in intervention coverage and frequencies among the different areas were not enough to produce differences in the rate of vectorial transmission. The landscape of the north region changed strongly during the last decade because of the increase in soya cultivation and rainfall increase during the last decades, that encouraged companies to buy extensive forest and shrub lands for soya plantation. This process produced two regional changes. Firstly, it expelled many of the rural populations occupying state-owned land (that emigrated to big cities). Secondly, the expansion of agriculture in the region led to infrastructural improvements (roads, electricity, etc), and changed the lifestyle of the rural population (from goat breeding to working in the soya farms). The landscape of the northwest area changed less, driven by the increase of cattle production (rainfall and soil quality are not good enough for soya exploitation). The west area is probably the one with least changes, undergoing eco-tourist developments with low environmental impact (Karlin et al., 1994; Zak and Cabido, 2002; Dayenoff et al., 2003; Zak et al., 2004).

The west area showed the lowest coverage and frequency of house visits by health agents for evaluation or insecticide spraying. As *T. cruzi* vectorial transmission has been markedly reduced for more than a decade in this area, and environmental changes have been less important than those occurring in the north area, it would appear that the vector control interventions have contributed to the strong decrease of the *T. cruzi* transmission in the area.

The northwest area showed the highest seroprevalence of *T. cruzi* infection in the younger age classes (2.3% or more in children younger than 4 years of age). The area is partially surrounding the Salinas Grandes (salt flats), where the environment only provides opportunity for goat breeding as a base economic activity. As shown for other parts of the Gran Chaco, goat corrals are one of the best refuges for *T. infestans*, because of the low efficiency of the traditional methodology of insecticide application with manual sprayers (Gürtler et al., 2004; Cécere et al., 2002). A number of families living in the northern part of this Córdoba region migrates annually southwards, where an artificial lake allows the operation of an artificial irrigation scheme. Living conditions of these families during the yield season are very basic and it is not rare that the temporarily occupied houses are infested by *T. infestans*. This living scheme has been repeated every year during the last decades, associated with the harvest of a number of products (cotton, garlic, olives, pepper, tomatoes, etc.).

The historically endemic region of the province of Córdoba shows a heterogeneous scenario of *T. cruzi* transmission, associated with differences in vector control interventions, land use and socioeconomic changes during the last decades. The results of this study show an overall low seroprevalence of *T. cruzi* in children below 15 years of age. The *T. cruzi* transmission is lower in the north and west, and higher in the northwest area. Reports of symptomatic acute Chagas cases during the last decade indicate that the vectorial transmission, although low, is still active in parts of the endemic area of Córdoba.

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