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CHAGAS DISEASE IN RURAL AREAS OF CHACO PROVINCE, ARGENTINA: EPIDEMIOLOGIC SURVEY IN HUMANS, RESERVOIRS, AND VECTORS

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Abstract. We studied the seroprevalence of antibodies against *Trypanosoma cruzi* in the human population along with domiciliary infestation by triatomine bugs in an area endemic for Chagas disease in the Chaco Province of Argentina. In addition, we carried out parasitologic surveys in patients, dogs, wild mammals, and vectors. The mean seroprevalence in humans was 27.81% (109 of 392) and 24.14% (63 of 261) in 1–15-year-old children. The minimum domiciliary infestation rate was 13.33%, with certain areas reaching 53.85%. The prevalence was 15.09% (16 of 106) in dogs and 35.71% (10 of 28) in opossums. Infection with *T. cruzi* was detected in 30.10% (59 of 196) of the *Triatoma infestans* tested. Compared with nationwide studies, our data suggest that 1) there are zones requiring immediate sanitary action, and 2) nationwide estimates are based on very heterogeneous epidemiologic situations. This heterogeneity emphasizes the importance of in-depth studies of restricted areas to provide additional information for a better understanding of the present status of Chagas disease in Argentina.

INTRODUCTION

Trypanosoma cruzi is the etiologic agent of Chagas disease, which affects several million people in Latin America. The disease still remains an important public health problem in certain zones of the endemic area in Argentina, in spite of all the efforts carried out by the National Chagas Service of Argentina.

In 1991, The Pan American Health Organization/World Health Organization instituted a regional program for the elimination of *Triatoma infestans* (the main regional vector) and control of blood banks.[1] In 1993, the average domiciliary infestation rate in Argentina was 25%,[2] and the inferred number of persons infected by *T. cruzi* was 2,300,000 (7.2% of the population).[3] During the last decade, important advances have been made in the control of Chagas disease. From 1993 to 1998, the National Control Program had 830,000 houses under surveillance.[4] In a serologic survey performed on 18-year-old men, a decrease in the seroprevalence from 5.8% in 1981 to 1.9% in 1993 was detected,[5] and four Argentinean provinces stopped the vectorial transmission of *T.* cruzi in 2001.[4] In Chaco Province, a decrease in seropositivity from 30.6% in 1981 to 13.5% in 1993 was also reported in 18-year-old men.[5] However, the epidemiologic situation in certain endemic areas of the country presents a different picture, as indicated by a seroprevalence of 43.83% in 1–15-year-old children and 45.83% in children less than five years old in rural areas of Chaco Province.[6]

The current data on mammal reservoirs of *T. cruzi* in Argentina are mainly from studies performed on dogs in endemic areas.[7–15] These studies pointed out the relevance of the dog as a reservoir in rural areas. Infection by *T. cruzi* has been demonstrated in wild mammals in a survey in which 32% of *Didelphis albiventris* (opossums), 5.5% of *Conepatus chinga* (skunks), and 100% (1 of 1) *Galictis cuja* (ferrets) were infected by the parasite.[16]

Since 1999, we have surveyed transmission circuits of different *T. cruzi* strains in rural areas of Chaco Province.[17] In this framework, we have obtained epidemiologic data that are presented in this report. These data include human seroprevalence, domestic infestation by triatomine bugs and parasitologic studies in patients, dogs, wild mammals, and vectors.

MATERIALS AND METHODS

Study area. The field work was carried out in Chacabuco and 12 de Octubre counties, Chaco Province, Argentina (Figure 1). Most of the samples were obtained within an area of 322 km^2, with the settlement of Tres Estacas (26°55′11″S, 61°37′42″W) as the central point, from January 1999 to November 2002. Based on its biogeographic characteristics, the area belongs to the Chaqueña Region[18] and exhibits patches of primary and secondary forest alternating with crop fields and dispersed human dwellings. The National Control Agency sprayed this area with deltamethrin in 1996 and 2000. There are 868 inhabitants in this area.

Serologic studies. A total of 392 persons voluntarily agreed to participate in this study. The age of the subjects ranged from 1 to 82 years, of whom 76.53% (300 of 392) were less than 20 years old and 66.58% (261 of 392) were less than 15 years old. Most of the children and teenagers were in elementary school. Informed consent was obtained from their parents. The Bioethic Commission of the Health Sciences Faculty (National University of Salta, Argentina) reviewed and approved the study procedures.

Blood samples were obtained by biochemists and primary health care agents from the Enrique V. de Llamas Hospital (Charata-Chaco). They were processed as follows: 1) 150 μL of peripheral blood was mixed with 250 μL of buffer provided with a commercial kit (Serokit®; Polychaco, Buenos Aires, Argentina) according to manufacturer's instructions, and 2) 5 mL of peripheral blood was centrifuged (3,000 rpm for 15 minutes) and the sera was recovered and stored at 4°C until use. These samples were studied using an indirect hemagglutination (IHA) test (Chagatest IHA; Wiener Laboratory, Rosario, Argentina) and an enzyme-linked immunosorbent assay (ELISA) (Chagatest ELISA recombinant version 3.0; Wiener Laboratory). Reactive samples at dilutions ≥ 1:16 were considered positive in the IHA assays. In the ELISA assay a cut-off value was established, based on the average

FIGURE 1. Study area in Argentina. The black area shows Chaco Province and the white square shows the area where samples were collected.

optic density (OD) of the negative control, plus 0, 300 OD units, according to the manufacturer's instructions. An indetermination zone, defined by the cut-off value ± 10%, was determined. Samples with an absorbance greater than the upper limit of the indetermination zone were considered positive.

Wild mammal trapping. Mammal trapping was carried out using Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, WI) for medium sized mammals, Sherman traps (H.B. Sherman Traps, Inc., Tallahassee, FL) for rodents, and mist nets for bats. Some mammals were captured manually.

Search for domiciliary triatomines. A total of 123 dwellings were searched for domiciliary triatomines. Those in which nymphs and adults triatomines were found were considered infested. A random sample of 13 dwellings from a 36-dwelling settlement was examined for the presence of triatomines. Each dwelling was examined by two different operators for 30 minutes. In the remaining settlements, dwellings were not randomly selected and searches were not performed systematically. For this reason, the percentage of domiciliary infestation was calculated as infected dwellings divided by the total number of dwellings in each settlement, and expressed as the minimum percentage of domiciliary infestation (MPDI).

Parasitologic examination. A total of 70 seropositive humans were examined by hemoculture. Five tubes per patient containing 2 mL of liver infusion tryptose medium (LIT medium) supplemented with 1% hemin, 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/mL of streptomycin were mixed with 200 μL of heparinized peripheral blood. Parasite growth was verified after 15, 30, 45, and 60 days of culture with an inverted microscope.

A total of 106 dogs and 74 wild mammals belonging to 11 different species were examined by xenodiagnosis using uninfected *T. infestans* nymphs. Medium-sized wild mammals and dogs were exposed to xenodiagnosis with 30 third-fifth instar *T. infestans* nymphs for 30 minutes. Rodents and bats were exposed to 10 *T. infestans* nymphs for 30 minutes. Bugs feces were microscopically examined (400×) for *T. cruzi* infection on days 30 and 60 after feeding. The feces of 196 *T. infestans* and 34 *T. guasayana* collected in the study area were also microscopically examined for *T. cruzi* infection.

RESULTS

Human seroprevalence. The mean seroprevalence was 27.81% (109 of 392) and seroprevalence in 1–15-year-old children was 24.14% (63 of 261). Concordance between IHA and ELISA was 91.07% (357 of 392). Discordant samples were considered negative and results were referred to the Regional Hospital with a recommendation for further analysis with a third serologic technique. Table 1 shows the seroprevalence at different age ranges for each locality studied.

Domiciliary infestation. A total of 29 dwellings were found to be infected by triatomines. The systematic search method identified 53.85% (7 of 13) of the dwellings with domestic infestation in the random sample. The general MPDI was 14.43% (29 of 201). The MPDI of the localities studied is shown in Table 2.

Parasitologic examination. Positive hemocultures were obtained from 6 (8.57%) of 70 patients studied. These parasitologically positive patients were from the localities of Tres Estacas (3) and Pampa Avila (3). The canine prevalence of infection, as determined by xenodiagnosis, was 15.09% (16 of 106). A total of 74 wild mammals belonging to 11 species were captured and examined by xenodiagnosis. Infection with *T. cruzi* was detected in 10 (35.71%) of 28 *D. albiventris* (opossums). None of the remaining 46 mammals was infected: 6 *C. chinga* (skunks), 1 *Monodelphys dimidiata* (short-tailed opossum), 8 *Chaetophractus vellerosus* (small armadillo), 3

TABLE 1
Human seroprevalence in the study area of Argentina

Localities	Mean seroprevalence	Seroprevalence at different age ranges (years) % (no. positives/no. examined)				
		1–4	5–9	10–15	16–20	> 20
Tres Estacas	26.99 (61/226)	6.67 (1/15)	15.62 (10/64)	30.43 (21/69)	50 (9/18)	33.33 (20/60)
Pampa Avila	35.58 (37/107)	16.67 (1/6)	44.44 (12/27)	30.00 (12/40)	18.18 (2/11)	47.83 (11/23)
El Picazo	12.12 (4/33)	0 (0/2)	10 (1/10)	7.14 (1/14)	50 (2/4)	0 (0/3)
Los Huaicos	23.08 (6/26)	25 (1/4)	33.33 (2/6)	25 (1/4)	16.67 (1/6)	16.67 (1/6)
Total	27.81 (109/392)	11.11 (3/27)	23/36 (25/107)	27.56 (35/127)	35.90 (14/39)	34.78 (32/92)

TABLE 2
Domiciliary infestation by triatomines*

Localities	Dwellings			MPDI
	Total	No. examined	No. infested	
Tres Estacas	71	56	10	14.8%
El Picazo	24	28	4	16.67%
Pampa Avila	70	26	8	11.43%
Las Tolderías†	36	13	7	19.4%
Total	201	123	29	14.43%

* MPDI = minimum percentage of domiciliary infestation calculated as the number of infected houses divided by the total house number of each locality.
† These values correspond to Barrio San Lorenzo only.

Tolypeutes matacus (southern three-banded armadillo), 5 *Oligoryzomys* sp. 1 (voles), 2 *Oligoryzomys* sp. 2 (voles), 3 *Akodon* sp. (voles), 2 *Callomys venustus* (voles), 1 *Felis geoffroyi* (wild cat), and 15 *Desmodus rotundus* (vampire bats). Infection with *T. cruzi* was observed in 59 (30.10%) of 196 *T. infestans*. None of the 34 *T. guasayana* examined carried the parasite.

DISCUSSION

Clear evidence for non-interruption or re-establishment of vectorial transmission in the study area was indicated by the observed seroprevalence in humans (mean = 24.14%, 27.81% in 1–15-year-old children and 11.11% in 1–4-year-old children) and dogs (15.09%) and the presence of *T. infestans* infected with *T. cruzi* (30.10%) in human dwellings. According to the Technical Report of the Chagas Disease National Control Program in Argentina presented in March 2003 at the XIIth Intergovernmental Meeting INCOSUR/Chagas,[4] the prevalence of infection by *T. cruzi* in 2001 among children less than 14 years old from endemic rural areas was 1.82% (569,033 children examined). The seroprevalence values (27.81%) we obtained for the same age range in this study area suggest that a great heterogeneity exists in the epidemiologic situation as related to Chagas disease in rural areas of Argentina.

Canine prevalence of infection with *T. cruzi* in the study area was relatively low. In a field survey carried out in a region with active vectorial transmission of *T. cruzi* adjacent to our study area, a canine prevalence of infection (determined by xenodiagnosis) of 32% (128 of 399) was observed.[19] In a rural area of Santiago del Estero, Argentina, the canine seroprevalence was 83.9% before the beginning of house spraying with residual insecticides; this decreased to 39.8% three years after spraying.[11] Compared with these data, our data suggest a decreased prevalence of canine infection in our study area as a consequence of the spraying with insecticides. However, most (73.58%, 78 of 106) of the dogs studied were born after the beginning of the spraying campaign in 1996. The prevalence of infection in dogs born after spraying was 11.54% (9 of 78), suggesting that *T. cruzi* transmission to this host was either not interrupted, or was re-established in the study area.

The prevalence of infection with *T. cruzi* in the *D. albiventris* population (35.71%), and the fact that these infections are due to strains belonging to *T. cruzi* I lineage in the study area,[17] a typically wild cycle–associated lineage, suggest the presence of a wild cycle of transmission in this area. This fact, and the presence of *T. guasayana* (a typically wild triatomine species) in the domiciliary environment, is of epidemiologic interest and should be taken into account in the planning of surveillance measures, since there is evidence of domestic and wild cycles overlapping in this rural area of the Chaco Province.[17]

The effort made and the success obtained during the antivectorial campaigns in Argentina are evident, as judged by the nationwide data. However, we believe that such data do not reflect the great heterogeneity of epidemiologic situations related to Chagas Disease in this country.[2,5] During the fieldwork period in the study area (January 1999–November 2002), we obtained clear evidence of active vectorial transmission of *T. cruzi* in domiciliary environments, and high levels of domiciliary infestation by *T. infestans*. This situation indicates a failure in the epidemiologic surveillance. In Argentina, this surveillance is carried out with community participation strategies, which imply transfer of responsibilities from central government institutions to municipalities or community leaders. We think that such a transfer should be accompanied by regular supervision by central government institutions involving qualified personnel.

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