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. In 1993, the average domiciliary infestation rate in Argentina was 25.7%, and the inferred number of persons infected by T. cruzi was 2,300,000 (7.2% of the population). 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. 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, and four Argentinean provinces stopped the vectorial transmission of T. cruzi in 2001. 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. 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.

The current data on mammal reservoirs of T. cruzi in Argentina are mainly from studies performed on dogs in endemic areas. 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.

Since 1999, we have surveyed transmission circuits of different T. cruzi strains in rural areas of Chaco Province. 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 km², 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 Chaquen Region 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
optic density (OD) of the negative control, plus 0, 300 OD units, according to the manufacturer’s instructions. An indetermi-
mination 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 posi-
tive.
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 system-
atically. For this reason, the percentage of domiciliary infes-
tation 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 hu-
mans were examined by hemoculture. Five tubes per patient
containing 2 mL of liver infusion tryptose medium (LIT me-
dium) 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 un-
infected 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 chil-
dren 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 ob-
tained from 6 (8.57%) of 70 patients studied. These parasito-
logically 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.
ingha (skunks), 1 Monodelphys dimidiata (short-tailed opos-
sum), 8 Chaetophractus vellerosus (small armadillo), 3

<table>
<thead>
<tr>
<th>Localities</th>
<th>Mean seroprevalence</th>
<th>1–4</th>
<th>5–9</th>
<th>10–15</th>
<th>16–20</th>
<th>≥ 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tres Estacas</td>
<td>26.99 (61/226)</td>
<td>6.67 (1/15)</td>
<td>15.62 (10/64)</td>
<td>30.43 (21/69)</td>
<td>50 (9/18)</td>
<td>33.33 (20/60)</td>
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<tr>
<td>Pampa Avila</td>
<td>35.58 (37/107)</td>
<td>16.67 (1/6)</td>
<td>44.44 (12/27)</td>
<td>30.00 (12/40)</td>
<td>18.18 (2/11)</td>
<td>47.83 (11/23)</td>
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<td>El Picazo</td>
<td>12.12 (4/33)</td>
<td>0 (0/2)</td>
<td>10 (1/10)</td>
<td>7.14 (1/14)</td>
<td>50 (2/4)</td>
<td>0 (0/3)</td>
</tr>
<tr>
<td>Los Huáicos</td>
<td>23.08 (6/26)</td>
<td>25 (1/4)</td>
<td>33.33 (2/6)</td>
<td>25 (1/4)</td>
<td>16.67 (1/6)</td>
<td>16.67 (1/6)</td>
</tr>
<tr>
<td>Total</td>
<td>27.81 (109/392)</td>
<td>11.11 (3/27)</td>
<td>23/36 (25/107)</td>
<td>27.56 (35/127)</td>
<td>35.90 (14/39)</td>
<td>34.78 (32/92)</td>
</tr>
</tbody>
</table>
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, 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. albiven-tris population (35.71%), and the fact that these infections are due to strains belonging to T. cruzi I lineage in the study area, 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.15

The effort made and the success obtained during the anti-vectorial 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 infection 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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