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A SEROEPIDEMIOLOGIC SURVEY OF CHAGAS' DISEASE IN TWO PARAGUAYAN VILLAGES[1]

Antonieta Rojas de Arias,[2] María Idalia Monzón,[3] Graciela Velázquez de Saldivar,[3] Elizabeth Guillen,[3] and Nestor Arrua T.[4]

A survey was conducted at two widely separated Paraguayan villages during February-May 1982 in order to help define the Chagas' disease problem in that country. This article reports the results of that survey.

Introduction

As in many Latin American countries, Chagas' disease is a serious endemic public health problem in Paraguay. Vector studies conducted in various parts of the country through the mid-1970s found the principal Chagas' disease vector, *Triatoma infestans,* to be present in all the areas surveyed, with the recorded house infestation indexes generally being in the 20-30% range *(1).* More recently, surveys of 18 villages in or around 1979 found *T. infestans* present in 57% of 451 homes inspected, and also found 23% of the captured vector insects to contain the Chagas' disease agent, *Trypanosoma cruzi.* Similarly, 1980 surveys of 616 homes in 20 villages found a *T. infestans* infestation index of 61%, with 25% of the captured insects testing positively for *T. cruzi (2).*

Regarding human infections, during the 1970s sera from 140 Indians living in the Paraguayan Chaco were tested for *T. cruzi* antibodies by the direct agglutination and indirect immunofluorescence methods. These two respective techniques obtained positive results with 72.9% and 73.6% of the test sera *(3).* It should be noted, however, that these are the highest rates of positivity ever reported from serologic testing for Chagas' disease antibodies in Paraguay. Much lower rates, averaging 9.7% positive, have been obtained by J. A. Cerisola through hemagglutination testing of sera from 6,000 military recruits 18 years of age from all parts of the country. When the recruits were grouped according to their geographic areas of residence, those from the department of Boquerón (38 samples, 18.4% positive) and the department of Cordilleras (931 samples, 16.2% positive) yielded the highest levels of positive response *(1).*

Despite these research efforts, however, in large areas of Paraguay little is known about vector infection and infestation levels, human serologic responses, and the socioeconomic impact of the disease. Therefore, in seeking to obtain a better understanding of the problem, it was decided that seroepidemiologic surveys should be conducted in two rural settlements that were separated geographically and relatively small, with not over 60 homes or 500 inhabitants. The aim was to survey both villages in their entirety for triatomid infestations and *T. cruzi* infection of the population. This survey was carried out in February-May 1982.

Materials and Methods

The Survey Areas

Two villages meeting the aforementioned criteria (Guazu-Cuá in the southwestern de-

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[2] Biologist-entomologist, Health Sciences Research Institute, Río de la Plata y La Gerenza, Casilla de Correo 2511, Asunción, Paraguay.

[3] Immunologist, Health Sciences Research Institute.

[4] Cardiologist, Health Sciences Research Institute.

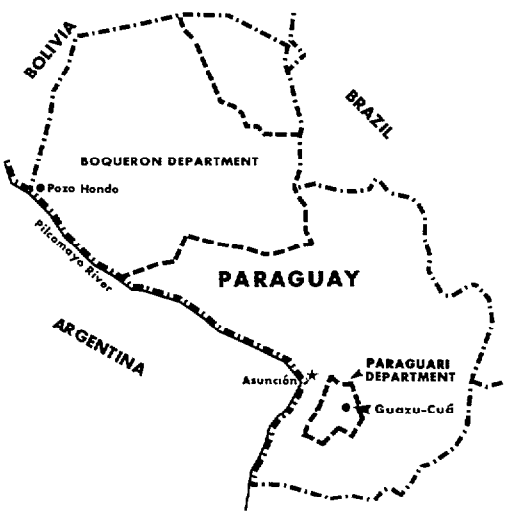
partment of Paraguarí and Pozo Hondo in the extreme west of the Chaco region) were selected at random. The location of these villages is shown in Figure 1. The village of Pozo Hondo in the Chaco region has no difficulty obtaining water because of its close proximity to the Pilcomayo River. However, the immediate environment is characterized by drought, poor soil, and constant wind that together create conditions unfavorable for most vegetation. As a result, the natural cover is dominated by tree-like cacti and large shrubs, most of which possess thorns for protection and small, leathery leaves to reduce transpiration. The total number of plant species present is small, and the general appearance of the landscape is monotonous. The area is used for grazing animals, particularly goats, but overgrazing has denuded the dry clay soil in places and has increased problems of erosion. The principal industrial product of the area is tannin.

Located in the Lower Chaco near the borders of Bolivia and Argentina, about 800 km from Asunción, Pozo Hondo also provides the grounds for a naval facility operated by the Paraguayan Armed Forces. However, some 50 transient military personnel living at the naval facility were not considered comparable to the rest of the village population. Therefore, data on these personnel were excluded, and the Pozo Hondo data considered were limited to the population (65 residents) living in the 14 homes within 45 km of the facility.

To the south, about 90 kilometers southeast of Asunción, the village of Guazu-Cuá is situated in a deciduous tropical forest. The area is dry, and during the dry season the deciduous vegetation looks open; however, during times of rain the plant growth becomes lush. The generally flat area of plains away from the local stream grows only a few tree-like plants, but the gallery forests along the stream-banks enjoy high humidity and support a rich vegetation. Most of the territory is farmland devoted to raising corn, cassava, and cattle fodder. The village itself, at the time of our survey, had 282 inhabitants and 60 residences.

Both Pozo Hondo and Guazu-Cuá were surveyed in their entirety. That is, efforts were made to capture triatomids at all homes, and blood samples were concurrently obtained at each residence.

Figure 1. A map of Paraguay showing the locations of the survey villages in the departments of Boquerõn and Paraguarí.



Collection Methods

The house inspections for triatomid vectors were conducted by a team of four people who searched all rooms of each house (including the walls, floors, attached structures, boxes, furnishings, household goods, and so forth) for 30 minutes. The triatomids were located and captured with lamps and tweezers and were placed in plastic containers. The containers were then covered with gauze and labeled with a survey number and the family's surname.

Additional survey data, which were obtained by one person, included personal data about the residents (name, age, sex, and place of birth), the structural characteristics of the home, and the places in the home where triatomid bugs were captured.

To collect blood samples from the 65 Pozo

Hondo and 282 Guazu-Cuá residents, special three-page "books" were used. The first page, made of ordinary paper, was used to enter the survey subject's name and other data. The second and third pages, made of Whatman No. 1 filter paper with a pre-marked nine square centimeter area, were used to collect the samples.

Two workers collected these samples by fingerprick, according to the procedure indicated by Camargo *(4)*. Then, after the impregnated filter-paper pages were dry, the samples were placed in polyethylene bags, the bags were put into thermoinsulating boxes, and the boxes were transported to the laboratory. The recommendations of Marinkelle *(5)* and Guimaraes *(6)* were followed with regard to collection, shipment, and storage of the samples.

Laboratory Tests

Detection of T. cruzi *in captured triatomids.* Following completion of the survey in each village, the captured *T. infestans* were transported to the laboratory to determine whether they were infected with *T. cruzi.* This determination was made by examining fresh feces (obtained by applying abdominal pressure to the bugs) that had been suspended in physiologic saline. Identification of observed flagellates was confirmed by noting the flagellates' growth patterns and morphologic characteristics in vitro, by examination of Giemsa-stained metacyclic forms *(7)*, and by removal and examination of the salivary glands from collected *T. infestans* yielding a negative result.

Serologic tests. The blood samples collected on filter-paper were cut from the rest of the filter-paper pages and placed in test tubes with phosphate-buffered saline (PBS) at pH 7.2. The tubes were put aside for over an hour, until the filter-paper became clear. The paper was then withdrawn and serologic tests were conducted with the eluate. Since each paper was considered to have absorbed approximately 0.08 ml of blood, equivalent to 0.05 ml of serum, placing the blood sample in 2 ml of PBS yielded a dilution of approximately 1:40, which was used for indirect immunofluorescence testing. For purposes of indirect hemagglutination testing, a lesser dilution of approximately 1:30 was employed *(8)*.

The indirect immunofluorescence tests were performed using the technique recommended by Dr. Mario Camargo of the Institute of Tropical Medicine of São Paulo. A diamond point was used to inscribe eight circles of 0.5 cm diameter in each of the microscope slides used. Gamma globulin labeled with fluorescein isothiocyanate was obtained from the Biomerieux Laboratory in France. The optimal fluorescein titer for the conjugate was 1:60. The microscope employed was an American Optical (USA) model 2071 with dark-ground illumination, a 10 × eyepiece, a 40/0.66 dry objective, and a 1.25 dark-field condenser. Ultraviolet light was provided by an OSARAM HBO 50-watt lamp. Observations of fluorescence were made in a darkroom.

The indirect hemagglutination test was performed according to the technique recommended by the immunology laboratory of the Institute of Tropical Medicine of São Paulo *(9)*. The tests were conducted with hemagglutination kits from TRILAB® (Brazil) using microtitration plates with U-shaped wells. The antigens used in these tests came from the same batch as those used in the indirect immunofluorescence tests.

Positive and negative control sera were used in both of these tests. The positive control sera were obtained from patients with a clinical and epidemiologic history of Chagas' disease and a positive xenodiagnosis. The negative control sera were obtained from healthy individuals with no clinical or epidemiologic history of the disease.

Examination of survey subjects. Subsequently, 68 (82.9%) of the 82 Guazu-Cuá subjects yielding positive serologic test results were given a general physical examination at the Paraguarí Department Health Center. (Those over 15 years of age were also given a chest X-ray and an ECG.) The results of these

examinations are to be discussed in a later work.

Results

The house inspections in the two towns surveyed detected *T. infestans* in 47 (78.3%) of the 60 Guazu-Cuá houses and seven (50%) of the 14 Pozo Hondo houses (Table 1).

Not all the triatomids captured could be examined for *T. cruzi* because 102 of the 435 captured in Guazu-Cuá died en route to the laboratory. However, all of the 19 *T. infestans* captured at Pozo Hondo and the 333 Guazu-Cuá triatomids that reached the laboratory alive were examined for the parasite. These examinations showed that four (21%) of the triatomids captured at Pozo Hondo and 68 (20.4%) of those examined from Guazu-Cuá were positive for *T. cruzi.*

As indicated in Table 2, the places where *T. infestans* were most commonly found were in the walls and among the household objects of infested homes.

Regarding house construction, 54 (90%) of the 60 dwellings inspected in Guazu-Cuá had thatched roofs, 52 (87%) had wooden walls, and 48 (80%) had earthen floors. Aside from the wood walls, these observations are consistent with more general data from the 1972 census for Paraguarí Department which classified 31,577 of the department's 38,203 homes as "rural" and indicated that 82% had straw-thatched roofs, 34% had "stucco" walls, and 79% had earthen floors (*14*).

During the Pozo Hondo survey it was observed that nine (64.3%) of the inspected dwellings had wattle-and-daub roofs, seven (50%) had adobe walls, and 13 (92%) had earthen floors. These data were likewise consistent with data from the 1972 census in Pozo Hondo's department (Boquerón), which classified 3,052 of the department's 4,734 homes as "rural" and indicated that 58% had wattle-and-daub roofs, 44% had adobe walls, and 70% had earthen floors (*14*).

With regard to serologic tests, the immunofluorescence tests indicated that 82 (29%) of the 282 Guazu-Cuá subjects and 13 (20%) of the Pozo Hondo subjects were positive for

Table 1. Rates of *T. infestans* house infestations at Guazu-Cuá and Pozo Hondo, and rates of *T. cruzi* infection of *T. infestans* captured in the two villages in February-May 1982.

Village	No. of houses inspected	Houses infested with *T. infestans*		No. of *T. infestans* captured	*T. infestans* examined for *T. cruzi*		*T. infestans* positive for *T. cruzi*	
		No	%		No.	%	No.	%
Guazu-Cuá	60	47	78.3	435	333	76.6	68	20.4
Pozo Hondo	14	7	50	19	19	100	4	21.1
Total	74	54	73	454	352	77.5	72	20.5

Table 2. Principal locations within Guazu-Cuá and Pozo Hondo residences where *T. infestans* specimens were captured.

Village	No of homes where the principal place harboring *T. infestans* was as indicated:				
	Walls[a]	Household belongings	Beds	Chicken coops	Floors
Guazu-Cuá	22	14	7	3	1
Pozo Hondo	9	2	2	–	1

[a]Including walls of peridomiciliary structures as well as walls of homes.

T. cruzi antibodies (Tables 3 and 4). A total of 79 of the Guazu-Cuá subjects yielded positive results in both the immunofluorescence and hemagglutination tests, indicating 96.3% agreement between the two test procedures. All 13 Pozo Hondo subjects yielding positive results by one method also yielded positive results by the other.

The highest serum titers yielding a positive result by immunofluorescence testing are shown in Table 5. Most (51%) of the positive Guazu-Cuá sera yielded positive results only at the lowest (1:40) level, and three-quarters fell into the 1:40-1:160 range. In contrast, nearly half (46%) of the positive Pozo Hondo sera yielded positive results at the highest titer (1:640), and over three-quarters were in the 1:320-1:640 range. Reflecting this disparity,

Table 3. Guazu-Cuá subjects whose sera tested positively for *T. cruzi* antibodies, according to the immunofluorescence test results, by age group and sex.

Age group (in years)	No of study subjects	No of subjects with positive sera			% of subjects yielding positive results
		Females	Males	Total	
0-4	38	2	3	5	13
5-14	90	11	7	18	20
15-24	52	9	8	17	33
25-34	20	2	2	4	20
35-54	45	9	9	18	40
55-64	21	3	4	7	33
65-88	16	6	7	13	81
Total	282	42	40	82	29

Table 4. Pozo Hondo subjects whose sera tested positively for *T. cruzi* antibodies, according to the immunofluorescence and hemagglutination test results, by age group and sex.

Age group (in years)	No. of study subjects	No. of subjects with positive sera			% of subjects yielding positive results
		Females	Males	Total	
0-4	25	–	–	–	–
5-14	7	2	1	3	43
15-24	11	1	1	2	18
25-34	11	3	2	5	45
35-54	8	1	–	1	13
55	3	1	1	2	67
Total	65	8	5	13	20

Table 5. Distribution of titers obtained with sera yielding positive responses to the immunofluorescence test, by village of origin.

Village	Total positive sera	Highest titers obtained with the indicated sera (No. and %)					Geometric mean titer
		1:40	1:80	1:160	1:320	1:640	
Guazu-Cuá	82	42 (51%)	11 (13%)	9 (11%)	8 (10%)	12 (15%)	1:93.94
Pozo Hondo	13	2 (15%)	–	1 (8%)	4 (31%)	6 (46%)	1:303.38

the geometric mean titer of the positive Guazu-Cuá sera was 1:93.94, while the geometric mean titer of the positive Pozo Hondo sera was 1:303.38.

Discussion

The vector *T. infestans* is known by the native Guaraní name of *chichá-guazú* in both Guazu-Cuá and Pozo Hondo. In Pozo Hondo, where most of the inhabitants are of Argentine origin, it is also known by the name *vinchuca.*

The entomologic survey results in both towns showed high levels of *T. infestans* infestation. Furthermore, bugs collected at nine of the infested Guazu-Cuá homes and three of the Pozo Hondo homes were found to harbor *T. cruzi,* so that an active potential for disease transmission existed in at least those homes. This proven potential for Chagas' disease transmission in at least 22% of the homes inspected (19% of those in Guazu-Cuá, 43% of those in Pozo Hondo) leads to the conclusion that the disease is highly endemic in both places *(10, 11).* The house walls, typically constructed of wood in Guazu-Cuá and adobe in Pozo Hondo, were the house locales where *T. infestans* were most often captured. It should also be mentioned that Guazu-Cuá residents commonly had a chicken coop installed as part of the home's common living space, and this often shared a wooden wall with the main bedroom. In contrast, the residents of Pozo Hondo did not build chicken coops, even in the space outside the home, and so the potential for transmitting *T. cruzi* from chickens in such coops to local residents did not exist.

In general, proliferation of triatomids was encouraged in these villages by the prevailing methods of housing construction, as well as by climatic factors and poor hygiene. Also, the inhabitants of the two villages had received little or no health education and were unaware of the relationship between *T. infestans* and Chagas' disease; this lack of understanding was therefore another factor favoring disease transmission.

T. cruzi antibodies were found among subjects in all the age groups shown in Tables 3 and 4 in both villages, with the sole exception of subjects 0-4 years of age in Pozo Hondo. Both towns were sprayed during antimalaria campaigns about two years before the survey; however, according to Guazu-Cuá residents, the triatomids staged a massive reinfestation of Guazu-Cuá homes about one year later. Reinfestation appears to have been less marked in Pozo Hondo because some inhabitants, particularly the Argentines, had continued to apply the insecticide Gamexame (hexachlorocyclohexane) after the campaign-related spraying ended.

The results of the immunofluorescence test used to detect exposure to *T. cruzi,* and the hemagglutination test used to confirm those results, both indicated that at least 20% of the study subjects in each of the two villages had been infected. Although other conditions, notably leishmaniasis, can sometimes induce false-positive serologic responses, the high prevalences of *T. cruzi* found in the captured triatomids and the fact that neither the Paraguayan Chaco nor Paraguarí Department are considered areas with endemic leishmaniasis made the occurrence of such false-positive responses unlikely. In 1982 only three leishmaniasis cases were reported from Paraguarí Department, and none were reported from the Paraguayan Chaco *(12).*

Regarding the various serum titers yielding positive immunofluorescence test responses (see Table 5), it seems evident that most of the Pozo Hondo sera yielded responses at considerably higher titers than did most of the positive Guazu-Cuá sera. These relatively high Pozo Hondo titers, which could have been due to recurrent stimulation of the subjects' immune systems by repeated infection with *T. cruzi (13),* suggests that repeated *T. cruzi* transmission may have been more common at Pozo Hondo than at Guazu-Cuá.

SUMMARY

To help define the Chagas' disease problem in Paraguay, a survey was carried out in February-May 1982 at two randomly selected and widely separated villages—one along the Pilcomayo River of northwestern Paraguay and the other to the south about 90 kilometers from Asunción. Residences in the two villages were visited by four-person teams that inspected the homes for triatomid bugs, collected basic data about the homes and their inhabitants, and obtained serum samples from the inhabitants. Captured triatomids were later examined in the laboratory to determine whether they contained the disease agent, *Trypanosoma cruzi,* and the serum samples were subjected to immunofluorescence and hemagglutination tests for the presence of *T. cruzi* antibodies.

The house inspections revealed the Chagas' disease vector *Triatoma infestans* in half the 14 residences visited at one village (Pozo Hondo), and in 47 of the 60 residences at the other village (Guazu-Cuá). Over 20% of the triatomids from each village that were subjected to laboratory examinations were found to contain *T. cruzi,* and sera from a high proportion of the residents (20% at Pozo Hondo and 29% at Guazu-Cuá) tested positively for *T. cruzi* antibodies. Responses to the immunofluorescence test indicated that the positive Pozo Hondo sera contained markedly higher levels of *T. cruzi* antibodies, on the average, than did the positive Guazu-Cuá sera, suggesting that recurrent transmission of the disease agent may have been more common in the former village.

In general, prevailing styles of home construction, climatic conditions, poor hygiene, lack of health education, and ignorance of the connection between the triatomid bugs and Chagas' disease all tended to encourage proliferation of the bugs and transmission of *T. cruzi* in both villages. Prevailing triatomid infestation levels had apparently been reduced temporarily in both places by spraying connected with an antimalaria campaign two years before the survey, and reinfestations may have been discouraged somewhat in Pozo Hondo as a result of continued insecticide applications by local residents.

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POST-ERADICATION SURVEILLANCE OF SMALLPOX

During 1983, 19 reports on suspected smallpox cases were recorded in the global smallpox rumor register. Eleven of the resulting investigations were closed by the end of 1983, and another five were closed during the first quarter of 1984. Investigation results are still pending in three cases.

A report from Kenya caused some public concern because the patient, an ex-variolator, died three days after having developed a rash. Prompt reporting and laboratory investigation of specimens collected by the Kenyan health services proved within a week that the suspect case was actually one of chickenpox.

In contrast, another report required nine months of investigation. In June 1983 a government official attending an international meeting said that in March 1983 there had been outbreaks of smallpox, including several deaths, in areas of Niger bordering Nigeria. On 10 June 1983 WHO asked an epidemiologist stationed in Niger to investigate. In July the epidemiologist indicated there was no smallpox in the Niger provinces in question, but that chickenpox was present.

In January 1984 the same informant told WHO that someone from Mali had reported the death of a relative from a smallpox-like disease in Bamako in April 1983, and that smallpox deaths were occurring in the town of Sejure in Upper Guinea. On 21 March 1984 the WHO Program Coordinator in Mali reported that he had investigated two children and that they suffered from measles; there was no evidence of smallpox in Bamako. This brought the investigation to a close.

In accord with World Health Assembly Resolution WHA 33.4 of 1980, WHO has encouraged and will continue to encourage the reporting of smallpox rumors so that they may be investigated promptly. It is important, however, that such reports give particulars of the rumors in question—including the name of the person who saw or diagnosed the patient, the date involved, the place involved, etc.—in order to facilitate the investigation.

Source. World Health Organization, *Weekly Epidemiological Record* 59(19):142-143, 1984