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Keywords

Chagas disease, epidemiology.
Poverty areas.
Ecology, vectors.

Abstract

Introduction

A descriptive, entomological and seroepidemiological study on Chagas disease was conducted in a place of recent occupation on the outskirts of Cochabamba, Bolivia: Avaroa/Primer de Mayo (population: 3,000), where the socio-economic level is low and no control measures have been made available.

Methods

The immunofluorescent antibody test (IFAT) was used for IgG and IgM anti-*Trypanosoma cruzi* antibodies in filter paper bloodspot eluates from 128 subjects (73 females, 55 males) selected by systematic sampling. Concerning each subject age, gender, birthplace, occupation, duration of residence and building materials used in their houses were recorded. Vectors were captured both in domestic and peridomestic environments.

Results

Seropositive, 12.5% (16/128): females, 15.1% (11/73); males, 9.1% (5/55). Average time of residence: 6.1 years for the whole population sample and 7.4 years for the seropositive subjects. Most houses had adobe walls (76.7%, n = 30), galvanized iron roofs (86.7%) and earthen floors (53.4%) 80% of the walls had crevices. One hundred forty seven specimens of *Triatoma infestans* were captured, of which 104 (70.7%) were domestic, and 1 peridomestic *Triatoma sordida*. Precipitin host identification: birds, 67.5%; humans, 27.8%; rodents, 11.9%; dogs, 8.7%; cats, 1.6%. House infestation and density indices were 53.3 and 493.0 respectively. We found 21 (14.3%) specimens of *T. infestans* infected with trypanosomes, 18 (85.7%) of which in domestic environments.

Discussion

The elements for the vector transmission of Chagas disease are present in Avaroa/Primer de Mayo and the ancient custom of keeping guinea pigs indoors adds to the risk of human infection. In neighboring Cochabamba, due to sub-

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standard quality control, contaminated blood transfusions are not infrequent, which further aggravates the spread of Chagas disease. Prompt action to check the transmission of this infection, involving additionally the congenital and transfusional modes of acquisition, is required.

Descritores

Doença de Chagas, epidemiologia.
Áreas de pobreza.
Ecologia de vetores.

Resumo

Introdução

Estudo descritivo, entomológico e soropidemiológico da infecção chagásica em área de ocupação recente da periferia de Cochabamba, Bolívia: Avama/Primer de Mayo, onde o nível socioeconômico da população é baixo e não foram instituídas medidas de controle.

Métodos

Por meio da reação de imunofluorescência indireta (RIFI), de anticorpos IgG e IgM anti-Trypanosoma cruzi em sangue capilar absorvido em papel-filtro, foram pesquisadas 128 pessoas (73 femininas e 55 masculinas) selecionadas por amostragem sistemática dentre 3.000 habitantes. Registraram-se idade, sexo, naturalidade, ocupação, tempo de moradia e características de construção das casas.

Resultados

Verificou-se que 12,5% (16/128) foram sororreagentes: 15,1% (11/73) do sexo feminino e 9,1% (5/55) masculino. O tempo médio de moradia foi de 6,1 anos para toda a amostra da população e de 7,4 anos para os sororreagentes. Predominaram construções de adobe (76,7%), teto de zinco (86,7%) e piso de terra (53,4%). Observaram-se gretas nas paredes de 80,0% das casas (n = 30). Capturaram-se 148 exemplares de triatomíneos: 147 de *Triatoma infestans*, 104 (70,7%) intradomiciliares, e 1 de *T. sordida*, peridomiciliar. Os índices de infestação e de densidade por casa visitada foram, respectivamente, 53,3 e 493,0. A identificação dos hospedeiros dos triatomíneos (reações de precipitina) foi: aves, 67,5%; humanos, 27,8%; roedores, 11,9%; cães, 8,7%; e gatos, 1,6%. Os adultos e ninfas de *T. infestans* infectados por tripanossomas foram: 21 (14,3%), dos quais 18 (85,7%) intradomiciliares.

Conclusões

Na área periférica de Cochabamba estudada foram encontrados todos os elementos da cadeia de transmissão vetorial: pessoas e vetores infectados e cobaios criados no interior das casas. A proximidade de Cochabamba, onde as transfusões de sangue são freqüentes, representa um risco adicional de transmissão. É necessária a pronta instituição de medidas profiláticas e trabalho educativo sobre as vias de transmissão da doença de Chagas, inclusive transfusional e congênita.

INTRODUCTION

As is often the case in South America, Chagas disease remains a serious public health problem in Bolivia. It is one of the consequences of poor standards of housing, education and hygiene prevailing in the rural, peri-urban and urban places inhabited by more than half the population of the country. Unemployment is frequent and the distribution of land inequitable. On account of the lack of adequate quality control, blood transfusions contribute to the further spread of the infection^{13,16}.

Triatoma infestans occurs in 7 of the 9 Departments into which Bolivia is divided, viz La Paz, Cochabamba, Tarija, Chuquisaca, Potosí, Santa Cruz and Beni. These departments add up to 84% of the Bolivian territory, and include valleys and lowlands (llanos), at altitudes between 300 and 3,500 meters²³. No vector triatomines have been found in the Departments of Pando and Oruro, at altitudes of less than 300 and 4,000 meters respectively^{4,13}. Local researchers place great emphasis on investigating such problems, posed by Chagas disease in peri-urban areas, as congenital transmission,

the sylvatic cycle of *T. infestans*, the action of insecticides and specific treatment⁴.

The present work aims at gathering entomological and seroepidemiological information about Chagas disease in a peri-urban area of the city of Cochabamba: Villa Avaroa/Primer de Mayo, established 16 years ago. Its inhabitants are willing to do their part in programs intended to evaluate and eventually control the transmission of Chagas disease in their community. It has been understood that this implies an improvement in their housing and living conditions.

METHODS

Study area - Avaroa/Primer de Mayo is a peri-urban locality of the city of Cochabamba (17°14'24"S and 66°5'24"W), Provincia Cercado, to the south of the Department of Cochabamba, Bolivia. Situated in a valley at an altitude of 2,400 meters, Avaroa/Primer de Mayo has a mild climate. Its population (3,000), besides having no guidance as to the prevention and control of naturally acquired Chagas disease, lives at a low socio-economic level.

By systematic random sampling 128 subjects aged one year or over were selected and the name, age, gender, birthplace, duration of residence in the locality, and occupation of each of them were recorded. The overall state of the houses and the characteristics of the walls (including the presence of cracks), flooring and roofing were also recorded, in view of the potential for colonization by triatomines. This survey was conducted during July 1996 (winter in the southern hemisphere).

Serology - Blood samples, collected by finger puncture, were absorbed into Whatman #3 filter paper strips (stapled together into booklets)⁸. By using a technique suggested by Ferreira & Carvalho⁹, the amount of blood per cm² of bloodspot surface area was estimated as 11.2 µl. The quantitative immunofluorescent antibody test (IFAT) as described elsewhere²⁰ was undertaken, using total *Trypanosoma cruzi* antigens and anti-IgG and anti-IgM human conjugates (Salck, S. Paulo, Brazil) standardized in terms of maximum reactivity. On the basis of previous experience, a cut-off titer of 16 was adopted. Positive and negative controls were supplied by a hospital specialized in cardiology²⁰.

Entomology - Triatomines in the egg, nymph and adult stages were captured, both inside and around 30 houses, for assessment of their density, house infestation indices and proportions of insects infected with *T. cruzi*¹⁹. Vector gut contents (stained according to the May Grünwald-Giemsa technique) were examined for metacyclic trypomastigotes, as described by Rodrigues et al.¹⁸, 1992. Wistar albino rats, aged 14 days on the average, were inoculated intraperitoneally with samples of gut contents from infected triatomines. A blood sample was transferred from each of the animals which developed parasitemia

within 7 to 21 days of inoculation to 3 test tubes, each containing 5 ml of the artificial Warren medium and incubated at 26.6° C. Xenodiagnosis was performed on the inoculated animals by using 10 *T. infestans* nymphs (4th or 5th instar).

The precipitin reaction to identify feeds from human, bird, marsupial (opossum), dog, cat, pig and goat hosts was performed in capillary tubes¹⁸. All anti-sera had been previously demonstrated to react with homologous sera at 1:10,000 dilution and not to react at 1:10 dilution with heterologous sera.

Blood sample collection and search for triatomines had been authorized beforehand by the occupants of the respective houses. Dawson-Saunders & Trapp⁷ was referred to for statistical guidance.

RESULTS

Serology

Positivity - Table 1 shows the distribution of the subjects by age, gender and seropositivity. Anti-*T. cruzi* IgG antibodies were detected in 12.5% (n = 128) of the subjects tested: 15.1% (n = 73) females and 9.1% (n = 55) males, proportions which did not differ significantly at the 5% confidence level (*ts* = 1.035, *p* = 0.300). No seropositives were detected among the 42 subjects (32.8% of the sample) aged under 10 years, although 9 of them (21.4%) were residents of houses in which triatomines infected with *T. cruzi* had been found (Table 5).

Among the 75 subjects aged from 10 to 49 years (58.6% of the sample) were found 87.5% of all seropositives (14 out of 16), 90.9% (10 out of 11) females and 80.0% (4 out of 5) males. Titers for IgG antibodies varied between 8 and 256 (geometric mean, 45.25). No cases positive for IgM antibodies were found, even at a low dilutions.

Time of residence in the region - The average time of residence was 6.1 years for the whole population sample and 7.4 years for the seropositives (median = 5.0 years, mode = 4.0 years). For the seronegatives the average time of residence was 5.8 years (median = 4.5 years, mode = 2.0 years).

Origin - Among the seropositives, 8 (50.0%) had come from endemic (Cochabamba, Capinota and Sucre), 7 (43.8%) from non-endemic areas (Oruro and Potosí) and 1 (6.3%) was of unknown origin. Among the seronegatives, 55 (49.1%) were born in endemic (Cochabamba, La Paz and Santa Cruz) and

Table 1 - Distribution by age group, gender and seropositivity for anti-*Trypanosoma cruzi* IgG antibodies by the indirect fluorescent antibody test (IFAT), of 128 inhabitants of Avaroa/Primer de Mayo, Cochabamba, Bolivia, 1996.

Age (years)	Females			Males			Males + Females		
	Total	Positive	%	Total	Positive	%	Total	Positive	%
0 - 4	7	0	0.0	8	0	0.0	15	0	0.0
5 - 9	12	0	0.0	15	0	0.0	27	0	0.0
10 - 14	15	3	20.0	8	2	25.0	23	5	21.7
15 - 19	4	1	25.0	2	0	0.0	6	1	16.7
20 - 29	12	1	8.3	8	0	0.0	20	1	5.0
30 - 39	12	4	33.3	3	1	33.3	15	5	33.3
40 - 49	7	1	14.3	4	1	25.0	11	2	18.2
≥ 50	4	1	25.0	7	1	14.3	11	2	18.2
Total	73	11	15.1	55	5	9.1	128	16	12.5

51 (45.5) in non-endemic areas (Llallagua, Catavi, Oruro and Potosí) and 6 (5.4%) were of unknown origin.

Occupation – The population sample was chiefly composed of schoolchildren (27.3%, n = 35), and housewives (17.2%, n = 22). There were also 2 farmhands, 2 tailors, 6 traders, 1 dressmaker, 1 contractor, 1 seamstress, 1 electrician, 1 housemaid, 1 mechanic, 1 motor vehicle driver, 5 bricklayers, 1 schoolteacher and 1 secretary. Among the seropositives, 43.8% (n = 7) were schoolchildren and 18.6% (n = 3) housewives, besides 1 farm-hand, 1 bricklayer and 4 subjects with no occupational record.

Housing Conditions

Thirty houses were visited to investigate their potential for breeding triatomines, according to building materials and maintenance: adobe walls predominated (76.7%); brick, 6.7%; cement, 3.3%; 13.3% of the walls were plastered. Corrugated galvanized iron was the commonest roofing material (86.7%); straw, 3.3%; other, 6.7%. Flooring: earthen, 53.4%; cement, 33.3%; wooden, 3.3%; other, 10.0%. Cracks were found in the walls of 80.0% of the houses.

Entomology

Table 2 shows the distribution, according to developmental stage, habitat and infection with trypanosomes, of the triatomines captured: 147 specimens (nymphs included) of *T. infestans* and 1 of *T. sordida*, respectively 99.3% and 0.7% (n = 148). Triatomines were found infesting 53.3% (n = 30) of the houses (30% in domestic environments); 63 nymphs and 41 adults were captured inside, 23 nymphs and 20 adults around the houses. A significant difference between these proportions (5% confidence level) was found ($t_s = 7.34$, $p < 0.05$).

Entomological indices³ (× 100)

Density per house visited	$(148/30) \times 100 = 493.3$
Confinement (per infested house)	$(148/19) \times 100 = 779.0$
Infestation (per house visited)	$(16/30) \times 100 = 53.3$
Intradomiciliary infestation	$(9/30) \times 100 = 30.0$
Trypanosome-triatomine infection	$(21/148) \times 100 = 14.2$
Rodent hosts (infected triatomines)	$(10/15) \times 100 = 66.7$

Table 2 - Distribution of *Triatoma infestans* captured in Avaroa/Primer de Mayo, Cochabamba, Bolivia, 1996, according to developmental stage, habitat and infection with trypanosomes.

Developmental stage	Habitat						Total		
	Domestic			Peridomestic			Number	Positive	%
	Number	Positive	%	Number	Positive	%			
Adult males	14	4	28.6	16	1	6.3	30	5	16.7
Adult females	27	5	18.5	4	1	25.0	31	6	19.4
5th nymph	23	6	26.1	8	1	12.5	31	7	22.6
4th nymph	23	1	4.4	7	0	0.0	30	1	3.3
3rd nymph	11	2	18.2	7	0	0.0	18	2	11.1
2nd nymph	2	0	0.0	0	0	0.0	2	0	0.0
1st nymph	4	0	0.0	1	0	0.0	5	0	0.0
Total	104	18	17.3	43	3	7.0	147	21	14.3

Table 3 - *Triatoma infestans*: precipitin bloodmeal identification of nymphs and adults in Avaroa/Primer de Mayo, Bolivia, 1996.

Developmental stage	Number of specimens tested	Host					Identified	
		Man	Bird	Rodent	Dog	Cat	Total	%
Nymph	86	25	54	10	4	2	76	88.4
Adult	54	10	31	5	7	0	50	95.6
Total	140	35	85	15	11	2	126	90.0

Table 4 - *Triatoma infestans* infected with trypanosomes: identification of hosts by precipitin bloodmeal reaction, by site of capture (inside or around the houses) and developmental stage. Avaroa/Primer de Mayo, Bolivia, 1996.

Site of capture	Developmental stage	Specimens	Bloodmeal identifications	Man	Bird	Rodent	Cat
Inside	Nymph	10	9	4	2	4	1
	Adult	8	4	1	0	3	0
Around	Nymph	1	1	0	0	1	0
	Adult	2	2	0	0	2	0
Total		21	16	5	2	10	1

Table 5 - Distribution of children aged 1 to 9 years, living in 5 of the 8 houses in which triatomines (domestic and peridomestic) infected with *T. cruzi* had been found and had their bloodmeals identified. Avaroa/Primer de Mayo, Bolivia, 1996.

Children		House	Triatomines	
Age (yrs)	Time of residence (yrs)		Habitat	Bloodmeal
1	1	1	Domestic	Human, bird
4	2	2	Domestic	Human, bird
8	2			
9	2			
4	4	3	Domestic	Cat, bird
7	7			
5	4	4	Domestic	Rodent
7	7	5	Peridomestic	Rodent
8	8			

Table 3 shows precipitin bloodmeal identification of *T. infestans*. The triatomines which had their hosts identified (n = 126) had fed mainly on birds (67.5%), humans (27.8%) and rodents (11.9%). None had fed on marsupials, pigs or goats.

Table 4 shows precipitin bloodmeal identification of nymphs and adults of *T. infestans*, infected with trypanosomes, by site of capture (inside or around the house) and developmental stage. The triatomines in which host identification succeeded (n = 16) had fed mainly on rodents 10 (62.5%) and humans (31.3%). None had fed on marsupials, pigs, goats or dogs. The gut contents of most triatomines (80.3%) reacted with only one kind of antigen, 19.0% with 2 kinds.

No gut contents could be collected from 7 of the 147 specimens of *T. infestans* captured. None of the available antisera reacted with the gut contents of 14 specimens.

Between the 30th and the 45th days of feeding, 70.0% of the nymphs used in xenodiagnosis were positive for trypanosomes. Parasitemia was first observed within 15 days of the inoculation of rats with infected triatomine gut contents and lasted for 35 days. Growth and maintenance of trypanosomes in the Warren artificial medium were satisfactory.

DISCUSSION

Mummies of Wankarani Indians, found in northern Chile, provided evidence of their infection with *T. cruzi* and their migration, earlier than 500 years BC, from Bolivia^{1,12}. The first cases of Chagas disease in Bolivia were reported by Arthur Neiva, in Potosí (1916), followed by Veintemillas, in La Paz (1931), Mazza & Chacon, in Cochabamba (1937) and Martins & Macedo, in Santa Cruz (1941)²¹.

It is now generally accepted that the center of dispersion of triatomines in South America was the region of the Bolivian Interandean Valleys where Cochabamba is located. The caves inhabited by pre-Columbian man presumably offered microclimatic conditions similar to those of rodent burrows, a habitat to which triatomines had already been adapted¹⁰. Further dispersion of the vectors resulted in the spread of Chagas disease among the human population of South America¹.

Data from the 1990s show a prevalence of *T. cruzi* infection ranging from 9.0% to 45.0% (average 40.3%) in the Bolivian departments situated in endemic areas. The average prevalence in the Department of Cochabamba is 31%²³. However, in such localities as Tabacal, prevalence values as high as from 70% to 100% have been observed in the segment of the population aged over 35 years¹⁵. Valencia (1990)²¹ estimates that, all age groups included, this proportion must be around 55.2%, varying between 41.3% and 59.0%.

Information about some peri-urban areas of Cochabamba is both scant and preliminary. About 17.5% of the general population and 7.2% of the segment aged between 1 and 4 years are seropositive⁴. In Avaroa/Primer de Mayo 12.5% were found to be seropositive, but none in the 1-9 age group, despite the fact that 9 (21.4%) of the latter were residents of houses in which triatomines infected with *T. cruzi* had been found. An independent observation conducted by the authors in Avaroa/Primer de Mayo, using blood samples from children aged 5 to 9 years (n = 80), showed 2 (2.5%) to be seropositive. Although this locality was founded 16 years ago, the average time of residence of its inhabitants is 6.1 years (mode and median less than or equal to 5 years). The risk of human infection was demonstrated by the capture of triatomines, which had fed on human hosts, infected with trypanosomes. This risk can also be inferred, in regions of recent occupation, by the proportion of seropositive children rather than seropositive adult migrants (who might have acquired the infection elsewhere). Thus the children born in this locality should also be followed up and investigated to rule out the possibility of congenital transmission, as was done by Azogue & Darras². Although the proportions of seropositive reactions showed no significant differences between the genders, the percentage of female seropositivity (15.1%) is a cause for concern. Azogue & Darras²

found 15.5% of the newborn children of seropositive mothers to be seropositive.

Migration from endemic areas of the country favors vector transmission of the infection²³. About half the seropositive inhabitants of Avaroa/Primer de Mayo came from endemic areas of Bolivia, which indicates that they are possibly not autochthonous cases. On the other hand, the occurrence of seropositive subjects born in areas labeled non-endemic (as Potosí) is not necessarily a rare event, observed by Carrasco et al.⁶ (1990), who found high proportions of seropositivity among prospective blood donors from this region. It is therefore necessary to investigate more accurately the epidemiological situation of some "non-endemic" regions. The fact that no IgM-seropositive subjects were found does not preclude the possibility of transmission in this region; these antibodies are detectable only during the initial phases of the infection^{5,17}. In Cochabamba, Chagas disease is frequently acquired through blood transfusion. It has been estimated that, during 1988, blood transfusion contributed 5 new cases of Chagas disease per day⁴. As a great number of infected individuals live in the city, the danger of infection through blood transfusion should not be underrated. In Avaroa/Primer de Mayo *T. infestans*, infected with trypanosomes, is often found in domestic and peridomestic environments. They feed on humans and animals such as birds, rodents, and dogs, living in or around their dwellings. In a preliminary observation, carried out in the outskirts of Cochabamba, *T. infestans* was found more often inside than around the houses (29% and 16% respectively)⁴. The season during which this investigation was undertaken was not recorded. In Avaroa/Primer de Mayo *T. infestans* was also found more frequently inside than around the houses (17.3% and 7.0%, respectively). The observations of this study were carried out in winter, when the activities of triatomines are expected to be reduced¹¹.

People involved in massive migration from rural environments go to the outskirts of big cities, where they live in shacks which provide optimal conditions for triatomine breeding. Insects brought along with the belongings of migrating families can colonize dwellings still not infested. The guinea pig (*Cavia cobaya*), a notorious reservoir of *T. cruzi*¹⁴, is frequently kept inside the dwellings, a habit dating back to pre-Columbian times and still followed in the Andean regions^{4, 20}. Thus the significance of the ob-

servation that the infected triatomines had fed predominantly on rodents should not be overlooked. Most of the houses visited were of recent occupation, which calls for prompt action against the vector, considering that Chagas disease has been diagnosed both in children and adults, and a relatively high proportion of triatomines feed on human or rodent hosts.

The occupations of the inhabitants of Avaroa/Primer de Mayo are typically urban, rural activities being exceptions. Students and housewives, the ma-

jority of this sample, deserve special attention, because they are in a position to cooperate with public health personnel in checking the spread of vector-transmitted Chagas disease.

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