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Infestation by triatomine bugs in indigenous communities of Valledupar, Colombia

ABSTRACT

OBJECTIVE: To calculate triatomine infestation indices in indigenous communities in Colombia.

METHODS: A descriptive study was carried out in 19 communities in Valledupar Municipality, Cesar Department, Colombia. During June to December, 2007, triatromine bugs were collected from their resting places in households. Taxonomic identification was made according to the keys by Lent & Wygodzinsky. An infection process in animal model and isozyme analysis of triatomine feces were performed.

RESULTS: *Rhodnius prolixus* showed a density index of 154.7%, for *Triatoma dimidiata* was 102.45%, *T. maculata* 109.25% and *Panstrogylus geniculatus* 0.3%. The mean infestation index was 40.54%, and mean *Trypanosoma* infection index was 9.4%. Of five hemocultures positive for *T. cruzi,* three were enzimatically identified as *T. cruzi* group I. Biopsies revealed few pathologic characteristics of infective process with these strains isolated from domiciliary triatomine bugs.

CONCLUSIONS: The high triatomine infestation indices in households and the *T. cruzi* infection index are evidence of active transmission of Chagas disease. The situation merits a vector control program and serological survey of the population at risk. The genetic characterization of *T. cruzi* strains as group I agrees with other findings on strains in this region of Colombia.

DESCRIPTORS: Indigenous Population. *Trypanosoma cruzi*, isolation & purification. Chagas Disease, prevention & control. Triatominae, domiciliary infestation.

INTRODUCTION

Chagas disease is a problem for rural populations living in substandard housing and poor socioeconomic conditions. Residents of these regions were not reached by vector control programs since 2007 and have not received therapeutic treatment due to particular cultural conditions.[2,4,23] At the end of the 1990s, the Program for Control of *Trypanosoma cruzi* Transmission and Child Cardiomyopathy in the main Endemic Areas of Colombia was initiated, including in Cesar and Guajira Departments. The goal was to determine priority indices for municipal attention.[a] Nonetheless, indigenous communities of Valledupar municipality (Cesar) were not incorporated in the program.

[a] Angulo VM, Tarazona Z, Sandoval CM, Reyes A, Romero R. Programa Nacional de prevención y control de la infección por *Trypanosoma cruzi,* agente causal de la enfermedad de Chagas y cardiopatía infantil, en las principales áreas endémicas de Colombia. Departamentos de Cesar y Guajira. Nodo Nor-oriental CINTROP-UIS. Informe final. Diciembre 2000.

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The communities of Arhuacos, Wiwas and Koguis that have traditionally inhabited the northeastern slope of the Sierra Nevada of Saint Martha (SNSM) build their houses with local resources, such as wood and mud for walls, palm and straw for the roof and earth for the floor. These materials deteriorate with time and create abundant crevices conducive to development of *T. cruzi* triatomine vectors. Therefore, the house becomes the most important risk factor for transmission of Chagas disease. Although this region was not included in the national Chagas program, local investigations have described the epidemiologic situation of the indigenous communities, in Gogtsezhi and Kemacumumake[18] with a report of 40% *T. cruzi* seroprevalence in 75 Amerindians, and Bunkwimake[7] reports 19% in 94 Amerindians. Both studies mentioned *Rhodnius prolixus, Triatoma dimidiata, T. maculata* and *Panastrongylus geniculatus* as triatomine species identified in houses.

Recently, cases of acute infections have been observed in residents of Seynimin, generating concern in the community over deaths that could eventually occur. Residents solicited medical care for patients and vector control measures from the authorities in health departments. This study sought to establish household entomological indices of communities in order to define the most suitable strategies for vector control and entomologic surveillance.

METHODS

The SNSM is located in the northeastern limits of Colombia, between 10 and 11 degrees north latitude and 72 and 74 degrees west longitude. It consists of a mountainous chain in pyramidal shape with a triangular base of 120 km, extending from the Caribbean plain at sea level to 5,775 m of altitude at Bolívar and Colón peaks. The total land area of 21,158 km^2 is divided by its slopes: the north slope, bordered by the plains of Guajira and the Caribbean Sea; the eastern slope delimited by the large alluvial plain of Magdalena river and the Ciénaga Grande de Santa Marta; the southwestern slope demarcated by the Racheria and Cesar rivers. It is has a special and unique geography in the world, with a high elevation, reaching a height of 5,700 m over a horizontal distance of 47 km. It has all the thermal zones and six biomes of Colombia: humid forests with hot, temperate and cold thermal zones; and páramo, superpáramo and nival plain, engendering a wide diversity of floral and faunal species, with various endemic species.[b] The indigenous populations studied reside in the rural area of Valledupar municipality: Donachui, Isrwa, Piñamake, Sabana de Crespo, Seynimin, Tamacal, Timaca and Jugaka, of Arhuaca ethnicity; Arwamake, Auyamal, Cherua, Dungakare and Surimena, of Wiwa ethnicity; Avingue, Pueblo Hernández, Nakalindua, Sabanas de Higueron, Sarachui and San José de Marwamake of Kogui ethnicity.

Fieldwork was performed between June and December of 2007, in 19 localities of the three indigenous groups in Valledupar municipality. The indigenous personnel of the Entomology Laboratory of the Cesar Department Secretary performed systematic captures in the 19 communities, excluding houses whose occupants were not present at time of collection. There were also non-systematic capture of triatomines in some houses by residents. Specimens were given to personnel who transported them to the Entomology Laboratory in Valledupar.

A portion of the collected entomologic material was labeled, packed and sent to the Entomology Laboratory of the National Institute of Health (INS) for confirmation of taxonomic identification, using the key by Lent & Wygodzinsky.[15] To detect infection, the *T. cruzi* parasite was isolated by examining fresh triatomine feces, in a 0.85% sterile saline solution under a light microscope with 40X magnification.[8]

Inbred ICR mice were raised in the INS animal facility and used as a biologic model for the development and characterization of the *T. cruzi* infective process. A volume of 0.3 ml of homogenized material obtained from each infected triatomine was inoculated in two mice by intraperitoneal injection. A total of 22 mice were exposed. Follow-up of the acute phase of parasitemia in mice was performed weekly for two months, by the crude drop method: a drop of blood, from the end of the tail, was examined under a light microscope with 40X magnification, analyzing 100 random fields to detect epimastigotes.

After two months, all blood was extracted (approximately 0.8 ml) by intracardial via and cultured under sterile conditions in Tobie media by triplicate. The cultures were maintained at 25°C for parasite development.

The eleven mice inoculated with homogenized trypomastigotes (*T. cruzi*) were sacrificed. Visceras (liver, heart, lower large intestine), brain, and skeletal muscle were extracted under sterile conditions in a laminar flow cabinet fixed in formaldehyde and processed in the Pathology Laboratory to be observed under a light microscope for analysis of histopathology.[19]

From the cultures in Tobie and LIT (liver infusion tryptose) media, three strains of *T. cruzi* were isolated and analyzed for isozymes by cellulose acetate electrophoresis, running five enzyme systems, MDH, GPI, GDH, ME and IDH, according to the method by Godfrey & Kilgour.[11]

[b] Instituto Geográfico Agustín Codazzi. Diccionario geográfico de Colombia. 3. ed. Bogotá: Horizonte Impresores; 1996. Tomo 4.

The population of Seynimin was selected for the serological screening. During 2006 and 2007 respectively, 244 and 28 blood samples were collected on filter paper, from patients probably infected. This sample was sent to the Parasitology Laboratory of the INS for confirmation of *T. cruzi* antibodies by the indirect immunofluorescence (IF) method.[3]

A database was created in Excel® for information storage. Only entomologic indices are considered as risk factors associated with *T. cruzi* transmission. Factors associated with the house were not analyzed due to logistic difficulties in their sampling. Results are presented in percentages and indices.

RESULTS

The percentage of houses sampled in each community varied from 12% to 100%. In total 1,431 houses were inspected: 394 of Kogui ethnicity, 179 of Wiwa ethnicity and 858 of Arhuaca ethnicity; of the total, 438 were infected by triatomines (43.7%). The triatomine species identified by the Entomology Laboratory were *R. prolixus, T. dimidiata, T. maculata,* and *P. geniculatus*. The entomological indices for the different populations are presented in Table 1, which shows *R. prolixus* as the predominant species with a density index of 154.7%, followed by *T. maculata* with 109.25% and *T. dimidiata* with 102.45%. Infestation by *P. geniculatus* was very low, and its density index was 0.3%. In the 19 localities inspected, the average triatomine infestation index was 40.5%, with a colonization index of 32.0%. The *T. cruzi* infection index in the sample sent to INS was 9.4%.

It was not possible to observe development of any parasitemia in the blood of mice inoculated with the infectious homogenized trypomastigotes. The mice behaved and appeared healthy while infected. Nonetheless, cultures inoculated with intracardial blood from five mice developed parasite epimastigotes: two mice infected with homogenized material of *R. prolixus,* from Sarachui (Kogui ethnicity); one mouse infected with homogenized material of *R.prolixus* from Isrwa (Arhuaca ethnicity); and two mice infected with homogenized material of *T. maculata,* from Sabanda de Crespo and Isrwa (Arhuaca ethnicity).

Of the five blood cultures that presented *T. cruzi* epimastigotes, three were classified by isoenzymes as *T. cruzi* group I, belonging to *R. prolixus* and *T. maculata* collected in homes of people in Isrwa and Sabana de Crespo (Arhuaca ethnicity).

Biopsies of mice inoculated with infectious homogenized *T. cruzi* trypomastigotes were analyzed by light microscope. All strains had similar histopathological behavior (Table 2). Four mice presented myositis and myocarditis, characteristic of the infective process. Among the 11 inoculated mice, only two presented intracellular *T. cruzi* amastigotes, from feces of *R. prolixus* collected in Sarachui locality (Kogui ethnicity); nonetheless, no mice presented a significant lesion in histopathological analysis.

In samples analyzed by IF from the population of Seynimin, positivity levels of 0.8% (2/244) and 28% (8/28) were obtained for 2006 and 2007, respectively.

DISCUSSION

The entomological indices for infestations (average 40.5%) and colonization (average 32.0%) demonstrate that infestation by triatomines is a widespread and important problem in 16 of the 19 indigenous locations inspected in Valledupar municipality. Although an evaluation of housing conditions was not performed, an association can be established between the perishable materials used for construction and the proximity to the ecosystems as factors that allow the development of dense triatomine populations. Recently, other high risk factors for triatomine infestation have been considered, such as the number of inhabitants per household in communities, storage density of household objects and the presence of barns and domestic animals,[5] but these were not evaluated in this study. In examining the association between housing, triatomine infestation and the transmission of *T. cruzi*, it is necessary to consider the cultural and religious meanings that the house has for indigenous communities of the SNSM. When designing an intervention and vector control program, any modification in type of construction material should be discussed with the community.

The triatomine species identified in this study are the same registered by other municipalities in Cesar.[a] The process for dispersion of *R. prolixus, T. maculata, T. dimidiata* and *P. geniculatus* probably has not faced barriers of biogeography and/or the type of culture among indigenous communities in the region.[17,18] *R. prolixus* was found in greatest density, followed by *T. maculata* and *T. dimidiata.* The two later species had a significant and important dispersion, considering the large proportion of houses inspected.

Parasitological diagnosis reveals a high number of triatomines infected by *T. cruzi* (infection index of 9.4%), especially *R. prolixius* and *T. maculata.* These findings confirm that the risk for Chagas disease is high, although a fraction of the total sample of triatomines was analyzed. In Seynimin community, the percentage of Chagas disease in children, corresponds to a point estimate for the indigenous population of SNSM, although children close the parasite transmission cycle and communities emphasize that Chagas disease begins at an early age in the Amerindians of these communities

Table 1. Entomologic indices of triatomines in populations of Arhuaca, Wiwa and Kogui ethnicities. Valledupar, Colombia, 2007.

Locality	Total houses	Date 2007	Houses sampled	Infestation Index (%)	Colonization Index(%)	Density indices by species[a]			Triatomines with *T. cruzi*[b] (%)
						Rhodnius prolixus (%)	*Triatoma maculata (%)*	*Triatoma dimidiata (%)*	
Arhuaca									
Yugaka	200	Sep	68	30,9	23,5	67,64	48.5	114.7	0,0
Donachui	400	Oct	82	45,1	37,8	130,5	6.1	91.5	0,0
Isrwa	80	Oct	64	64,1	51,6	70,3	220.3	26.6	8,8
Piñamake	140	Nov	102	13,7	10,8	2,0	112.0.	0,0	1,3
S. Crespo	450	Oct	358	15,6	13,4	2,0	106.5	0,0	1,0
Seynimin	360	Jun	134	57,5	47,0	176,9	37,30	188,0	8,2
Tamacal	70	Oct	18	44,4	33,3	44,45	133,3	16,7	0,0
Timaca	250	Ago	32	40,6	31,3	137,5	0,0	181,25	21,43
Kogui									
Avingue	300	Sep	125	48,8	32,8	324,0	0,0	0,0	2,7
P.Hernández	300	Dic	75	0,0	0,0	0,0	0,0	0,0	0,0
Nakalindua	53	Dic	45	0,0	0,0	0,0	0,0	0,0	0,0
S. Higueron	53	Dic	45	0,0	0,0	0,0	0,0	0,0	0,0
Sarachui	100	Oct	57	52,6	35,1	416,0	0,0	0,0	12,0
San José de Marwamake	65	Nov	47	6,4	6,4	130,5	0,0	91,5	30,0
Wiwa									
Arwamake	150	Nov	28	64,3	60,7	118,0	210,7	121,4	0,0
Auyamal	70	Nov	24	33,3	33,3	417,0	0,0	71,0	4,8
Surimena	30	Oct	30	66,7	53,3	413,3	0,0	110,0	3,6
Cherua	150	Oct	70	31,4	24,3	171,4	0,0	114,3	0,0
Dungakare	50	Sep	27	33,3	18,5	122,0	0,0	0	0,0
Total /average	3271		1431	40,5	32,0	154,7	109,3	102,45	9,4

[a] Indices calculated over the total triatomines captured
[b] Triatomines examined in the Parasitology Laboratory of the Colombian National Institute of Health

Table 2. Biochemical pathologic behavior of isolates from feces of domiciliary triatomines in indigenous populations. Valledupar, Colombia, 2007.

Isol N°	Locality, Ethnicity	Triatomine Species	Development in mice	Histopathology	Cultures	Isoenzymes
1	Sarachui, Kogui	*R.prolixus*	48 days	Sporadic evidence of intracellular Trypanosoma amastigotes, myositis, acute myocarditis type lymphomonocytosis.	YES scarce	NO
2	Sarachui, Kogui	*R.prolixus*	52 days	Evidence of intracellular Trypanosoma amastigotes with formation of pseudocytosis, myositis and acute myocarditis type lymphomonocytosis.	NO	NO
3	S. Crespo, Arhuaca	*T.maculata*	68 days	Acute myocarditis type lymphomonocytosis, amastigotes not recognized; cardiac muscle, liver, intestine without histopathologic changes, reactive hyperplasia in splenic white pulp.	YES	YES, Five enzymatic systems
4	Sarachui, Kogui	*R.prolixus*	60 days	Light lymphomonocytosis myositis, reactive hyperplasia in splenic white pulp, heart without histopathologic changes.	NO	NO
5	Isrwa. Arhuaca	*R.prolixus*	68 days	Acute myocarditis with moderate lymphomonocytosis, reactive hyperplasia of splenic white pulp, liver, heart and intestine without histopathologic changes. Amastigotes not recognized.	YES	YES
6	Sarachui, Kogui	*R.prolixus*	60 days	Heart, liver without histopathologic changes, low reactive hyperplasia. Amastigotes not recognized.	NO	NO
7	Isrwa, Arhuaca	*T.maculata*	55 days	Light acute myocarditis, lymphomonocytosis, reactive lymphoid hyperplasia in kidney and intestine segment. Liver without histopathological changes, amastigotes not recognized.	YES	YES
8	Dungakare, Wiwa	*R.prolixus*	97 days	Moderate acute myocarditis lymphomonocytosis, liver, heart, intestine, brain without histopathologic changes, amastigotes not recognized.	YES scarce	NO
9	Auyamal, Wiwa	*R.prolixus*	70 days	Acute myositis and myocarditis with light lymphomonocytosis, kidney, reactive lymphoid hyperplasia, brain, liver without histopathologic changes.	NO	NO
10	Surinema, Wiwa	*R.prolixus*	75 days	Acute myositis and myocarditie with light lymphomonocytosis, renal lymphoid reactive hyperplasia, liver, intestine without histopathologic changes, amastigotes not recognized.	NO	NO
11	Isrwa, Arhuaca	*R.prolixus*	48 days	Acute myositis lymphomonocytosis, heart, liver, intestine without histopathologic changes, amastigotes not recognized.	NO	NO

The parasitological test of feces and the histopathological characterization of biopsies from infected inbred ICR mice, together with the isoenzymatic analysis of blood cultures for parasites, produced similar results as obtained in experimental conditions of strains from a distinct origin.[20] The results allow for identification of the group and some characteristics of histopathological behavior of *T. cruzi* from the triatomines collected in these indigenous communities.

In regards to the distribution of *T. cruzi* strains, molecular characterization studies performed in Latin American countries have recognizes two well differentiated lineages. *T. cruzi* II is located in the south of the continent (Paraguay, Chile, Argentina and part of Brazil), with human isolates,[10,14,22] and *Triatoma infestans* as the main domiciliary vector.[22] In Central American countries and northern South America, the main vectors are *R. prolixus* and *T. dimidiata,*[22] and most isolates come from humans, corresponding to *T. cruzi* group I.[1,6,14] Considering this distribution, the three strains of *T. cruzi* from triatomines in Cesar Department coincide with group I *T. cruzi,* circulating in humans. These results agree with recent findings for strains isolated from diverse hosts in different regions of Colombia, indicating that this group of *T. cruzi* predominates in Colombia.[9,12,13,16,21]

The biological and parasitological behavior described in the post-inoculation phase, without apparent parasitemia in mouse periphery blood could be defined as another characteristic specific to strains belonging to *T. cruzi* I and confirm the findings of a previous study.[2]

On the other hand, histopathological results show myositis as a common lesion in mice inoculated with infectious homogenized *T. cruzi* and support the pathological behavior previously identified,[2,4,23] using mice as an animal model for Chagas disease.

Although these strains of *T. cruzi* group I do not produce parasitemia visible in peripheral blood, they do present a positive tropism in cardiac tissue of mice and produce light lesions in this organ that do not lead to host death. Therefore, they are classified as strains poorly adapted to the host, a situation previously observed by other investigators in Colombia.[c]

In considering the careful follow-up of parasite cultures, the *T. cruzi* clones that produced the histopathological traits are the same that grew in the culture media. Nonetheless, there is a possibility that post-inoculation clonal selection of *T. cruzi* may have occurred, a process described by Botero et al[4] as the result of the host immune system, in this case ICR mice.

In conclusion, infestation by triatomines infected with *T. cruzi* is a serious problem in the houses studied, impacting on transmission of Chagas disease as verified by some human cases. There is an urgent need for appropriate and concerted vector control strategies in these communities. Entomologic surveillance and serologic screening should be expanded to all 104 indigenous communities in SNSM in order to reach environmental and epidemiological coverage of the entire transmission foci.

T. cruzi group I, a parasite that infects triatomines in the houses of these Amerindians, coincides with the predominant group in Central America and northern South America, commensurate to the vector dispersion. This finding is striking considering the relative cultural and bio-ecological isolation of indigenous communities in SNSM.

Lastly, when selecting techniques for detection, multiplication and classification of parasites from vectors, it is reasonable to consider that some *T. cruzi* strains found in triatomine feces may not infect vertebrate hosts since the immunologic system exerts a selective action against certain lines.

[c] Vallejo G, Laboratory of Tropical Parasitology Investigations, University of Tolima, Colombia, personal communication.

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