Eco-Epidemiology of Chagas Disease in an Endemic Area of Colombia: Risk Factor Estimation, *Trypanosoma cruzi* Characterization and Identification of Blood-Meal Sources in Bugs

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Abstract. The Sierra Nevada de Santa Marta (SNSM) is a mountainous area in Colombia that is highly endemic to Chagas disease. We explored some eco-epidemiological attributes involved in the Chagas disease transmission scenario in three Indigenous communities. An epidemiological survey was done, where parasite infection in reservoirs and insects, *Trypanosoma cruzi* genotyping, identification of blood-meal sources in intradomiciliary insects using the high-resolution melting technique, and some risk factors were evaluated. The results suggest that several dwelling conditions such as thatched palm roofs and mud walls carried the highest risk of finding intradomiciliary *Rhodnius prolixus*, which 56.41% were infected with *T. cruzi* and fed with human blood. Moreover, *T. cruzi* Ia was the most frequent haplotype found in insects. These results indicate the existence of a domestic *T. cruzi* transmission cycle that does not overlap with the sylvatic cycle, and highlight the need for efficient entomological control focused to this area.

INTRODUCTION

Trypanosoma cruzi, aetiological agent of Chagas disease is a kinetoplastid parasite that circulates between vectors of the Triatominae subfamily and mammals. To understand the great variability of the parasite, six discrete typing units (DTU) has been described for *T. cruzi* (TcI to TcVI).[1] It has been postulated that the main DTU circulating in Colombia is TcI, with few reports of other DTUs.[2–5] Within TcI, a mini-exon-based classification was described where haplotype Ia is associated with domestic environments, whereas haplotypes Ib and Id are associated with peridomestic and sylvatic environments, respectively.[6,7]

Colombia is one of the most endemic countries to Chagas disease, with an estimated 700,000 people infected, representing 5% of the population living in endemic areas.[8] Indigenous communities are particularly at risk for infection as a result of their social, ecological, environmental, and cultural conditions, which are advantageous for the establishment of the insect vectors and the possible *T. cruzi* transmission.

The Sierra Nevada de Santa Marta (SNSM) is an isolated mountain mass, which rises abruptly from the tropical lowlands on the north coast of Colombia. Earlier studies showed in the North Slope infection rates of *T. cruzi* > 47% in ethnic groups inhabiting this region and an increase according to age (32% in individuals between 10 and 19 years of age, and 89% in individuals > 70).[9–12]

Although, two transmission cycles are commonly described to explain the *T. cruzi* transmission, in this particular zone an overlapping of domestic and sylvatic cycles occurs, resulting in a continuous flow of *T. cruzi* genotypes between vectors and animal populations.[11,13] Together, these studies suggest that the epidemiological scenario of Chagas disease in the SNSM area is very complex.

In this work, we studied the epidemiological situation on the southeast slope of SNSM, where the Wiwa indigenous community lives, and where a previous pilot study revealed a human seroprevalence of 33.5%.[14] In this sense, epidemiological and entomological surveys were carried out, with *T. cruzi* characterization and blood-meal source identification in bugs, with the aim to determine the transmission dynamics and several risk factors and vectors involved in this epidemiological scenario of Chagas disease in Colombia. The results indicate the existence of a domestic *T. cruzi* transmission cycle clearly differentiated from the sylvatic one, highlighting the need for efficient entomological control in this area and permanent surveillance of the sylvatic cycle.

MATERIAL AND METHODS

Study area. This study was conducted in the Marocazo River basin on the southeastern slope of SNSM located in San Juan del Cesar municipality, La Guajira department, Colombia, where the indigenous communities of Marocazo (10°59′09″ N and 73°06′59″ W), Sabana de Joaquina (10°58′08″ N and 73°09′39″ W), and Seminke (10°56′41″ N and 73°11′13″ W) are established (Figure 1). These communities have 407, 335, and 328 inhabitants, respectively, all of them belonging to the Wiwa ethnic group, who occupy disperse dwellings across a wide area in the Marocazo river basin. The Seminke community is inhabited by a traditional indigenous group, whereas the others have indigenous people with some degree of acculturation. The settlement pattern in the communities is dispersed into individual parcels (farms), with a congregation center composed with dwellings that usually are uninhabited.

Triatomine samples. An entomological survey was conducted during the dry seasons in three different time points of 9 days each during 2010 in March and August, and 2011 in July. The samplings were scheduled jointly with indigenous health authorities to visit the three communities each time. For extra-domiciliary captures, we used live bait traps with chickens.[15] The capture effort deployed 64 trap-nights: 24 in Marocazo, 16 in Sabana de Joaquina, and 24 in Seminke distributed in the three samplings for each community. The traps were left overnight in possible places of triatomine presence as caves, rock piles, and trees. In intra-domiciliary environments, we visited 28 houses from Marocazo, 17 from Sabana de Joaquina, and 28 from Seminke (73 total houses) and left plastic containers (one per house in each community) to be returned to us with vectors captured by the residents in the next 3 or 4 days after delivery. The triatomines collected were

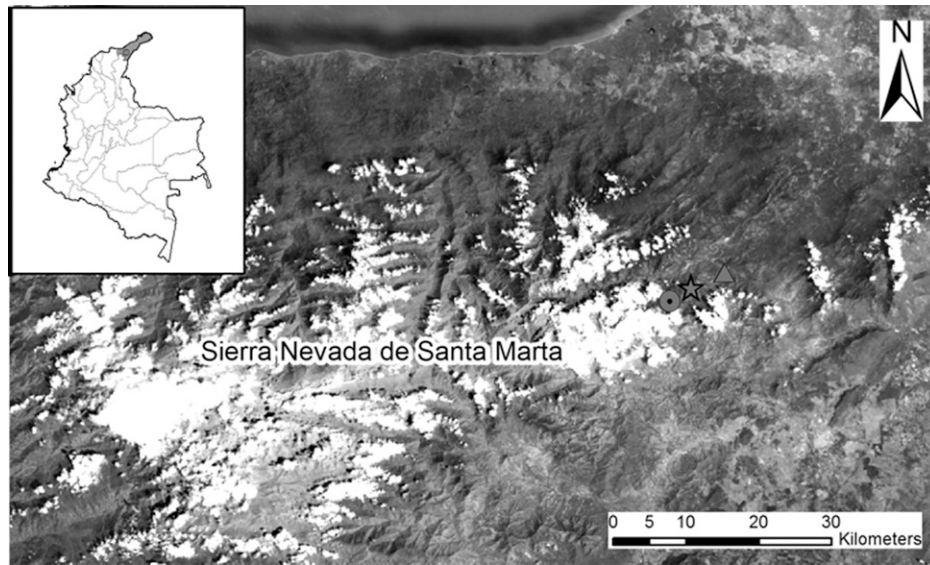* Address correspondence to Omar Triana-Chávez, Calle 70 52-21 Medellín 1226. E-mail: otriana@gmail.com

Figure 1. Location of the study area and distribution of communities The box points to the location of the Sierra Nevada de Santa Marta in Colombia where the gray area represents La Guajira department. Communities are indicated by figures as follows: Triangle: Marocazo, star: Sabana de Joaquina, circle: Seminke.

taken to the laboratory for analysis, where they were classified according to triatomine taxonomic keys.[16]

T. cruzi diagnosis. To detect *T. cruzi* infection in triatomines, bug feces were collected from live insects, diluted in 100 µL phosphate buffered saline (PBS) and 10 µL were examined for parasites under the microscope. The DNA was extracted from the remaining feces and analyzed by polymerase chain reaction (PCR).

To screen the prevalence of *T. cruzi* infection in mammals, we collected from domestic dogs and sylvatic mammals 3 mL to 5 mL of blood samples, depending on the size of the animal. Dogs were included in the study if the inhabitants of the house recognized them as their property. A portion of the collected blood (200 µL) was stored with an equal amount of guanidine chloride (6M-EDTA 0.2M, pH: 8.0) for molecular diagnosis by PCR. The remaining portion of blood was processed in hemocultures prepared with an NNN-medium combined with liver-infused tryptose (LIT) medium, supplemented with 20% fetal bovine serum. The culture was checked periodically over 3 months.

Sylvatic mammals were captured using Tomahawk live trap® (Hazelhurst, WI) of 26 × 9 × 9 in. The bait was composed of a mixture of banana, peanut butter, vanilla, oat, and fish. The traps were placed in wild areas surrounding houses by ~100 m in a sampling effort of 210 trap-nights. Captured animals were anesthetized (50 mg/kg body weight of ketamine, administered by intramuscular injection) and bled by cardiac puncture. Additionally, a xenodiagnosis was performed using 10 individuals of *Rhodnius prolixus* fifth instar nymphs from insectary colonies allowing them to feed on animals for 20 minutes. The collected blood was analyzed in hemocultures, as detailed previously and the insects were transported to the laboratory for molecular detection of *T. cruzi*.

Ethics approval (Act no. 53, 30/06/2009) for analyzing animal specimens was obtained from the animal ethics Committee of the University of Antioquia, Medellin, Colombia.

DNA extraction and amplification. The DNA was extracted from animal blood samples mixed with guanidine chloride 6M-EDTA 0.2 M solution and from triatomine feces samples

diluted in PBS. Extractions were performed with phenol-chloroform.[17] Purity and DNA concentration were measured using a Nanodrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Trypanosoma cruzi molecular detection was carried out by PCR amplification of the satellite region of 195 base pair (bp) using the primers TcZ1 and TcZ2.[18,19] The reaction was performed as described previously.[20] The amplification products were analyzed by electrophoresis on 1.5% agarose gel in 1 × TBE, stained by ethidium bromide and detected by UV light. The DNA of *T. cruzi* was used as the positive control and water instead of DNA with the other PCR reagents was used as the negative control.

T. cruzi genotyping. To identify *T. cruzi* I (TcI) or other DTUs in positive samples, a multiplex PCR on an intergenic spacer from a mini-exon gene was performed according to primers and conditions described by Souto and others.[21] Amplicons of 350 bp for TcI and a 300-bp band for TcII, TcV, or TcVI were expected.[22] The amplification products were analyzed as previously mentioned.

Samples genotyped as TcI were then examined to identify circulating haplotypes inside this DTU in the study area. The mini-exon region was amplified with primers described by Falla and co-workers[7] to distinguish between haplotype Ia, Ib, and Id, respectively, with amplification products of 228 pb, 250 pb, and 200 pb for each of them. The DNA from parasites of DTUs TcI and TcII and DNA of parasites belonging to each haplotype were used as the positive control for DTU and haplotypes identification, respectively; water instead of DNA jointly with a PCR mixture was used as the negative control.

Identification of blood-meal sources. To identify blood-meal sources in insect vectors, we carried out a cytochrome *B* gene high-resolution melting analysis from bug feces. Briefly, the *CytB* gene was amplified using the primers forward 5′-CCC CTC AGA ATG ATA TTT GTC CTC A-3′ and reverse 5′-CCA TCC AAC ATC TCA GCA TGA TGA AA-3′.[23] Reaction conditions and thermal profile were performed as described previously.[24] The standard species used in this study

were opossum (*Didelphis marsupialis*), mouse (*Mus musculus* L.), cow (*Bos taurus*), goat (*Capra aegagrus*), dog (*Canis lupus familiaris*), sheep (*Ovis aries*), horse (*Equus caballus*), pig (*Sus scrofa*), rabbit (*Oryctolagus cuniculus*), cat (*Felis catus*), rat (*Rattus norvegicus*), donkey (*Equus asinus*), human (*Homo sapiens* L.), and chicken (*Gallus gallus* L.). The species were selected by DNA availability, closeness to human environments, and ecoepidemiological importance.

Risk factors. Additionally, as another ecoepidemiologic approach we developed a cross-sectional study to identify the main risk factors associated with the presence of triatomines in human dwellings. With this objective in mind, the same 73 household heads that received the containers were surveyed based on the questionnaire designed for the National Program to Prevent and Control Chagas Disease and Children's Heart Disease in Colombia (PNPCECCI, according to its initials in Spanish),[25] with some modifications for sanitary services, public services, building materials, and the outdoor environment to adapt it to the communities' living conditions. The items included in the questionnaire were family name, knowledge of the vector species, vector sightings inside the house (where and when); the house's construction materials (walls and roof), peridomicile characteristics (i.e., pig pens or chicken coops near the house), pet ownership, and sightings of wild animals near the houses.

Unfortunately, there is no real census about the number of houses in the study area, some aspects as uninhabited houses and number of houses on the congregation center makes it difficult to estimate an approximate number. For this reason, the survey was conducted house by house including all inhabited houses according to information provided by indigenous health promoters, except for two or three houses not sampled because of their difficult access.

Statistical analyses. Taking into count that most of the data analyzed for this work are binary or categorical qualitative variables, the statistical analyses were performed in two stages. First, the data were analyzed for possible associations between dependent and independent variables. Second, a multivariate analysis was performed with the variables that were significant in the previous analysis.

To find relevant associations among different risk factors, the survey's results were analyzed through contingency tables with the χ^2 statistic; considering the positive report of vector sightings inside a dwelling and last sighting as dependent variables. The last vector sighting had five categories: < 1 day, < 3 days, < 1 week, < 1 month, and > 1 month. When contingency tables had one or more squares with a value < 5, a Fisher exact test was used. Additionally, the odds ratio (OR) and confidence interval (CI) were calculated, except when a square with a value of 0 was present. The multivariate analysis was done with a principal components analysis (PCA) of the most important variables according to the contingency tables and previous knowledge of eco-epidemiology and other issues concerning Chagas disease. Other associations were sought between risk factors and other variables such as blood-meal sources.

All calculations were done in the Statgraphics plus 5.1 statistics packages (Manugistics, Inc., Dallas, TX) and Epidat 3.1 (Pan American Health Organization, Washington, DC and the Consejería de Sanidad de la Junta de Galicia, La Coruña, Spain).

RESULTS

Capture attempts using bait traps were ineffective in the study area. Some attempts of manual search also were unsuccessful; possibly because the insects live inside the palm
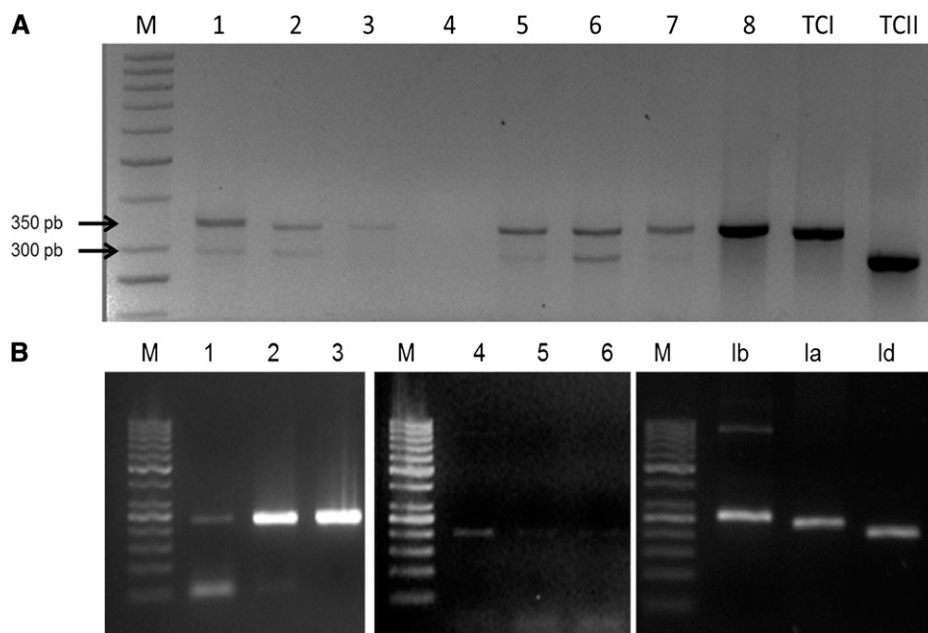

FIGURE 2. Genotyping of parasites from triatomine feces obtained in Sierra Nevada de Santa Marta (SNSM). (**A**) Mixed infection with discrete typing unit (DTU) TcI and TcII–TcVI, 2% agarose gel stained with ethidium bromide. Lanes 1, 2, 5, 6, and 7: insect samples showing mixed infection with DTU TcI and TcII–TcVI; Lanes 3 and 8: samples with TcI infection exclusively; TcI: positive control for TcI DTU; TcII: positive control for TcII DTU. (**B**) Identification of haplotypes of TcI in domestic vectors, 1.5% agarose gel stained with ethidium bromide. Lanes M: 50 pb ladder; 1, 2, and 3: Samples with presence of haplotype Ia; 4, 5, and 6: Samples with haplotype Id; Ib: Haplotype Ib control; Ia: Haplotype Ia control; Id: Haplotype Id control.

TABLE 1
Percentages of blood-meal sources of captured insects in the southeastern slope of SNSM*

	Single specie blood-meal source				Mixed species blood-meal source					Not determined	
	Human	Chicken	Rat	Dog	Human-rat	Chicken-rat	Chicken-UH	Dog-human	Human-UH	UH	Not amplified
All insects	53.03	9.09	0	1.52	4.54	1.52	3.03	1.52	1.52	3.03	21.21
Infected insects Only	48.72	10.26	0	0	7.69	2.56	2.56	0	0	5.13	23.08

*Values are given as percentages.
SNSM = Sierra Nevada de Santa Marta; UH = unidentified host.

roof where it is inaccessible to search manually. Therefore, the collection method was through plastic containers left for the people of the communities. Twenty houses returned the containers, which had a total of 84 bugs. Seven insects captured were identified as *Triatoma dimidiata*, four were captured inside homes (in different nymphs instar) and the other three, consider sylvatic, were collected in areas without human activity. One insect was classified as *Panstrongylus geniculatus* and the remaining vectors were *R. prolixus*.

Seventy-one insects were tested for *T. cruzi* infection, of which only seven were positive by microscopy and 43 (60.56%) were positive by PCR, all corresponding to the *R. prolixus* species but one sylvatic *T. dimidiata*. The 13 remaining insects could not be used for molecular analysis because they were delivered by inhabitants in advanced status of decaying.

Based on the insects collected, the Seminke community had the highest proportion of dwellings with infected insects with 86% of its houses positive, followed by Marocazo with 55%, and Sabana de Joaquina with 50%.

The most frequently reported wild mammals near houses according to the epidemiological surveys were opossums (63.01%), foxes (32.88%), agouti (8.22%), and oncillas (6.85%). However, only two mammals were captured, one *D. marsupialis* and one *Metachirus nudicaudatus*. Both were

positive for *T. cruzi* infection according to xenodiagnosis and they were classified as DTU TcI by PCR (data not shown). Out of 10 domestic dogs sampled, none was positive for *T. cruzi* by hemoculture or PCR.

Out of 43 *T. cruzi*-positive DNA samples extracted from triatomine feces, 27 (62.79%) amplified the 350-bp mini-exon gene, corresponding to DTU TcI. Eleven (25.58%) of the 27 also amplified a 300-bp band indicating mixed infection with other DTUs (Figure 2A). The remaining samples did not amplify, possibly because the DNA was not good enough for PCR because it was extracted from feces.

Moreover, 12 of the 27 *T. cruzi*-positive insect samples amplified the band corresponding to TcI haplotype Ia (Figure 2B). Five of those 12 samples also amplified the band corresponding to haplotype Id, two corresponding to Sabana de Joaquina, and three to Seminke (Figure 2B). None of the samples amplified the band from haplotype Ib. The remaining samples did not amplify. Neither the sylvatic sample nor the reservoir samples amplified in three different PCR reactions.

Sixty-six insects (61 *R. prolixus* and five *T. dimidiata*) from the three communities studied were assessed to identify blood-meal sources. Among them, 14 samples (21.21%) did not amplify and 5 (3.03%) individuals fed on unidentified host (Table 1). We found that humans, chickens, rats, and dogs
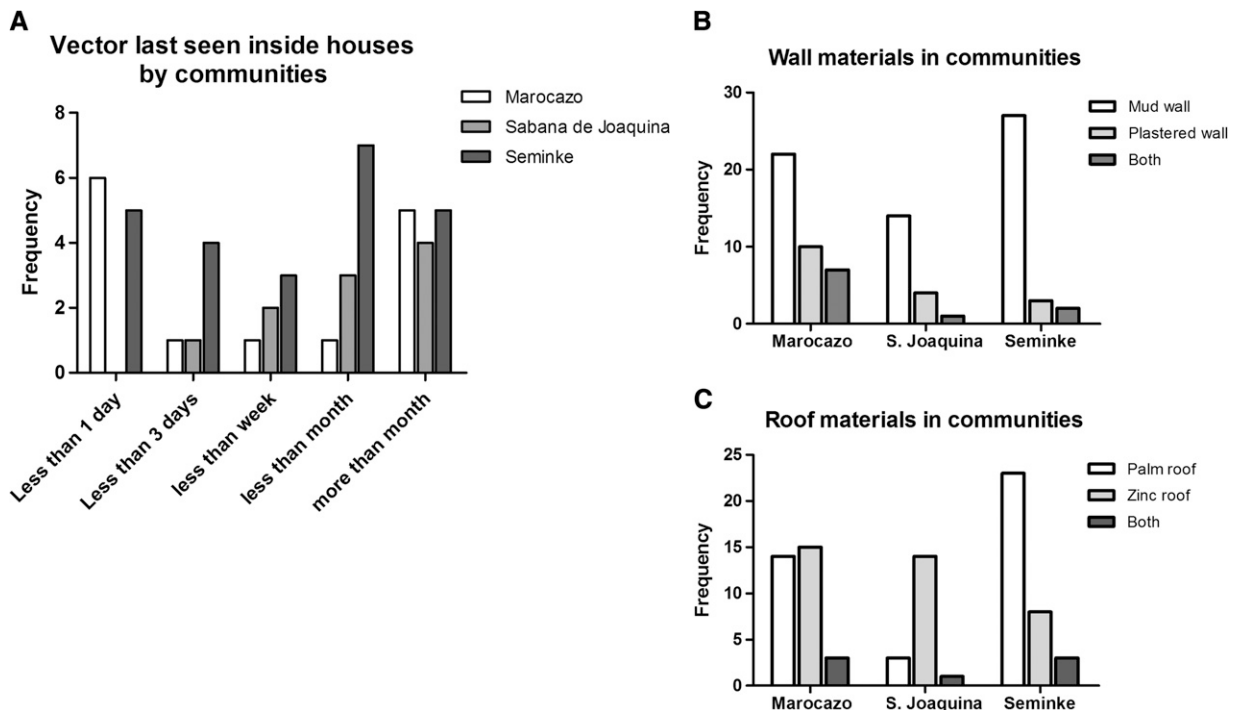

FIGURE 3. Epidemiological surveys carried out in the three communities of the study area (**A**) Most recent vector sighting inside houses with five categories distributed by community. (**B** and **C**) Frequency of houses with different wall types and roof types across the communities in the study area. S. Joaquina: Sabana de Joaquina community.

TABLE 2
P value, OR (odds ratio), and 95% confidence intervals according to contingence tables for risk factors*

	See vector	Less than 1 day	Less than 3 days	Less than 1 week	Less than 1 month	More than 1 month
Mud wall house	***0.0019***	0.5543	0.3084	0.3084	0.1516	0.9632
	OR: 8.1666	OR: 1.9038	–	–	–	OR: 1.0400
	CI:1.86–35.77	CI: 0.22–16.61				CI: 0.19–5.49
Plastered wall house	0.0633	0.3850	0.1549	0.1549	***0.0451***	0.6263
	OR: 0.3482	OR: 1.8071	–	–	–	OR: 1.3846
	CI: 0.11–1.08	CI: 0.47–6.96				CI: 0.37–5.15
Palm roof house	***0.0192***	***0.0298***	0.5419	0.5419	0.9856	0.4780
	OR: 3.4737	OR: 5.1667	OR: 1.7222	OR: 1.7222	OR: 0.9882	OR: 0.6629
	CI: 0.40–3.68	CI: 1.04–25.56	CI: 0.29–10.05	CI: 0.29–10.05	CI: 0.27–3.58	CI: 0.21–2.07
Pig pen	0.5631	0.1837	0.6404	0.1266	0.2067	0.2085
	OR: 0.6382	–	OR: 1.7143	OR: 5.0833	–	OR: 2.6500
	CI: 0.14–2.95		CI: 0.17–16.85	CI: 0.76–33.75		CI: 0.55–12.64
Chicken coop	0.6789	0.4225	0.5884	0.2394	0.4373	0.3577
	OR: 0.8067	OR: 0.5892	OR: 0.6167	OR: 2.7857	OR: 1.6615	OR: 0.5740
	CI: 0.29–2.23	CI: 0.16–2.16	CI: 0.10–3.60	CI: 0.48–16.28	CI: 0.45–6.03	CI: 0.17–1.89
Sighting oncillas	0.6913	0.2857	***0.0329***	0.5244	0.8644	0.2578
	OR: 1.5789	–	OR: 17.000	–	OR: 1.2222	–
	CI:0.16–15.30		CI:1.74–166.2		CI:0.12–12.25	
Sighting opossum	0.8219	0.3590	0.2897	0.8254	0.3590	0.1540
	OR: 0.8461	OR: 2.6757	–	OR: 0.7674	OR: 2.6756	OR: 0.3589
	CI: 0.19–3.63	CI: 0.04–3.28		CI: 0.07–8.11	CI: 0.04–3.28	CI: 0.08–1.52

*Only the most relevant variables are shown, with domestic, peridomestic, and sylvatic variables defined in the study as dependent. "Vector sightings inside the house" and the most recent vector sighting had five categories: < 1 day, < 3 days, < 1 week, < 1 month, and > 1 month. The significant variables are highlighted. P values are in the top portion of the boxes. Boxes without OR represent squares without a value.

OR = odds ratio; CI = confidence interval; – = no value.

were the blood-meal sources, with humans the most common (54.05%). However, 60 meals were found in 52 insects, indicating that eight insects showed mixed feeding sources between human and rat (three samples), chicken and rat (one sample), human and dog (one sample), chicken and an unidentified host (two samples), and human and an unidentified host (one sample) (Table 1).

Of the 66 insect samples assessed to identify blood-meal sources, 39 were infected with *T. cruzi*. The blood-meal sources found in infected insects were human, chicken, rat, and an unidentified host. Five (12.82%) of the 39 positive samples for *T. cruzi* infection showed feeding sources between human and rat (three samples), rat and chicken (one sample), and chicken and an unidentified host (one sample). None of the infected insect samples showed a blood meal from dogs (Table 1). In addition, samples from *T. dimidiata* captured inside a house were positive for a chicken blood meal.

Seventy-three household heads were surveyed across the three communities. All reported recognition of the vector species and identified it with the common name *pito*; the most frequently recognized species was *R. prolixus*. Out of the 73 household heads, 60.71% of those living in Marocazo (*N* = 28), 58.82% Sabana de Joaquina (*N* = 17), and 89.28% Seminke (*N* = 28) reported seeing the vector inside houses. Furthermore, as an additional estimation, the categorical variable "last vector sighting" with five categories was analyzed. The Seminke community reported frequent sightings with uniform frequencies through the time evaluated; Marocazo reported the highest frequencies in the "< 1 day" and "> 1 month" categories, whereas the Sabana de Joaquina community presented more frequent sightings in the category "> 1 month" (Figure 3A).

Among the variables analyzed for vector presence inside the house, the construction materials of the house, specifically mud walls and palm roofs, were the most statistically significant variables (*P* = 0.0019 and *P* = 0.0192, respectively). The presence of these materials increases the risk of vector

sightings inside the house 8.16 and 3.47 times, respectively (Table 2). Distribution of these materials in communities is presented in Figure 3B and C, where it is shown that the mud wall and the palm roof are more frequently distributed in Seminke, followed by Marocazo, and then Sabana de Joaquina, according to the sighting of vectors reported by the community in the surveys.

The PCA included the most important variables according to the contingency tables. Nine components were generated, of which the two plotted explain 48.97% of total variability (Table 3). Their respective equation values are shown in Table 4. Plotting the principal components shows that two of the principal variables that facilitate the presence of the vector inside the house, mud walls, and palm roofs, are more related to the "vector sightings inside the house" variable (Figure 4), as seen with first component analyses.

Finally, for *T. cruzi* genotypes and blood-meal sources, a significant relationship was found between rats and TcI (data not shown). Between blood-meal sources and the main risk factors, two significant associations were found between humans as blood-meal sources and houses with palm roofs

TABLE 3
Total components, self-value of each component, variance percentage explained by each component, and cumulative percentage of explained variability

Component number	Self-value	Variance percentage	Accumulative percentage
1	2.67761	29.751	29.751
2	1.72981	19.220	48.971
3	1.1891	13.212	62.184
4	1.08624	12.069	74.253
5	0.719503	7.994	82.247
6	0.615373	6.837	89.085
7	0.550759	6.120	95.204
8	0.273992	3.044	98.249
9	0.157609	1.751	100.000

TABLE 4
Values obtained with four components from the principal components analysis for each variable included in the analysis

	Component 1	Component 2	Component 3	Component 4
Vector seen inside house	0.309146	0.30195	0.267871	0.0844107
House with mud wall	0.369647	0.489091	−0.00907763	0.0730819
Brick house	−0.158481	−0.4207	−0.590579	0.101281
House with plastered wall	−0.318098	−0.228628	0.630712	0.0663809
House with palm roof	0.478512	−0.228863	0.0496285	0.254642
House with zinc roof	−0.499711	0.213601	0.170508	−0.214997
Wild animals seen	−0.186535	0.0826562	−0.0372327	0.817885
House with chicken coop	−0.321985	0.336373	−0.0713877	0.387716
House with pig pen	−0.155988	0.471565	−0.378896	−0.20626

(P = 0.0249), and between the unidentified host blood-meal source and houses with plastered walls (P = 0.0254) (Table 5).

DISCUSSION

In this study, we report a new epidemiological scenario in a hyperendemic area of Chagas disease where previous work on the northern slope of SNSM showed a complex scenario where sylvatic and domestic cycles are overlapping.[11] The finding of two separated different transmission scenarios within the same mountain area is an example of the importance of describing each transmission panorama, because each one requires different control strategies that are not often applicable everywhere.

The fact that more than a half of captured insects living inside dwellings were infected indicate that people in the area are in high risk of infection with *T. cruzi*. The main triatomine blood-meal source was human, which explains the high *T. cruzi* seroprevalence previously reported in the area.[14] Likewise, analysis of blood-meal sources revealed that almost half of the *T. cruzi*-infected *R. prolixus* collected in our study had fed on humans. This result, in addition to the fact that none of the insects in the study had fed on the opossum, a synanthropic species,[26] or extra-domiciliated dogs, supports the hypothesis that the domestic and sylvatic transmission cycles do not- or minimally overlap in the area, showing a different pattern from those observed on the northern slopes of SNSM.[11,12]

Additionally, 71.23% of households heads reported seeing the insects inside houses. Although such studies are rare, results from different countries showing similar results have used this methodology to help describe the impact of the disease in the region.[27–29] In Colombia, this is particularly important given that previous studies found 23.26% of surveyed

FIGURE 4. Principal components analysis of main risk factors of *Trypanosoma cruzi* transmission.

households reported having seen the vector, which gives an idea of the magnitude of the problem in the study area.

Some of the infected insects in this study had a mixed infection of DTU TcI and TcII–TcVI, which is rare in Colombia,[5] and although TcII circulates in Colombia in low proportions,[2–5,30] it had never been reported in this area. This result could be explained by the fact that we isolated *T. cruzi* DNA directly from the feces of triatomines having a higher representation of the clonal population from vector species, which is affected in the isolation process, as reported previously.[31]

According to a report by Falla and others[7] *T. cruzi* haplotype Ia is associated with the domestic cycle. However, five insects were found with mixed infection with Ia and Id haplotypes. This last haplotype has been associated with sylvatic environments, indicating the sporadic presence of sylvatic bugs inside houses or passive transport from other regions. However, previous works have discussed the risk of classification based only on the mini-exon region, therefore associations between haplotypes and transmission cycles may be artificial.[32,33] According to these works, novel methodologies should be designed to correlate parasite's groups with epidemiological features with more reliability; meanwhile, the mini-exon region is the only approach to do this.

The finding of vectors with mixed infection of DTUs may be intriguing, especially because we did not find conclusive evidence of overlapping between transmission cycles. However, inhabitants often travel to other regions of the SNSM where overlapping of cycles occurs frequently[11,13] and the sporadic presence of sylvatic bugs inside houses could contribute to this phenomenon of DTUs diversity.

The main risk factors for vector presence inside the house were mud walls and palm roofs. These building materials have been previously identified as risk factors for Chagas disease in Colombia and other countries[34–40] and might explain the proportions of houses with presence of vectors found in these communities because the number of houses constructed with these materials is higher in Seminke, followed by Marocazo, and finally by Sabana de Joaquina.

Although palm leaves used for roofs are a common construction material, a striking feature is the absence of palm trees in the area. However, residents reported that they were easily and frequently transported from other localities of the SNSM because these abound in other nearby areas. Interestingly, palm trees have been described as the natural ecotopes of triatomines of the genus *Rhodnius*[41] and are considered a risk factor for triatomine infestation of houses[42–44] and the unique wild habitat of *R. prolixus* found in Colombia.[45,46] Therefore, the infestation of dwellings and absence of palm trees in the study area suggest that insects were transported to
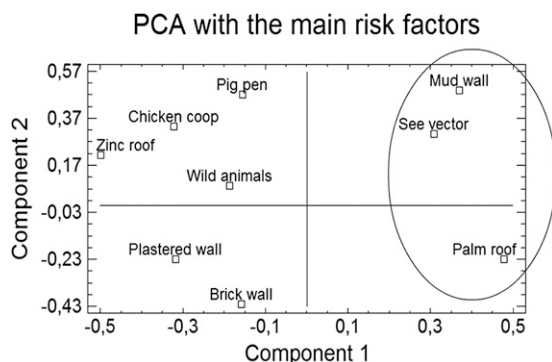
TABLE 5
Relationship between blood-meal sources and main risk factors, as found in a risk factor survey of houses*

	Mud wall	Palm roof	Plaster wall	Chicken coop
Human	0.6549	**0.0249**	0.3796	0.3255
	OR: 0.7727	OR: 0.2217	OR: 0.2973	OR: 1.9250
	CI: 0.08–7.66	CI: 0.05–0.87	CI: 0.02–5.15	CI: 0.52–7.18
Chicken	0.9023	0.0943	0.2293	0.5253
	OR: 0.8648	OR: 3.4091	OR: 0.2000	OR: 0.6261
	CI: 0.08–8.01	CI: 0.77–15.05	CI: 0.28–88.52	CI: 0.15–2.67
Rat	0.4870	0.7544	0.6704	0.2250
	–	OR: 0.6889	–	OR: 0.2564
		CI: 0.06–7.19		CI: 0.02–2.65
Unidentified host	0.2971	0.4211	**0.0254**	0.2250
	OR: 0.2857	OR: 2.2857	OR: 15.0000	OR: 0.2564
	CI: 0.02–3.43	CI: 0.29–17.90	CI: 0.74–303.74	CI: 0.02–2.65

*P value, OR (odds ratio), and 95% confidence interval values. P value on top of each square.
OR = odds ratio; CI = confidence interval; – = without OR because presents data without values in the contingency table.

the southern slope of SNSM from other areas of the SNSM and are restricted to domestic environments in the study area. Thus, they might be eradicated through chemical control, housing improvements, and adequate entomological surveillance in the area. This is particularly important because this species is the most important vector transmitting the parasite in the area, as confirmed by captures, infection percentage, and blood-meal source analysis.

Interestingly, four *T. dimidiata* individuals of different nymphs instar were feeding on chickens and captured in a *R. prolixus*-free house with mud walls and a zinc roof and, although the *R. prolixus* were found in palm-roofed houses, these were found to be feeding on humans and infected with *T. cruzi*. This result could indicate different blood-meal preferences and environmental characteristics in the two species, with *T. dimidiata* favored only by mud walls, whereas *R. prolixus* is promoted by the mud wall and the palm roof. This can explain the weaker relationship found by the palm roof in the PCA, because the mud wall supports the infestation of any triatomine species and the palm roof only supports the infestation by *R. prolixus*. Another possible explanation is that *T. dimidiata* is not established inside houses in this zone and comes from the extra-domiciliary environment.

These results lead us to suggest an epidemiological scenario on the Wiwa communities where the main risk factor for triatominae infestation is correlated with a palm roof, which is responsible for *R. prolixus* infestation, the main *T. cruzi* vector there. This insect can be infected with other genotypes than TcI, which could complicate the epidemiological scenario regarding pathogenesis, clinical manifestations, and treatment of the disease. The distribution of risk factors in the communities suggests increased risk of *T. cruzi* transmission in the Seminke community, followed by Marocazo and Sabana de Joaquina. It should be noted that although remaining respectful of traditions and laws of Wiwa ethnic communities, which do not allow taking samples from patients for spiritual reasons, it was possible to carry out a study on Chagas disease without taking a single human blood sample that yielded sufficient conclusive evidence to define the factors associated with vector presence in the inhabitants' houses, vector incrimination, and the dynamics of transmission. However, we cannot exclude the hypothesis that other infection routes may provide part of the seroprevalence found there, such as oral transmission because some animals such as opossum are often consumed. Moreover, it is important to highlight the importance of using techniques such as high-resolution melting analysis of cytochrome *B* to study blood-meal sources to describe the epidemiologic panorama in an area because it describes vector–host contact with high accuracy.

Finally, the results suggest the existence of a domestic *T. cruzi* transmission cycle that is not or minimally overlapping with a wild cycle of transmission. This is supported by the following data: a high number of *R. prolixus* insects positive to *T. cruzi* captured inside houses, high seroprevalence in humans found in a previous study,[14] a high rate of bugs fed with human blood, no infection in dogs or infected insects fed on dog blood, and no presence of palm trees, the natural ecotope of *R. prolixus*. Additionally, it is possible that an extra-domestic cycle exists, with *T. dimidiata* as the main vector that feeds mainly on chickens and thus is not infected with the parasites. However, the presence of Tc Id genotypes could indicate that some insects from the wild environment enter houses, but is not enough evidence to suggest cycle overlapping. Thus, this work provides sufficient knowledge to the community and to health authorities to implement vector control strategies and prevent transmission of the parasite.

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