VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 22, Number 9, 2022
Mary Ann Liebert, Inc.
DOI: 10.1089/vbz.2021.0089

ORIGINAL ARTICLES

Evaluation of the Effectiveness of Chemical Control for Chagas Disease Vectors in Loja Province, Ecuador

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Abstract

The objective of this study was to evaluate the effectiveness of selective and community-wide house insecticide spraying in controlling triatomines in the subtropical areas of Loja Province, Ecuador. We designed a quasi-experimental pre–post-test without a control group to compare entomological levels before and after spraying. The baseline study was conducted in 2008. Second, third, and fourth visits were conducted in 2010, 2011, and 2012 in three rural communities. Out of the 130 domestic units (DU) visited, 41 domestic units were examined in each of the four visits. Selective and community-wide insecticide interventions included spraying with 5% deltamethrin at 25 mg/m^2 active ingredient. At each visit, a questionnaire was administered to identify the characteristics of households, and DUs were searched for triatomine bugs. In addition, parasitological analysis was carried out in life triatomines. One and two rounds of selective insecticide spraying decreased the probability of infestation by 62% (pairwise odds ratios [POR] 0.38, 95% confidence interval [CI] 0.17–0.89, $p=0.024$) and 51% (POR 0.49, 95% CI 0.23–1.01, $p=0.054$), respectively. A similar effect was observed after one round of community-wide insecticide application in Chaquizhca and Guara (POR 0.55, CI 0.24–1.25, $p=0.155$) and Bellamaria (POR 0.62, CI 0.22–1.79, $p=0.379$); however, it was not statistically significant. *Trypanosoma cruzi* infection in triatomines ($n=483$) increased overtime, from 2008 (42.9% and 8.5% for *Rhodnius ecuadoriensis* and *Panstrongylus chinai*, respectively) to 2012 (79.5% and 100%). Neither of the two spraying methodologies was effective for triatomine control in this area and our results point to a high likelihood of reinfestation after insecticide application. This underscores the importance of the implementation of physical barriers that prevent invasion and colonization of triatomines in households, such as home improvement initiatives, accompanied by a concerted effort to address the underlying socioeconomic issues that keep this population at risk of developing Chagas disease.

Keywords: Chagas disease, *Trypanosoma cruzi*, triatomine, control, insecticide, Ecuador

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Introduction

Chagas disease, or American trypanosomiasis, is caused by an infection with the protozoan hemoflagellate *Trypanosoma cruzi* (Chagas, 1909) and transmitted by blood-sucking insect vectors of the subfamily Triatominae (Jeannel, 1919). Currently, 16 species of Triatominae have been reported in Ecuador, distributed in 20 of the 24 provinces (Abad-Franch et al, 2001). Chagas disease is endemic in Loja Province (Black et al, 2007, Grijalva et al, 2005), located in the southern Andean region, where widespread triatomine household infestations were recently reported in 68% of 92 rural communities examined (Grijalva et al, 2015).

This study found an 80% colonization rate indicating a high bug adaptation to domestic and peridomestic environments. *Rhodnius ecuadoriensis* (Lent and León, 1958) and *Triatoma carrioni* (Larrousse, 1926) were the most common species. However, this study also found well-established colonies of *Panstrongylus chinai* (Del Ponte, 1929) and *Panstronylus rufotuberculatus* (Champion, 1899) in the domicile.

Additional studies conducted in this area have demonstrated that *R. ecuadoriensis* is present in wild habitats throughout the province (Grijalva et al, 2012), associated with the nests of the white-naped squirrel *Simosciurus nebouxii,* previously lumped with the Guayaquil squirrel *Sciurus stramineus*. Analyses indicate that the abundance of the wild *R. ecuadoriensis* correlates with the abundance of domestic/peridomestic infestation (Grijalva et al, 2015; Grijalva et al, 2012). In addition, previous results suggested that sylvatic and domicile/peridomicile populations of *R. ecuadoriensis* shared phenotypic characteristics suggesting a continuous flow of individuals between both environments (Villacis et al, 2010).

Current vector control recommendations include periodic vector surveillance, followed by deltamethrin spraying of domestic and peridomestic structures of infested domestic units (Grijalva et al, 2005, Schofield, 2001, WHO, 2017; WHO, 2002). However, some studies have also demonstrated a variable effectiveness of long-term spraying strategy (Hashimoto et al, 2006, Yoshioka et al, 2015). The evolution of insecticide resistance (or tolerance) in triatomines is a process that negatively affects the progress in the control of these vectors. Triatominae resistance to pyrethroids has been reported in *Rhodnius prolixus* in Venezuela and in *Triatoma infestans* in Brazil, Argentina, and Bolivia (Alarico et al, 2010, Lardeux et al, 2010, Picollo et al, 2005, Roca Acevedo et al, 2011, Vassena et al, 2000).

Added to this important problem of resistance, the reinfestation of insecticide-treated households by wild vectors is common. Insecticide spraying was shown to have only short-term effectiveness in the coastal region of Ecuador, likely due to the ubiquitous presence of wild triatomine populations that readily recolonize domestic and peridomestic structures soon after the residual effect of the insecticide subsides (Grijalva et al, 2011). Because of the presence of wild triatomine populations in southern Ecuador, it is extremely important to know triatomine response to insecticides.

This study aims to evaluate the effectiveness of selective and community-wide house insecticide spraying in controlling triatomines in an area with wild triatomine populations. This approach could help to identify the need to implement alternative control methods in this area.

Materials and Methods

Study area

This study was conducted in Loja province, which is in the south of the Ecuadorian Andes and shares a border with Peru. The temperature varies from 12°C to 24°C (INHAMI, 2013). Study locations included three rural communities on Calvas county: Chaquizhca (888–1323 meters above sea level [masl], $n = 54$ domestic units [DU]), Guara (1064–1450 masl, $n = 38$ DU), and Bellamaria (1000–1384 masl, $n = 38$ DU) (Supplementary Fig. S1).

Study design

To measure the effectiveness of insecticide spraying, we designed a quasi-experimental pre–post test without a control group to compare entomological levels before and after spraying. The initial visit for household survey and triatomine collection were conducted in 2008 to establish the baseline for the study. Second, third, and fourth visits were conducted in 2010, 2011, and 2012 in Guara and Chaquizhca communities, whereas Bellamaria was only surveyed in 2011 and 2012. Two intervention strategies were applied immediately after the triatomine collections and were evaluated: (1) selective insecticide application: only infested DUs at the baseline and 2010 and (2) community-wide insecticide application in all searched houses in 2011 and 2012. We visited all existing DUs in each community and the households were included in the study if their inhabitants agreed to participate. The exclusion criteria were those households inhabited or closed.

Household survey

Each household was georeferenced and given an individual code as previously described (Grijalva et al, 2015). At each visit, a questionnaire was administered to the head of the household to obtain information about construction materials, crowding, sewage, domestic animals, and self-conducted and vector control program insecticide spraying.

Triatomine collection

Domiciles and peridomiciles were searched for triatomine using the one-man-hour method as previously described (Grijalva et al, 2005) and conducted by two-person skilled teams (30 min in domestic habitats and the 30 min in peridomestic habitats) from the national or provincial vector control programs under the supervision of project personnel. Collected triatomines were placed in individually labeled plastic containers and transported to either the field laboratory or the insectary of the Center for Research on Health in Latin America (CISeAL).

Details of place of capture (intra- or peridomicile and microhabitat), species, number of insects found dead or alive, and insects' developmental stages and sex (of adults) were noted by each team in the field and corroborated by trained entomologists at the field laboratory (Carcavallo et al, 1998). Species identification was performed using a dichotomous key (Lent and Wygodzinsky, 1979). Triatomines were collected under Ecuadorian collection permit number 002-07 IC-FAU-DNBAPVS/MA, number 006-IC-FLO-DTL-MA, number 016-07 IC-FAU-DNBAPVS/MA, and number 008-IC-INSEC-DTL-MA.

Insecticide application

The selective and community-wide interventions included the spraying of all environments (domicile and peridomicile) of infested and searched dwellings, respectively, as described by Schofield (2001). In brief, kitchenware, food, bedding, clothes, and personal items were removed from domiciles before complete indoor and outdoor spraying of all surfaces of the dwellings and peridomestic structures (<100 meters). Spraying was done with 5% deltamethrin wettable power at a target application dose of 25 mg/m^2 by trained personnel from the former National Chagas Control program using Hudson X-pert sprayers (H.D. Hudson Manufacturing Co., Houston, TX). At the end of the day, each team stored the remaining insecticide and the water used to wash the spraying equipment in a plastic container for use the following day (Schofield, 2001).

Natural infection with trypanosomes

The parasitological analysis was carried out in 426 life triatomines, representing >40% of the total collected in each visit. Triatomines were randomly selected, trying to include samples from each collection point. Triatomines were washed in White's solution (HgCl 0.8 mM, NaCl 111 mM, HCl 0.125%, and 25% v/v of ethanol 95%) before being dissected under a stereo microscope. Feces and intestinal content were mixed with 200 μL of sterile phosphate buffered saline. All samples were tested for the presence of trypanosomatids by microscopy (Grijalva et al, 2012) and differential detection of *T. cruzi* and *Trypanosoma rangeli* (Tejera, 1920) was carried out by PCR amplification of kinetoplast DNA using the S35/S36 primer set (Vallejo et al, 1999) and the D7a divergent domain of the large subunit of the ribosomal RNA gene (D75/D76) (Souto et al, 1999).

Each amplification set was run with a control for *T. cruzi*, *T. rangeli*, and negative. Results were considered valid if control samples amplified the expected bands. The infection index (100 × number of infected individuals/total number of analyzed individuals) was calculated based on PCR results for each type of microhabitat.

Data analysis

We assessed the effectiveness of insecticide spraying by comparing the entomological data obtained before and after each round of spraying. We calculated the infestation indices by species and habitat for every time point (2008, 2010, 2011, and 2012). A DU was considered infested when at least one live triatomine nymph or adult was found and the following entomological indices were calculated: Infestation rate (100 × number of DU infested/number of DU searched), density (number of triatomines captured/number of DU searched), crowding (number of triatomines captured/number of DU infested), and colonization index (100 × number of DU with nymphs/number of DU infested) (WHO, 2002).

As a measure of insecticide spraying effectiveness at the house level, the percentages of DU infested and sprayed at time t that were again found infested at time $t+1$ (apparently persistently infested) were calculated for each insecticide spraying round. We use generalized estimating equations to evaluate the spraying strategy on the probability of infestation throughout the study period. Pairwise odds ratios (PORs) and 95% confidence interval (CI) were the effect measure that allowed us to assess whether DUs infected at a given time are more likely to be infested at latter assessments. Analysis was performed using STATA v.11.0.

Ethics approval

The protocol was approved by the institutional review boards of Ohio University (Protocol 01X021) and Pontifical Catholic University of Ecuador (Reference No. 5-11-2008). Written informed consent to participate in this study was obtained from the head of each household.

Results

Study population

Out of the 130 DU visited during the period 2008–2012, 120 (92.3%) were examined at least once and were included in this study. Of 73 DU visited in 2008, 20 (27.4%) were not searched (closed = 15.1%, uninhabited = 11.0% or declined to participate in the study = 1.4%). Some houses were vacated between consecutive surveys (1–8%), whereas new houses searched represented 4–7%. In addition, some households refused searches for triatomines through the follow-up (0–11%). Characteristics of households are described in Supplementary Tables S1 and S2.

Infestation indices at baseline

Out of 53 DUs searched at baseline in Guara and Chaquizhca communities, 22 (41.5%) were infested. *R. ecuadoriensis* and *P. chinai* bugs were found mainly in the domicile rather than in the peridomicile (Table 1). In the peridomestic habitats, *R. ecuadoriensis* was associated with chicken nests (100%) and *P. chinai* with bricks (65.2%), chicken nests (26.1%), and duck nests (8.7%). The overall density of triatomines was 12.4 per searched DU; the triatomine crowding was 29.8 bugs per infested DU and the colonization index was 68.2% (Table 1). In the Bellamaria community (2011), 6 (17.1%) out of 35 searched houses were infested. *R. ecuadoriensis* was mainly found in the peridomicile associated with chicken nests (100%), whereas *P. chinai* were found in the bedroom inside houses (93.7%).

Infestation indices postspray

In 2010, 53 DUs were searched and 11 (20.8%) were infested in the Chaquizhca and Guara communities. In 2011, a total of 101 DUs were searched, and the prevalence of house infestation was 23.8%. In the last evaluation (2012), triatomines were found in 15.3% of 111 DUs examined (Fig. 1 and Table 1). Of the 41 DUs examined four times, a total 47% reduction in infestation index was observed through the period 2008–2012 (Table 2). The triatomines density decreased by 51.6%, whereas crowding and colonization decreased by 11%.

Of the 31 DUs examined twice in the Bellamaria community (2011–2012), a total 17% reduction in infestation was observed after one round of community-wide intervention. In addition, triatomine density, crowding, and colonization decreased >50%. In 2012, *R. ecuadoriensis* was only found in the peridomestic habitat, whereas *P. chinai* was mainly found inside houses (Table 3).

One and two rounds of selective insecticide spraying decreased the probability of infestation by 62% (POR 0.38, 95%

TABLE 1. ENTOMOLOGICAL AND PARASITOLOGICAL INDICES, IN DOMICILE AND PERIDOMICILE HABITATS, OF THE SPECIES OF TRIATOMINES FOUND IN DOMESTIC UNITS OF GUARA, CHAQUIZHCA DURING THE YEARS 2008–2012 AND BELLAMARIA DURING 2011–2012

Year	*2008*			*2010*			*2011*			*2012*		
Houses surveyed	n = *53*			n = *53*			n = *101*			n = *111*		
Habitat	*D*	*P*	*Total*	*D*	*P*	*Total*	*D*	*P*	*Total*	*D*	*P*	*Total*
Rhodnius ecuadoriensis												
Infestation index (%)	28.3	20.0	28.3	9.4	11.3	17.0	11.9	9.9	19.8	3.6	8.1	9.9
Density	6.0	16.3	10.6	0.4	1.2	1.6	0.5	3.2	3.8	0.2	0.8	1.0
Crowding	21.3	81.3	37.5	4.4	10.5	9.4	4.3	32.8	19.0	5.8	10.1	10.4
Colonization index	60.0	100.0	60.0	100.0	66.7	100.0	91.7	90.0	90.0	25.0	88.9	81.8
Parasite analysis (*n*)	33	2	35	11	18	29	19	143	162	16	67	83
Trypanosoma cruzi (%)	45.5	0.0	42.9	81.8	16.7	41.4	73.7[a]	85.3[a]	84.0	87.5	77.6	79.5
Trupanosoma rangeli (%)	3.0	0.0	2.9	0.0	11.1	6.9	10.5[a]	21.7[a]	20.4	6.3	3	3.6
Panstrongylus chinai												
Infestation index (%)	20.8	5.7	24.5	7.5	1.9	9.4	9.9	1.0	9.9	3.6	3.6	7.2
Density	1.3	0.4	1.8	0.2	0.0	0.2	0.8	0.0	0.8	0.1	0.1	0.2
Crowding	6.4	7.7	7.2	2.0	2.0	2.0	7.6	1.0	7.7	4.0	1.5	2.8
Colonization index	81.8	66.7	76.9	75.0	0.0	60.0	70.0	100.0	70.0	50.0	25.0	37.5
Parasite analysis (n)	33	14	47	8	2	10	53	3	56	1	3	4
T. cruzi (%)	9.1	7.1	8.5	50.0	0.0	40.0	69.8	100.0	71.4	100.0	100.0	100.0
T. rangeli (%)	0.0	7.1	2.1	0.0	0.0	0.0	1.9	0.0	1.8	0.0	0.0	0.0
Total												
Infestation index (%)	37.7	11.3	41.5	17.0	13.2	20.8	17.8	9.9	23.8	6.3	10.8	15.3
Density	7.3	5.0	12.4	0.6	1.2	1.8	1.3	3.3	4.5	0.4	0.9	1.2
Crowding	19.5	44.5	29.8	3.3	9.3	8.6	7.1	32.9	19.0	5.6	8.1	8.0
Colonization index	90.0	83.3	68.2	88.9	57.1	90.9	94.4	90.0	95.8	42.9	66.7	64.7
Parasite analysis (n)	66	16	82	19	20	39	72	146	218	17	70	87
T. cruzi (%)	27.3	6.3	23.2	68.4	15.0	41.0	70.8	85.6	80.7	88.2	78.6	80.5
T. rangeli (%)	1.5	6.3	2.4	0.0	10.0	5.1	4.2	21.2	15.6	5.9	2.9	3.4

[a] Include mixed infections.
D, domicile; P, peridomicile.

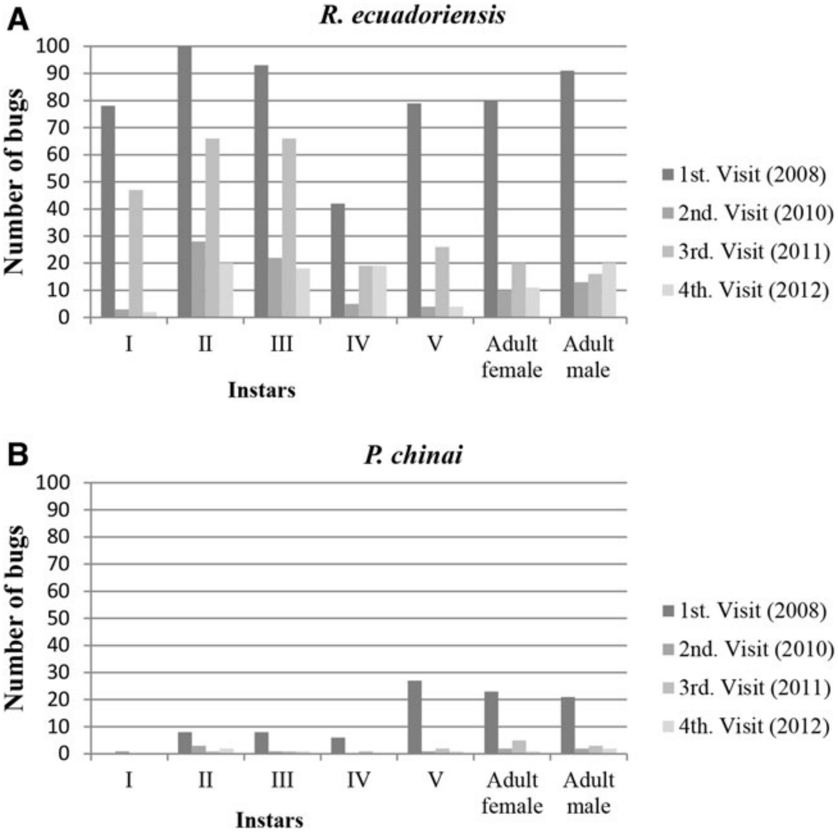

FIG. 1. Population structure of triatomines collected in Chaquizhca and Guara communities of Loja Province, Ecuador at baseline and during the follow-up visits. Number of live nymphs I–V and adult female and male bugs collected in domestic units. **(A)** *Rhodnius ecuadoriensis*, **(B)** *Panstrongylus chinai*.

TABLE 2. ENTOMOLOGICAL INDICES OF TRIATOMINE INFESTATION BY SPECIES IN CHAQUIZHCA AND GUARA COMMUNITIES OF LOJA PROVINCE, 2008–2012

	Rhodnius ecuadoriensis				Panstrongylus chinai				*Both triatomines*			
Entomological indices	*Baseline (2008)*	*Second visit (2010)*	*Third visit (2011)*	*Fourth visit (2012)*	*Baseline (2008)*	*Second visit (2010)*	*Third visit (2011)*	*Fourth visit (2012)*	*Baseline (2008)*	*Second visit (2010)*	*Third visit (2011)*	*Fourth visit (2012)*
Houses infested	12	7	9	6	9	5	5	5	17	9	11	9
Live triatomines captured	65	74	33	54	64	10	11	7	129	84	44	61
Number of DUs with nymphs	7	7	8	5	7	3	3	2	12	8	10	6
Infestation index (%)	29.3	17.1	22.0	14.6	22.0	12.2	12.2	12.2	41.5	22.0	26.8	22.0
Density index	1.6	1.8	0.8	1.3	1.6	0.2	0.3	0.2	3.1	2.0	1.1	1.5
Crowding index	5.4	10.6	3.7	9.0	7.1	2.0	2.2	1.4	7.6	9.3	4.0	6.8
Colonization index (%)	58.3	100.0	88.9	83.3	77.8	60.0	60.0	40.0	70.6	88.9	90.9	66.7

Includes only 41 domestic units that were examined in each of four visits. A selective spray intervention was applied in 2008 and 2010. In 2011, a community-wide insecticide intervention was applied. DU, domestic units.

CI 0.17–0.89, $p = 0.024$) and 51% (POR 0.49, 95% CI 0.23–1.01, $p = 0.054$), respectively. A similar effect was observed after one round of community-wide insecticide application in Chaquizhca and Guara (POR 0.55, CI 0.24–1.25, $p = 0.155$) and Bellamaria (POR 0.62, CI 0.22–1.79, $p = 0.379$); however, it was not statistically significant.

Population structure

During the initial 2008 visit to the Chaquizhca and Guara communities, more *R. ecuadoriensis* nymphs II and III and male adults were found (Fig. 1A). After one round of selective insecticide application, the number of nymphs and adults was reduced. In 2011, after two rounds of selective spray, the number of nymphs and adults increased compared with 2010; however, these numbers did not reach the levels found at the baseline. Finally, after one round of community-wide spray (2012), the proportion of nymphs I–III, V, and female adults decreased in relation to the previous evaluation. For *P. chinai*, a predominance of nymphs V and adults (male and females) were found at baseline. These proportions of all nymphs and adults decreased through the follow-up (Fig. 1B).

The Bellamaria community at baseline (2011) showed more *R. ecuadoriensis* nymph IV and V and adults (female and male) (Fig. 2A). After one round of community-wide spray (2012), no nymphs III–V and few female and male adults were found. For *P. chinai,* we found a predominance of nymph V and no nymph I or female adults were found (Fig. 2B). The proportions for all nymphs declined in the next evaluation, but the number of male and female adults slightly increased.

New and persistent infestations

Of the 17 infested DUs at baseline in Chaquizhca and Guara communities, 3 (17.6%) were reinfested after one round of selective insecticide spraying. After two rounds of selective spraying, 5 (29.4%) DUs were reinfested (Fig. 3A and B). In 2012, after a round of community-wide insecticide spraying, infestation persisted in four (23.5%) DUs (Fig. 3C). In Bellamaria community, which only experienced one community-wide insecticide spray, the persistent infestation was detected in one (16.7%) of six infested DUs. Four DUs (16.0%) where no bugs were found initially were subsequently found to be infested despite community-wide spraying (Table 3).

TABLE 3. ENTOMOLOGICAL INDICES OF TRIATOMINE INFESTATION BY SPECIES IN BELLAMARIA COMMUNITY OF LOJA PROVINCE, 2011–2012[a]

	Rhodnius ecuadoriensis		Panstrongylus chinai		*Both triatomines*	
Entomological indices	*Baseline (2011)*	*Second visit (2012)*	*Baseline (2011)*	*Second visit (2012)*	*Baseline (2011)*	*Second visit (2012)*
Houses infested	4	2	3	3	6	5
Live triatomines captured	120	20	64	15	184	35
Number of DUs with nymphs	3	1	3	1	5	2
Infestation index (%)	12.9	6.5	9.7	9.7	19.4	16.1
Density index	3.9	0.6	2.1	0.5	5.9	1.1
Crowding index	30.0	10.0	21.3	5.0	30.7	7.0
Colonization index (%)	75.0	50.0	100.0	33.3	83.3	40.0

In 2011, a community-wide insecticide intervention was applied.
[a] Includes only 31 domestic units that were examined in each two visits.

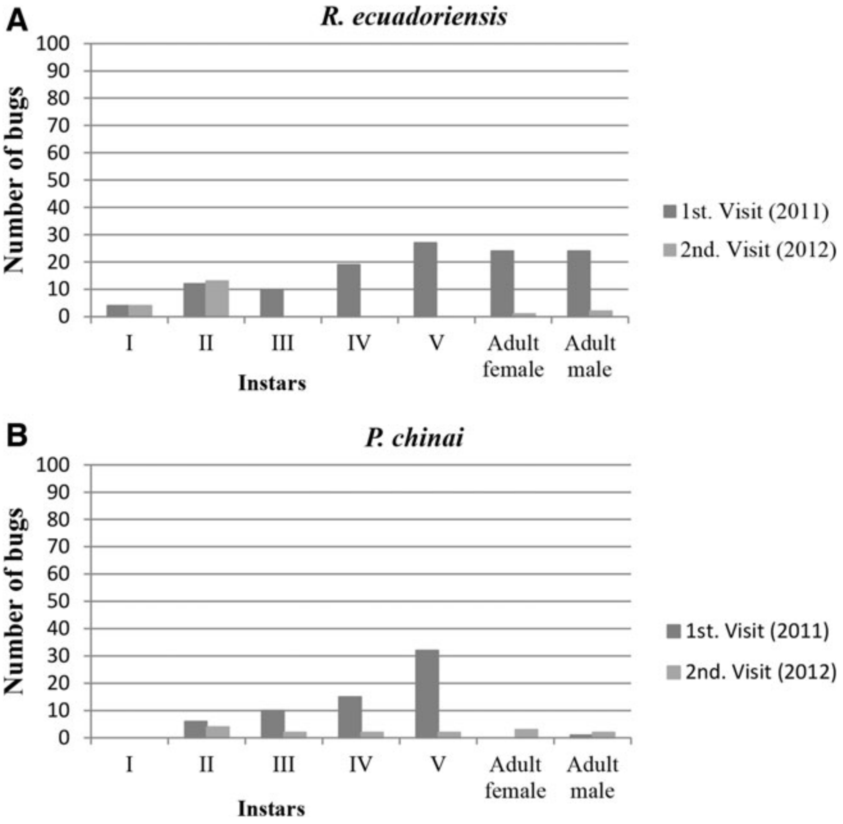

FIG. 2. Population structure of triatomines collected in Bellamaria community of Loja Province, Ecuador at baseline and during the follow-up visits. Number of live nymphs I–V and adult female and male bugs collected in domestic units. **(A)** *Rhodnius ecuadoriensis*, **(B)** *Panstrongylus chinai*.

Natural infection with trypanosomes

We analyzed the intestinal content of 426 triatomines (*R. ecuadoriensis, n* = 309; *P. chinai, n* = 117) collected in the domicile (*n* = 174) and the peridomicile (*n* = 252). The presence of trypanosome-like parasites by microscopy was detected in only 10% of the samples (*n* = 44); however, by PCR we found >70% infection rate of the two expected species of *Trypanosoma* (*T. cruzi* and *T. rangeli*). Overall infection rates showed that *T. cruzi* infects 66% of the triatomines analyzed, whereas 9.6% were infected with *T. rangeli*. Regarding the habitat of capture, 56% of *T. cruzi* infection was found in triatomines captured in the domicile and >70% in the peridomicile.

Along the 5-year study period, lower infection rates with *T. cruzi* were found at the baseline visit in 2008, in domicile (27.3%) and peridomicile (6.3%). However, these rates dramatically increased during following visits, in both domicile and peridomicile, reaching infection rates >80% in 2011 and 2012 (Table 1). Comparing infection between triatomine species, we observed higher infection rates in *R. ecuadoriensis* (42.9%) than in *P. chinai* (8.5%) during the baseline visit. However, during the following visits, both species presented infection rates >40% in 2010 and raised to >70% in 2011 and 2012 (Table 1).

Discussion

Both methods (selective spraying and community-wide spraying) were not effective for the control of triatomines in three rural communities of Loja province. Indeed, the reinfestation of triatomines in treated and untreated houses is frequent under either spraying schedule.

Although selective spraying initially reduced the triatomine infestation, the overall triatomine infestation and triatomine density remained the same thereafter despite yearly selective or community-wide insecticide spraying. *R. ecuadoriensis* infestation on the bedroom walls and bed inside the house, and chicken nests in the peridomicile were the most common, which is consistent with our previous reports (Grijalva et al, 2015; Grijalva et al, 2005). Moreover, higher colonization indices were found for *R. ecuadoriensis* at all subsequent visits, which indicate the capacity of this species to rapidly invade and be established in domestic and peridomestic habitats.

Considering that our previous experimental results indicated that the *R. ecuadoriensis* life cycle takes 181.3 ± 6.4 days (Villacis et al, 2008) from egg to adult, the fact that nymphs IV and V were present in visits 3 and 4 strongly suggests that reinfestation occurred right after insecticide application. These results also support our hypothesis that sylvatic triatomines are the main source of *R. ecuadoriensis* domiciliary infestation in southern Ecuador. Attraction of adult triatomine bugs to artificial light, radiant heat, or human odors have been documented (Castro et al, 2010). These typical traits of human residences may, therefore, play a key role also in household invasion by adult sylvatic triatomines.

Interestingly, Bellamaria community, which joined the study in 2011 and only received community-wide spraying, showed a complete reduction of intradomiciliary infestation with *R. ecuadoriensis* but a persistent infestation with *P. chinai*. This could be explained because *P. chinai* is an annual species (one generation per year), which means that it is likely an univoltine species (Mosquera et al, 2016), and the insecticide has a negligible residual effect that permits the

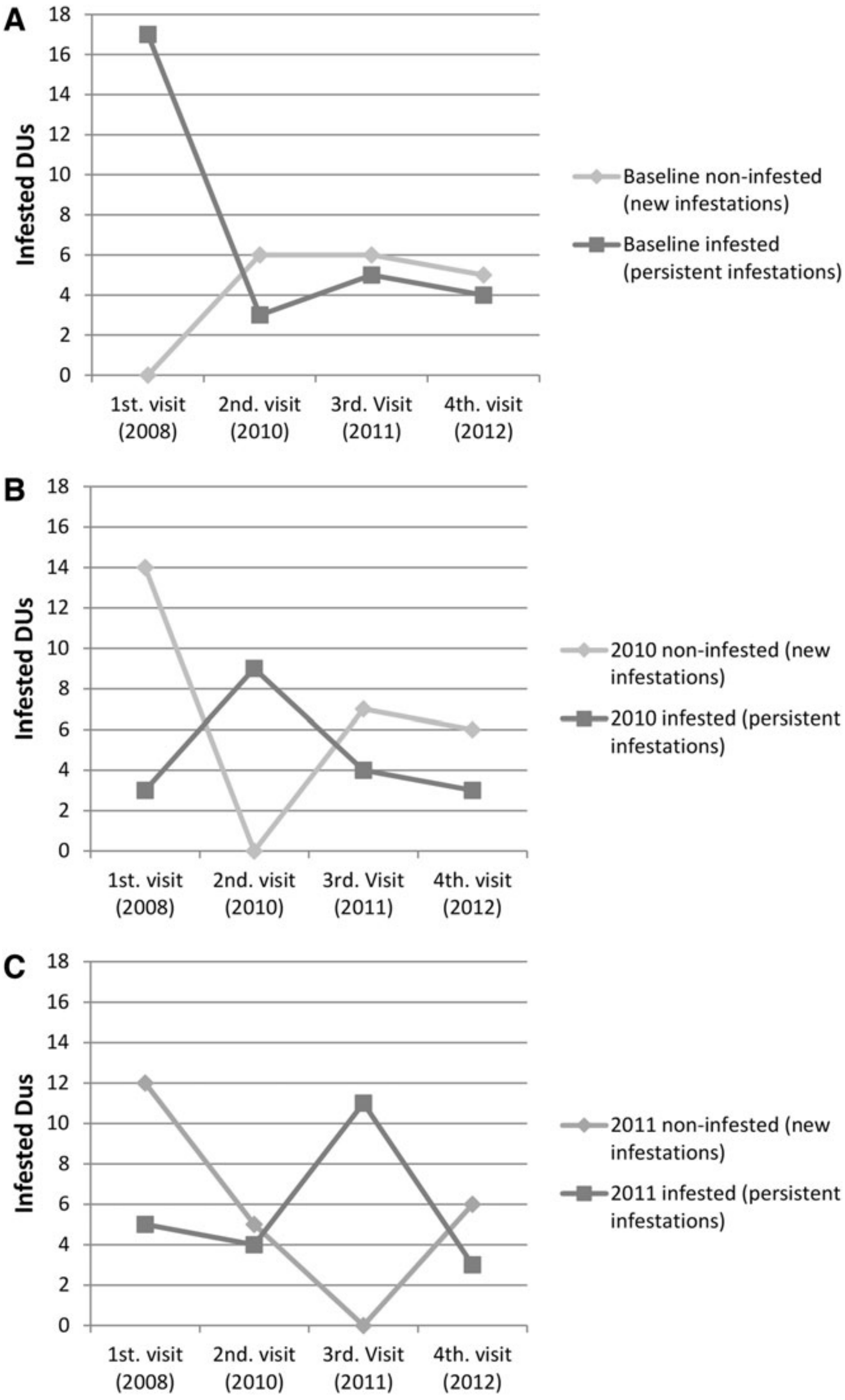

FIG. 3. Domestic Units infested at baseline and follow-up visits. Percentage of the infestation with *Rhodnius ecuadoriensis* and *Panstrongylus chinai* of 41 DUs examined four times between 2008 and 2012. Infested DUs that were not infested in the preceding visit are considered new infestations. Infested DUs that were infested in the preceding visit are considered persistent infestations. Houses infested and not infested in a particular year **(A)** 2018, **(B)** 2010, and **(C)** 2011, as they compare with other years. DU, domestic units.

survival of pre-existing eggs or triatomines that invade bedroom areas after treatment (Gurtler et al, 1999).

Other studies have also demonstrated a variable effectiveness of long-term spraying strategy. Yoshioka et al (2015) demonstrated that house infestation levels of *Triatoma dimidiata* were drastically reduced after the first insecticide treatment, but no significant changes were found after the second spray treatment. This study concluded that large-scale insecticide spraying would not be effective where *T. dimidiata* infestation levels are relatively low. A previous study showed that highly infested villages were likely to remain infested after multiple rounds of insecticide spraying (Hashimoto et al, 2006). It is also important to recognize the behavior of each triatomine species. Hashimoto et al (2006) mentioned a reduction of indoor infestation index of *T. dimidiata*, because this species is mostly domiciliary in this area. Conversely, in our study, the principal problem of *R. ecuadoriensis* are the sylvatic populations (Grijalva et al, 2012).

Some factors related to insecticide application may diminish substantially the effects of insecticide spraying, such as (1) the quality of insecticide spraying, (2) preservation of the active ingredient, (3) preparation of the mix, (4) maintenance of the sprayers, (5) the technique of application on walls and other surfaces, and other details that could generate low effectiveness. This could be unlikely given that the fieldwork was closely supervised by project personnel.

In addition, we could consider possible bias in the method due of the limited sensitivity of the man-hour manual search, that is, when the bugs are captured early during the search, the personnel continue with greater enthusiasm, favoring success, as Monroy et al (1998) mentioned. Although our results point to reinfestation from wild populations, we cannot discard the possibility that triatomines in treated DUs originated from populations that survived to the exposure to the insecticide as observed in other studies (Lardeux et al, 2010, Picollo et al, 2005, Vassena et al, 2000). Therefore, insecticide resistance should be monitored.

Although infestation was higher inside the houses at baseline, in subsequent visits infestation was generally higher in peridomestic habitats. This suggests that the peridomicile could serve as a stepping-stone in the process of intradomicile infestation. The preference that both triatomine species show for chicken nests, as evidenced by another study in the same area (Ocana-Mayorga et al, 2021), dictates the necessity of a modification of the practices associated with chicken husbandry. Moreover, Ocana-Mayorga et al showed that triatomines that contained chicken blood were found infected with *T. cruzi*. These findings show that triatomines move from one microenvironment to another, thus, the dispersal of triatomines between the intradomicile, peridomicile, and sylvatic cycles might play an important role in transmission (Ocana-Mayorga et al, 2021).

High percentage of the typical rural houses in Loja province and Calvas county are constructed of adobe walls (INEC, 2010) with deep cracks, which provide shelter for triatomines. In addition, frequent inhabitant crowding and clutter of personal items in walls, under the beds, and hanging from the rafters create a multitude of intradomiciliary microhabitats suitable for triatomine colonization. These habits have been previously associated with a higher presence of triatomines and seropositivy for *T. cruzi* (Black et al, 2007, Grijalva et al, 2015). Our results support the fact that without a systemic approach that addresses this underlying association through the infrastructural improvement of living environments and promotion of Chagas disease protective practices, insecticide-based interventions alone are not effective to control Chagas disease.

The richness of the data requires a deeper look at the new and persistent infestations. A successful vector control program aims to achieve a complete elimination of intradomestic triatomines. Considering the prevalence of sylvatic triatomine infestations found in this area, new infestations are to be expected in houses that did not receive insecticide spraying under the selective application protocol. Indeed, many studies in this province demonstrate that the wide distribution of sylvatic *R. ecuadoriensis* populations is a risk in the effectiveness of control campaigns conducted to eliminate synanthropic populations of this species (Grijalva et al, 2012).

Also, the high *T. cruzi* infection rates found in sylvatic *R. ecuadoriensis* populations in southern Ecuador could constitute a danger for house reinfestation and persistent long-term Chagas disease transmission in the region (Grijalva et al, 2012, Ocana-Mayorga et al, 2010). However, our results demonstrate that reinfestation occurred even in houses that were treated with insecticide during all visits, at levels compared with those of the houses that did not receive any treatment at baseline. This tendency continues when examining houses that were found to be infested and not infested at the second and third visits.

The fact that community-wide spraying failed to prevent infestation of houses previously found to be triatomine-free further reinforces the notion that this vector control approach is ineffective in this region of Ecuador. These results are like those we have observed with *R. ecuadoriensis* in the coastal region of Ecuador (Grijalva et al, 2011). Some molecular tools and morphometric analyses have not had enough discriminatory resolution to establish the origin of the reinfesting triatomines (Villacis et al, 2017; Villacis et al, 2010). However, a recent study using new techniques such as 2b-RAD genotyping evidenced that triatomine movement from the sylvatic to domestic and peridomestic habitats does occur at a high rate (Hernandez-Castro et al, 2022; Hernandez-Castro et al, 2017).

This study has some limitations. The assessment of intervention effectiveness comes from a before/after comparison and, therefore, provides weaker evidence than a randomized controlled trial. Another limitation included the relatively small number of DUs that were searched in the four visits.

These results support the need for programs aimed at creating physical barriers to reduce contact between people and triatomines along with risk-reducing uses of the space, (removing clutter, relocation of domestic animals, etc.). Other programs in Latin America have implemented infrastructure improvement measures for reducing triatomine infestation and the risks for Chagas disease transmission (Dias, 2007, Lardeux et al, 2015, Lucero et al, 2013). These programs are typically focused on wall plastering and improving illumination and ventilation systems, but to our knowledge, none of them has looked at the underlying causes leading to house construction practices prone to triatomines presence. Decision-making regarding housing is a particular process that must be understood in the context of social, economic, and cultural factors influencing populations at risk (Bates et al, 2020, Lucero et al, 2013, Nieto-Sanchez et al, 2019).

In this context, our group formulated the Healthy Living Initiative (HLI), a research-based effort aimed at understanding the socioeconomic conditions of rural communities exposed to Chagas disease in southern Ecuador to propose effective and sustainable practices that could prevent the disease and promote health in a comprehensive way. HLI's main project is Healthy Homes for Healthy Living, a health promotion effort focused on designing, building, and evaluating living environments to deter triatomine presence in intra- and peridomestic areas of these communities (Bates et al, 2020, Nieto-Sanchez et al, 2019; Nieto-Sanchez et al, 2015). These efforts have been conducted as examples of translational research, aimed at contesting the dynamics of neglect characteristic of diseases that, such as Chagas, remain unattended due to different forms of marginalization, poverty being the most definitive of them.

Conclusion

Our investigation provides evidence that the use of deltamethrin spraying is not effective to control triatomine populations in Loja province and our results point to a high likelihood of reinfestation after insecticide application interventions. However, it is also necessary to carry out studies on resistance to insecticides in triatomines in this study area. The application of effective methods of disease control that incorporate routine monitoring of domestic and peridomestic triatomine infestations plus technical insecticide application, either through community-based or control program personnel efforts is paramount at this juncture.

These activities plus community education campaigns (for both children and adults) are important to reduce the risk of Chagas disease transmission. However, it is time to go beyond these efforts and promote governmental and private investment in the implementation of concerted efforts aimed at the underlying social and economic conditions and promote large-scale implementation of anti-triatomine houses in southern Ecuador, such as those proposed by HLI.

Data Availability Statement

Data are available from the corresponding author upon request.

Acknowledgments

Technical assistance was provided by Darwin Guerrero, Alejandra Zurita, Amber McDonald y Fernanda Aguirre, María Victoria Suárez and Marco Orozco from the CISeAL, Pontifical Catholic University of Ecuador. The personnel and insecticide were provided by the National Chagas Control Program, National Service for Malaria Eradication (SNEM), Ecuadorian Ministry of Health. Inhabitants from the participating communities; participants of the 8th, 10th, 11th and 12th community-based Tropical Disease Research Program in Ecuador, Ohio University.

Authors' Contributions

M.J.G., A.G.V., S.O.-M., and A.L.M. conceived the study and wrote the draft of the article. A.G.V., S.O.-M., C.A.Y., C.N.-S., and E.G.B. performed field and laboratory work. S.O.-M. and A.L.M. performed data analysis. All authors have read and approved the final article.

Author Disclosure Statement

The authors declare no conflict of interest.

Funding Information

Financial support was received from The National Institute of Allergy and Infectious Diseases, Division of Microbiology and Infectious Diseases, Academic Research Enhancement Award (1R15AI077896-01); National Institutes of Health, Fogarty International Center, Global Infectious Disease Training Grant (1D43TW008261-01A1); Pontifical Catholic University of Ecuador G19017, G19111, H13174, I13048, J13049; Ohio University 1804 Fund; Plan Internacional Ecuador; and Children's Heartlink (Minneapolis, MN). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Figure S1

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