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The long-term impact of an Ecohealth intervention: Entomological data suggest the interruption of Chagas disease transmission in southeastern Guatemala

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ABSTRACT

Triatoma dimidiata is the main vector of Chagas disease in southern Mexico, Central America and northern South America. As a native vector, it moves readily among domestic, peri-domestic and sylvatic environments, making it difficult to control only using insecticide as this requires regular application, and re-infestation frequently occurs. Other social innovation alternatives such as those based on Ecohealth principles can be used to tackle the dynamics of the disease in an integral way. We asked whether an Ecohealth intervention, implemented beginning in 2001 in a highly infested village, 41.8%, in southeastern Guatemala, was sustainable in the long term. This intervention included initial insecticide treatments, followed by making low-cost house improvements to eliminate transmission risk factors such as repairing cracked walls, covering dirt floors with a cement-like substance and moving domestic animals outside. We assessed the long-term sustainability through entomological and house condition surveys, as well as an analysis of community satisfaction. We found over a 19-year period, infestation with *T. dimidiata* was reduced to 2.2% and maintained at a level below the level (8%) where vector transmission is unlikely. This long-term maintenance of low infestation coincided with a large proportion of villagers (88.6%) improving their houses and completing other aspects of the Ecohealth approach to maintain the village at low risk for Chagas transmission. There was unanimous satisfaction among the villagers with their houses, following improvements using the Ecohealth method, which likely played a role in the long-term persistence of the modifications. Although the infestation has remained low, 11 years following the last intervention and as the population grew there has been an increase in the proportion of "at-risk" houses, to 33%, pointing out the necessity of maintaining vigilance. The Ecohealth approach is a low-cost, sustainable approach for the long-term control of vector-borne Chagas disease. We recommend this approach including ongoing community monitoring and institutional response for the long-term, integrated control of Chagas disease.

Abbreviations

ES	Entomological Survey(s)
HCS	House Condition Surveys
MoH	Ministry of Health
VCP	Vector Control Program
WHO	World Health Organization
SS	Satisfaction Survey
JICA	Japan International Cooperation Agency.

1. Introduction

Chagas disease causes life-threatening, chronic cardiac and gastric pathologies. Approximately 6 million people are infected with *Trypanosoma cruzi*, the parasitic causative agent, and 70 million live at risk of contracting the disease (WHO, 2017). *Triatoma dimidiata* is the main native vector of Chagas disease over a wide geographic area covering much of southern Mexico, Central American and northern South America. Exclusive use of insecticides has been the traditional approach to control Chagas transmission and indeed was quite successful in

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eliminating transmission by the introduced, domestic vector, *Rhodnius prolixus,* through a massive, eight-year (2000 to 2008) insecticide campaign across Guatemala. This campaign was conducted jointly with JICA (Japan International Cooperation Agency) and the Guatemalan Ministry of Health (MoH), using Deltamethrin, 5% water-soluble powder (Hashimoto and Schofield, 2012).

However, this campaign, although successful against *R. prolixus*, was ineffective against the main native vector, *T. dimidiata*. Many studies have shown that following insecticide application populations of *T. dimidiata* rebound, even as quickly as four months (Dumonteil et al., 2004; Hashimoto et al., 2006; Yoshioka et al., 2015; Helms-Cahan et al., 2019). Reinfestation can be explained as field studies have shown that *T. dimidiata* is well adapted to domestic, peridomestic and wild environments (Monroy et al., 2003; Bustamante et al., 2009) and genetic studies have shown it moves readily between these environments and nearby localities (Dorn et al., 2003; Stevens et al., 2015; Helms-Cahan et al., 2019). Because of the necessity of repeated application, frequent insecticide spraying is costly, kills beneficial insects, and fails to mitigate the underlying risk factors supporting the continuing presence of triatomine vectors in the houses (Bustamante et al., 2009, 2015; Monroy et al., 2009). Finally, it was concluded that exclusive use of insecticides is not a viable strategy for the control of domestic *T. dimidiata*, especially at low infestation levels (Yoshioka et al., 2015).

The rapid reinfestation of *T. dimidiata* motivated the development of an innovative Ecohealth control strategy based on the elimination of risk factors for *T. dimidiata* colonization, which was developed with consideration of community customs including gender roles (Monroy et al., 2009; Rodríguez-Triana et al., 2016; Castro-Arroyave et al., 2020). We developed the Ecohealth intervention against Chagas disease over several years determining the main risk factors for infestation (Monroy et al., 1998a; Bustamante et al., 2009, 2015), the traditional customs that could be helpful towards their mitigation (Castro-Arroyave et al., 2020) and considering the land use effect and anthropogenic change on triatomines (Gottdenker et al., 2011). Focused on the most important risk factors, in consultation with the community, we developed a low-cost, sustainable approach using local materials, "Ecohealth", and implemented it in phases: (1) an initial insecticide application to reduce the infestation, (2) wall plastering to avoid cracks, which serve as hiding places for the vector, followed by, (3) dirt floor improvement to provide a cement-like floor and remove hiding places for nymphs and eggs, (4) construction of outdoor wire chicken coops to avoid animals inside the houses, which amplify the vector population, and finally, (5) reforestation using native fruit trees to replace wild habitat for the vectors. A sixth component, (6) education in Chagas disease transmission and prevention was conducted throughout the process. These interventions serve to make houses refractory to the insect vectors by removing refugia and blood sources from houses as well as re-establishing a suitable wild habitat for the vectors.

The Ecohealth approach was implemented from 2001 to 2008 in La Brea, Quesada, Jutiapa, Guatemala, a region of high Chagas infestation, >40% of the homes infested on a 2001 survey by the MoH (Lucero et al., 2013), and designated as a high-risk Chagas disease transmission region in the Chagas Control Initiative in Central America and Mexico (IPCAM) (Pons et al., 2015). As this was our initial implementation of the intervention and due to the initially high, and then residual infestation following the first partial insecticide spraying (2001, only infested houses), we added two additional insecticide applications (2003 and 2005, all houses). We have found it is important to first reduce the infestation before making the house improvements. The current protocol is to start with one comprehensive insecticide treatment (inside and outside all houses). Over the medium-term (seven years) the effectiveness of the Ecohealth approach for interrupting Chagas disease transmission was demonstrated by infestation reduction to less than or equal to 3%, below the level that would trigger insecticide spraying by the VCP, and a significant reduction in "at risk" houses (62% in 2002 to 15% in 2009, Lucero et al., 2013).

In addition to these gains, it is essential to assess the long-term persistence of vector control interventions to see how they hold up over time. First, it is important to determine if the reduced level of *T. dimidiata* infestation following the intervention is maintained without further interventions by researchers as the human population, and the built and natural environment continue to change. Second, as a community-engaged intervention, it is important to know if the Ecohealth improvements are being maintained and knowledge communicated to new arrivals so that existing houses remain and new houses are constructed to be low risk for infestation. Third, for broad implementation it is helpful to understand the perceptions of benefits of the intervention by the community. We undertook this study to evaluate long-term changes in the risk of Chagas transmission 19 years after an Ecohealth intervention began in a locality of previously high transmission in Guatemala. For this assessment, we conducted entomological and house condition surveys, and evaluated householders' perceptions of the impact of the Ecohealth intervention on their quality of life over time.

2. Methods

2.1. Study design

To assess the impact of an Ecohealth intervention for reduction of risk factors for transmission of Chagas disease over the long term we conducted the following 19-year study (Fig. 1). An initial Entomological Survey (ES) in 2001 in the village of La Brea by the Vector Control Program (VCP) of the MoH showed a high proportion of infested homesteads. Only infested houses were sprayed with the insecticide Deltamethrin by the VCP. Due to the high remaining infestation, two additional insecticide applications were performed inside and outside all houses, regardless of infestation (2003 and 2005). Plastering interior walls began in 2005, and construction of a cement-like floor and outdoor chicken coops began in 2008 (Lucero et al., 2013). Reforestation using native fruit trees began in 2006. Education in Chagas disease transmission and prevention has been included since 2005. For assessment, ES and House Condition Surveys (HCS) were conducted 2001, 2002, 2004, 2006, 2008, and post-intervention in 2009, 2011, 2013 and 2020 with an additional Satisfaction Survey (SS) conducted in 2020. Since 2008, the VCP has followed their protocol, visiting 20 houses annually and spraying only the colonized houses (nymphs present). Some interim data were reported previously (Lucero et al., 2013).

2.2. Study site

La Brea is a rural village located in the Quezada municipality, Department of Jutiapa, Guatemala (14.329692, −90.063103) (Fig. 2). Inhabited by mestizos (mixed race, Spanish and indigenous); the villagers speak Spanish and the economy is mainly agriculture. La Brea is surrounded by forest and agricultural land; the nearest village is approximately 4 km away (Monroy et al., 2009; Lucero et al., 2013). During the study, La Brea's population grew by 57% and the number of dwellings more than doubled, from 2001 (610 inhabitants in 98 dwellings) to 2020 (957 inhabitants in 222 dwellings).

2.3. Entomological and house condition surveys

The Ecohealth intervention started with entomological and house condition surveys (ES and HS) in 2001. Before starting any survey, verbal, informed consent was obtained from the head of household, providing authorization for participation in the study. ES were performed by experienced personnel from the VCP using a two person-hour collection method (two people, 30 min inside the house and 30 min in the surrounding peridomestic area) in all the homesteads where the homeowners gave consent. Researchers accompanied the VCP personnel to document infestation over the six years of entomological evaluations,

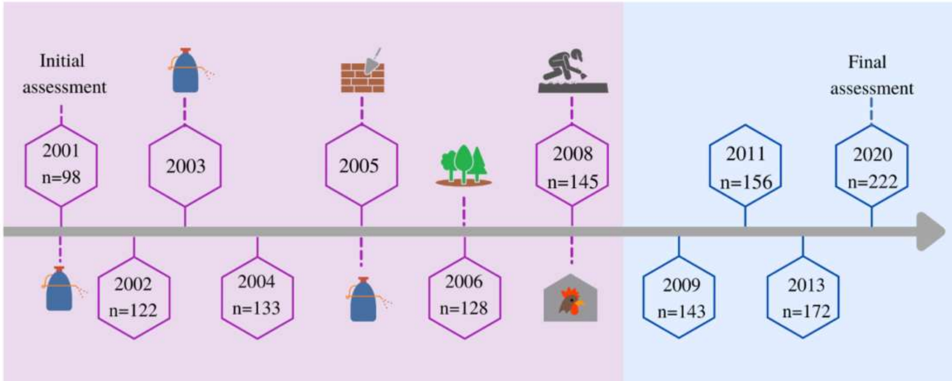

Fig. 1. Ecohealth intervention in La Brea village, during intervention (purple) and follow up assessment (blue). n = number of homesteads surveyed; ( ) insecticide application; ( ) wall plastering; ( ) native tree reforestation; ( ) floor improvement; ( ) chicken coop construction.

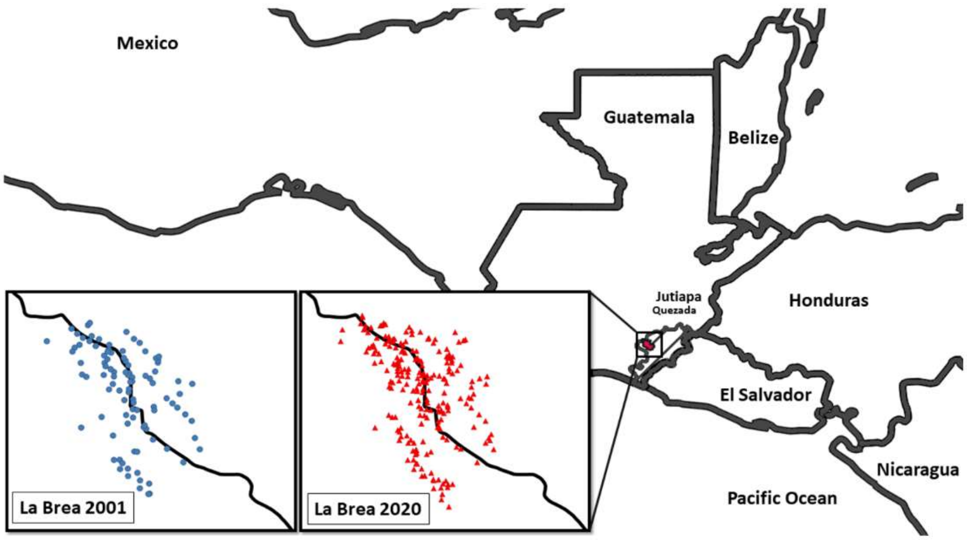

Fig. 2. Homesteads surveyed in the study area, La Brea village, Jutiapa province, in southern Guatemala. 98 homesteads were surveyed in the first assessment in 2001(blue dots, left inset) and 222 homesteads surveyed for the final assessment in 2020 (red triangles, right inset) spread out along a road (black line).

2001, 2002, 2004, 2006, 2008 and 2009 (for details, see Lucero et al., 2013). In the follow up assessment, the same person-hour collection method was used for entomological evaluations conducted in 2011, 2013 and 2020, in 156, 172, and 222 homesteads respectively (Fig. 2). In 2020, entomological data was collected electronically through ONA Data electronic forms V2.0.15 using ODK collect V1.30.0 app for Android devices (ONA, 2021) instead of the usual paper surveys, to reduce COVID-19 risk. Entomological indices were calculated based on World Health Organization (WHO) recommendations (WHO, 2002).

At the same time as the ES, the HCS was performed by researchers. Since 2001 houses have been classified using an A (low risk), B (medium risk) and C (high risk) scale (Lucero et al., 2013). Survey criteria evolved from very basic and qualitative (Monroy et al., 2009) to more detailed, quantitative and weighted as the relative importance of particular risk factors for infestation was determined (Bustamante et al., 2015; Penados et al., 2020; Monroy et al., 2022). Wall condition and degree of wall plastering is the most heavily weighted factor, since a high proportion of *T. dimidiata* are found in cracks in the walls (Monroy et al., 1998b). Other risk factors recorded included presence or evidence of vectors, a chicken coop inside or attached to the house, other animals inside the house, the accumulation of wood or construction materials, house age more than six years, a dirt floor, and clutter. Only houses of B or C risk classification were improved. As with the ES, in 2020 HCS data was collected electronically.

2.4. Insecticide application

The initial insecticide spraying (2001, Deltametrin, 5% water-soluble powder, at 25 mg of active ingredient per m^2) was performed by VCP personnel inside houses only where triatomines were captured. Due to an initial high infestation (41.8%,) and residual infestation (26.2%), after the first “infested house only” treatment in 2001 (Table 1, Lucero et al., 2013), in 2003 and 2005 insecticide was sprayed inside and outside all houses regardless of infestation to ensure a low infestation before the house improvements were begun (Monroy et al., 2009; Lucero et al., 2013). Since 2008, VCP personnel have rarely sprayed only a few houses, following their protocol, searching 20 houses randomly per village annually and spraying only those colonized, i.e., triatomine nymphs found (MSPAS, 2012). In the cases where we found domestic colonies of triatomine bugs, we reported this to the VCP staff, they then inspected the houses and if they found nymphs, sprayed the houses inside and out. Householders generally cannot afford to spray their own homes so insecticide treatment by villagers is rare.

2.5. House improvements, animal management, reforestation

In 2004, traditional community house construction and improvement practices were studied and a modified plaster mixture, made from local materials, was developed and tested. This plaster, "revocado," was applied according to traditional practice, where mainly women plaster the walls using their hands (Monroy et al., 2009). We then instructed villagers living in higher risk houses (B and C, see below) in Ecohealth methods 2005 to 2008. After vector refugia were plastered over, walls were finished by painting them with lime (begun in 2005). Then, dirt floors were leveled and three layers of soil, volcanic ash and lime added to make a cement-like floor as described (Monroy, 2013) beginning in 2008. At the same time, we supplied chicken wire and instructed the villagers in the construction of outdoor chicken coops (Monroy et al., 2009). Restoration of the forest by planting native fruit trees began in 2006. As villagers undertook the improvements when they had the time, this was an ongoing process and a C house may have been improved to B condition in one year and finally to A condition in a subsequent year. All improvements done to homesteads after 2008 were done at the initiative of the villagers.

2.6. Sustainability of wall improvements

To analyze the longevity and maintenance of the house improvements over the 19 years, we selected the 75 houses improved using the Ecohealth method and evaluated each survey, 2001 to 2020. To determine in which years the wall condition differed year-to-year a Cochran's Q test followed by Dunn's test post hoc test was performed, using packages "RVAideMemoire" (Hervé, 2021) and "FSA" (Ogle et al., 2021).

The house infestation risk for the whole village (all houses surveyed) in each survey was recorded, and the proportion of high, medium and low risk houses was calculated over time. We then analyzed if there was at least one significant difference in risk factors for home infestation between years. Since the number of houses surveyed are so variable over time and they were not always surveyed in all the years of assessment (may have been closed, uninhabited or destroyed), we randomly selected 50 improved houses by the Ecohealth method (were initially medium, type B, and high type C, risk) and 50 unimproved houses (type B and C houses that did not make improvements to their homes, houses with initially low risk, type A and some new houses).We compared *T. dimidiata* infestation risk between them using the Friedman test followed by multiple post hoc comparisons, specifically using the Wilcoxon test with a signed rank with Bonferroni correction. This is done using the packages "tidyverse" (Wickham et al., 2019), "ggpubr" (Kassambara, 2020a) and "rstatix" (Kassambara, 2020b) using the R 4.0.3 software (R Core Team, 2020).

2.7. Satisfaction Survey

In 2020 a qualitative Satisfaction Survey, was included and results collected electronically. Only villagers who claimed to have made improvements to their houses with the Ecohealth method were surveyed. Villagers were asked a series of questions to determine what benefits they perceived from the Ecohealth interventions, in addition to fewer Chagas vectors in their homes. To distinguish their level of satisfaction before and after home improvements, open-ended questions were asked, e.g., "How did you feel about your house before completing the house improvements?" with the surveyor summarizing the responses with "satisfied, dissatisfied or indifferent". Likewise, each respondent was asked whether they themselves had learned the Ecohealth method of home improvements (someone else in the household or a previous owner may have been the one trained), if they would continue to maintain their home using this method, and if they would recommend that others do so as well.

3. Results

3.1. Effects of Ecohealth intervention on entomological indices

T. dimidiata infestation of homesteads was substantially reduced with the Ecohealth intervention (2001–2008) and this reduction was maintained over the remaining 11 years of the study (2009–2020) (Fig. 3). An initial high infestation of nearly 42% of homesteads dropped to <7% following the application of three rounds of insecticide. Importantly, following completion of the Ecohealth intervention (2008) the level of infestation was maintained at <6% of homesteads for the 11 years that followed (Table 1) rather than the usual rebound of the population as

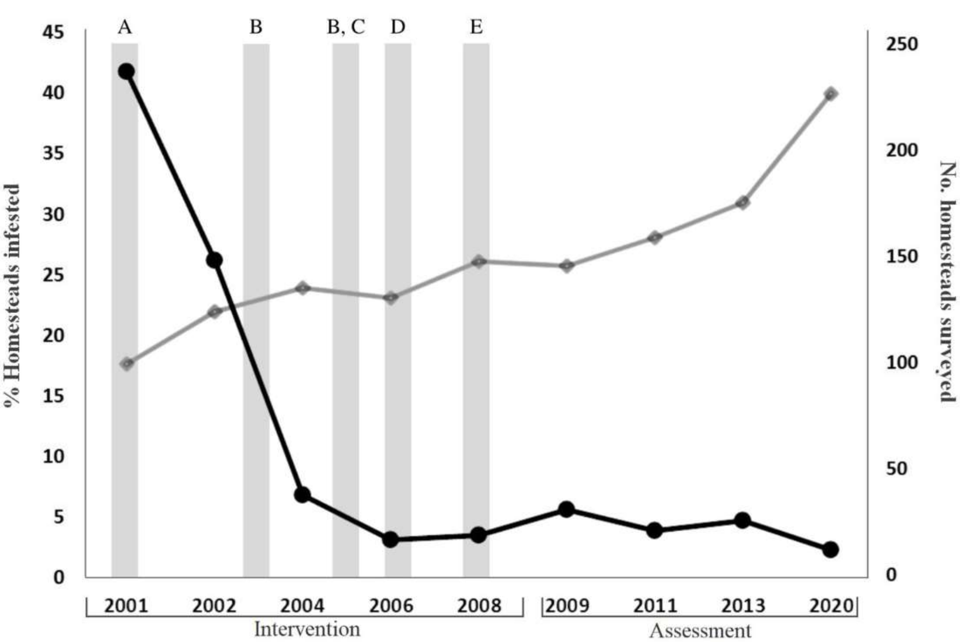

Fig. 3. Reduction in percentage of homesteads with triatomines (black dots), and increase in number of homesteads surveyed (gray diamonds) over 19 years of evaluation in La Brea. The bars indicate the interventions in the village: (A) insecticide sprayed by VCP personnel inside houses where triatomines were found, (B) insecticide sprayed inside and around all houses regardless of infestation, (C) beginning of wall plastering, (D) beginning of reforestation, (E) beginning of cement-like flooring and construction of outdoor chicken coops.

Table 1
Entomological indices from each survey conducted during and following the Ecohealth intervention.

	2001 *Intervention*	2002	2004	2006	2008	2009	2011 *Assessment*	2013	2020
No. homesteads surveyed	98	122	133	128	145	143	156	172	222
No. homesteads infested	41	32	9	4	5	8	6	8	5
% Homesteads infested	41.8	26.2	6.8	3.1	3.4	5.6	3.8	4.6	2.2
% Intradomiciliary infestation	33.7	17.2	5.3	0.8	2.8	4.2	2.6	2.9	0.4
% Peridomiciliary infestation	10.2	10.6	1.5	2.3	0.7	2.1	1.9	1.7	1.8
% Colonization	80.5	62.5	44.4	75.0	80.0	50.0	66.6	87.5	100
% Intradomiciliary colonization	58.5	34.4	33.3	25.0	60.0	37.5	33.3	62.5	20.0
% Peridomiciliary colonization	21.9	34.4	11.1	50.0	20.0	25.0	33.3	25.0	80.0
Crowding	6.7	5.6	1.3	11.0	12.0	5.9	28.8	7.2	7.0

% Homesteads infested = (number of houses with vectors / number of houses surveyed)*100;.
% Intradomiciliary infestation = (number of houses with indoor vectors/number of houses surveyed)*100;.
% Peridomiciliary infestation = (number of houses with peridomicile vectors/number of houses surveyed)*100;.
% Colonization = (number of houses with nymphs/number with house with vectors)*100;.
% Intradomiciliary colonization= (number of houses with nymphs in the house/number of houses with vectors)*100;.
% Peridomiciliary colonization= (number of houses with nymphs in the peridomicile/number of houses with vectors)*100;.
Crowding = (number of vectors collected in the village/number of houses with vectors found).

has been documented many times in several localities. This reduction in infestation was seen inside (intradomiciliary) as well as outside houses, in the peridomestic habitat, and importantly occurred during the time that the number of houses in the village more than doubled (98 to 222, Fig. 3) and the population increased 57% (610 to 957 inhabitants, 2001–2020).

Interestingly, the few houses that remained infested (2–6% of the houses, 2009–2020, Table 1) showed high levels of colonization (nymphs present) inside and outside the houses suggesting the vectors are reproducing in the few infested homesteads. The majority of these houses (>75%) were only partially improved or unimproved. A similar pattern was observed with the crowding index (Table 1). A high number of vectors are maintained in the few houses with vectors.

3.2. Householder participation and changes in risk condition of houses

The high participation in the house improvements (88.6% of type B and C houses) resulted in a substantial reduction in the percentage of houses categorized as the highest infestation risk (type C, Fig. 4). Before the house improvements, the majority of houses in La Brea were categorized as high risk for *T. dimidiata* infestation (type C, 59 - 72%). There were fewer medium (type B, 16% - 27%) and low risk houses (type A, 12% - 14%) (Fig. 4). By 2006, just one year after the beginning of wall plastering, the percentage of type C houses had fallen to 17% ($p< 0.05$, Wilcoxon signed-rank test). A low percentage was maintained until 2013 (7%) when it began to rise over the next seven years to 33% ($p< 0.05$, Wilcoxon signed-rank test) at the final assessment in 2020. Of the 88.6% of type B and C houses that were improved, the majority of householders used the Ecohealth approach to improve their walls and/or floors (82.9%), while many fewer (17.1%) had cement professionally applied, the latter mostly to the floors. More than 3/4 of households (77.9%) built wire chicken coops using the Ecohealth approach and moved the chickens outside the houses. By 2020, there was more than double the number of houses compared to 2001. Many new houses continue to be built with the traditional adobe (79.3% by 2020 are adobe), and there was an increase in the percentage of cement block houses (20.7%) compared to 2001 (8.2%). By 2020, 11 years after the last intervention, the percentage of the adobe houses that had walls and/or floors improved using the Ecohealth method had decreased from 88.6% in 2008 to 42.6%, and 25% were unimproved

3.3. Sustainability of wall improvements over time

The wall improvements performed as part of the Ecohealth intervention proved durable or were maintained by residents as assessed by following the wall condition of 75 homes that were visited each and every survey year (Fig. 5). On average, the wall condition of 82% of the houses was maintained without cracks, over the 15 years following the wall plastering instruction. This difference in wall condition between before (2001–2004) and after (2006–2020) wall plastering instruction was statistically significant ($p < 0.0001$, Cochran's Q test, X^2 (8) = 202.8). There was no significant difference in the wall condition within each window, before or after wall plastering, ($p > 0.05$, Dunn's test).

3.4. Satisfaction and additional benefits of Ecohealth

Of those surveyed in 2020, there was unanimous satisfaction among the 55 respondents with their houses, following improvements using the Ecohealth method. This is in sharp contrast to how they reported feeling about their houses before the intervention (5.5% satisfied). The vast majority said that they planned to maintain the improvements in the future (98%) and that they would recommend implementing the Ecohealth approach to others (94%). Low cost (91%, Fig. 6A) was the most cited reason to choose the Ecohealth approach, followed by ease of maintenance (69%). All inhabitants noted additional benefits realized, beyond fewer Chagas vectors in the house. Namely, that the house was easier to clean (94.5%, Fig. 6B) and their status in the village improved (67.3%).

4. Discussion

Infestation of homesteads with *T. dimidiata* dropped significantly following the Ecohealth intervention and importantly, was maintained without further insecticide applications for an additional 15 years demonstrating the long-term effectiveness of the Ecohealth intervention in reducing Chagas transmission risk (Fig. 3). Crucially, village-wide infestation stayed below 8%, the level below which vectorial transmission is unlikely (Aiga et al., 2012). This maintenance of low infestation is in marked contrast to the usual reinfestation of *T. dimidiata* following insecticide spraying (Nakagawa et al., 2003; Hashimoto et al., 2006; Manne et al., 2012; Yoshioka et al., 2015; Helms-Cahan et al.,

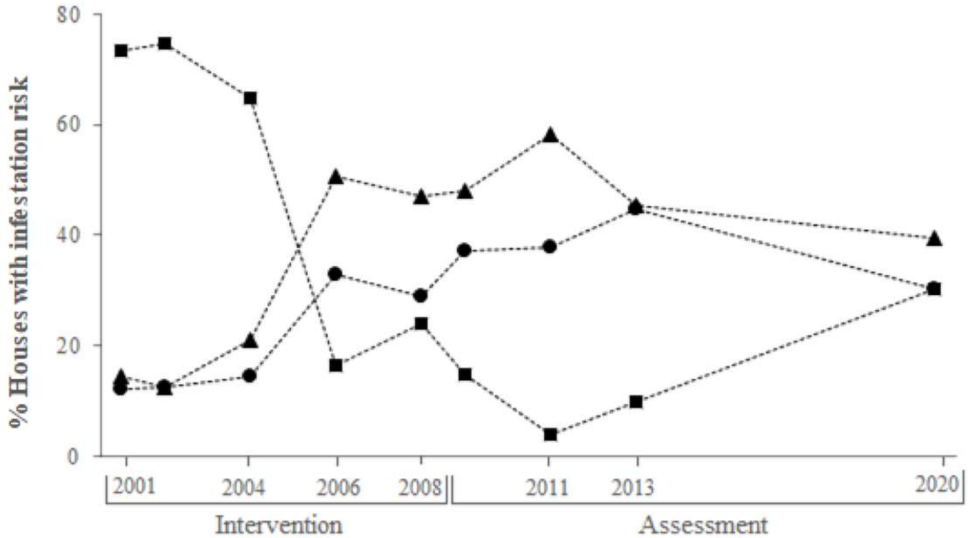

Fig. 4. Change in percentage of houses in La Brea, identified by risk categories during the Ecohealth intervention and follow up assessment. The *T. dimidiata* infestation risk is classified as high, type C (squares), medium, type B (triangles) or low, type A (dots).

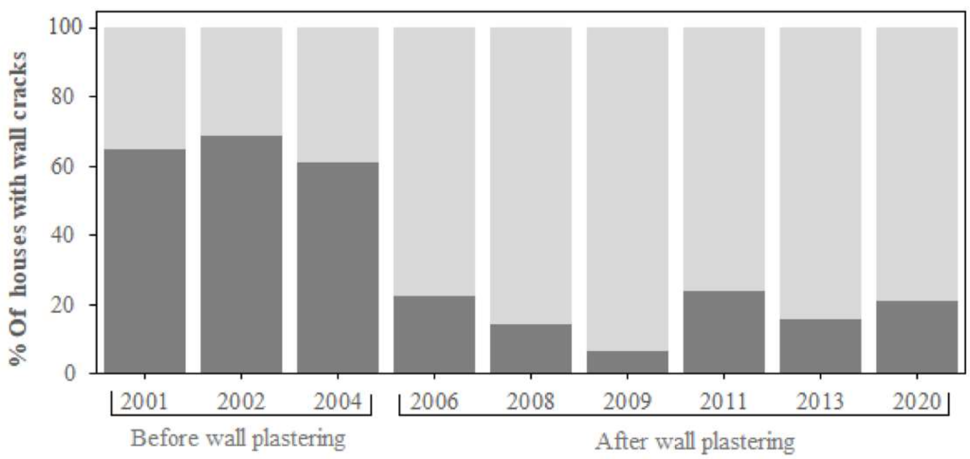

Fig. 5. Wall condition of 75 houses improved with the Ecohealth approach over the course of the study, with (dark) or without (light) cracks. Difference between before and after wall plastering, $p < 0.001$.

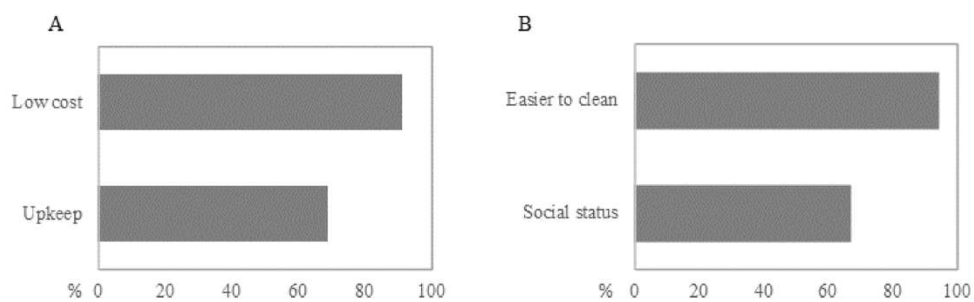

Fig. 6. Qualitative analysis of motivations and additional benefits from the Ecohealth intervention. (A) Most cited reasons for implementing the Ecohealth improvements. (B) Additional benefits of Ecohealth intervention, in addition to fewer Chagas vectors in houses.

2019), which can occur as quickly as four months following spraying (Dumonteil et al., 2004). Insecticides are known to have a short half-life, especially in peridomestic habitats (Cecere et al., 2002). The rapid reinfestation of *T. dimidiata* is driven by migration between habitats and nearby dwellings or villages and insecticide survival, especially of immature stages (Zeledón et al., 1970; Dorn et al., 2003; Stevens et al., 2015, Helms-Cahan et al., 2019). The Ecohealth approach removed the risk factors for infestation (wall cracks, dirt floors, animal blood sources, etc.) making the houses refractory to reinfestation. Several studies have documented short-term reduction in infestation of *T. dimidiata* following Ecohealth intervention (Monroy et al., 2009; Pellecer et al., 2013; Lucero et al., 2013; Monroy et al., 2022). This is the first study to show that these reductions in infestation can last more than a decade following the intervention and importantly, this occurred while the number of houses more than doubled and the population increased >50%. The integrated Ecohealth approach results in a long-term reduction in infestation with *T. dimidiata*. This can be a low cost, sustainable alternative alleviating the need for repeated insecticide application.

Underscoring the importance of maintaining houses refractory to the vectors is the persistence of the vector in a very few houses, in relatively high numbers and with ongoing reproduction, which can serve as foci for reinfestation. However, even with this focal persistence, infestation village-wide stayed low, demonstrating the effectiveness of the Ecohealth intervention in avoiding reinfestation by *T. dimidiata* in the village. Given the reality that there will always be some householders that do not improve their houses and that *T. dimidiata* is a highly mobile vector species well-adapted to several habitats, maintaining a high proportion of refractory houses is essential to reducing the overall transmission risk in the village. The Ecohealth intervention was well-accepted by the community with very high rates of participation and resulted in a dramatic improvement in the risk condition of the houses. Nearly 90% of householders improved their houses; a level of participation that would not be possible without community engagement and empowerment including prior knowledge of social and cultural practices that impact implementation and sustainability (Monroy et al., 2009; Soto et al., 2019). The dramatic improvement in the risk condition of the houses can be noted especially with the reduction in type C houses (Fig. 4). The Ecohealth approach is a sequenced process over time and villagers perform the steps as they have time. So as type C houses decrease, type B and A houses increase year-by-year. The long-term maintenance of the walls of the improved houses, the most important risk factor for *T. dimidiata* infestation (Bustamante et al., 2009), is evident in the subset of 75 improved houses that were included in each survey and showed a highly significant difference in risk status before and after the intervention ($p < 0.0001$, Fig. 5). The long-term maintenance of the walls was excellent; >80% of walls remained refractory showing sustainability of the plastering method and maintenance by villagers (Fig. 6). This is notable because it is a long enough time frame (up to 15 years) that some houses would be expected to deteriorate so would need repair. This maintenance of house condition coincided with the dramatic and sustained decrease in *T. dimidiata* infestation over the same timeframe (Table 1).

Our data extends previous results that documented medium-term (5–6 years since last pesticide application) sustained reduction in *T. dimidiata* infestation in the same village (Monroy et al., 2009; Lucero et al., 2013) and in a nearby village (Pellecer et al., 2013; Lima-Cordon et al., 2018). Interestingly, in the latter we also studied blood meal sources which showed a marked reduction in proportion of *T. dimidiata* that had fed on humans following the intervention or were infected with *T. cruzi* (Pellecer et al., 2013).

Although the reduction in *T. dimidiata* infestation has been maintained over the 11 years since the last intervention, the proportion of houses at higher risk for infestation is increasing. This is not surprising given that the population has increased >50% and the number of houses has more than doubled. New houses are often built in the traditional fashion using mud and vegetable material (adobe and bajareque, nearly 80% of houses by 2020), which eventually results in wall cracks. It appears that many of the new arrivals are not learning the method from fellow villagers. Some houses are still being improved by the Ecohealth method (42.6%), however, it is clear that 11 years after the last intervention, reinforcement is needed to sustain the involvement, commitment and empowerment of the community which are essential for the success of the strategy in the long term (Castro-Arroyave et al., 2020). In this regard, public policies that support and reinforce the interventions are essential. An example of this occurs in Colombia where, by law, funds are allocated to improve rural housing in municipalities with high epidemiological risk of Chagas disease (Guhl, 2007). In Guatemala, the VCP of the MoH play a key role in entomological surveillance both by ES and also monitoring collection boxes they place in the villages where villagers leave vectors they find in their houses. As there is rapid turnover of MoH personnel it should be a priority to instruct new personnel in theory and procedures for Ecohealth integrated control of Chagas disease.

Finally, the Ecohealth intervention resulted in high satisfaction levels among the villagers, where 100% reported being satisfied with their houses after the intervention, in sharp contrast to only 5.5% satisfied beforehand. The low cost was one of the main motivations for the villagers to use the Ecohealth method. Use of local materials keeps the cost down to a level affordable by villagers, most of whom are unable to pay for cement and professional application; close proximity of needed materials facilitated the improvements. Ease of maintenance, which they are also able to do on their own using traditional practices, was also valued. Villagers noted additional benefits such as ease of keeping the house clean and an increase in social status. The cement-like floor was considered a step up in social status and this served as a motivator in the sequenced intervention. Completion of the wall improvements was a requirement for receiving the supplies for improving the floors. Some villagers also obtained economic benefits from planting native trees, starting new businesses producing the native fruits, nances and jocotes. Another economic benefit accrued to the MoH, since low infestation was maintained with the Ecohealth intervention, insecticide application throughout the village was no longer necessary. Lack of frequent insecticide application may also aid beneficial insects such as pollinators. Additionally, changing the dirt floor for the cement-like floor and increased cleanliness, and favors the elimination of other parasites, such as soil-transmitted helminths (Romero-Sandoval et al., 2017). This is especially important in Guatemala, which has the highest incidence of trichuriasis and ascariasis in Latin America and Caribbean, contributing to malnutrition of many children living in poverty (Hotez et al., 2008; Nolan et al., 2021). The commitment of community leaders and their participation influenced the adoption. It will be interesting to study how and why Ecohealth knowledge is communicated to new arrivals and to include a more in-depth satisfaction and quality of life analysis of the villagers who have participated in Ecohealth interventions to learn about other elements that may contribute to sustaining the interventions.

T. dimidiata has a very large geographic range, from south Mexico to Colombia (Stevens et al., 2015), with differences in vector habitat preference, and completely different ecological, political and social scenarios (Lima-Cordon et al., 2018). Investigators are beginning to implement this Ecohealth method across its range as a long-term solution for controlling infestation of this vector implementing adaptations to these unique conditions. In Yucatan, Mexico, the Ecohealth method has been adapted to the intrusive, non-domicilated *T. dimidiata* using window screens, insecticide impregnated curtains and removing clutter from the peridomicile to reduce Chagas risk (Waleckx et al., 2015). In other studies, reinforcing traditional practices like sleeping in hammocks could potentially reduce human/vector contact (Waleckx et al., 2016). A scaled-up study addressed the unique ecological, political and social characteristics of areas of Guatemala, El Salvador and Honduras; characteristics that must be taken into account to effectively apply the method (Lima-Cordon et al., 2018). Additionally, the Ecohealth method was applied to a new hotspot of disease in Guatemala, and this approach included serology and treatment of infected patients (Monroy et al., 2022). The Ecohealth method is also proving effective against other native species, such as *T. infestans* in El Chaco, Bolivia (Gonçalves et al., 2021).

There are limitations to this study. The manual person-hour ES, although it is the most commonly used assessment tool, shows low sensitivity (Monroy et al., 1998b), which can affect the accuracy of the infestation, especially with respect to small nymphs. However, given the multiple surveys and the large and sustained reduction in infestation following the intervention, this suggests that even if some infested houses were missed, the overall trends are supported. We also lack complete records of when the VCP has sprayed; they report visiting 20 houses annually and spraying those where they find nymphs. Given the insensitivity of the survey method for nymphs and the lack of VCP resources (fuel for vehicles, insecticide), we don't expect that this has affected our results as they likely spray very few houses. In addition, in this study we infer interruption of transmission based on published data showing that transmission is unlikely when domestic *T. dimidiata* infestation is 8% or less (Aiga et al., 2012). However, we have no evidence of human infection below infestation in the village. We expect to have direct tests of human transmission in the future. Serology of children born after the intervention is underway. This will be crucial and will complement our current evidence on transmission interruption. Likewise, *T. cruzi* prevalence in *T. dimidiata* was not part of this study, however, we did measure this in a previous study in a nearby village and documented a reduction in *T. cruzi* prevalence in vectors following the Ecohealth intervention (Pellecer et al., 2013). The lack of a control village followed long term and treated solely with insecticide spraying is a limitation. However, many studies nearby and in other areas have documented rebounding of *T. dimidiata* populations with the "insecticide only" approach (Nakagawa et al., 2003; Dumonteil et al., 2004; Hashimoto et al., 2006; Manne et al., 2012; Yoshioka et al., 2015; Helms-Cahan et al., 2019). Furthermore, it would be unethical to follow a control village long term, knowing that reinfestation is likely after spraying. We also acknowledge there is limited data available on the effects of reforestation and the economic impact of the Ecohealth intervention and would encourage further studies in these areas.

Overall, the data presented in this article supports the hypothesis that the Ecohealth intervention is a long-term solution for controlling *T. dimidiata* infestation and reducing the risk factors for Chagas transmission in an endemic region. Using local materials, adapting traditional customs and engaging the community led to high participation and sustained community engagement and long-term reduction in infestation. Community satisfaction was likely a major factor in the sustainability of the interventions performed. The changes however, are not indefinite and reinforcement, especially implemented at the institutional and policy level, is essential to maintain these villages at low risk for Chagas transmission.

Ethics approval and consent

Study received clearance from San Carlos University Bioethics committee (AC-010–2018), Guatemala City, Guatemala.

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CRediT authorship contribution statement

Fredy Manolo Pereira: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Daniel Penados:** Software, Resources, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Patricia L. Dorn:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Belter Alcántara:** Investigation, Resources, Data curation. **María Carlota Monroy:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. F. Pereira et al.

Data Availability

Data will be made available on request.

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