

## Triatomine Infestation in Guatemala: Spatial Assessment after Two Rounds of Vector Control

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**Abstract.** In 2000, the Guatemalan Ministry of Health initiated a Chagas disease program to control *Rhodnius prolixus* and *Triatoma dimidiata* by periodic house spraying with pyrethroid insecticides. The aim of this study was to characterize infestation patterns and analyze the contribution of programmatic practices to these patterns. Spatial infestation patterns at three time points were identified using the Getis-Ord  $G_i^*(d)$  test. Logistic regression was used to assess predictors of reinfestation after pyrethroid insecticide administration. Spatial analysis showed high and low clusters of infestation at three time points. After two rounds of spray, 178 communities persistently fell in high infestation clusters. A time lapse between rounds of vector control greater than 6 months was associated with 1.54 (95% confidence interval = 1.07–2.23) times increased odds of reinfestation after first spray, whereas a time lapse of greater than 1 year was associated with 2.66 (95% confidence interval = 1.85–3.83) times increased odds of reinfestation after first spray compared with localities where the time lapse was less than 180 days. The time lapse between rounds of vector control should remain under 1 year. Spatial analysis can guide targeted vector control efforts by enabling tracking of reinfestation hotspots and improved targeting of resources.

### INTRODUCTION

Chagas disease (CD) is among the most prevalent neglected diseases in Latin America, with approximately 8–10 million people infected and as many as 15,000 deaths per year.<sup>1,2</sup> Caused by the protozoan parasite *Trypanosoma cruzi*, CD is transmitted primarily by vectors of the subfamily Triatominae that infest homes in poor communities throughout this region. In addition, it is currently estimated that CD is attributed with the loss of about 670,000 disability-adjusted life-years per annum globally.<sup>3</sup> The two available treatments for CD, Benznidazole and Nifurtimox, are associated with frequent adverse effects,<sup>4</sup> particularly when administered in adults. Therefore, prevention, most often through vector control, remains the most common public health strategy for addressing this disease.

Local and later, national efforts to control CD began as early as the 1940s, although the most widely recognized regional attempt to control the disease commenced in 1991 when Brazil, Argentina, Chile and Paraguay, Uruguay, and Bolivia formed the Southern Cone Initiative (Iniciativa de Salud del Cono Sur [INCOSUR]).<sup>5–8</sup> With the support of this regional collaboration and the Pan American Health Organization (PAHO), a majority of INCOSUR member countries effectively strengthened their national vector control programs, improving house spraying and control of blood-borne transmission as well as diagnosis and treatment initiatives. These strategies resulted in the reduction of disease incidence by 60–95% in each country and the certified interruption of disease transmission by *Triatoma infestans*, the main vector in South America, in several Southern Cone nations including Brazil, Chile, and Uruguay as well as parts of Paraguay and Argentina.<sup>2,8</sup>

Using the successful Southern Cone Initiative as a model, the Japan International Cooperation Agency (JICA) partnered with the Guatemalan Ministry of Health (MoH), in collaboration with the PAHO, in establishing a National Chagas Disease Control Project in 2000. The project's primary aim was to

implement periodic domestic spraying with pyrethroid insecticides (deltamethrin) in specific areas where the disease was believed to be endemic to decrease infestation. In cross-sectional surveys estimating seroprevalence of disease that were conducted by the Universidad del Valle de Guatemala from 2000 to 2001, it was found that some municipalities had an estimated seroprevalence of CD in children under 14 years of age greater than 20%.<sup>9,10</sup> With a national prevalence of 5% as of 2007, it is estimated that approximately 730,000 Guatemalans are infected with CD.<sup>11,12</sup> The project aims were to eliminate *Rhodnius prolixus* and bring under control domestic infestation with *T. dimidiata*, specifically maintaining the prevalence of intradomestic infestation below 5%.<sup>11,12</sup> These two vectors have distinct ecological characteristics, although they have similar rates of natural infection.<sup>13</sup> The former is a nonnative species that the PAHO officially regards as eliminable from Central America, because they can only survive in domestic settings.<sup>8</sup> The latter can live in both the natural environment and domestic settings, making it especially difficult to control and impossible to eliminate. Because *R. prolixus* is considered eliminable and because evidence suggests that it is a more efficient vector, it has become the priority for vector elimination.<sup>8,11</sup> In addition to house spraying, other primary emphases of the program included local capacity building, community education, and community participation.<sup>14–16</sup>

In 2005, JICA's involvement in the project officially ended, but the project continued under local government administration and with informal technical and financial support from JICA. In 2008, the World Health Organization certified Guatemala for the interruption of transmission by *R. prolixus*. Although a new official second phase of collaboration between JICA and the MoH was launched in 2009, the long-term work of the initiative has been affected by increasingly scarce resources. There has not yet been an evaluation of the vector control program initiated in 2000 across the entire coverage area, although several previous studies in the departments of Zacapa and Jutiapa have showed the efficacy of one round of spraying in reducing domestic infestation with *T. dimidiata* as well as the continued presence of localities with high infestation indices after several rounds of house spraying in the state of

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Jutiapa.<sup>15,17-19</sup> This evaluation allows for a comprehensive assessment of the efficacy of the program, and it provides crucial information for targeting resources and improving spray operations in the future.

Our objective was to appraise the spatial pattern of vector infestation in the areas targeted by the CD control project at three time points: (1) 2000–2002, a baseline period before any spraying was initiated, (2) 2003–2006, when an assessment was conducted after the first round of house spraying with pyrethroid insecticides, and (3) 2005–2007, when an assessment was conducted after the second round of house spraying with pyrethroid insecticides. These analyses aim to offer concrete recommendations to improve vector control interventions to allow for better planning, monitoring, and evaluation of program activities.

MATERIALS AND METHODS

**Study area.** Guatemala is divided into 22 states, commonly referred to as departments, that are subdivided into smaller administrative units called municipalities that contain individual communities called localities. The coverage area of the intervention includes 45% of the departments of Guatemala (Figure 1), a majority of which are located on the eastern side of the country, comprising a population at risk of approximately 4.1 million.<sup>11</sup>

**Description of spray activities and data on CD.** This study used data on domestic infestation from the Guatemalan Chagas Disease Control program as recorded by national and departmental staff of the MoH’s Program on Malaria, Dengue and Chagas Disease. The program conducted a baseline survey of vector infestation between 2000 and 2002 in five departments (Zacapa, Jutiapa, Jalapa, Santa Rosa, and Chiquimula) and between 2002 and 2004 in an additional five departments (Alta Verapaz, Baja Verapaz, El Progreso, Quiche, and Huehuetenango). The baseline survey consisted of a search of selected houses with thatched roofs or mud walls distributed throughout the locality. In the majority of localities, between 10 and 20 houses were surveyed, regardless of the total number of houses in the locality. In localities where greater than 5% of houses searched were found to be infested, all houses in the locality with mud walls and/or thatched roofs underwent residual spraying with pyrethroid insecticides. Post-spraying vector survey was performed after both the first and second spray and included search of houses that were previously infested.<sup>17</sup>

The variables collected by the program included the index of infestation (defined as  $100 \times \text{number of infested houses} / \text{number of houses evaluated}$ ) observed in each locality,<sup>20</sup> the date when spraying occurred, and the date that house evaluation of infestation occurred. Mean indices of infestation at different time points were compared using non-parametric Wilcoxon sign rank tests. Domestic vector infestation was

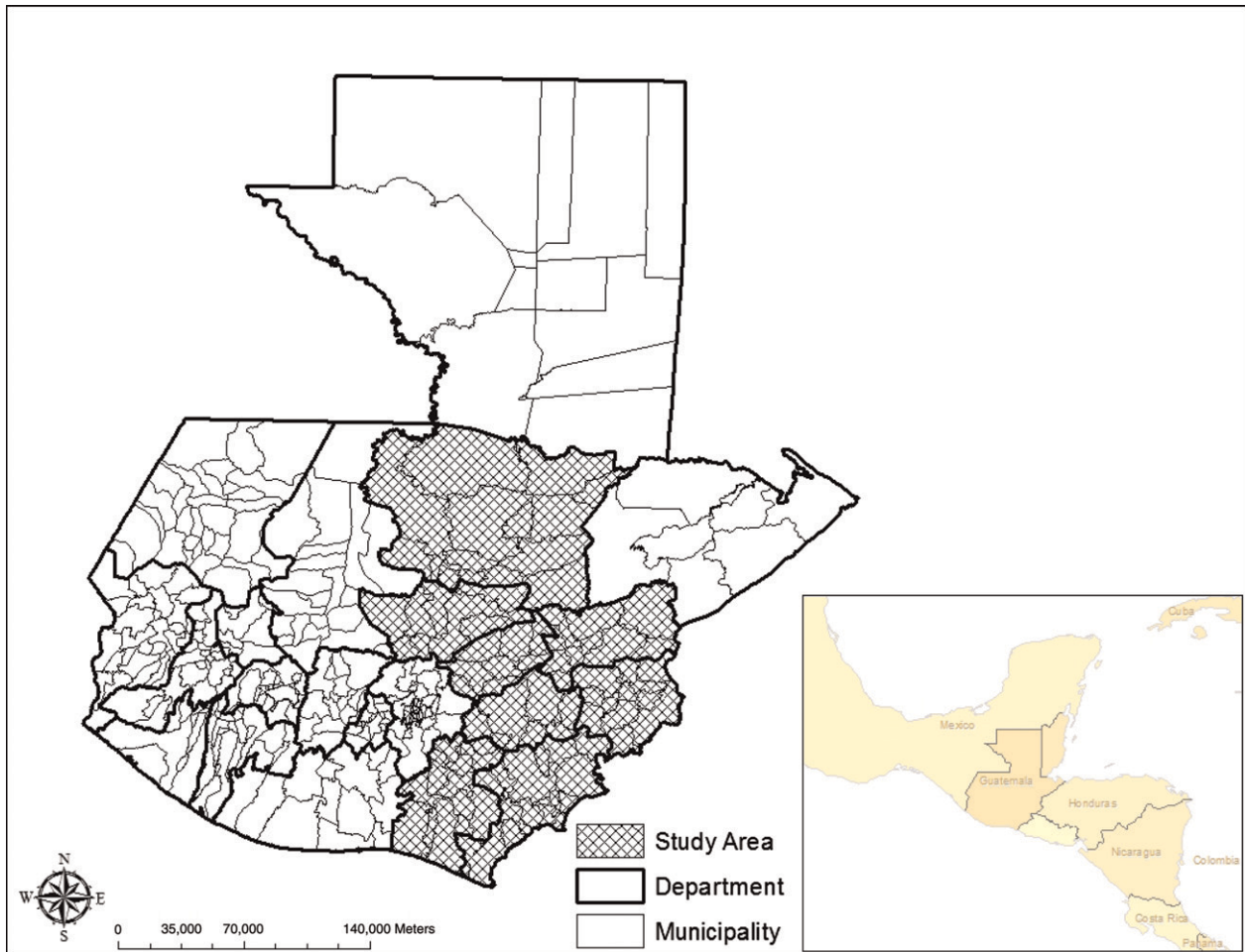


FIGURE 1. Guatemalan National Chagas Disease Control Program coverage area.

defined by the presence of bugs inside the home or in the peridomestic areas attached to the home. The data were collected for 4,419 localities in 10 departments during the baseline survey, and a subset of these localities was followed-up over the first two rounds of house spraying that occurred between 2002 and 2007. The locality was chosen as the unit of analysis, because no house-level data were available. Localities from two departments, Huehuetenango and Quiché, were excluded, because no geographical coordinates were recorded. The remaining eight departments included 3,987 localities, among which 3,571 had been surveyed at baseline and 2,317 had correct geographical coordinates. It was possible to obtain geographical coordinates for 402 localities based on their name and the information available on the United States Geological Survey's Earth Explorer (<http://edcns17.cr.usgs.gov/EarthExplorer/>). These 2,719 localities were then used in the analysis at baseline. However, not all localities were followed up over the course of the intervention because of insufficient resources. Thus, only 1,252 localities were followed up after the first spray, and 950 localities were followed up after the second spray.

We defined reinfestation as having a locality-level index of infestation greater than zero after spray. We then defined the time lapse between spray and evaluation in categories, reflecting the efficacy of the pyrethroid insecticides used by the program as per empirical data available and recommendations made by other CD vector control programs.<sup>21,22</sup> As such, we defined these groups as short (time lapse less than 180 days), medium (time lapse of 180–360 days), and long (time lapse of 360 days or more), with the short time lapse group serving as the reference group in our regression models.

In spray 1, 380 (30%) of 1,252 localities were missing information about the time lapse between spray and evaluation, whereas in spray 2, 334 (35%) of 950 localities were missing this information. As such, missing data were imputed using the municipality mean time lapse in days. After this imputation, the first spray was missing time lapse information for only 23 localities, whereas the second spray was missing this time information for 32 localities. In both cases, no time data had been recorded for the municipality. Moreover, 80 localities that were sprayed and evaluated in spray 2 did not have data recorded for spray 1, leaving 1,228 localities that contained all information relevant for inclusion in the regression analysis at spray 1 and 838 localities that contained all relevant information for inclusion in regression analysis at sprays 1 and 2. Finally, after incorporating the season in which spray was administered for the models of reinfestation after spray 1 and reinfestation after spray 2, these models were left with 1,156 localities and 663 localities, respectively.

**Statistical analysis.** We used the Getis-Ord  $G_i^*(d)$ , a local indicator of spatial autocorrelation, to assess if domestic infestation indicated any clustering pattern across the intervention area. This test enables one to delineate clusters of high or low values within a specified radius  $d$  of a given location  $i$ .<sup>23</sup> In this test, we defined the neighborhood as a fixed distance band of 24 km based on methodological and conceptual considerations. Specifically, because the evaluation after first and second rounds of spraying did not follow-up all locations surveyed at baseline, it was necessary to set the distance band for these tests at 24 km to ensure that each index location had at least one neighbor within the specified neighborhood. The minimum number of neighbors at spray 1 was 1 and the maximum

number of neighbors at spray 2 was 48, whereas at spray 1, the minimum number of neighbors was 1 and the maximum number of neighbors was 29. This distance would also be sufficient to capture the potential flight range of a vector up to 1,500 m as well as carriage by animals or humans.<sup>24</sup>

The results were corrected for multiple testing through the false discovery rate (FDR) procedure.<sup>25,26</sup> Location of clusters of high and low infestation was compared over time to assess persistence and changes in spatial patterns of vector presence after initiation of control. This comparison allowed us to create three additional variables: (1) high infestation cluster at baseline, indicating that a locality had fallen in a high infestation cluster at baseline according to the Getis-Ord  $G_i^*(d)$ , (2) high infestation cluster after first residual insecticide spray, indicating that a locality had fallen in a high cluster after the first round of spraying had been administered, and (3) high infestation cluster after second residual insecticide spray, indicating that a locality had fallen in a high cluster after the second round of spray with pyrethroid insecticides had been administered. High infestation cluster at baseline was meant to capture the effect of differences between localities that existed at baseline before the initiation of house spraying. Finally, categorical variables representing the quarter of the year in which spray was administered were created and included in the models of reinfestation after first residual insecticide spray and reinfestation after second residual insecticide spray. In these models, administration of spray in quarter 2 (April–June), quarter 3 (July–September), and quarter 4 (October–December) were each compared with the reference group, which was quarter 1 (January–March).

We used logistic regression to analyze how the time lapse between spray and evaluation affected infestation after the first and second rounds of spray. The association was evaluated in two ways. First, we used reinfestation as the dependent variable. Second, we used the variables high infestation cluster after first residual insecticide spray and high infestation cluster after second residual insecticide spray as dependent variables. Because we had data at three points in the intervention, we compared baseline and time after first spray as well as first and second sprays. In total, four different logistic regression models were used: (1) model 1 examined the outcome of reinfestation after first residual insecticide spray, (2) model 2 examined the outcome of high infestation cluster after first residual insecticide spray, (3) model 3 examined the outcome of reinfestation after second residual insecticide spray, and (4) model 4 examined the outcome high infestation cluster after second residual insecticide spray. Reinfestation was defined as having a locality-level index of infestation greater than zero. In all models, fit was assessed through examination of the log likelihood. Interaction terms were tested but not statistically significant.

## RESULTS

Infestation across the study area significantly decreased after the first round of spraying compared with baseline. The mean index of infestation at baseline was 15.33 and fell to 4.77. However, similar gains were not realized between the first and second sprays; the index of infestation decreased from 4.77 after spray 1 to 4.16 after spray 2, but this decline was not statistically significant. In addition, the difference in mean index of infestation at baseline between those localities

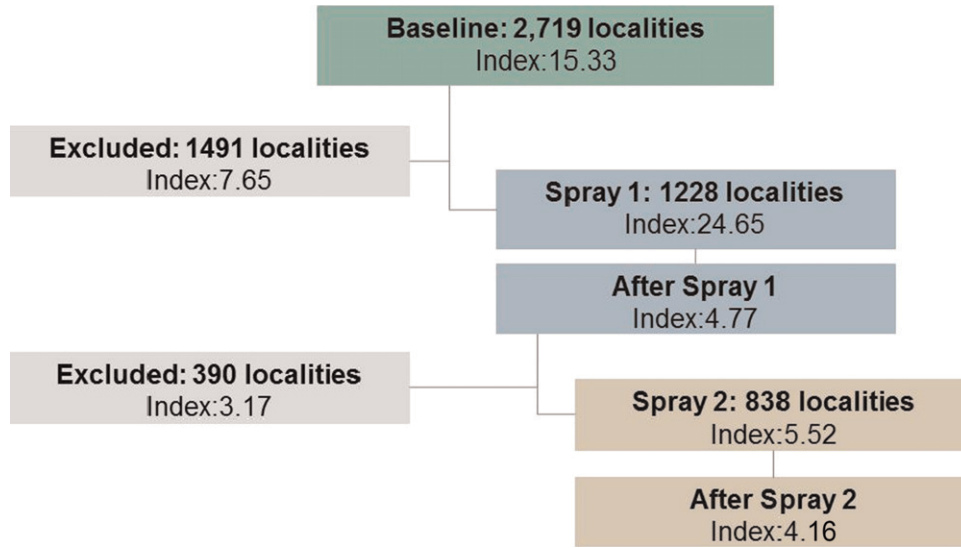


FIGURE 2. Progression of localities in the Guatemalan National Chagas Disease Program.

that were followed-up in the first round of spray (24.65,  $N = 1,228$ ) and those localities that were not followed-up (7.64,  $N = 1,491$ ) was statistically significant ( $P < 0.01$ ) and suggests the choice of which localities to follow-up in the program may have been targeted to areas of higher infestation. The same was true after the first residual spray, because those localities that were sprayed in the second spray administration had a higher mean index of infestation (5.52,  $N = 838$ ) than those localities that were not included in this second spray (3.20,  $N = 390$ ). Figure 2 depicts the progression of localities in the intervention. Finally, the mean number of days between spraying and evaluation differed notably by department for both spray 1 and spray 2 (Figure 3).

Maps of the results of the  $G_i^*(d)$  test at baseline and first and second sprays show the presence of high infestation clusters in the departments of Jutiapa, Alta Verapaz, and Chiquimula (Figure 4). After the first spray, the clusters became dispersed

throughout six of eight departments, but high infestation cluster areas remained most concentrated in Jutiapa, Alta Verapaz, and El Progreso. Finally, after the second spray, areas of high infestation remained concentrated in Jutiapa, with a large area of low infestation in Chiquimula. A summarized comparison of the clustering pattern over time indicated that 455 localities fell in same category of the  $G_i^*(d)$  test (not significant, high, or low) at both baseline and first spray and that 632 localities fell in the same clusters at both first and second sprays. Also, 178 localities fell in a high infestation cluster at all three time periods. This finding shows that there are localities that experience persistent infestation and may provide an indication of potential infestation hotspots that should be more intensely surveyed.

Logistic regression showed a strong effect of time between spraying and evaluation on both outcomes for the first spray (Tables 1 and 2). In this case, both a time lapse between spray

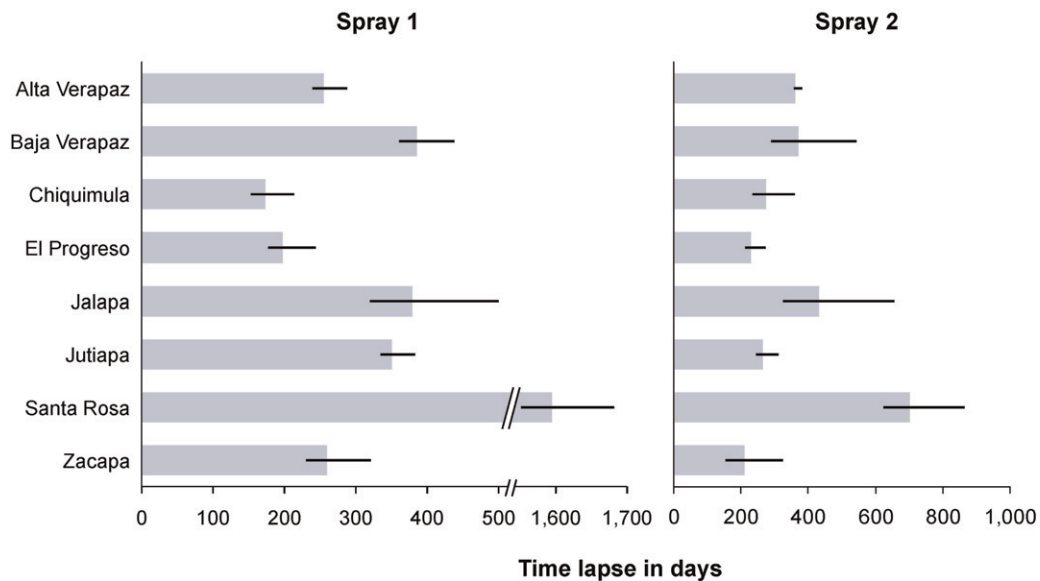


FIGURE 3. Mean time lapse between spray and evaluation at sprays 1 and 2 by department.



## Clustering of *Triatoma* Infestation in Guatemala

Source: National Chagas Disease Program,  
Ministry of Health

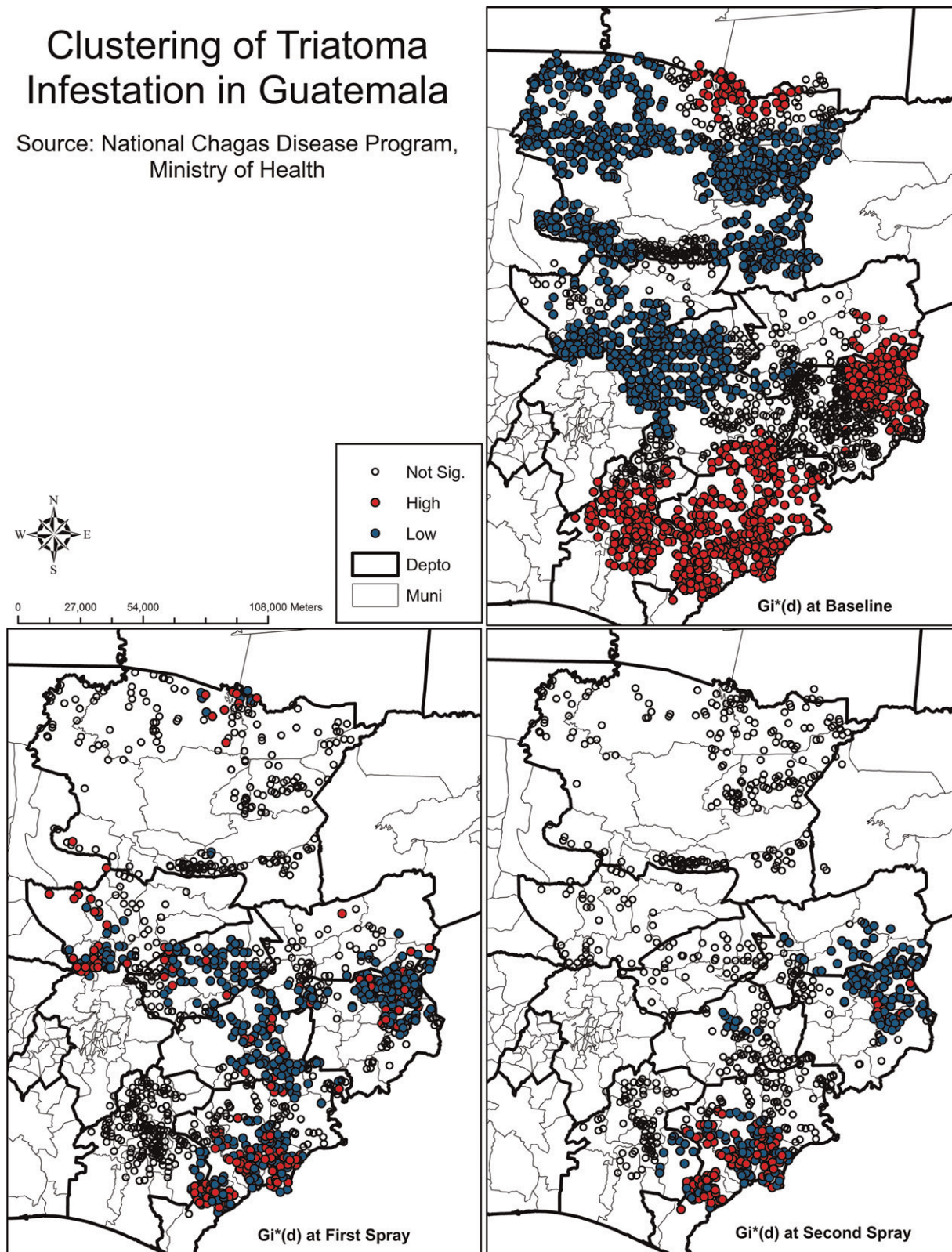


FIGURE 4. Results of Getis-Ord  $G_i^*(d)$  statistical test at baseline, spray 1, and spray 2.

and evaluation of 180–360 days (odds ratio [OR] = 1.54, 95% confidence interval [CI] = 1.07–2.23,  $P = 0.022$ ) and a time lapse of greater than 360 days (OR = 2.66, 95% CI = 1.85–3.83,  $P < 0.001$ ) were associated with statistically signifi-

cant increased odds of being reinfested compared with localities where this time lapse was less than 180 days. In addition, administering spray in the second quarter had a protective effect on the risk of reinfestation after first spray (OR = 0.68,

TABLE 1

Relationship between reinfestation after first residual insecticide spray and time lapse during first spray

Model 1 (N = 1,156)	OR	95% CI	P value
Time lapse 180–360 days	1.54	1.07–2.23	0.022
Time lapse > 360 days	2.66	1.85–3.83	< 0.001
High cluster at baseline	1.17	0.90–1.53	0.220
Quarter 2 vs. quarter 1	0.68	0.48–0.97	0.034
Quarter 3 vs. quarter 1	1.07	0.75–1.53	0.696
Quarter 4 vs. quarter 1	0.77	0.51–1.17	0.229

Quarter 1 = January to March; quarter 2 = April to June; quarter 3 = July to September; quarter 4 = October to December.

95% CI = 0.48–0.97,  $P = 0.034$ ), although administering spray in the third and fourth quarters had no statistically significant effect. These same independent variables had a more pronounced association with the outcome of falling in a high cluster after first spray. A time lapse between spray and evaluation of 180–360 days had an OR of 3.14 (95% CI = 1.85–5.35,  $P < 0.001$ ), a time lapse between spray and evaluation of greater than 360 days had an OR of 5.95 (95% CI = 3.57–9.92,  $P < 0.001$ ) and falling in a high cluster at baseline had an OR of 3.60 (95% CI = 2.61–4.96,  $P < 0.001$ ). This finding indicates that, although there is an association between baseline characteristics and the odds of being reinfested, the time lapse between spray and evaluation is also an important factor associated with reinfestation.

In the second spray (Tables 3 and 4), both a medium and long time lapse between spray and evaluation were not significantly associated with the odds of being reinfested after the second residual insecticide administration, whereas having been reinfested after first spray was a statistically significant predictor of subsequent reinfestation after second spray (OR = 2.12, 95% CI = 1.50–3.01,  $P < 0.001$ ). Quarter in which spray was administered was highly non-significant in the second spray. In contrast, a time lapse of 180–360 days was significantly associated with increased odds of falling in a high cluster after second residual insecticide spray (OR = 1.90, 95% CI = 1.36–2.63) as was having been reinfested after the first spray (OR = 2.56, 95% CI = 1.82–3.59,  $P < 0.001$ ), whereas a long time lapse was associated with a protective effect on this outcome (OR = 0.52, 95% CI = 0.37–0.72,  $P < 0.001$ ).

DISCUSSION

Our analysis indicated that the Chagas Disease Control Program of Guatemala has achieved overall and significant declines in domestic vector infestation after two rounds of house spraying. However, the results show that areas of high infestation have persisted over time, suggesting the presence of hotspots that should be more intensely surveyed. We also showed a significant impact of programmatic practices on reinfestation. Specifically, our results indicate that the time

TABLE 2

Relationship between falling in a high infestation cluster after first residual insecticide spray and time lapse during first spray

Model 2 (N = 1,228)	OR	95% CI	P value
Time lapse 180–360 days	3.14	1.85–5.35	0.000
Time lapse ≥ 360 days	5.95	3.57–9.92	0.000
High cluster at baseline	3.60	2.61–4.96	0.000

TABLE 3

Relationship between reinfestation after second residual insecticide spray and time lapse during second spray

Model 3 (N = 663)	OR	95% CI	P value
Time lapse 180–360 days	1.37	0.96–1.58	0.084
Time lapse > 360 days	1.17	0.81–1.68	0.411
Reinfested after spray 1	2.12	1.50–3.01	< 0.001
Quarter 2 vs. quarter 1	1.10	0.68–1.80	0.699
Quarter 3 vs. quarter 1	0.80	0.477–1.34	0.400
Quarter 4 vs. quarter 1	1.32	0.74–2.33	0.346

Quarter 1 = January to March; quarter 2 = April to June; quarter 3 = July to September; quarter 4 = October to December.

between the rounds of house spraying may have been too long, particularly in the first spray, which contributed to the occurrence of reinfestation in several localities.

There were statistically significant clusters of high infestation at each point in the intervention, and 178 localities remained in high infestation clusters throughout this period of the intervention, which may be relevant when targeting of program resources is necessary. Specifically, the areas of high infestation at baseline were primarily located in Chiquimula, Jutiapa, and Baja Verapaz, with a subset of localities in Chiquimula and Jutiapa persisting as high infestation clusters throughout both the first and second spray.

Our analysis also shows heterogeneity in the time lapse between spraying and evaluation and consequently, the time between rounds of spray, department, and its impact on the efficacy of the first round of pyrethroid insecticide administration. Long time lapses between spraying and evaluation resulted in similarly long time lapses between subsequent rounds of house spraying. We could show that those localities in which greater than 1 year passed between spraying and evaluation after the first round of house spraying had a substantially increased risk of reinfestation after the first spray. These findings are consistent with studies showing that the residual efficacy of deltamethrin insecticide decreases dramatically by 3 months after spray.<sup>21</sup>

Both prior experience from INCOSUR as well as existing international guidelines for CD vector control programs point to the importance of systematizing the time lapse between rounds of house spraying. In the case of INCOSUR, the guidelines governing the first phase of the vector control program in all participating countries prescribed two consecutive rounds of spraying performed 6 months apart in all houses, regardless of whether they were found by the program to be infested. These first rounds of spraying are considered an attack phase in which the goal is to control infestation to the greatest degree possible. This attack phase is then followed by a sustained surveillance phase in which evaluation is ongoing, and additional house spraying is performed in areas where infestation is found. In addition, the technical guidelines for CD control programming that are provided by the PAHO suggest that the post-spray evaluation should take place 3–6 months after spray, because

TABLE 4

Relationship between falling in a high infestation cluster after second residual insecticide spray and time lapse during second spray

Model 4 (N = 838)	Odds ratio	95% CI	P value
Time lapse 180–360 days	1.90	1.36–2.63	0.000
Time lapse ≥ 360 days	0.52	0.37–0.72	0.000
Reinfested after spray 1	2.56	1.82–3.59	0.000

this time frame is consistent with studies suggesting that the residual effect of pyrethroids tends to be highest during the first month after spray, with efficacy almost completely diminished in 3–6 months after spray is administered or sooner in peridomestic settings.<sup>21,22,27,28</sup> The results of this study, thus, confirm that failing to follow-up within 6 months of spray does, in fact, increase the risk of reinfestation and highlight the critical nature of these recommendations.

The effect of this inconsistent time lapse between spray and evaluation in the intervention also has implications for evaluation. Specifically, in areas where the time to post-spray evaluation was less than 6 months, estimates of reinfestation may be masked by a lack of follow-up in the period after the insecticide efficacy diminished. In contrast, in localities where this time to post-spray evaluation far exceeded the period of insecticide efficacy, reinfestation can be measured, although it is difficult to determine whether the bugs were exterminated during the first round of spraying and subsequently returned or whether the insecticide was not effective in fully eliminating bugs from the house. In addition to residual insecticide efficacy, it has been suggested that proximity to untreated foci is a cause of domestic reinfestation in the case of *Triatoma* vectors.<sup>29</sup>

Spraying in the second quarter of the year was protective for reinfestation after first spray. This result confirmed previous findings indicating that a maximum reduction in triatomine abundance is achieved when insecticide spray is administered in early April, just as the transient seasonal infestation with *T. dimidata* begins on the Yucatan peninsula.<sup>30,31</sup> In these previous studies, the effectiveness of spray decreased slightly if administered in May or June and dropped to a much lower level during the remaining month of the year. Although this effect of season of spray administration was present in the first spray, season did not impact reinfestation after second spray.

This work offers additional evidence of the use of spatial analysis for planning and evaluation in an infectious disease control program, specifically in regards to targeting resources to areas with persistent infestation. We show a straightforward approach to assess hotspots or areas of concern in terms of reinfestation after spray in the context of an extensive vector control intervention and highlight potential gains that can be captured through simple alterations to programmatic practices. It has been shown previously that spatial analysis can be used to improve and evaluate CD control programs by optimizing spraying strategies,<sup>32,33</sup> monitoring reinfestation,<sup>34</sup> and targeting screening for *T. cruzi* in children<sup>35</sup> as well characterizing discordant results of tests for *T. cruzi* infection.<sup>36</sup>

In the future, it is critical that the time between spray and evaluation be systematized in accordance with the technical guidelines provided by the PAHO such that houses in need of subsequent sprays be administered insecticide in a timely manner.<sup>27</sup> Furthermore, more consistent data should be collected to determine the true infestation status of all localities. It may be advantageous for the program to consider seeking resources for a second national infestation survey in which accurate spatial coordinates for all localities are collected and the sampling of infestation is either exhaustive or performed using a consistent sampling fraction. If performed, such a framework should be maintained in future surveillance and evaluation. This sampling would allow the intervention to gauge existing hotspots as accurately and completely as possible across the coverage area. Although such a survey would require an initial investment of time, personnel, and resources, it would

provide new national estimates on which future surveillance, monitoring, and evaluation could be conducted.

This study has several limitations. First, not all localities were followed over time, resulting in a decrease in points that could be included in the analysis over the course of the two sprays and limiting the generalizability of the results. Second, missing spatial coordinates and information about the time lapse between spray and evaluation was another limitation, although we made an effort to overcome this limitation through manual matching of latitude and longitude and imputation of missing time data through use of the municipality average time lapse. Third, the data do not provide information about vector species across all time points and locations, which prevented a separate analysis of reinfestation by each of the two major vectors over the intervention. Although not a limitation to the analysis contained here, such information would have been useful in further illuminating the accomplishments of and challenges to the intervention. Fourth, survey of vector infestation was not performed using a consistent sampling fraction per locality, a fact that may have resulted in underestimation of the true level of infestation, particularly in larger localities where a smaller proportion of homes were sampled.

The significance of having been in a high infestation cluster at baseline on subsequent reinfestation after first spray suggests that there are other factors that may be contributing to the distribution of baseline infestation and in turn, the variation in reinfestation across space after first spray. In addition, we could not show a similar affect for the second time lapse as for the first spray, which again, may indicate the importance of other factors that were not captured in our analysis. Additional investigation into socioeconomic and environmental variables, such as house material type, elevation, and seasonality of spray,<sup>30</sup> are warranted to characterize these factors. Also, in this context, the effect of sequential spray strategies on reinfestation seems of interest; however, conducting such an analysis using standard longitudinal methods bares the risk of introducing time-varying confounding. Future studies may use methods, such as marginal structural models, that allow for the estimation of unbiased results in such situations.

In addition to these limitations, it is important to note that the clustering pattern of infestation found using the Getis-Ord  $G_i^*(d)$  test was not fully consistent with expectations based on a baseline seroprevalence survey in children undertaken by the Universidad del Valle de Guatemala in 1998–2000 before the initiation of spraying.<sup>9</sup> Although this inconsistency may seem anomalous given the known relationship of infestation and infection,<sup>37</sup> these findings may have been caused by inconsistency in the sampling frames of the seroprevalence study, the school-based study, or the vector infestation data collection, a house by house sampling design, or the insensitive nature of standard methods for bug detection.

In conclusion, two important policy recommendations can be offered based on the results of this study. First, the timing between rounds of house spraying should be systematized in accordance with existing technical guidelines to both increase the potential efficacy of the vector control program and ensure accurate evaluation of the program in the future. Although this timing requires careful consideration of obstacles, such as resource shortages and other acute disease outbreaks, evidence indicates that it would greatly improve the effectiveness of the program. Second, this analysis provides support for a recommendation that the program consider implementing a more



extensive monitoring and evaluation system and a suggestion that this system may be based on the use of spatial analysis. By using spatial methods such as those methods used here, the program would be able to track hotspots of reinfestation and optimize targeting of resources to those areas.

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Note: Supplemental Appendix appears at [www.ajtmh.org](http://www.ajtmh.org).

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