# Comparative Field Trial of Alternative Vector Control Strategies for Non-Domiciliated *Triatoma dimidiata*

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*Abstract.* Chagas disease is a major vector-borne disease, and regional initiatives based on insecticide spraying have successfully controlled domiciliated vectors in many regions. Non-domiciliated vectors remain responsible for a significant transmission risk, and their control is a challenge. We performed a proof-of-concept field trial to test alternative strategies in rural Yucatan, Mexico. Follow-up of house infestation for two seasons following the interventions confirmed that insecticide spraying should be performed annually for the effective control of *Triatoma dimidiata* ; however, it also confirmed that insect screens or long-lasting impregnated curtains may represent good alternative strategies for the sustained control of these vectors. Ecosystemic peridomicile management would be an excellent complementary strategy to improve the cost-effectiveness of interventions. Because these strategies would also be effective against other vector-borne diseases, such as malaria or dengue, they could be integrated within a multi-disease control program.

## INTRODUCTION

 Chagas disease is a vector-borne parasitic disease widely distributed in Latin America that affects 9.8 to 11 million people; approximately 60 million individuals are at risk of infection. 1,2 It is caused by the protozoan parasite *Trypanosoma cruzi* , which is transmitted to humans primarily by hematophagous triatomine vectors. Prevention of Chagas disease relies primarily on vector control strategies and the screening of blood donors; several regional initiatives in the Americas have helped in the implementation of these strategies. Vector control interventions are based on the elimination of domiciliated triatomines by indoor residual insecticide spraying associated, when possible, with housing improvements. This has led to a large reduction in house infestation by triatomines (particularly *Triatoma infestans* and *Rhodnius prolixus* ), and a corresponding reduction in Chagas disease transmission to humans. $1-3$ 

 However, several triatomine species do not establish permanent domestic colonies and can occasionally infest domestic habitats by immigration from peridomestic and/or sylvatic habitats. These include *R. prolixus* in Venezuela,<sup>4</sup> Triatoma *brasiliensis* and *Triatoma pseudomaculata* in Brazil,<sup>5</sup> Triatoma mexicana in central Mexico,<sup>6</sup> and *Triatoma dimidiata* in the Yucatan peninsula, Mexico, and Belize.<sup>7,8</sup> With the control of domiciliated triatomines well underway, the control of house infestation by non-domiciliated triatomine vectors is becoming a key challenge for Chagas disease control.<sup>2,9–11</sup> Indeed, conventional insecticide spraying has a rather limited and short-lived effect on house infestation because of the rapid re-infestation by immigrating peridomestic and/or sylvatic insects. 4,12,13 In addition, insecticide resistance is emerging as a threat to conventional vector control interventions. 14 Accordingly, it is of key importance to investigate the potential of alternative strategies, such as insect screens, long-lasting

insecticide-impregnated curtains, or ecosystemic environmental management.<sup>11,15</sup>

 We previously used mathematical modeling to optimize the control of non-domiciliated *T. dimidiata* in Yucatan, Mexico.<sup>13</sup> In this region, house infestation occurs on a marked seasonal basis because of the influx of sylvatic and peridomestic insects to the houses. 7,16–19 We found that optimum insecticide efficacy required spraying just at the beginning of the house infestation season, even though such application was predicted to be effective only until the following infestation season. 13 Alternatively, insect barriers aimed at reducing bug entry were predicted to provide a reduction in house infestation proportional to exclusion efficacy and would be sustainable for several years. 13 This was in agreement with the identification of insect screens as a significant protecting factor against house infestation by *T. dimidita* in an urban area in the Yucatan.<sup>20</sup> Long-lasting insecticide-impregnated curtains may also represent an alternative (chemical) barrier strategy, and they have been suggested to have potential against non-domiciliated *Rhodnius* spp. in a 30-day pilot field trial<sup>21</sup>; however, no long-term evaluations have been performed to date, so their efficacy remains to be assessed.

 Conversely, ecosystemic approaches are gaining increasing support in public health and suggest that appropriate environmental management may allow for an effective and sustained control of a variety of vector-borne diseases.<sup>22-24</sup> Ecosystemic approaches based on peridomicile cleaning or housing improvements for the control of *T. dimidiata* have been quite promising. 25,26 In the case of non-domiciliated *T. dimidiata* , the identification of the backyard/peridomicile and their characteristics as major risk factors for house infestation<sup>20</sup> lend further support to the feasibility of peridomicile management for vector control.

 In this study, we present the first long-term comparative evaluation of alternative vector control strategies to provide proofof-concept data on their efficacy to reduce house infestation by non-domiciliated *T. dimidiata* . We compared insect barriers, including insect window screens and long-lasting insecticideimpregnated curtains, environmental management of peridomiciles, and conventional indoor insecticide spraying for their efficacy at controlling *T. dimidiata* over a 2-year period.

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## MATERIAL AND METHODS

 **Study area and design.** The study was conducted in four rural villages about 15 to 20 km apart in the state of Yucatan, Mexico and located in the region presenting the highest risk of *T. cruzi* transmission. 27 The villages, Bokoba (21.01°N, 89.07°W), Sanahcat (20.77°N, 89.21°W), Sudzal (20.87°N, 88.98°W), and Teya (21.05°N, 89.07°W), were comprised of 494, 381, 297, and 518 households, respectively, and possessed very similar housing and socioeconomic conditions (see later). We tested four different vector control interventions, which were applied to different subsets of houses from volunteer participants in the villages of Bokoba, Teya, and Sudzal. Interventions included conventional indoor residual insecticide spraying  $(N = 264)$ houses in Bokoba), window insect screens  $(N = 19)$  houses in Teya), long-lasting insecticide-impregnated curtains  $(N = 25$ houses in Teya), and peridomicile management ( $N = 50$  houses in Sudzal). The remaining houses from these villages served as control houses without vector control intervention. The village of Sanahcat was used as an additional control without intervention. The presence of triatomine in houses with and without interventions was monitored continuously during the 8 months before the interventions were applied and during the 20 months after their implementation. We were thus able to evaluate efficacy over two consecutive house infestation seasons.

 **Housing characteristics.** Residents from 15 participating households were randomly selected in each village and were interviewed using a standardized questionnaire to gather data about the house, its surroundings, the inhabitants, and their habits. 20 Variables included the number of rooms, the number of bedrooms, roof, wall and flooring types and materials, the presence of insect screens on windows and doors, the type of adjacent premises (inhabited house, park, abandoned lot, etc.) the size of the backyard, the type of wall around the property, the presence of trees and vegetation, the presence of junk or trash material in the yard, indoor or outdoor insecticide spraying by the households and, if applicable, spraying frequency, and the number and species of domestic animals. Variables related to the inhabitants and their habits included the number of adults and children, their places of birth, the amount of time they had lived in the house, the profession of the head of the family, each family member's sleeping habits (bed or hammock), whether they had been bitten by a triatomine, and their knowledge of Chagas disease.

 **Insecticide spraying.** For insecticide spraying, participating houses from the village of Bokoba were sprayed with 50 mg/m<sup>2</sup>

of cyfluthrin (Solfac 10 WP, Bayer Environmental Science, Mexico). Because previous modeling studies indicated that the timing of insecticide spraying is critical, 13 it was performed between March 15 and March 30, 2007, just as the seasonal house infestation period was beginning, to maximize its efficacy. Three teams of two sprayers each were recruited among the villagers. Sprayers were initially trained for two days by specialized personnel from the Department of Vector Control of the Yucatan Health Services to ensure spraying quality. Following training, they sprayed 15–20 houses/day, under the constant supervision of vector control personnel from the Ministry of Health. Spraying covered the walls and ceilings of the living room and bedrooms of each home.

 **Insect screens and insecticide-impregnated curtains.** Insect screens were constructed by a local woodworker and consisted of standard mosquito plastic mesh fixed on a wooden frame. Frames were then nailed to the outside of the bedroom windows, with an average of three screens per house (Figure 1A). For insecticide-impregnated curtains, we used wool fabric, which has been reported as highly absorbent and able to maintain the insecticide residual activity for the longest period of time. 28 Curtains of the appropriate size of each window were custom-made and impregnated with  $600 \text{ mL/m}^2$  of a solution containing K-O Tab 1-2-3 tablets (Bayer Environmental Science) to achieve a deltamethrin dose of 30 mg/m<sup>2</sup>. This formulation provides a wash-resistant residual activity for at least 3 years. 29 Curtains were dried on a flat surface and were then taken to each house and hung from metal wire on the inside of the bedroom windows and doors (Figure 1B). Both screens and curtains were installed in the houses during the month of April 2007. Two houses received a combination of both insect screens and impregnated curtains because of their specific structures.

 **Peridomicile management.** This strategy consisted of the cleaning of the peridomicile followed by insecticide spraying to reduce or eliminate potential triatomine refuges and colonies from this area. Volunteer households from the village of Sudzal were requested to eliminate all junk, wood or rock piles, trash, and other unnecessary objects from their yards, and the collection of all discarded materials was organized through the local authorities. All places where objects had been removed, and potential animal burrows and the stone walls surrounding the peridomiciles, were sprayed with 50 mg/m<sup>2</sup> of cyfluthrin (Solfac 10 WP) from April 2–10, 2007, as described previously for indoor insecticide spraying.

 **Entomologic surveillance and monitoring.** Throughout the entire study, entomologic monitoring was performed through



FIGURE 1. Examples of insect screens and impregnated curtains used. A, Insect screens were fixed on the outside of the houses. **B**, Long-lasting impregnated curtains were hung from a metal wire on the inside of the windows. This figure appears in color at www.ajtmh.org .

community participation. 7,18 Public workshops open to all interested inhabitants were organized in the study villages during July 2006 to provide information on Chagas disease, the vector, and the project. Volunteer households were instructed to collect triatomines found inside their homes in plastic vials/ bags without directly touching the bugs, to label the vials/bags with their name, address, and date of capture, and to deposit the vials/bags at the local health center in their village. Although we attempted to collect additional information on the insects (dead/alive, location, time of capture, etc.), we obtained limited and unreliable information that could not be taken into account in the analysis. Insects were stored at each health center, together with a hard copy database of the collected insects. To maintain household interest and participation throughout the study, additional workshops were held every 4 to 6 months in each village, including special awareness workshops performed in primary schools. Chagas disease awareness posters were also permanently displayed in public places, and leaflets were distributed at the local health center. All of the collected insects were taken to the laboratory every 2–3 weeks, from August 2006 to November 2008, for further identification (species, sex, stage) and analysis. Geographic coordinates of all inhabited houses from the villages (including infested houses) were obtained with a hand-held global positioning system (GPS). All data were imported into a geographic information systems (GIS) database in ArcView 3.2 (Environmental Systems Research Institute, Redlands, CA). Maps showing the distribution of houses with vector control interventions, and houses with infestation, were produced and used to calculate distances between them. The distance of each house to the bushes/agricultural land/forest areas surrounding the village was also calculated.

 **Household interviews.** Subsets of 15 households from each vector control intervention group were interviewed using a structured questionnaire to investigate their perception and acceptance of the intervention at the end of the first infestation season.

 **Data analysis.** Comparisons of household characteristics between villages were performed by  $\chi^2$  tests and analysis of variance (ANOVA). Triatomine collection data were used to calculate the abundance of triatomine in houses from the different control intervention groups, and from control houses without intervention, on a bi-monthly basis so that the natural

seasonal variations in triatomine abundance could be taken into account. Confidence intervals (95%) of triatomine counts were calculated based on a Poisson distribution. Efficacy of the interventions was defined as the percentage of reduction in triatomine abundance in the respective intervention groups, as compared with triatomine abundance in the absence of intervention. Efficacy was calculated for each infestation season and it is given with its 95% confidence interval. For spatial analysis of the interventions, we determined the number of infested houses surrounding (within 200 m) houses with or without vector control interventions. We also calculated the average distance of infested houses from houses with or without vector control interventions. Comparisons between groups were performed by the Wilcoxon test and by an empirical permutation test.<sup>30</sup> The cost of the interventions was determined by recording the cost of all the materials required and estimating the labor time associated with each intervention per house.

## RESULTS

 **Housing characteristics.** We first determined the housing characteristics of the four study villages (Table 1). Households from all four villages had similar characteristics, with no significant differences between villages. A typical household consisted of about 5 to 6 people, living in a house of 2 to 3 rooms with 1 to 2 bedrooms. The majority of houses (75%) were made of concrete or blocks; only 25% were made of adobe/wooden stick walls and thatched roofs. A few houses had insect screens on the windows (30%), and sporadic indoor insecticide use was common (72%). Most houses were surrounded by relatively large yards  $(400-800 \text{ m}^2)$  with vegetation, often enclosed by piled-stone walls (58%). Yard maintenance (cleaning, trimming) was generally poor and infrequent. Most households had domestic animals in their yard (90%), and most had a corral enclosure for them (60%). About half of the households reported having seen triatomines in their house before, and half mentioned having been bitten as well. Thus, overall living conditions were very comparable between the different villages.

 **Efficacy of vector control interventions.** Vector control interventions, including conventional indoor residual insecticide spraying, peridomicile management, and the installation of





\* Continuous data are presented as mean ± SEM.

insect screens and insecticide-impregnated curtains, were then applied to subsets of houses, and triatomine abundance in the houses was monitored for up to two consecutive infestation seasons. First, because of each house's location in the village, and particularly each house's distance from the surrounding forest/bushes, is a key factor for house infestation by non-domiciliated triatomines,<sup>31</sup> we ensured that houses receiving control interventions were located at a comparable distance from the periphery as houses without vector control interventions (permutation test  $P = 0.13$ ,  $P = 0.39$ , and  $P = 0.10$ in the villages of Sudzal, Teya, and Bokoba, respectively) and thus were exposed to a comparable risk for infestation.

 Insect collections in houses without interventions showed a typical seasonal infestation pattern as described previously 7,17 and data from control houses from all four villages were thus pooled (Figure 2). Most infestation occurred during the months of March–July, and very few bugs were collected during the rest of the year. As expected, indoor insecticide spraying was able to almost completely eliminate *T. dimidiata* from sprayed houses very quickly after spraying in late March (Figure 2A). This corresponded to a reduction of 75% [58.7–86.8] in triatomine abundance during the first infestation season. Because we were unable to reliably determine if the bugs collected after insecticide application were dead or alive, this was likely an underestimation of insecticide efficacy. Furthermore, vector control by insecticide spraying appeared to only last until the following infestation season, when sprayed houses were reinfested to a level comparable to houses without interventions (Figure 2A).

 Insect screens ( Figure 2B ) and insecticide-impregnated curtains (Figure 2C) were able to quickly reduce triatomine abundance in the houses by over 96% ([82.6–100] and [81.8–100], respectively) after their installation. However, this effect was

well sustained for the second infestation season, and triatomine abundance remained reduced by over 87% during that time ([67.8–96.5] and [76.4–97.7], respectively). Reinfestation of houses with screens was mostly concentrated in a single house, which turned out to be colonized, as evidenced by the collection of nymphal stages in the house and in the peridomicile. Reinfestation occurred in five houses with impregnated curtains but was limited to very few insects.

 Contrary to previous interventions, the effect of peridomicile cleaning and partial insecticide spraying was not immediate, as there was a delay of about 2–3 months before observing a reduction in triatomines inside the houses (Figure 2D). Because of this time lag, overall reduction in triatomine abundance only reached 52% [31.4–67.7] during the first infestation season. However, some effect of the intervention was still observed during the second infestation season, during which an efficacy of about 62% [36.8–79.9] appeared to be maintained.

 **Spatial analysis of house reinfestation and efficacy.** To evaluate if house reinfestation following vector control was affected by a house's location in the villages, we compared the distance to the surrounding bushes and forests from houses with control interventions that were reinfested or not. Following insecticide spraying in Bokoba, reinfested houses were located significantly closer to the surrounding bushes than non-reinfested houses ( $125 \pm 14$  versus  $148 \pm 6$  m, respectively, permutation test,  $P = 0.043$ ). Reinfestation following peridomicile management in Sudzal and insect screening and impregnated curtains in Teya also tended to occur in houses closer to the surrounding bushes, but this did not reach statistical significance, possibly because of the small number of reinfested houses (93  $\pm$  22 versus 107  $\pm$  9 m in Sudzal, and  $141 \pm 42$  versus  $148 \pm 16$  in Teya, respectively).



FIGURE 2. Temporal variations in triatomine abundance. Bimonthly variations in triatomine collections per house with the 95% confidence interval (CI) based on a Poisson distribution is shown for houses with (closed circles) and without (open circles) vector control interventions. **A** , Insecticide spraying, **B** , insect screens, **C** , long-lasting impregnated curtains, and **D** , peridomicile management. The arrows indicate the time when interventions were applied. Shaded areas indicate the seasonal infestation period used to calculate the efficacy of each intervention, which is also given with its 95% CI.

Village	Intervention	Number of infested houses $< 200 \text{ m}$	Distance to infested houses $(m)$
Bokoba	No intervention	$5.1 \pm 0.2$	$554 + 2$
	Insecticide spraying	$5.1 \pm 0.2$	$559 + 2$
Teva	No intervention	$3.4 + 0.1$	$537 + 1$
	Screens/curtains	$3.4 \pm 0.3$	$547 \pm 4$ ±
Sudzal	No intervention	$5.1 \pm 0.1$	$467 + 2$
	Yard management	$4.4 \pm 0.4$	$480 \pm 5$ ±

TABLE<sub>2</sub> Effect of control intervention on surrounding houses\*

\* Data are presented as mean SD.

 $\ddot{\tau}$  and  $\ddot{\tau}$  indicate a significant difference (Wilcoxon test,  $P = 0.040$ ; and permutation test,  $P = 0.010$ ; respectively.

 We also explored the potential effect of the different vector control interventions on surrounding houses. For this, we compared the average number of infested houses within 200 m of houses with or without vector control interventions, and the average distance of infested houses from these. Indoor insecticide spraying had no effect on infestation in surrounding houses, as a similar number of infested houses and average distance to infested houses were observed for houses with or without insecticide treatment (Table 2). The use of insect screens and/or impregnated curtains also had no effect on the number of infested houses around the treated houses. Nonetheless, it resulted in infested houses being somewhat further from houses with screens/curtains compared with houses with no interventions, suggesting a small reduction in infestation around houses with screens/curtains (Table 2). Interestingly, peridomicile management appeared to have a clear effect on surrounding houses. Indeed, there were significantly less infested houses around houses with cleaned peridomiciles compared with houses with no peridomicile management (Wilcoxon test, *P* = 0.040). Furthermore, infested houses were located further from houses with managed peridomiciles than from houses without interventions (Table 2, permutation test,  $P = 0.010$ ).

 **Household acceptation and cost.** We studied household perception of the vector control interventions by conducting interviews on a subset of 15 households from each intervention group at the end of the first infestation season. Most households (> 93% [14/15]) for each intervention were pleased with the interventions, accepted them well, and reported seeing less insects and particularly triatomines in their houses. For insecticide spraying, only 6% (1/15) felt that spraying had been ineffective, 13% (2/15) complained about the bad smell of the insecticide when sprayed, and another 13% (2/15) complained about the trouble of having to remove and re-install their belongings to allow for spraying. Satisfaction and acceptance were excellent (100%, 15/15) for both insect screens and impregnated curtains, and the only complaints were related to the quality of the materials used (20% of households [3/15]). For peridomicile management, 26% (4/15) of households declared that the intervention had no effect and that they still found triatomines in their houses as before the intervention.

We also assessed the costs of the different strategies (Table 3). Insecticide spraying and peridomicile management were the cheapest interventions, with an estimated cost of \$5–6/house. Insect screens had a somewhat lower material cost than impregnated curtains but required more labor for their manufacture and installation, so that both had an overall cost of \$40–50/house. However, for houses with already installed curtains, the cost would be limited to the impregnation, which was approximately \$2–3/house (Table 3).

TABLE 3

Cost of interventions				
Intervention	Materials	Labor	Total*	
Insecticide spraying	$$2-3/house$ 2 h/house		$$5-6/house$	
Insect screens	$$20-30/house$ 7 h/house		\$40-50/house	
Impregnated curtains	$$30-40/house$ 2 h/house		$$40-50/house$	
		\$1-2/house† 30 min/house†	$$2-3/house$	
Peridomicile management \$2–3/house 2 h/house			$$5-6/house$	

 \* Total cost was estimated based on a wage of \$1.5/h, which represents about 3 times the Mexican minimum wage.

 † For impregnated curtains, cost was also calculated without the manufacture and installation of curtains, i.e., considering only impregnation.

## DISCUSSION

 The control of non-domiciliated triatomines presents a major challenge. We performed the first long-term evaluation of alternative vector control strategies to provide proofof-principle data on their usefulness. Our data clearly showed that indoor residual insecticide spraying can provide over a 75% reduction in house infestation by triatomines after application, but this effect was not sustained during the subsequent infestation season, even though insecticide was applied at the optimum time of year, as determined previously, $13$  confirming that effective insecticide spraying would need to be annual. On the other hand, the use of physical and chemical barriers (insect screens and long-lasting impregnated curtains, respectively) provided a high level of reduction in triatomine abundance (> 87%) during at least two infestation seasons. These results are in very good agreement with our previous modeling predictions<sup>13</sup> and confirm the usefulness of such modeling approaches to optimize vector control interventions. Although the actual efficacy of insect screens or impregnated curtains was difficult to predict, our field data clearly show that they are able to considerably reduce the influx of immigrant triatomines inside houses. This is in agreement with studies of greenhouse screens, which have been found to be able to exclude over 90% of insects. 32 On the other hand, we observed that the efficacy of impregnated curtains was similar to that reported previously against domiciliated *T. infestans* in Argentina<sup>33</sup> and somewhat higher than that observed against non-domiciliated *R. prolixus* in Venezuela (60%). 21 These differences in efficacy may be caused by differences in insecticide and dose used for impregnation (30 mg/m<sup>2</sup> in our study versus 12.5 mg/m<sup>2</sup>,<sup>21</sup> or  $1-200$  mg/m<sup>233</sup>).

 Peridomicile management, which aims at reducing or eliminating peridomestic triatomine colonies acting as sources for house infestation, resulted in only a partial reduction in insect abundance in the houses (about 50%), mostly because of a time lag in its effect during the first infestation season. This seems less effective than a peridomestic ecological control intervention in Costa Rica, which resulted in a strong reduction in house infestation over a 1-year period.<sup>34</sup> The lower efficacy we observed may be explained by a direct influx of sylvatic bugs into the houses, independent from peridomestic populations. Indeed, population genetics analysis indicated that house infestation was caused by an influx of both peridomestic and sylvatic insects, although the relative contribution of each source remained unclear.<sup>16</sup> However, in agreement with the Costa Rican study, $25$  we found that the reduction in house infestation achieved by peridomicile management may be sustained, as bug collections remained somewhat lower during the second infestation season as well. In addition, spatial analysis revealed that peridomicile management had an additional protective effect on the houses surrounding the treated peridomicile. This suggests that a colonized yard may serve as a source of infestation for many houses surrounding it.

 The evaluation of the acceptability of the intervention and satisfaction of householders performed at the end of the first infestation season indicated that all of the alternative interventions were usually well accepted by the participants, and their perceptions of their efficacy matched well with the actual reduction in bug infestation observed. Indeed, only in the case of peridomicile management did some householders complain about the inefficacity of the intervention to reduce house infestation. These data suggest that large-scale implementations of these strategies may be feasible, provided appropriate community participation can be fostered, in agreement with previous studies. 21,34,35

 Although we made no attempt at optimizing costs at this stage, our study provides some relevant information for implementation. Insecticide spraying had a cost within the lower range of that reported in other studies (\$7-48/house).<sup>26,36-40</sup> However, it should be noted that we did not include transportation costs in our estimate. Impregnated curtains and insect screens had a higher cost, as do most housing improvement strategies, <sup>26,35</sup> but their sustainability would likely make them more cost-effective in the long term. Furthermore, because many houses already have curtains in place, a program based on insecticide impregnation alone would have a much lower cost (\$2–3/house), comparable to that of bednet impregnation, 40–42 and would thus be very affordable. Peridomicile management was also rather economical, as noted before.<sup>34</sup>

 In conclusion, our trial confirmed previous modeling predictions that insecticide spraying would need to be annual for an effective control of non-domiciliated *T. dimidiata* , but that insect screens or long-lasting impregnated curtains may represent good alternative strategies for a sustained control of these vectors. 13 Ecosystemic peridomicile management, while not effective enough on its own, would be an excellent complementary strategy to improve the overall cost-effectiveness of interventions. It is also important to stress that peridomicile management, as well as insect screens and impregnated curtains (and variants of long-lasting impregnated materials), are also effective against other vector-borne diseases, such as malaria $41,43$  or dengue, $44$  so that such strategies may be integrated within a multi-disease control program that would further optimize its cost effectiveness.

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