PREVENTION OF THE TRANSMISSION OF CHAGAS’ DISEASE WITH PYRETHROID-IMPREGNATED MATERIALS

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Abstract. Interrupting the transmission of Chagas’ disease using insecticide-treated materials could be a cost-effective option, particularly for sylvatic vectors, which enter houses at night. A randomized trial was undertaken that included all houses in two communities in regions endemic for this disease, one in Venezuela (50 houses) and one in Colombia (47 houses). After a baseline study (including a short questionnaire survey, entomologic assessment, and Chagas’ disease serology), each household was randomly allocated to either the intervention group, which used pyrethroid-impregnated bed nets, or the control group, which used unimpregnated bed nets. Serologic analysis of children in the baseline study showed active transmission of Chagas’ disease in the Venezuelan community (10.7% of 103 children were positive), but none in the Colombian community (0% of 100 children were positive). Vectors were sylvatic and bugs entered the houses at night in both communities. The efficacy of pyrethroids against triatominine vectors was tested in Venezuela by exposing Rhodnius prolixus to lambdacyhalothrin-impregnated fabrics and in Colombia by residual house spraying with deltamethrin. This randomized trial showed that in both countries users of impregnated bed nets were well protected from vector bites (immediate benefit). The long-term effect on the community was high vector mortality. In Venezuela, all 62 vectors detected (mainly R. robustus) died within 72 hours of contact with impregnated bed nets. In houses that used unimpregnated nets, only 24.5% (13 of 53) of the vectors died (P < 0.001). The vectors most likely came from infested palm trees and they maintained transmission of the disease in this community (28.1% of 629 R. robustus were positive for Trypanosoma cruzi). Bioassays showed that the mortality rate of R. prolixus was 100% on lambdacyhalothrin-impregnated materials. In addition, in Colombia (the main vector was R. prolixus), the effect of repellent on vectors (driving them away from infested nets) was significant. Thus, users of impregnated bed nets are well protected from transmission of Chagas’ disease, and vector reduction or elimination can potentially be achieved in areas infested with R. prolixus and R. robustus.

INTRODUCTION

Eliminating the transmission of Chagas’ disease, along with substantially reducing its vectors, has a highly favorable cost-benefit ratio.1,2 This has been largely successful through residual house spraying and housing programs in the Southern Cone countries of Latin America (Argentina, Chile, Paraguay, and Uruguay), where a strictly domesticated vector species, Triatoma infestans, prevails.3,4 However, it relies on large-scale funding for insecticides and careful planning,5 as well as secure access to remote areas. These conditions are not always present in regions north of the Southern Cone countries.6,7 The main species of Chagas’ disease vectors in Venezuela and Colombia have widespread sylvatic ecotopes and enter houses at night, particularly Rhodnius robustus and less frequently Rhodnius prolixus.8–11 Therefore, residual house spraying and housing programs may be less successful here than in the Southern Cone region.12,13

Both Venezuela and Colombia have reported an important decrease in Chagas’ disease over the last decades;11,14 however, in Venezuela approximately four million inhabitants (16% of the population) are still at risk of infection; the seroprevalence in rural areas is 9.2% and the seroprevalence in blood donors is 0.78%.14 In Colombia, the respective proportions are 20% of the population is at risk, 5% is infected, and 1.6% of blood donors are seropositive.11,15

Pyrethroid-impregnated materials (including bed nets, curtains, and screens) could be a complementary or alternative control measure. However, the protective efficacy of insecticide-treated materials in reducing Chagas’ disease transmission and eliminating the vector population has yet to be demonstrated. For these reasons, we tested the protective efficacy of pyrethroid-impregnated mosquito nets against R. prolixus in an experimental hut study.16 The results showed that not only was the net user protected, but that 95% of the insect vectors were killed. This suggested that impregnated materials could reduce the transmission of Chagas’ disease and help eliminate its vectors.

Except for a small study that demonstrated the protective efficacy of beta-cypermethrin–impregnated screens fixed under the roof of a house infested with Triatoma infestans in Argentina,17 community-based studies have not yet been carried out. Therefore, we conducted a randomized controlled trial using as principal outcome measures 1) the level of protection that impregnated bed nets provide against vectors of Chagas’ disease, particularly the repellent effect (driving vectors away from beds, which is an immediate benefit to users), and 2) the effect of such bed nets on vector mortality (long-term benefit to communities).

MATERIALS AND METHODS

Study sites. The study was conducted over a period of 18 months in 1998 and 1999 in Venezuela (Zaragoza, Trujillo State) and Colombia (Puerto Nuevo, Norte de Santander State). No regular vector control activities have been conducted in these areas during the previous three years. The two study communities, which are approximately 300 km apart, have similar environmental conditions. The main triatomine was known to be R. prolixus, but other vectors had also been observed by local entomologists.9,18 The study was reviewed and approved by the Universidad de los Andes (Nucleo Trujillo, Venezuela) and Colombian Institute of Tropical Medicine (Medellin, Colombia).

Baseline study. A census of 50 Venezuelan and 47 Colombian houses was completed. This included a short questionnaire interview about the inhabitants, their housing conditions, use of bed nets, and domestic animals. Finger prick
blood was taken from children (<15 years old), stored on filter paper, and later analyzed in the laboratory for antibodies to *Trypanosoma cruzi* by an immunofluorescence test, an enzyme-linked immunosorbent assay, and a direct agglutination test. Results were considered positive when the results of two or three of these tests were above the threshold values. This provided a sensitivity of approximately 99% and a specificity of approximately 95%. Two entomologic auxiliary workers in both communities examined each house for 30 minutes (i.e., one-person-hour) for triatomine infestation.

**Randomized trial.** Houses in both communities were numbered and then randomly allocated using computer-created random numbers into two groups: 1) intervention houses, in which all beds were protected with insecticide-impregnated polyester bed nets (with an average of three per house), and 2) control houses, in which beds were protected with unimpregnated bed nets. The sample size calculation took into account one vector per house with an expected difference of vector mortality between intervention and control houses of 90% versus 10%18, 80% power, and α = 0.05. This resulted in eight vectors or eight infested houses in each group (total of 16 houses). The number was doubled due to the expected design effect of more than one vector per house (calculated with Epi-Info, Version 6.04; Centers for Disease Control and Prevention, Atlanta, GA). Inhabitants were unaware if their nets were impregnated. The insecticide used for impregnation in Venezuela was lambdacyhalothrin (ICON 2.5 CS®, target concentration = 2.5 mg/m²; donated by Syngenta, Basel, Switzerland). The insecticide used for impregnation in Colombia was deltamethrin (K-Othrine 25 CS®, target concentration = 25 mg/m²; donated by Agrevo, Frankfurt, Germany). These insecticides were used based on recommended concentrations for net fabrics.20 It was assumed that the insecticidal effect of the two chemicals was similar.20 In houses in which the bedroom had a floor of earth or wood, it was covered with plastic sheets to aid collection of the insects. Each morning between 5:00 AM and 8:00 AM, the houses in Venezuela were visited by researchers and those in Colombia were visited by trained local leaders (due to security reasons) to collect the vectors from the inhabitants and to check the room for remaining vectors. The place where vectors were found was recorded and it was noted if they were alive or dead. The vectors were stored in small containers with a piece of wet filter paper. These containers were covered with a net fabric and transported to the laboratory for observation (within 72 hours) and further analysis.

After 30 days of observation, all houses were sprayed with insecticides available from the vector-borne disease control office (organophosphorus compounds in Venezuela and deltamethrin in Colombia). The remaining triatomines were then collected and counted. All unimpregnated nets were then impregnated.

**Palm tree study.** This was conducted only in Venezuela. Fourteen of 90 palm trees (*Acrocomia aculeata*) at the study site were cut down with the permission and help of the owners. The triatomines detected under the leaves were collected and examined in the laboratory for infection with *Trypanosoma cruzi* using the technique reported by Lugo de Yarbuh.21

**Residual efficacy of pyrethroids against triatomine vectors.** In Venezuela, three groups of 10 laboratory-bred and starved *R. prolixus* third stage nymphs reared from the natural population (second generation) were exposed to lambdacyhalothrin-impregnated fabrics for one hour using the petri dish method.22,23 As suggested by Vassena and others,24 younger nymphs, rather than those recommended by the World Health Organization,22 were used due to the excessive control mortality of older *R. prolixus* nymphs. They were then observed for 72 hours. A control group was exposed to unimpregnated fabrics for one hour and then observed for 72 hours. The knock-down effect and mortality were recorded. In Colombia, residual house spraying was done in the three houses in which the vector behavior study was conducted (next section). *Eratyrus mucronatus* was then observed for 72 hours and vector mortality was recorded.

**Vector behavior study.** This study was conducted only in Colombia. Three houses infested with triatomines were given deltamethrin-impregnated bed nets. For three consecutive nights, two pairs of entomologic auxiliary workers were present in each house from 8:00 PM to 1:00 AM (first pair) and from 1:00 AM to 6:00 AM (second pair). One person from each pair was under the bed net and the other was outside of it. The individual under the bed net marked the vectors with fluorescent powder (Dayglo Color Corporation®, Chunna-hatti, India) so that they could be observed with an ultraviolet hand lamp and have their contact times recorded. The person outside the bed net collected both marked and unmarked vectors and stored them in a receptacle for later observation. The vectors were sorted according to the time of collection. The mortality of vectors was monitored over a 72-hour period. After three nights, the houses were sprayed with deltamethrin and the triatomine vectors were collected and observed for 24 hours. Since only *E. mucronatus* was found, this study could not be included in the randomized trial, but it served as a complementary study. The behavior of *R. prolixus* on impregnated bed nets had been observed in our previous study.16

Entomologic analysis was conducted by professional entomologists in Venezuela and Colombia. For the differentiation of *R. prolixus* and *R. robustus*,25,26 the *Atlas of Chagas' Disease Vectors in the Americas*25 was used and the results were verified by one of its authors (I. Galindo).

**Statistical analysis.** Data analysis was completed in the United Kingdom. For testing statistical significance (with Epi-Info Stat Calc), the chi-square test with Yates’ correction and Fisher’s exact test were used. The number of degrees of freedom in the 2 × 2 tables was 1; whenever the cell value was less than 5, results of Fisher’s exact test were used.

**RESULTS**

**Study communities, endemicity of Chagas' disease, and triatomine vectors.** The background information on the two study communities shown in Table 1 indicates that these are rural communities with a high crowding index (5.1 and 4.1 persons per house, respectively). All houses have domestic animals, particularly dogs and chickens, and less frequently, turkeys and pigs. Approximately half of the population was using unimpregnated bed nets for protection against mosquitoes throughout the year during the night and during naps after lunch.

The distance between the houses ranged from 20 to 50 meters. Palm trees in the gardens and on the fields were kept mainly for protection against sunshine; they were located 5–500 meters from the houses. Housing conditions were different at the two sites. In Venezuela, a government housing
program had replaced poor rural dwellings with prefabricated housing materials, including the roofs, to eliminate vectors of Chagas’ disease.13 In Colombia, 42.5% of the houses were of poor quality, built with walls of mud and thatched with palm leaves that are a preferred habitat for some intradomestic triatomines.27,28 The climate was similar: an average temperature of 30°C in Venezuela and 32°C in Colombia, and a relative humidity of approximately 70%.

In the Venezuelan community, active transmission of Chagas’ disease was occurring: 10.7% of the children were infected, including 12.5% of those less than five years old (Table 1). In the Colombian community, no active transmission of Chagas’ disease was taking place (0% of the children were infected). Consistent with this finding, a much higher proportion of houses in the Venezuelan community (80.0%) was infested with triatomine vectors compared with the Colombian community (19.1%; \( \chi^2 = 40.7, P < 0.001 \)). In both areas, approximately 75% of the insects were captured indoors and 25% in the peridomitory area.

The main triatomines were R. robustus, R. prolixus, and Triatoma maculata in Venezuela and R. prolixus, Panstrongylus geniculatus, and E. mucronatus in Colombia (Table 1). The median vector density per dwelling was two in Venezuela and one in Colombia.

Further evidence for transmission of Chagas’ disease in the Venezuelan community. Analysis of the 14 palm trees (A. aculeata) in Venezuela showed that all were infested with R. robustus, with a mean of 45 vectors per tree (Table 2). Of the 629 vectors collected, 28.1% were positive for Trypanosoma cruzi. All seropositive people lived in houses at a distance of less than 35 meters from the palm trees.

Protective efficacy of impregnated bed nets against bites by Chagas’ disease vectors and vector mortality. Twenty-two houses in Venezuela and 22 in Colombia had impregnated bed nets and 28 houses in Venezuela and 25 in Colombia had unimpregnated bed nets (Table 3). During the 30-day observation period in houses with impregnated bed nets, triatomines were found more frequently than in those with unimpregnated nets (86.4% versus 75.0% in Venezuela and 27.3% versus 12.0% in Colombia) but the differences were not statistically significant.

In Venezuela, the impregnation of bed nets with lambda-cyhalothrin showed a high protective efficacy (Table 3). In the 19 infested houses that had impregnated bed nets, dead vectors were encountered; this was the case in less than half (47.6%) of 21 infested houses with unimpregnated bed nets (\( \chi^2 = 13.7, P < 0.001 \)). Of the 62 vectors encountered in intervention houses, 82.2% were found dead in the morning while those that survived (all were encountered on the walls) died within 24 hours. In the houses with unimpregnated bed nets, significantly fewer vectors died (13 of 53) during the 72-hour observation period (\( \chi^2 = 33.4, P < 0.001 \)); half of the 40 living vectors were found on the bed nets or beds and half on the floor.

In Colombia, fewer vectors were encountered by the inhabitants. In 22 intervention houses, six were infested and dead vectors were found in one of these; in the control group (25 houses), only three were infested and in one of these the vectors were dead. However, all vectors identified as being alive when they were handed over to the trained local leaders to the researchers (Table 3) died before reaching the laboratory. Likewise, the 29 vectors identified as being alive in houses with unimpregnated bed nets died soon afterwards. This was probably due to poor handling. However, there was another indication of the effect of pyrethroid-treated bed nets on the vectors: as in Venezuela, the places where vectors were found in houses with impregnated and unimpregnated bed nets showed large differences. In the houses with unimpregnated bed nets, 29 R. prolixus were found on routes of entry; in the control group (25% of trees with 1 or more infected R. robustus) and their infection with Trypanosoma cruzi in the Venezuelan community

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of palm trees</td>
<td>14</td>
</tr>
<tr>
<td>Average height in meters (range)</td>
<td>12.3 (9–16)</td>
</tr>
<tr>
<td>% of palm trees with R. robustus</td>
<td>100</td>
</tr>
<tr>
<td>Mean number of R. robustus per tree (range)</td>
<td>44.9 (15–92)</td>
</tr>
<tr>
<td>% of R. robustus with T. cruzi</td>
<td>28.1 (177/629)</td>
</tr>
<tr>
<td>% of trees with 1 or more infected R. robustus</td>
<td>64.3 (9/14)</td>
</tr>
</tbody>
</table>

### Table 1

Type of housing, proportion of children seropositive for *Trypanosoma cruzi*, and house infestation with Chagas’ disease vectors in the study communities of Venezuela and Colombia*  

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Venezuela</th>
<th>Colombia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Houses</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Persons per house</td>
<td>5.1</td>
<td>~</td>
</tr>
<tr>
<td>Poor quality houses†</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Houses with domestic animals</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Houses with unimpregnated bed nets</td>
<td>26</td>
<td>52.0</td>
</tr>
<tr>
<td>Children (&lt;15 years old)‡ seropositive§</td>
<td>11/103</td>
<td>10.72</td>
</tr>
<tr>
<td>Houses infested in baseline study</td>
<td>40</td>
<td>80.0</td>
</tr>
<tr>
<td>Houses infested at the end of study (30 days later)</td>
<td>40</td>
<td>80.0</td>
</tr>
</tbody>
</table>

* The number of vectors encountered during 30 days of observation was as follows: in Venezuela, 130 *Rhodnius robustus*, 23 R. prolixus, and 12 Triatoma maculata; in Colombia, 36 R. prolixus, 9 Panstrongylus geniculatus, and 7 Eratyrus mucronatus.
† Due to a housing program in Venezuela, all houses were made of prefabricated materials (concrete walls and floors, and roofs or corrugated iron); poor quality houses in Colombia had thatched roofs, earth floors, and mud walls.
‡ In those less than five years old in Venezuela, 3 (12.5%) of 24 were seropositive.
§ Serologic results were positive when two or three of the following test results were above the threshold values: direct agglutination test = 1/64; immunofluorescence test = 1/64; enzyme-linked immunosorbent assay = 1/20.
Houses with un-impregnated bed nets (28 in Venezuela, 25 in Colombia)  

**R. prolixus**  
In Venezuela, 30 laboratory-bred onatus were collected thereafter and all of them died within the houses were sprayed with deltamethrin. Fifteen which none of them died. After three nights of observation, collected immediately and observed for 72 hours, during floor or remained paralyzed on the bed net. All of them were 2−45 minutes). Thereafter, some (18.7%) of them escaped, When they touched the bed net, they became irritated and down to the floor, and from there climbed up to the bed nets.  

**TABLE 3**  
Infestation of houses with impregnated and unimpregnated bed nets in Venezuela and Columbia (30 days of observation)  

<table>
<thead>
<tr>
<th></th>
<th>Venezuela</th>
<th>Columbia</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Houses with impregnated bed nets (22 in Venezuela, 22 in Colombia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Houses with vectors</td>
<td>19/22</td>
<td>86.4</td>
<td>6/22</td>
<td>27.3</td>
</tr>
<tr>
<td>Infested houses with dead vectors</td>
<td>19/19</td>
<td>100</td>
<td>1/6</td>
<td>16/6</td>
</tr>
<tr>
<td>Fraction of dead vectors</td>
<td>51/62*</td>
<td>82.2</td>
<td>1/23†</td>
<td>4.3</td>
</tr>
<tr>
<td>Houses with un-impregnated bed nets (28 in Venezuela, 25 in Colombia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Houses with vectors</td>
<td>21/28</td>
<td>75.0</td>
<td>3/25</td>
<td>12.0</td>
</tr>
<tr>
<td>Infested houses with dead vectors</td>
<td>10/21</td>
<td>47.6</td>
<td>0/3</td>
<td>0.0</td>
</tr>
<tr>
<td>Fraction of dead vectors</td>
<td>13/53*</td>
<td>24.5</td>
<td>0/29†</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* The other 11 vectors in intervention houses died within 72 hours but none died in the control houses.  
† All vectors in the intervention and control houses died within 72 hours.

**DISCUSSION**  
The results of our experimental hut study in Colombia, which had demonstrated excellent protection of users of deltamethrin-impregnated bed nets against *R. prolixus* bites and high insect mortality, were reconfirmed by the community study in Venezuela. Here, full protection of users of lambdacyhalothrin-impregnated bed nets and 100% mortality of *Rhodnius* vectors that had come into contact with impregnated bed nets was achieved. The vectors entered the house at night, apparently from palm trees. Since 28% of the vectors found in palm trees were positive for *Trypanosoma cruzi*, they are probably responsible for maintaining transmission of this parasite among humans. One adult in the community died of Chagas’ disease during the study period, which may explain the high seroprevalence among children. The housing program appeared to have no major effect on the transmission of Chagas’ disease by sylvatic vectors.

In Colombia, protection from triatomine bites by use of impregnated bed nets was effective because vectors were driven away from beds with impregnated bed nets and all the households were eager to get the impregnation done. However, this study did not provide the same conclusive answer on vector mortality as the Venezuelan study because the triatomines had to be collected by the inhabitants due to security reasons; the vectors all died after being improperly handled.

In the study on vector behavior in another community of Colombia, only a larger species, *E. mucronatus*, was found. Although its potential for transmission of *Trypanosoma cruzi* is unclear, its behavior when exposed to pyrethroid-impregnated materials is worth mentioning. It was hyperactive after coming into contact with the bed net and tried to escape, a phenomenon also described in an experimental study. However, *E. mucronatus* spent much more time (18 minutes) on deltamethrin-impregnated bed nets compared with the smaller species, *R. prolixus*, on nets impregnated with the same chemical (three minutes;†). This indicates that larger triatomine species need longer contact times before being knocked down or killed. This is consistent with the findings of studies that showed a higher susceptibility of *R.
prolixus to pyrethroids compared with the larger species of Panstrongylus megistus and Triatoma infestans.24,31 Many adult vectors showed the knock-down effect, but this did not lead to a higher vector mortality when they fell to the floor. Insufficient contact time may be the main reason. In this case, several contacts with the impregnated bed net seem to be necessary for killing the adult vectors of the larger species. The vectors in our study that remained paralyzed on the net could have absorbed a lethal dose if they had not been collected for observation. Nymphs are smaller and have a less developed cuticulum,32 and therefore need less contact time (47% mortality in our observational study in Colombia).

In conclusion, programs with insecticide-treated materials, which are generally cheaper than house spraying programs,33 can play an important role in eliminating transmission of Chagas’ disease in those areas where R. prolixus and R. robustus are the main vectors and have a sylvatic habitat and where the political commitment for large-scale house spraying programs is difficult to achieve.1,14

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