

## Impregnated Netting Slows Infestation by *Triatoma infestans*

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**Abstract.** We used sentinel animal enclosures to measure the rate of infestation by the Chagas disease vector, *Triatoma infestans*, in an urban community of Arequipa, Peru, and to evaluate the effect of deltamethrin-impregnated netting on that rate. Impregnated netting decreased the rate of infestation of sentinel enclosures (rate ratio, 0.23; 95% confidence interval, 0.13–0.38;  $P < 0.001$ ), controlling for the density of surrounding vector populations and the distance of these to the sentinel enclosures. Most migrant insects were early-stage nymphs, which are less likely to carry the parasitic agent of Chagas disease, *Trypanosoma cruzi*. Spread of the vector in the city therefore likely precedes spread of the parasite. Netting was particularly effective against adult insects and late-stage nymphs; taking into account population structure, netting decreased the reproductive value of migrant populations from 443.6 to 40.5. Impregnated netting can slow the spread of *T. infestans* and is a potentially valuable tool in the control of Chagas disease.

### INTRODUCTION

Chagas disease, caused by the unicellular parasite *Trypanosoma cruzi*, continues to affect millions of people in Latin America.<sup>1–3</sup> *T. cruzi* is carried by numerous species of blood-sucking triatomine bugs of the order Hemiptera (family Reduviidae). The parasite is usually transmitted to humans and other mammals when the vector's feces enter the host through the insect bite or mucous membranes.<sup>2</sup> Of the vector species, *Triatoma infestans* is the most important in southern South America and the sole vector in southern Peru. *T. infestans* is found most often in poor, rural households.<sup>4,5</sup> However, in Arequipa, a city of 860,000 people in southern Peru, *T. infestans* and Chagas disease have become urban problems.<sup>6,7</sup> The prevalence of *T. cruzi* infection among school-children was 4.7% in southwestern Arequipa.<sup>8</sup>

*Triatoma infestans* can be controlled through application of residual insecticides to households in affected areas. Since 1991, a concerted vector control campaign, known as the Southern Cone initiative, has successfully eliminated transmission of *T. cruzi* by *T. infestans* in Chile,<sup>9</sup> Brazil,<sup>10</sup> and Uruguay.<sup>1</sup> However, *T. infestans* control efforts are challenged, even in rural areas, by the return of the vector as the residual effect of insecticide diminishes.<sup>11,12</sup> In the city of Arequipa, where household density is very high,<sup>6</sup> and the vector can easily walk from one house to the next, re-dispersion of the vector may be faster than in rural areas. Residual vector populations have been found in 2–3% of households within 1 year of completion of insecticide application in Arequipa (Ministry of Health, unpublished data). Additional measures may be necessary to prevent re-infestation by *T. infestans* after insecticide application in the urban environment and to thereby prevent the re-establishment of vector-borne *T. cruzi* transmission.

Insecticide-treated nets (ITNs) have been shown to be ef-

fective in malaria control<sup>13</sup> and potentially in the control of leishmaniasis.<sup>14</sup> ITNs kill triatomines,<sup>15,16</sup> but no study has yet evaluated their use to prevent vector infestation and aid in the *T. infestans* elimination campaign. Vector infestation typically begins in peri-domestic animal enclosures,<sup>17</sup> and in Arequipa, guinea pig enclosures are particularly susceptible.<sup>6</sup>

We performed a longitudinal field trial to measure the rate of vector infestation in sentinel enclosures housing two guinea pigs with and without protective covers made of deltamethrin-impregnated netting. We use novel statistical methods to simultaneously estimate the effect of the netting on the rate of infestation while controlling for the effect of surrounding vector populations, taking into account both the density of these populations and their distance from the sentinel enclosures.

### MATERIALS AND METHODS

**Study area.** Nueva Alborada is an urban community at the eastern edge of the city of Arequipa, Peru (16.433° S, –71.492° W), surrounded by farmland on three sides. It was founded by landless and displaced families, many of whom migrated from rural areas to the city out of economic necessity or to escape terrorism from 1980 to 1995.<sup>18</sup> The community consists of 499 households located within a grid of 45 rectangular city blocks. Typical households consist of a human dwelling (bedrooms, kitchens, living rooms, and storage/work rooms) plus an enclosed yard. Houses are contiguous, and all share either two or three walls with their neighbors. The community had not undergone systematic insecticide application before this study.

**Study design.** The study was conducted in coordination with the vector control program of the Arequipa Ministry of Health. The use of vertebrate animals in the study was approved by the Johns Hopkins IACUC committee. In July 2006, 5 months before the initiation of vector control activities, trained entomologic collectors visited all households in the community. After receiving consent, collectors searched for triatomines with the aid of an aerosol insecticide spray

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containing tetramethrin (Sapolio “Mata Moscas” tetramethrin 0.15%), a flushing out agent for *T. infestans*.<sup>19</sup> Search was limited to the peri-domestic area of households, including all external walls and animal enclosures, and aimed only to establish whether there were vectors present. Collected vectors were removed from the household, but no attempt was made to control their population during the search. Households in which live *T. infestans* of any stage were encountered were invited to participate in the longitudinal sentinel enclosure study.

We took advantage of the grid structure of the city blocks in Nueva Alborada and assigned each block sequentially to one of three groups, following the numbering of the blocks that was assigned geographically by the Ministry of Health. The first group received a newly constructed guinea pig enclosure over which we placed a specially made long-lasting deltamethrin-impregnated net (PermaNet 2.0, 55 mg/m<sup>2</sup> target deltamethrin concentration, 25 holes/cm<sup>2</sup> mesh size; Vestergaard Frandsen, Lausanne, Switzerland). The second group received an identical new enclosure without a net; this group served as the control group. The final group of blocks did not receive anything and is not included in this study. In total, nine city blocks participated in the study: four in the intervention group and five in the control group.

Newly constructed guinea pig enclosures measured 1.4 × 0.8 m. The walls were constructed of metallic wire shown previously to be refractory to *T. infestans* colonization,<sup>6</sup> and the roof was of corrugated rubber. Impregnated nets measured 1.5 × 1.0 m (Figure 1). Enclosures were placed in the yards or roofs of participating households. Care was taken to place sentinel enclosures at least 2 m from sites of vector infestation, and the area surrounding the enclosures was re-examined at the time of placement of the enclosure for signs of triatomines. We placed two guinea pigs into each enclosure along with 25 bricks, which provide a habitat for *T. infestans*. Bricks were stacked loosely in the form of a “U” at one end of the enclosure. We visited participating houses every 2 weeks for 4 months and carefully examined the 25 bricks, the netting if present, and the area under the new enclosures for



FIGURE 1. A sentinel guinea pig enclosure protected by impregnated netting in Nueva Alborada, Arequipa, Peru. Twenty-five bricks have been placed inside the enclosure to provide habitat to vectors that navigate past the net. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

the presence of *T. infestans* and *T. infestans* eggs. All insects encountered were removed from the enclosures, staged, and sexed (for adults) at the National University of San Agustín.

Nueva Alborada underwent insecticide application, conducted by the Arequipa Ministry of Health Chagas Disease Control Program, from November 27, 2006 to January 18, 2007. Ministry of Health personnel sprayed each house and all peri-domestic structures with deltamethrin suspension concentrate (Delta 5% SC; Farmex Corp., Lima, Peru) diluted in water at a rate of 25 mg/m<sup>2</sup>. Immediately after insecticide application, two trained triatomine collectors systematically searched each room of the human dwelling, all animal enclosures, and remaining peri-domestic areas for a total of 1 person-hour. All sites of collection within the house were located on a high-resolution GoogleEarth image of the community after collection.<sup>20</sup> In households with a sentinel enclosure, the distances from all sites of vector collection to the sentinel enclosure were also measured by tape measure.

Triatomines captured from each site were stored separately on ice packs until processing at the National University of San Agustín. Vectors were counted by site, stage, and sex (for adults). Live and moribund third-, fourth-, and fifth-instar and adult triatomines were examined for *T. cruzi* as described by Gurtler and others.<sup>21</sup> Briefly, intestinal contents of the insects were extracted by applying pressure to the lower abdomen of the triatomine with forceps. Extracted material was diluted in one drop of saline solution and examined under a microscope at ×400 magnification. We examined insects consecutively for each site until 1 positive insect was found, 10 negative insects had been examined, or all available insects had been examined, as described previously.<sup>6</sup>

**Data analysis.** The latitude and longitude of all sites of collection and sentinel enclosures were determined from GoogleEarth and entered into a Geographic Information System (ArcView 3.3; ESRI, Redlands, CA). The distances from sites of collection to sentinel enclosures were calculated in ArcView. Comparison between distances determined through GoogleEarth and ArcView and those taken by tape measure showed a median discrepancy of 1.43 m; 90% of measures were within 3.6 m. When both computer-generated and manual measures of distance were available, the manual measures were used.

We calculated the total rate of infestation of sentinel enclosures by *T. infestans* as the number of insects collected in the enclosure per time in which the enclosure was exposed in the household (sentinel enclosure days). We compared the infestation rates in enclosures with and without impregnated netting using the  $\chi^2$  test. We also calculated the stage-specific estimated reproductive value of insects encountered in each type of enclosure. The reproductive value for each stage of insect is the product of the probability that the insect survives until adulthood and the expected number of eggs produced as an adult. We followed custom and only considered female insects. Sex was not determined for nymphs, so we divided the number of nymphs captured by 2 to calculate the reproductive value. For adult female insects, reproductive value decreases with time as an adult, which we could not measure. We therefore also halved the reproductive value of females, assuming all were at the middle of their reproductive lives on migration. All stage-specific survival values and adult fecundity were derived from life table statistics of *T. infestans* in the experimental colonies reported by Rabinovich.<sup>22</sup>

We used log-linear models to estimate the effect of netting on the infestation rate of vectors controlling for the density and distance of potential source populations. Because the variance of the log of infestation rates between enclosures was much greater than the mean, overdispersed Poisson log-linear models fit the data better than simple Poisson log-linear models based on the likelihood ratio test. We fit the overdispersed Poisson log-linear models following the methods described in Breslow<sup>23</sup> and implemented in the *dispmod* library of the R statistical package (R 2.1, [www.r-project.org](http://www.r-project.org)).

There were multiple potential sources of triatomines around all sentinel enclosures. We assumed that the effect of these populations on the rate of infestation of sentinel enclosures to be a function of both the density of each source population ( $S_j$ ), and the distance between the source population and the sentinel enclosure ( $d_{ij}$ ). We used kernel density estimators to summarize the distance and population density of all vector populations surrounding sentinel enclosures. Biologically, these kernel density functions represent the sum of the products of vector density of each source population and the probability of migration from that source to the sentinel enclosure. We assumed the probability of *T. infestans*

migration to decrease with increasing distance and modeled this decrease with different one-parameter kernel functions. We considered the exponential, Cauchy, and Gaussian kernels. We included the kernel density estimates into the overdispersed Poisson log-linear models as a covariate. The full log-linear models take the following forms:

$$Y_i \sim \text{Poisson}(\mu_i)$$

$$\mu_i = \text{Time} \times e^{\lambda_i}$$

$$\lambda_i = \beta_0 + \beta_1 \times \text{Net}_2 \times \sum k_{ij}$$

$$\text{Exponential}^{24}: \sum k_{ij} = \sum S_j \times e^{-d_{ij}^a}$$

$$\text{Gaussian}^{24}: \sum k_{ij} = \sum S_j \times 1/\sqrt{2\pi} \times e^{-1/2 \times (d_{ij}/b)^2}$$

$$\text{Cauchy}^{25}: \sum k_{ij} = \sum S_j \times 2c/\pi(c^2 + d_{ij}^2)$$

We identified the best fit model across a wide range of possible parameters of the spatial kernel estimators (for the exponential, the parameter is denoted as  $a$ ; for the Gaussian, the parameter is called the bandwidth and is denoted as  $b$ ; the parameter for the Cauchy kernel is  $c$ ) for each type of model.

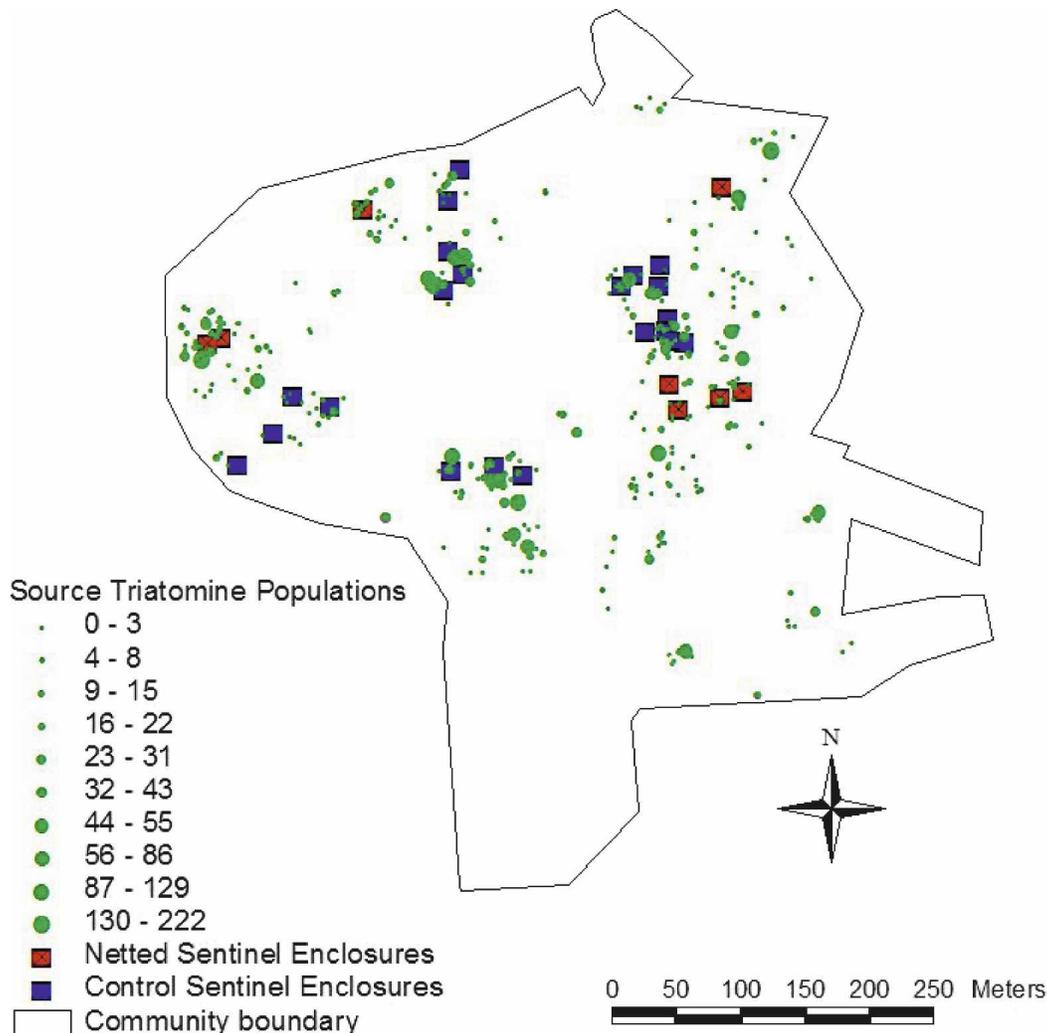


FIGURE 2. Map of sentinel enclosures with impregnated netting (squares marked with an “X”) and without (empty squares) placed in Nueva Alborada, Arequipa, Peru. All surrounding source populations of *T. infestans* are shown in circular points; the size of the points reflects the number of insects caught at the site. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

We compared the fit of the models using the Akaike information criterion (AIC). The model with the lowest AIC was accepted as the best fit.<sup>26</sup>

## RESULTS

Two hundred eighty-eight of 499 households in Nueva Alborada agreed to participate in the preliminary vector survey 5 months before insecticide application. Triatomines were captured in the peri-domestic areas of 56 (19.4%) households. Twenty-nine households agreed to participate in the longitudinal study (participation rate = 51.8%); by chance, 8 were located in city blocks assigned to the impregnated netting group and 21 to the control group without netting. Starting in November 2006, 454 households were sprayed by the Ministry of Health. At the time of spraying, vectors were encountered in 162 (32.5%) households and collected from 406 distinct sites (Figure 2). No triatomines (0% of 1,460; one-sided 97.5% confidence interval [CI], 0%, 0.25%) were infected with *T. cruzi*.

During the 4-month follow-up period, a total of 17 triatomines were found in netted enclosures over 998 days of exposure time (rate = 0.017 insects/d) and 189 triatomines were found in control enclosures over 2,594 days of exposure time (rate = 0.073 insects/d). The rate ratio of triatomines in netted enclosures compared with control enclosures was 0.23 (95% CI, 0.13–0.38;  $P < 0.001$ ). Thirteen dead or moribund insects were found in netted enclosures (0.013 insects/d), whereas seven were found in control enclosures (0.0027 insects/d). The rate of encounter of dead and dying bugs was 4.83 times greater in the netted enclosures (95% CI, 1.79–14.3;  $P < 0.001$ ).

The great majority of live insects collected were early-stage nymphs. Triatomine eggs, which are white or pink and fairly easy to spot, were encountered in only one enclosure, which did not have a net. The proportion of each stage of insect captured in the sentinel enclosures without netting very closely resembled the stable stage distribution of *T. infestans* measured in laboratory conditions (Figure 3).<sup>22</sup> In the netted enclosures, many more adult insects than immature stages were found dead or moribund. The average expected reproductive value of insects per enclosure was 10.9 times higher in control enclosures than netted enclosures (443.6 compared with 40.5). The majority of the reproductive value across all control enclosures could be attributed to first-instar nymphs (51.7%). Adults and fifth-instar nymphs contributed only 4.83% and 2.50% of the total reproductive value, respectively (Table 1).

In multivariate analysis, an overdispersed Poisson model with a Gaussian kernel function with a bandwidth of 44 m best fit the data (AIC = 35.783; Table 2). In this model, the rate of infestation in enclosures with nets was less than a quarter of that in control enclosures ( $P = 0.018$ ) after adjusting for the effect of the surrounding source populations. The estimated dispersion term in the model was 1.59, confirming that the data were indeed overdispersed. Similar overdispersed Poisson models with a Cauchy or exponential kernel density functions fit the models nearly as well (Cauchy: AIC = 36.540, parameter = 28; exponential: AIC = 36.315, decay parameter = 0.035). The choice of kernel density function did not greatly alter the estimate of the effect of the nets

nor the significance level of that estimate. Plots of the AIC of the full overdispersed Poisson models over all the parameters of the Gaussian, Cauchy, and exponential kernel density functions considered were continuous and had clear minima in all three cases (Figure 4). The estimated relation between migration rate and distance are shown in Figure 5.

## DISCUSSION

*Triatoma infestans* rapidly infested sentinel guinea pig enclosures in urban households in Arequipa, Peru. Vectors infested unprotected enclosures at a rate of one bug every 2 weeks. Controlling for the density of all potential source populations of triatomines and their distance from sentinel enclosures, we estimate that deltamethrin-treated nets slow the rate of vector infestation 4-fold. Impregnated netting may be a useful tool in Chagas disease control.

Using log-linear models including kernel estimates of vector density of surrounding vector populations, we estimated the relationship between vector migration rate and distance. A Gaussian function fit the data better than an exponential function, suggesting a more gradual decrease in migration rate per meter of distance over short distances, especially when source populations are within 10 m of sentinel enclosures. Our estimated function is qualitatively in line with data from light-trapping experiments, in which nymphs were observed to walk up to 42 m,<sup>27</sup> as well as evidence from blood meal analyses that nymphs can walk even greater distances.<sup>28</sup> We cannot compare the actual estimated rates of migration observed here to estimates from light trap studies because such studies to date have been limited to nights of high probability of vector flight. *T. infestans* adults have been observed to fly up to 2 km,<sup>29</sup> but such long-range flight was probably a negligible factor in vector migration on the scale of our study. The Cauchy function, which is “long-tailed,” may be a more

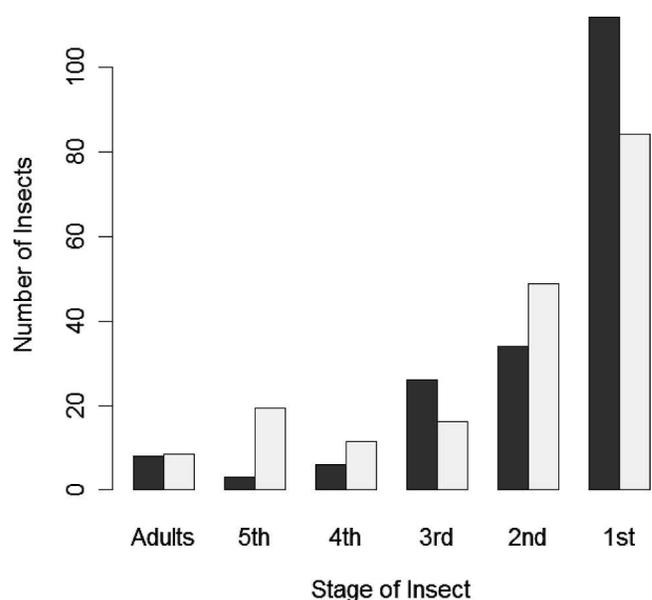


FIGURE 3. Population structure of insects collected over the course of the study in sentinel enclosures without nets (black) and stable population structure in laboratory colonies from Rabinovich<sup>22</sup> (gray).

TABLE 1

Reproductive value by stage for insects collected in sentinel guinea pig enclosures without protective netting placed in Nueva Alborada, Arequipa, Peru

Stage	Probability of surviving into adulthood	Reproductive value*	Number of females collected†	Total reproductive value among migrants	Percent of total reproductive value
First instar	0.596	88.86	56	4,975.93	51.70
Second instar	0.733	109.33	17	1,858.68	21.00
Third instar	0.772	115.03	13	1,495.36	15.30
Fourth instar	0.784	116.93	3	350.80	4.60
Fifth instar	0.838	124.86	1.5	187.29	2.50
Adult female	1.0	70.05	6	420.30	4.83

\* The reproductive value is the product of the probability of surviving into adulthood and the expected number of eggs produced as an adult, measured as 140.09 by Rabinovich.<sup>22</sup> We assume that adult female migrants are of average fecundity and will produce 70.05 eggs.  
 † The number of females in immature stages is estimated at one half of the total number of nymphs for each stage.

appropriate model of the relation between vector migration and distance when long-distance migration is more important.

Surprisingly, the great majority of insects collected from sentinel enclosures were early-stage nymphs. Vazquez-Prokopec<sup>27</sup> collected fifth-instar nymphs in light traps in northwestern Argentina and suggested that larger nymphs may play an important role in the local propagation of *T. infestans* within households, but no nymph below the fifth stage was collected in light traps. Our results suggest that, in terms of numbers and reproductive value, the early-stage nymphs are the most important agents of local dispersion of *T. infestans*. Unfed first-instar nymphs are very unlikely to be infected with *T. cruzi*. Thus, local propagation of vectors likely occurs before the spread of the parasite.

We observed no increased rate of infestation among adult insects compared with nymphs. The structure of the population in the control sentinel enclosures closely paralleled that of the stable stage distribution of *T. infestans* as measured in laboratory conditions.<sup>22</sup> Our results suggest that migration propensity is equal across all stages in *T. infestans*, even though only the adults are winged. The impregnated nets were especially effective against adult insects. Presumably, the larger insects were less able to fit through or under the mesh. Adult and late-stage nymphs have higher reproductive

values.<sup>22</sup> Taking into account the difference in population structure of insects caught in the netted enclosures, we calculated that the nets decrease the reproductive value of *T. infestans* > 10-fold. More data are needed on mortality of insects in enclosures with and without nets to fully estimate the effect of nets on *T. infestans* colonization potential and population growth in these enclosures.

The ultimate goal of the Southern Cone initiative is the elimination of *T. infestans*. Infestation by *T. infestans* typically begins in peridomestic structures,<sup>17</sup> and decreasing the susceptibility of these structures with impregnated netting or through other interventions might aid in the elimination of the vector. More complete analysis is necessary to determine the effect that netting might have on the number of sites a residual population of triatomines would infest, a measure known as the “ $R_0$ ”<sup>30</sup> of the vector in the community. A key determinate of the potential decrease in  $R_0$  is the decrease in migration from enclosures with nets. Future work is also needed to estimate the duration of the effect of ITNs against *T. infestans* in field conditions. Nets were damaged over the course of the study. Tougher insecticide-impregnated materials may be needed for use in the peri-domestic habitat.

TABLE 2

Overdispersed Poisson log-linear models of infestation rate in sentinel guinea pig enclosures, Nueva Alborada, Arequipa, Peru

	Rate ratio	P value	AIC
<b>Gaussian model*</b>			35.783
Nets present	0.25	0.018	
Kernel density of source populations	1.005454/m†	0.21	
<b>Cauchy model‡</b>			36.540
Nets present	0.27	0.024	
Kernel density of source populations	1.172104/m†	0.10	
<b>Exponential model§</b>			36.315
Nets present	0.26	0.020	
Kernel density of source populations	1.004355/m†	0.13	

\* The optimal bandwidth for the Gaussian kernel density function was 44 m. The dispersion parameter of the Gaussian model was 1.59.

† The rate ratio for the kernel density of source populations is dependent on the scale by which distance is measured in the density function. For instance, the estimated rate ratio for the Gaussian model would be 1,005.454 if measurements were in kilometers rather than meters.

‡ The optimal parameter of the Cauchy model was 28. The dispersion parameter of the model was 1.53.

§ The optimal decay parameter for the exponential kernel density function was 0.035. The “half-life” of the function is 1/0.035 or 28.6, meaning that the estimated probability of migration over distances < 28.6 m is equal to the estimated probability of migration over all distances > 28.6 m. The dispersion parameter of the model was 1.55.

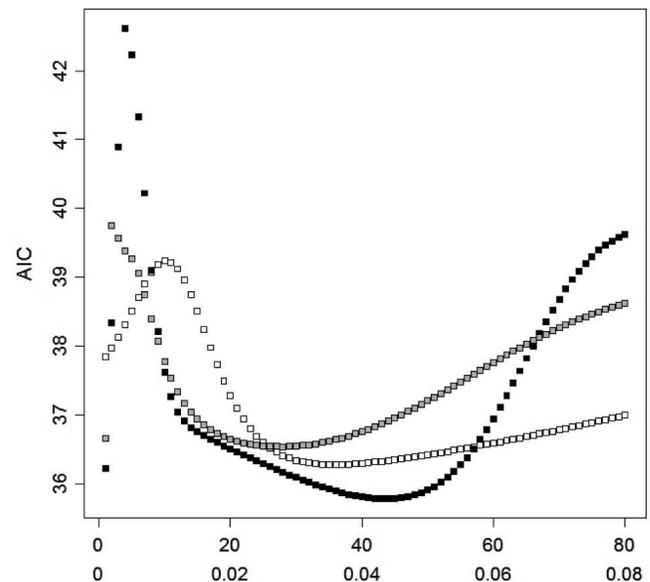


FIGURE 4. Relationship between the parameters of Gaussian (filled black square), Cauchy (filled grey square), and exponential (outlined square) spatial kernel functions used as covariates in the overdispersed Poisson log-linear models and the Akaike information criterion (AIC) of these models.

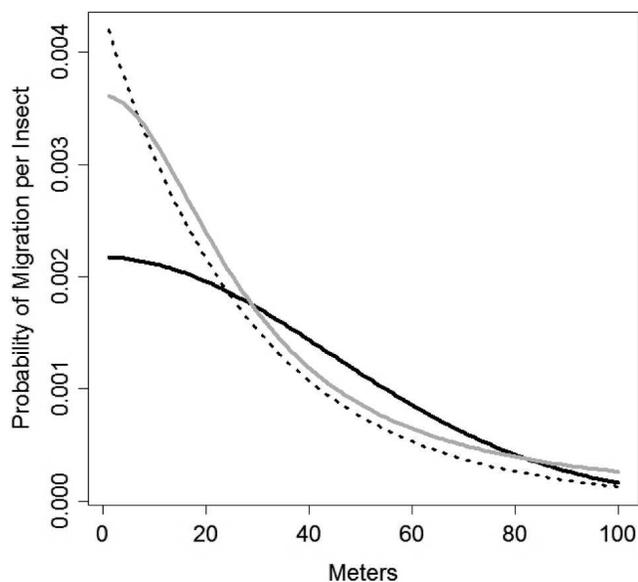


FIGURE 5. Estimated best-fit Gaussian (solid black line), Cauchy (solid grey line), and exponential (dashed line) kernel functions for migration of *T. infestans* by distance.

Previous studies have suggested that deltamethrin-impregnated nets might prevent *T. cruzi* transmission to humans.<sup>15,16</sup> Our results show that such protection would be partial, because vectors can navigate past the nets. However, in our study, most vectors that traversed the nets were early-stage nymphs, which are less likely to carry *T. cruzi*. Nets might thereby substantially decrease the force of infection to humans, even if they do not fully block the vector's access to its blood meal. Bednets might be especially effective against *T. infestans* because bugs would face the double jeopardy of passing the net as they approach their host to feed and again as they return to their colony. Bednets might thereby decrease domestic populations of triatomines.

Our study had a number of limitations. Because of the two-phase design, we had an unequal number of netted and control enclosures. The low participation rate for the initial vector collection led to a low sample size for the longitudinal sentinel enclosure study and decreased precision in our estimates of vector migration rate over distance. We placed 25 bricks in sentinel enclosures to provide habitat to infesting insects. We cannot exclude the possibility that this habitat was incompatible to insects of certain stages, adults particularly, which may prefer other materials. We were only able to examine the sentinel enclosures biweekly; natural predators of triatomines, especially chickens and ants, probably ate many dead and moribund triatomines. We were also not able to control for the presence of vectors in neighboring communities.

There are hundreds of urban and peri-urban communities similar to Nueva Alborada in the city of Arequipa. Although *T. cruzi* transmission was not observed in Nueva Alborada at the time of vector control, the parasite is quite common in similar communities.<sup>6</sup> Achieving elimination of vector-borne transmission of *T. cruzi* in the urban environment will not be easy and likely requires measures in addition to those shown to be successful elsewhere in rural areas covered by the Southern Cone initiative. ITNs have proven to be a low-cost

and effective intervention against malaria.<sup>13</sup> Further study is needed to establish whether they might have similar potential in the control of Chagas disease.

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