

# Selective Insecticide Applications Directed Against *Triatoma infestans* (Hemiptera: Reduviidae) Affected a Nontarget Secondary Vector of Chagas Disease, *Triatoma garciabesi*

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Received 6 August 2015; Accepted 1 October 2015

## Abstract

The control of nondomiciliated triatomine species adapted to peridomestic habitats represents a challenge because they are connected to sylvatic colonies, and pyrethroid insecticides have limited effects outdoors. The effects of residual insecticide spraying have rarely been assessed on secondary triatomines. *Triatoma garciabesi* (Carcavallo, Martinez, Cichero, Prosen & Ronderos, 1967) is a nontarget vector that inhabits the dry western Chaco region, and a member of the *Triatoma sordida* Stål 1859 complex. Little is known on the capacity of *T. garciabesi* to invade and establish viable domestic or peridomestic colonies, and on its response to residual insecticide sprays directed against *Triatoma infestans* Klug 1834. The presence and abundance of triatomines were assessed by timed manual collections annually or biannually (spring and fall) during 10 yr after a community-wide insecticide spraying campaign and selective insecticide sprays directed against *T. infestans* in a rural village of northwestern Argentina. *T. garciabesi* mainly occupied peridomestic habitats associated with chickens, and was unable to colonize human sleeping quarters. Trees with chickens occurred in nearly all houses and were infested in >25% of the occasions. The abundance of bugs at house-compound level was best explained by a generalized estimating equation model that included selective insecticide sprays during the previous semester (negative effects), chicken abundance (positive effects), seasonality, and their interactions. Our results suggest that insecticide applications targeting *T. infestans* affected the abundance of *T. garciabesi*, and reduced the likelihood of future infestation.

**Key words:** vector ecology, triatomine, pyrethroid insecticide

Several species of Triatominae, vectors of the etiologic agent of Chagas disease, are distributed along a habitat gradient of anthropogenic conditions that encompass human sleeping quarters, peridomestic structures housing domestic and commensal animals, and sylvatic habitats. The relevance of peridomestic triatomine populations rests primarily on their potential to (re)invade houses (Abad-Franch et al. 2010b). According to their adaptation to human dwellings, triatomine species have been classified as domestic, domiciliary, intrusive, and sylvatic (Noireau and Dujardin 2010), with the intent of better defining their potential importance as secondary vectors of *Trypanosoma cruzi* and to understand the domiciliation mechanisms (Schofield and Dias 1999). The control of most nondomiciliated triatomine species represents a challenge both because peridomestic populations remain connected to sylvatic populations

that can contribute to their viability (Waleckx et al. 2015), and because residual insecticide spraying has reduced efficacy in peridomestic habitats (Gürtler et al. 2004). The effects of residual insecticide spraying on nontarget triatomine species have rarely been investigated (Diotaiuti et al. 1998).

At least three species of triatomine bugs occur sympatrically in the dry (western) Chaco region: the main vector *Triatoma infestans* Klug, 1834 occupies domestic or peridomestic structures and is rarely found in sylvatic habitats, whereas *Triatoma guasayana* and *Triatoma garciabesi* (Carcavallo, Martinez, Cichero, Prosen & Ronderos, 1967) usually occur in both sylvatic and peridomestic areas (Canale et al. 2000; Vazquez-Prokopec et al. 2005, 2008; Ceballos et al. 2011). There is little evidence of vector-borne transmission of *Tr. cruzi* to humans by either *T. guasayana* or

*T. garciabesi*, which were thought to participate as bridge vectors between the sylvatic and domestic or peridomestic cycles (Cecere et al. 1999). In consequence, *T. infestans* is the only target of the regional elimination program “Southern Cone Initiative” since 1991 (Schofield and Dias 1999).

*T. garciabesi* (originally a member of the *Triatoma sordida* Stål 1859 complex) was later synonymized to *T. sordida* and revalidated again based on morphological, interbreeding, cytogenetic, ecological, and biochemical evidence (Jurberg et al. 1998). *T. sordida* is a secondary vector of *Tr. cruzi* originally endemic of the central Brazilian savannas (the “Cerrado” region), which has become widely distributed throughout the Gran Chaco region down to central Argentina (Schofield et al. 1991). Noireau et al. (1998) proposed that *T. sordida* may have suffered a recent cryptic speciation, originating morphologically similar populations that cannot interbreed. Recent studies bring new evidence in support of the niche differentiation hypothesis between *T. sordida* from Brazil and *T. garciabesi* from western Argentina, and the revalidation of *T. garciabesi* as a different species characterized by a darker color and a shorter head (Gurgel-Gonçalves et al. 2011). Why *T. sordida* has a strong tendency to invade and colonize domestic premises whereas *T. garciabesi* does not remains unknown.

*T. garciabesi* rarely attacked humans, and showed very low rates of infection with *Tr. cruzi* (0.2%) in a defined area of the Argentine Chaco (Cecere et al. 1999, Cardinal et al. 2006, Marcet et al. 2006). Preliminary data showed a strong association of *T. garciabesi* with the rugged bark of *Prosopis alba* or *Prosopis nigra* (Fabaceae) trees where chickens roosted, and chicken coops in peridomestic areas (Canale et al. 2000), and with nests of Furnariidae and Psittacidae, loose bark, and refuges of rodents or other mammals in sylvatic habitats (Carcavallo et al. 1988). Little is known about the capacity of *T. garciabesi* to invade and establish viable colonies in domestic and peridomestic habitats; whether bug population size is related to host abundance (as seen for other triatomine species, e.g., Villalobos et al. 2011), and whether residual insecticide applications targeting *T. infestans* may affect the abundance of *T. garciabesi* in peridomestic habitats. In this study, we investigated the effects of time-variable factors on the abundance of *T. garciabesi*, including the number of chickens in each house compound, seasonality, and selective insecticide sprays over the 6 mo that preceded each bug survey. Additionally, we sought to identify the key peridomestic ecotopes of *T. garciabesi*.

Based on preliminary findings (Canale et al. 2000), we hypothesized that peridomestic ecotopes used by chickens were primarily responsible for the presence of *T. garciabesi* colonies; chicken abundance was associated with bigger bug numbers, and insecticide sprays directed to *T. infestans* would exert negative effects on *T. garciabesi* abundance. The present study expands preliminary results by including the impacts of selective sprays with pyrethroid insecticides in a 10-yr time series of triatomine abundance, and a detailed study of habitat occupancy of *T. garciabesi*. This article illustrates a modeling approach of insecticidal effects that can be applied to other triatomine species and settings.

## Materials and Methods

### Study Area

Field studies were carried out in the rural village of Amamá (27° 12'33" S, 63° 02'10" W), Province of Santiago del Estero, Argentina. The community included 71 houses distributed over 140 ha. The area and the early history of house infestation by the

three main triatomine species have been described previously (Gürtler et al. 1999, Canale et al. 2000, Cecere et al. 2002, Vazquez-Prokopec et al. 2005). The area is semiarid, belongs to the dry Chaco region, and is characterized by a xerophytic secondary forest. The region has a dry period from May to October and a rainy season during the rest of the year. From 1950 to 2000, the average temperature was 25.2°C in November and 17.4°C in May, and the average annual rainfall was 598 mm (data from the “WorldClim” database, available for download from <http://www.worldclim.org>, last accessed 15 October 2015, Hijmans et al. 2005).

Most houses were made of adobe walls and thatched roofs, with one or two adjacent bedrooms. The peridomestic environment included structures that did not share a roof with bedroom areas, such as storerooms, chicken coops, various types of corrals, and other structures located within the area of human activity (Canale et al. 2000). All houses were identified with a numbered plaque and mapped in 1992. Peridomestic sites were also mapped with GPS (global positioning system) readings accurate to 1 m (GeoXM; Trimble Navigation Ltd, Sunnyvale, CA). New and abandoned structures were recorded and identified in every survey (see below).

All houses were sprayed with suspension concentrate deltamethrin by field personnel from the National Chagas Service (NCS) following standard procedures in 1985, 1992, and 2004 (Gürtler et al. 1994, 2007; Cecere et al. 2002). The surveillance phase, spanning from May 1993 until October 2002, included strong community participation in triatomine detection activities and insecticide application. Selective residual insecticide spraying of sites infested by *T. infestans* was conducted by NCS during 1993–1996, whereas treatments during 1997–2002 were conducted by the local health-care agent and some villagers, and included all domestic and peridomestic sites at each infested house compound. Because of their apparent lack of capacity to colonize human sleeping quarters and very low infection rates with *Tr. cruzi*, the finding of *T. garciabesi* or *T. guasayana* was not considered as a sufficient cause to treat a house with insecticide.

### Field Surveys

All domestic and peridomestic sites of each house compound were searched for triatomine bugs between 1993 and 2002, except for 2001. House compounds were searched for bugs in every fall and spring between 1995 and 1998; only in spring in 1993, 1994, and 2002, and only during fall in 1999 and 2000. During the 1994 fall, house compounds were searched for triatomines only indoors (domestic sites). Searches included timed manual bug collections (TMC) by two skilled persons from the NCS using 0.2% tetramethrin spray (Icona SA, Buenos Aires, Argentina) to flush out the insects from their hiding places (capture effort, one person-hour per house); by householders' bug collections; and by domestic sensor boxes placed on bedroom walls as described elsewhere (Gürtler et al. 1999, Cecere et al. 2004).

All triatomine bugs collected were identified to species and stage. The relative length of the second to the first rostral segment was the main character used to distinguish adults of *T. guasayana* and *T. garciabesi*; the second segment is more than twice as long as the first one in *T. garciabesi* or *T. sordida*, and less than twice as long in *T. guasayana* (Canale et al. 2000). The feces of adults and fourth- and fifth-instar nymphs collected between March 1998 and December 1999 were examined under optic microscope to determine the infection with *Tr. cruzi*. Only 5 of 1,124 *T. garciabesi* bugs collected between 1993 and 1997, in March 2000 and October 2002, were positive for *Tr. cruzi* infection (Cecere et al. 1999,

Cardinal et al. 2006, Marcet et al. 2006). Householders were asked about numbers of fowl and other domestic animals they owned in 9 of the 13 surveys to estimate host availability at each house at the time of triatomine surveys (Cecere et al. 2002). The date at which each site of each house compound was sprayed with insecticide were recorded individually in order to preserve the data at both site- and house-compound levels. Each level was used to answer questions at different scales.

### Data Management and Analysis

The information collected in the 13 surveys of sites inspected by TMC and in the additional survey of domestic sites only was used to characterize the distribution and abundance of *T. garciabesi* across all ecotopes within each house compound. Bugs collected by householders and sensor boxes mainly provided data on the presence of *T. infestans* inside bedrooms, where rarely *T. garciabesi* bugs were collected by any of the three methods.  $\chi^2$  tests were performed to examine the association between type of ecotope and the presence of *T. garciabesi*.

The term “colonized” is taken to mean the finding of at least one nymph of *T. garciabesi*. In total, 16 positive sites including 31 small nymphs in a poor condition for a reliable identification to species were excluded from the analysis. The relative importance of each type of ecotope was scaled according to the frequency of triatomine occupancy, frequency of bug colonies, and the frequency of occurrence of each type of peridomestic ecotope. Thus, ecotopes with stable bug colonies and ubiquitous across house compounds were more important for *T. garciabesi* than peridomestic ecotopes with transient colonies or infrequent. Using the frequencies of infested sites and bug colonies of *T. garciabesi* per ecotope, we constructed two separate tables that were tested by  $\chi^2$  tests; cells with small expected frequencies (<5) were combined until less than 20% of all cells had small frequencies.

The relation between the future presence of *T. garciabesi* and selective insecticide sprays at house-compound level was tested in a three-way contingency table. Data of consecutive surveys across house compounds were classified by insecticide application between time  $t$  and  $t + 1$  and the presence of *T. garciabesi* at  $t + 1$ , stratified by the current presence of *T. garciabesi* at time  $t$ . House compounds where the presence of *T. garciabesi* and *T. infestans* was not registered across the 10-yr period were excluded from this analysis. The effect of insecticide application on the future presence of *T. garciabesi*, controlling for the current presence of *T. garciabesi*, was tested by a Mantel–Haenszel test.

The relation between the relative abundance of *T. garciabesi* (total catch per unit effort) and time-variable factors for each house compound was analyzed by a generalized linear model with a correlation structure between repeated measures over the same house: generalized estimating equations (GEE). GEE is a marginal approach that produces more efficient and unbiased regression estimates in repeated-measures study designs with nonnormal response variables, accounting for the eventual correlation between observations within subjects (Zeger et al. 1988, Ballinger 2004). The number of chickens reported by each household and their peridomestic resting places varied widely with meteorological conditions, the specific needs and capacity of chicken production at household level, government-sponsored projects promoting poultry production (during 1997), and household economic conditions. The total number of trees with chickens roosting fluctuated substantially over time (see below). These variations affected the mean triatomine abundance, and therefore only total bug catch per house was considered for

analysis. Variations in the number of chickens registered in each house compound implied that both host numbers and ecotope quality were heterogeneous spatially and temporally.

In our GEE modeling, the dependent variable (total catch per unit effort) consists of count data described by a Poisson distribution, and a log link function. The number of insects collected in all sites and ecotopes from each house at a given survey occasion was pooled for analysis of the overall temporal pattern of infestation. Explanatory variables included in the model were the number of chickens (categorized in 0, 1–5, 6–20, and >20 chickens per house) to allow for the relative lack of precision of householders’ reports on the number of chickens they owned; absence of residual insecticidal spray at house-compound level over the previous 6 mo (a binary factor, with the reference level given by the prior occurrence of an insecticide spray); and seasonality (binary), to distinguish between surveys conducted during spring (October, November, and December) and fall months (May, June), the latter taken as the reference level. By adopting these reference levels, all regression coefficients represent changes in favor of increasing numbers of *T. garciabesi* bugs.

All possible interactions were considered. The data were registered on the same 61 identified house compounds over nine occasions (of the 13 surveys) for which the number of chickens was available.

The correlation over time was studied via an unstructured correlation matrix to estimate the correlation coefficients between every pair of repeated measures. We found that consecutive measures of the same house unit were more correlated than temporally distant ones, with the strength of the correlation declining in about one to two time steps. This result supported the decision of specifying a first-order autoregressive correlation structure as the working correlation matrix. To evaluate the goodness of fit of the fitted model, we calculated the Wald statistic (in which the estimate of the parameter is divided by its robust standard error estimated from the GEE model; Quinn and Keough 2002), and then compared nested full and reduced models. Additionally, we used a quasi-likelihood information criterion (QIC; analogous to Akaike’s information criterion but suitable for GEE models) and obtained similar results (not shown). The analyses were implemented on statistical platform R 3.1.0 (R Core Team 2014) using the geepack package and geeglm function (Yan 2002, Yan and Fine 2004, Højsgaard et al. 2006).

## Results

### Vector Distribution Across Ecotopes

In total, 3,756 sites of 17 types of ecotopes from 71 house compounds were inspected during 13 surveys conducted between 1993 and 2002, and 1,286 *T. garciabesi* were collected by TMC (Table 1). Over 89% of the bugs were collected in the three ecotopes with chickens associated. *T. garciabesi* bugs collected inside bedrooms or in other peridomestic structures with various resident hosts (e.g., latrines, mud ovens, and kitchens) represented <1% of the total bug collection and 37.4% of the total sites inspected. Only one adult *T. garciabesi* was found over 788 inspections of human sleeping quarters. The number of house compounds examined in each survey (mean, 57; SD, 5) varied slightly because of the construction of new houses, the destruction of previously registered ones, and the absence of householders at the time of the survey. The 70 *T. garciabesi* bugs examined were negative for *Tr. cruzi* infection; therefore, the overall prevalence of infection across the 10-yr period was 0.42% (5 of 1,194).

**Table 1.** Infestation by *T. garciabesi* in (peri)domestic ecotopes of Amamá, northwestern Argentina

| Ecotopes                    | No. of sites inspected | No. of sites positive (%) | No. of insects collected | Mean insect catch per survey (SD) | No. of sites colonized (%) | Maximum ecotope occurrence (%) <sup>a</sup> |
|-----------------------------|------------------------|---------------------------|--------------------------|-----------------------------------|----------------------------|---|
| Tree with chickens          | 745                    | 202 (27.1)                | 928                      | 71 (52)                           | 134 (18.0)                 | 93.8  |
| Tree without chickens       | 156                    | 23 (14.7)                 | 93                       | 7.2 (10.7)                        | 22 (14.1)                  | 53.8  |
| Chicken nest                | 82                     | 16 (19.5)                 | 82                       | 3.5 (7.6)                         | 11 (13.4)                  | 32.3  |
| Chicken corral              | 133                    | 6 (4.5)                   | 45                       | 6.3 (7.9)                         | 4 (3.0)                    | 40.0  |
| Pig corral                  | 419                    | 18 (4.3)                  | 44                       | 3.4 (4.4)                         | 12 (2.9)                   | 73.8  |
| Goat or sheep corral        | 308                    | 13 (4.2)                  | 40                       | 3.1 (6.5)                         | 9 (2.9)                    | 45.3  |
| Cow or horse corral         | 102                    | 4 (3.9)                   | 10                       | 0.8 (1.5)                         | 4 (3.9)                    | 16.9  |
| Piled material              | 146                    | 9 (6.2)                   | 19                       | 1.5 (3.2)                         | 7 (4.8)                    | 53.8  |
| Other                       | 24                     | 2 (8.3)                   | 8                        | 0.6 (1.7)                         | 2 (8.3)                    | 15.4  |
| Storeroom                   | 238                    | 4 (1.7)                   | 6                        | 0.5 (1.0)                         | 3 (1.3)                    | 50.8  |
| Low importance <sup>b</sup> | 615                    | 6 (1.0)                   | 10                       | 0.1 (0.6)                         | 4 (0.7)                    | 100.0                                       |
| Bedroom                     | 788                    | 1 (0.1)                   | 1                        | 0.1 (0.3)                         | 0                          | 100.0                                       |
| Total                       | 3,756                  | 304 (8.1)                 | 1,286                    | 197.5 (140.0)                     | 212 (5.6)                  |   |

The table includes information on the 13 surveys combined unless otherwise stated. An ecotope was considered positive when at least one live *T. garciabesi* was found by TMC, and colonized when at least one nymph of *T. garciabesi* was found by TMC (see text for details).

<sup>a</sup> Measures the commonness of each type of ecotope across all house compounds and surveys.

<sup>b</sup> Ecotopes that had very low importance for *T. garciabesi*: latrines, kitchens, orchard fences, nests, mud ovens, and sheds.

There was a substantial variation in the number of trees with chickens resting on them over time (Fig. 1A). Because of the uncertainties associated with a reported count variable (which is expected to be less accurate as numbers grow), we categorized the number of chickens according to interquartile ranks (Fig. 1B) for GEE modeling. Peridomestic areas were heterogeneous regarding both the type and the number of ecotopes. In total, 202 trees with chickens were infested (27.1% of the 745 trees inspected) and yielded 928 *T. garciabesi* (Table 1). Most house compounds (93.8%) had at least one tree with chickens at 10–31 m of human sleeping quarters. On average, the prevalence of infestation and total bug catch were similar in trees without chickens (14.7%, 93 insects) and in chicken nests (19.5%, 82 insects) across surveys. Half of the house compounds had at least one tree without chickens which had previously been used by chickens. Only 32.3% of the house compounds had chicken nests. Over 10% of the sites from each main ecotope (trees with chickens, trees without chickens, chicken nests) were colonized by *T. garciabesi*. The rest of the ecotopes (orchard fences, sheds [i.e., “ramadas”], kitchens not sharing a roof with bedroom areas, mud ovens, and latrines) represented less important habitats for *T. garciabesi* because: both their bug infestation and occurrence were infrequent; infestations only included adult bugs; the overall abundance of bugs was very low; and bug colonies did not persist over time. The frequency of infested sites and colonies of *T. garciabesi* differed among ecotopes in a highly significant way ( $\chi^2 = 418.66$  and  $254.84$ , respectively;  $df = 4$ ;  $P < 0.0001$ ). *T. garciabesi* bugs were not found in sensor boxes located in bedroom areas, and only five adults were caught by householders.

### Temporal Variations in Factors Affecting Bug Abundance

The total catch of *T. garciabesi* per survey in the main ecotopes showed high and low values throughout the 10-yr period (Fig. 2). The temporal fluctuations in the total abundance of bugs correlated closely with bug abundance in trees with chickens ( $r = 0.97$ ,  $P < 0.0001$ ).

Over the first 3 yr of follow-up (October 1993–May 1996), only seven house compounds were sprayed with pyrethroids during the

previous semester. House compounds were subsequently sprayed with insecticides on 463 occasions (Fig. 2). The peaks of *T. garciabesi* abundance were negatively related to the total number of insecticide applications during the semester preceding each house infestation survey (Fig. 3). The time points showing an extremely high frequency of pyrethroid sprays coincided with extremely low values of *T. garciabesi* abundance. The future presence of *T. garciabesi* bugs (at  $t + 1$ ) was significantly and negatively associated with a local insecticide application (between time  $t$  and  $t + 1$ ) after controlling for the current presence (at time  $t$ ) of *T. garciabesi* at house-compound level (Table 2; Mantel-Haenszel test,  $\chi^2 = 6.87$ ;  $df = 1$ ,  $P < 0.01$ ; odds ratio, OR = 0.55, 95% confidence interval, CI = 0.35–0.87). In house compounds uninfested with *T. garciabesi* at time  $t$ , the relative odds of future infestation was significantly reduced by insecticide applications directed against *T. infestans* (OR = 0.51, 95% CI = 0.28–0.94), whereas in houses with *T. garciabesi*, the relative odds of future infestation was less affected (OR = 0.61, 95% CI = 0.32–1.19).

### Effects of Host Availability, Insecticide Application and Seasonality

The total catch of *T. garciabesi* bugs per house compound in the nine surveys conducted between October 1993 and 2002 was best described by a saturated model that included the three explanatory variables and their interaction terms. The regression coefficients, their standard errors, Wald test statistics, and corresponding  $P$ -values are shown in Table 3. The saturated model was marginally significant ( $P = 0.047$ ); nonetheless, all reduced models without a three-way interaction term had a poor fit. House compounds with the lowest numbers of chickens, which were sprayed with pyrethroids during the preceding semester and were inspected for infestation during the fall season, had significantly lower abundance of *T. garciabesi* than house compounds with more than six chickens which had not been sprayed with insecticides over the preceding semester and were inspected during spring. When we explored the three-way interaction, there was a tendency toward lower triatomine catch when the house had previously been sprayed with insecticides, and on fall relative to spring surveys (Fig. 4).

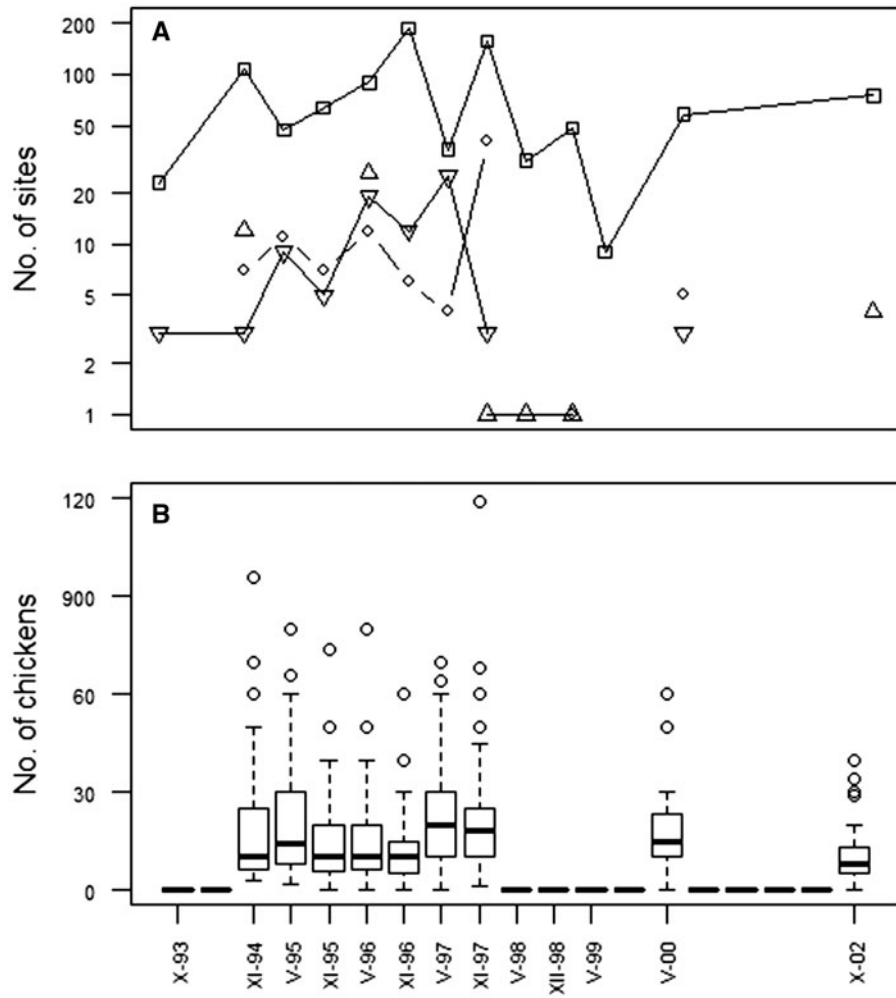


Fig. 1. Number of sites with chickens associated (A), and number of chickens per house compound over time as determined in nine surveys (B). In (A)—trees without chickens (diamonds), trees with chickens (squares), chicken nest (downward triangle), and chicken corral (upward triangle), in logarithmic scale. In (B)—the lower and upper limits of the boxes represent the 25- and 75-percentiles, respectively.

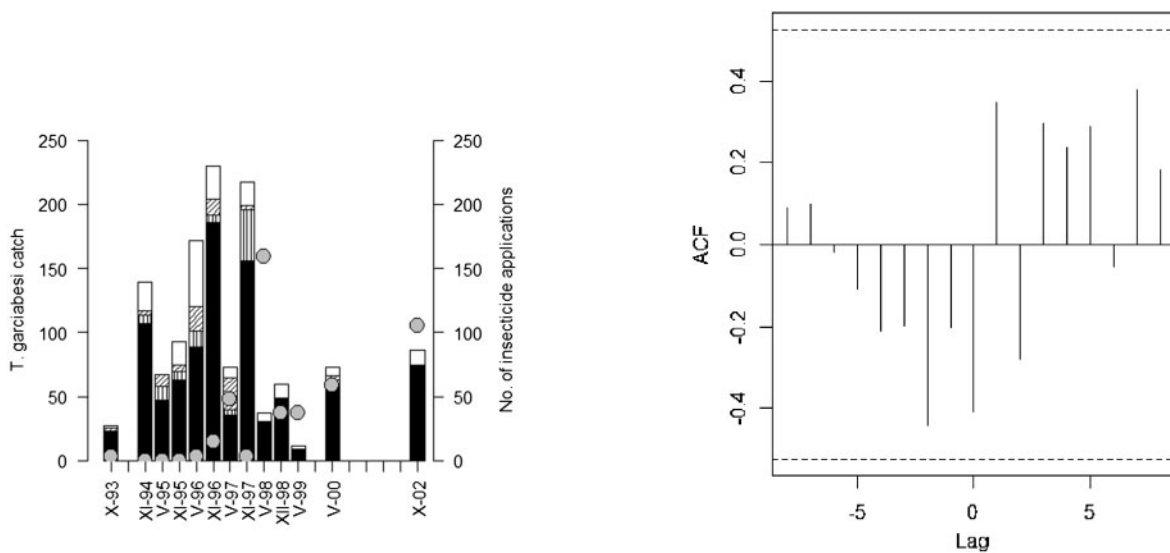


Fig. 2. Total catch of *T. garciabesi* per survey (bars) and number of insecticide applications at site level over the previous semester (circles). The height of bars represents the total bug catch across all ecotopes, and ecotope-specific catches in trees with chickens (black), trees without chickens (vertical stripes), and chicken nests (diagonal stripes).

Fig. 3. Cross-correlation between the total catch of *T. garciabesi* and the total number of insecticide sprays that occurred during the previous semester. Autocorrelation function (ACF) values in the Y axis. The dashed lines signal correlation values of 0.5 and -0.5.

**Table 2.** Relationship between current insecticide application (between time  $t$  and  $t + 1$ ) and future house infestation with *T. garciabesi* bugs (at time  $t + 1$ ), controlling for current infestation with this species (at time  $t$ ), for 638 consecutive pairs across the 10-yr period

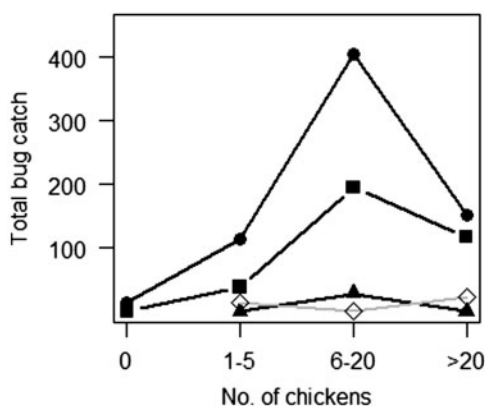
| <i>T. garciabesi</i> ( $t$ ) | Insecticide spray | <i>T. garciabesi</i> ( $t + 1$ ) Presence | <i>T. garciabesi</i> ( $t + 1$ ) Absence | Total |
|------------------------------|-------------------|---|--|-------|
| Presence                     | Yes               | 18  | 30                                       | 48    |
| Presence                     | No                | 84  | 86                                       | 170   |
| Absence                      | Yes               | 15  | 61                                       | 76    |
| Absence                      | No                | 112                                       | 232                                      | 344   |
| Total                        |                   | 229                                       | 409                                      | 638   |

**Table 3.** GEE analysis of the regression model for the total abundance of *T. garciabesi*

| Effects  | Estimate | SE     | Wald test | P        |
|--|----------|--------|-----------|----------|
| Intercept  | 0.0946   | 0.4588 | 0.04      | 0.8366   |
| Chicken numbers  | 0.3446   | 0.2095 | 2.70      | 0.1000   |
| No insecticide   | 2.7923   | 1.4104 | 3.92      | 0.0477*  |
| Seasonality  | 0.7220   | 0.5671 | 1.62      | 0.2029   |
| Chicken numbers $\times$ no insecticide                      | -1.6125  | 0.5966 | 7.31      | 0.0069** |
| Chicken numbers $\times$ seasonality                         | -0.1817  | 0.2602 | 0.49      | 0.4851   |
| No insecticide $\times$ seasonality                          | -1.8895  | 1.6272 | 1.35      | 0.2456   |
| Chicken numbers $\times$ no insecticide $\times$ seasonality | 1.3950   | 0.6980 | 3.99      | 0.0456*  |

The reference levels for each binary factor correspond to levels in which the abundance of *T. garciabesi* was expected to be the lowest (sprayed with insecticide and fall season).

Significance levels: "\*\*\*" 0.01, "\*\*" 0.05



**Fig. 4.** Total catch of *T. garciabesi* bugs according to the abundance of chickens per house compound. The data used include nine surveys in which chicken numbers at house-compound level were determined (total, 402 data points). House compounds not sprayed with insecticides during the semester preceding the triatomine survey and inspected during fall ( $n = 152$ ) are plotted in circles, and those inspected during spring ( $n = 219$ ) in squares. Compounds that were sprayed during fall ( $n = 24$ ) are plotted in triangles, and during spring ( $n = 7$ ) in grey diamonds.

## Discussion

Our study shows that the peridomestic abundance of *T. garciabesi* per compound was significantly modified by the interaction between season (spring versus fall), chicken abundance (positive effects), and selective insecticide applications targeting *T. infestans* (negative

effects) in peridomestic structures. The infestation with *T. garciabesi* was restricted to peridomestic ecotopes, particularly in those occupied by chickens, in line with our hypothesis and preliminary findings.

Trees with chickens were the key peridomestic ecotope of *T. garciabesi*. The maximum prevalence of infestation and bug abundance, and the maximum number and persistence of bug colonies were found in trees where chickens roosted, followed by chicken nests and by trees which had previously been occupied by chickens. Other ecotopes harbored minor bug infestations, transient bug colonies, or only adult bugs, and therefore were of marginal relevance. The trees with chickens were the most important habitats because they apparently combined the best conditions for site occupancy and productivity of *T. garciabesi*: building materials and structures closely related to sylvatic ecotopes (Gajate et al. 1996) and host availability (Gürtler et al. 2014). The infested trees with no current chicken occupancy revealed both the fluctuations of host availability and the persistence of bug colonies despite of the absence of the local host. In contrast, chicken nests usually had many hosts but the building materials and structure differed substantially from the tree barks where *T. garciabesi* is usually found in sylvatic habitats.

Almost every peridomestic area of each house compound had at least one tree with chickens. Therefore, both the preferred host and best-quality habitat for *T. garciabesi* coincided. In addition, the abundance of *T. garciabesi* was positively associated with the abundance of chickens in a house compound. In agreement with these findings, the presence of *Triatoma maculata* was positively related to the number of chicken nests in Venezuela (Sanchez-Martin et al. 2006), and so was domestic *T. infestans* in northern Argentina (Cecere et al. 1997). The 1996 and 1997 surveys showing the highest densities of *T. garciabesi* exactly coincided with the years in which the federal rural development agency (INTA) distributed chickens among all study households to promote a food security program. The median abundance of chickens peaked during these years, and later returned to equilibrium chicken abundance. Another factor that likely contributed to the larger abundance of *T. garciabesi* during 1996 and 1997 is the near absence of insecticide applications since the last community-wide house spraying campaign conducted in 1992.

Human sleeping quarters were rarely invaded and failed to be colonized by *T. garciabesi* over the entire 10-yr period despite of the concurrent loss of natural habitats through deforestation and other land-use changes, and the near absence of domestic *T. infestans*. These results are consistent with earlier observations (Wisnivesky-Colli et al. 1993) that did not register *T. garciabesi* in 43 bedroom areas of Amamá and neighboring villages between 1982 and 1990. Therefore, *T. garciabesi* appears to be unable to replace *T. infestans* in domestic premises of the study area, and suggests the former at best may have a marginal role in peridomestic transmission cycles given their strong association with chickens, which are refractory to *Tr. cruzi* infection.

Seasonality affects the probability of individual bug catch because low temperatures depress or halt triatomine activity, more so in certain habitats with structures and building materials that are intrinsically more difficult to search for bugs (e.g., thatched roofs, cracked walls), and in which catches depend primarily on bug mobility in response to the dislodging aerosol. Seasonal variations of temperature affect different ecotopes in different manners: the more the site is modified, the more the temperature is buffered (Vazquez-Prokopec et al. 2002). The establishment of new colonies of *T. infestans* between fall and spring in Amamá was significantly more frequent than between spring and fall, but it was not possible to

distinguish if this pattern was due to seasonal establishment, the low sensitivity of the bug collection method when temperatures are low, or a combination of both mechanisms (zu Dohna et al. 2007). In our study, ecotopes usually occupied by *T. garciabesi* were either slightly or not modified relative to its sylvatic habitats (i.e., trees, nests without roof), and they were exposed to seasonal and daily weather variations. The probability of bug detection outdoors might be substantially reduced as the fall season proceeds, and so does bug abundance, which tended to peak during spring likewise *T. guasayana* (Vazquez-Prokopec et al. 2005, 2008). Timed manual collections have limited sensitivity (Gürtler et al. 2001), with multiple searches of the same site increasing the probability of bug detection (Abad-Franch et al. 2010a). On the flip side, all searches for bugs were as exhaustive in ecotopes occupied almost exclusively by *T. garciabesi* as in those that are more typical of *T. infestans*.

Analysis of correlated data under a population-averaged approach (GEE) has limitations and strengths. Unregistered host availability information in 4 of the 13 surveys restricted the analysis to a subset of the 10-yr data, and determined that some survey events were not equally spaced in the temporal sequence. Although the first-order autoregressive correlation matrix specified in the model assumes equal spacing between repeated measures, the GEE method is robust to misspecifications of the initial relationship of the within-subject correlation (Liang and Zeger 1986). GEE estimation can handle missing data only if such data are missing completely at random (Zorn 2001). All missing values in the time series of *T. garciabesi* most likely were generated at random. Despite of these limitations, the versatility of GEE for longitudinal data analysis allowed us to treat nonnormally distributed data and within-subject correlation when intercluster correlation is a problem and not of substantive interest by itself (Ballinger 2004), as in subject-specific approaches which are generally analyzed via random-effects multilevel models. Another limitation of our GEE-based analysis at the house-compound level is that (re)infestation occurs at a finer level (habitat- or site-specific) which is hard to observe. Assessing the precise number of hosts at each peridomestic site at a relevant time scale entails a sizable research effort, and the site presence and abundance of chickens may change rather fast.

Despite the lower effectiveness of pyrethroid insecticides in peridomestic habitats (Gürtler et al. 2004, Cecere et al. 2013), *T. garciabesi* was negatively affected by selective pyrethroid sprays targeting *T. infestans*. Furthermore, insecticide sprays apparently reduced the likelihood of future infestations with *T. garciabesi*: infestations were less frequent in house compounds that were sprayed with insecticide during the previous semester than in untreated house compounds. In line with these findings, *Rhodnius ecuadoriensis* was also affected by insecticide applications directed against *Aedes* mosquitoes (Grijalva et al. 2010), and *T. sordida* was successfully controlled in peridomestic areas with an annual application of pyrethroids (Diotaiuti et al. 1998).

Vazquez-Prokopec et al. (2005) suggested that house reinfestation by *T. guasayana* in Amamá village originated from sylvatic bug colonies through frequent flight dispersal (this species is a well-known flier) and recurrent house invasion, and not by peridomestic residual foci that survived insecticide application. In contrast, *T. garciabesi* has rarely been collected by light traps or householders (Wisnivesky-Colli et al. 1993, Gürtler et al. 1999). Therefore, peridomestic reinfestation with this species may have originated from residual foci that survived the selective insecticide applications, especially because trees were sprayed approximately up to 2.5 m height, leaving a large surface of the bark untreated. Other likely sources of reinfestation bugs were from neighboring sylvatic areas

(although they were subject to increasing deforestation during the study period), and from undetected and unsprayed peridomestic foci. The pattern is similar to those displayed by *Triatoma pallidipennis* and *Triatoma barberi* after a community-wide insecticide spraying in Mexico, in which peridomestic foci detected postintervention most likely were residual (Ramsey et al. 2003). Similarly, peridomestic habitats most likely were the origin and main source of the *T. infestans* bugs that fueled domestic reinfestation after insecticide spraying (Cecere et al. 1997, 2004). Our results suggest that insecticide applications targeting *T. infestans* affected the abundance of *T. garciabesi*, and reduced the likelihood of future infestation. In line with the goal of keeping peridomestic areas free from triatomines to prevent the transmission of *Tr. cruzi* (Guhl et al. 2009), our findings suggest that the presence and abundance of secondary triatomine vectors in peridomestic habitats are significantly reduced though not suppressed by standard insecticide application procedures.

## Acknowledgments

We thank Dr Roberto Chuit and Abel Hurvitz and his staff at the National Control Service (Argentina) for providing active support during fieldwork; María Moyano and Omar Sitatti for field accommodation; and the residents of Amamá for their participation in insect searches. The Amamá database is the product of a sustained collaborative effort between researchers from the University of Buenos Aires (R.E.G.); the Directorate of Epidemiology (Ministry of Health and Social Action, Argentina) and National Chagas Service (Roberto Chuit); and Rockefeller University (Joel E. Cohen) between 1992 and 2000. This project was supported by Agencia Nacional de Promoción Científica y Técnica (Argentina) and University of Buenos Aires awards to R.E.G.

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