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Monitoring house reinfestation by vectors of Chagas disease: a comparative trial of detection methods during a four-year follow-up

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Abstract

Domestic reinfestations by triatomine bugs were monitored after application of deltamethrin and apparent elimination of *Triatoma infestans* in Amamá and other nearby rural villages, north-west Argentina, from 1992 to 1996. The five methods used were sensor boxes, sheets of pink typing-paper, timed manual catches by a skilled three-person team aided by a flushing-out agent, collections by house-dwellers, and knockdown using insecticide fumigant canisters. In bedrooms, house-dwellers collected *T. infestans* significantly more frequently than the flushing-out method, but the reverse occurred in peridomestic sites. Both methods and sensor boxes revealed the frequent invasion of adult *Triatoma guasayana* and *T. infestans*, but neither *T. guasayana* nor *Triatoma sordida* colonized bedroom areas in spite of their rising abundance in nearby peridomestic sites. Sensor boxes were significantly more sensitive than the matched paper-sheets in three of five cross-sectional surveys. On average,

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each box recorded 2.0–3.2 times more triatomine fecal smears than each paper sheet. The frequency of dejecta in sensor boxes correlated positively with the proportion of houses where *T. infestans*, *T. guasayana* or *T. sordida* were captured by any method in bedroom areas. Triatomine fecal smears in sensor boxes were the earliest and most frequent sign of domiciliary infestation, followed by dwellers' collections of adult bugs. Analyzing the data prospectively, we provide a quantitative, predictive understanding of detection methods and review the validity and interpretation of the different signs of infestation obtained. The most sensitive and cost-effective combination of detection methods for vector surveillance in domestic areas was the use of sensor boxes and house-dwellers collections. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Sampling; *Triatoma infestans*; *Triatoma guasayana*; *Triatoma sordida*; Argentina; Chagas disease; Sensitivity; Specificity

1. Introduction

Early detection of houses reinfested by triatomine bugs is essential to monitor the effects of control actions and to establish the need for additional operations. Detecting the presence of triatomines in mud-and-thatch houses is a difficult task that may be carried out by active or passive methods. Active methods include the search for bugs and capture: (i) by house-dwellers during prolonged and undefined periods (Schenone et al., 1979; Marsden and Penna, 1982); (ii) by trained personnel aided with a flushing-out agent during a fixed searching time per house (Schofield, 1978; Pinchin et al., 1981); (iii) after application of an insecticide with knockdown power (Gürtler et al., 1993); and (iv) during partial or complete house demolition (Rabinovich et al., 1995). Passive methods involve use of different designs of cardboard boxes (Gómez-Núñez, 1965; Wisnivesky-Colli et al., 1988), and sheets of typing-paper or calendars (García-Zapata and Marsden, 1993). All such devices are fixed to bedroom walls for long periods and usually detect infestations through indirect signs of bugs, such as triatomine fecal smears, but the boxes also allow the collection of exuviae, eggshells or bugs.

Timed manual capture (with or without flushing-out agents) has long been the standard reference for triatomine surveillance, but it has been gradually replaced by passive methods. Comparisons between Gómez-Núñez boxes and the flushing-out method yielded variable results, probably owing to large variations in the methodology used (Schofield, 1978; Pinchin et al., 1981). In several long-term studies, however, sensor boxes or paper-sheets were at least as sensitive as and more cost-effective than the flushing-out method (García-Zapata et al., 1988; Chuit et al., 1992). In a short-term comparative trial in a densely infested community, sensor boxes were individually more sensitive than matched paper-sheets at low and intermediate densities of *Triatoma infestans* (Gürtler et al., 1995). No comparative field trial of their performance after application of residual insecticides, when bug abundance is very low, has been carried out even though these methods are recommended for surveillance activities.

As part of a larger project, in this study our objective was to test whether sensor boxes were more sensitive than paper-sheets in detecting domestic reinfestations by triatomine bugs in rural villages that had been sprayed with deltamethrin. Because of the likelihood that the sensing devices might reflect the presence of non-target sylvatic triatomine species that invade but rarely colonize houses (*Triatoma sordida* (Stål) and *Triatoma guasayana* Wygodzinsky and Abalos), results obtained were rigorously compared with those obtained by other active methods. Analyzing the data prospectively, we provide a quantitative, predictive understanding of detection methods and review the validity and interpretation of the different signs of infestation obtained. The present results contribute to the choice of a low-cost, simple and effective method, or combination of methods, for monitoring domestic bug reinfestations at district or regional levels.

2. Materials and methods

2.1. Study area

Field studies were carried out in the rural villages of Amamá, Trinidad, Mercedes, Villa Matilde and Pampa Pozo (27°S, 63°W), Province of Santiago del Estero, Argentina. The area and their history of infestation by *T. infestans* were described before (Gürtler et al., 1994). In mid-October 1992, all 93 houses and their peridomestic outbuildings were sprayed thoroughly with deltamethrin following standard procedures, after which search for residual foci of *T. infestans* and selective deltamethrin treatments were carried out (Cecere et al., 1997).

2.2. Survey design and entomologic methods

A comparative prospective study of matched pairs of sensor boxes and paper-sheets was initiated at Amamá in December 1992, extended to the other villages in October 1993 to increase the sample size, and ended on May 1995 in view of the results obtained.

The vector collection methods used were described before (Gürtler et al., 1995). In mid-December 1992, an average of three (range, 2–5) sensor boxes (Biosensor, Biocientífica de Avanzada®, Buenos Aires) were placed indoors or in the veranda of each house to monitor reinfestation by triatomine bugs. A total of 259 sensor boxes were tacked to bedroom walls of 87 houses. The location of each box was recorded on a sketch map of each house. In 47 houses from Amamá, the boxes were paired with sheets of non-absorbent pink typing-paper (33×22 cm) to assess the relative sensitivity of both devices. Each device was marked with the installation date, house and pair number. In October 1993, the matching procedure was extended to 53 houses from Trinidad, Mercedes, Villa Matilde and Pampa Pozo. House-dwellers were told not to touch or move the devices and to replace them if they fell down. New houses built after December 1992 were included in the study in the first round in which they were censused.

All sensing devices were inspected for evidence of bug infestation each 6 months from May 1993 to May 1997. On each round, for each house we recorded for each sensing device: (i) the number, instar and species of any triatomine bug found; (ii) their exuviae or eggs; and (iii) the number and location of fecal smears considered to be typical of triatomine bugs, as determined with the key of Schofield et al. (1986), or dubious. In this study, 'triatomine fecal smears' refers to typical triatomine dejecta unless otherwise stated. The same person judged fecal smears in the presence of reference samples of dejecta voided by different arthropods and instars of triatomines in the laboratory. Every smear was marked with ink and dated to avoid its inclusion on the following round. *T. infestans* eggs could be differentiated from other local triatomine eggs based on their size, but the identity of exuviae was less firmly established. Absent or deteriorated sensing devices were replaced in each round, and all sensor boxes were substituted by new ones in November 1995.

Every 12 months from October 1993 to November 1996, three skilled bug collectors from the National Chagas Service searched for triatomines in all bedroom and peridomestic areas using 0.2% tetramethrin as flushing-out agent. During 30 min per house, two men searched bedrooms (one person/h) while another man searched peridomestic sites (1/2 person/h per house). Additional searches for bugs were carried out at peridomestic sites each May from 1995 to 1997. The total number of inhabited houses inspected by flushing-out in each round increased from 87 to 109 houses, from October 1993 to May 1994, due to the inclusion of Villa Matilde and Pampa Pozo. Every 6 months thereafter until May 1997, the number of houses inspected by flushing-out was 103, 114, 111, 106, 113 and 118 houses, respectively; variations were due to inclusion of newly built houses and loss of a few that were closed during the survey weeks or indefinitely.

In May 1993 and thereafter, a labeled self-sealing plastic bag was provided to each household to contain any triatomine that they could capture in domestic or peridomestic sites. At each visit, house-dwellers were asked for their bag collections, the place where they had captured the bugs, and the presence or sighting of triatomines in bedrooms or peridomestic areas. Dwellers were shown pinned specimens of *T. infestans* nymphs and adults to check the identity of the species they reported. All bugs were later identified to species and stage at the field laboratory following the keys of Lent and Wygodzinsky (1979) and Brewer and Garay (1989). Species determination were always made regardless of the identity of adult triatomines or older nymphs in each colony. The relative length of the second rostral to the first segment was the main character used to distinguish between adults of both species. Difficulties in distinguishing between species for first, second and third instar nymphs of *T. sordida* and *T. guasayana* were frequent. In a few cases, the small nymphs were in poor conditions or identifications were considered unreliable; these bugs were excluded from the database.

As a further check of triatomine infestation in bedrooms, 30 houses that had triatomine fecal smears in sensor boxes at least once from November 1994 to November 1995 were intensively searched for bugs after applying insecticides with knockdown power in November 1995. Treatments included one or two fumigant

canisters (Agufog, Aguvac, Buenos Aires) per bedroom in 28 houses, and a standard spraying with deltamethrin in two houses that were too open for an effective treatment with fumigant canisters. The knocked down bugs were collected 3–5 h after treatment. All sites with *T. infestans* nymphs collected were treated with deltamethrin (in both domestic and peridomestic areas) or insecticide fumigant canisters (indoors) by the staff (1993–1995) or house-dwellers (1996–1997).

For flushing-out or knockdown searches the terms 'infested' or 'positive' were taken to mean the finding of at least one live or moribund triatomine of the species under consideration; for sensor boxes, these terms meant at least one sign of infestation (i.e. triatomine fecal smears or *T. infestans* bugs, eggs or exuviae); and for paper sheets, the finding of one or more triatomine fecal smears. A house was 'colonized' when nymphs were found in it; nymphs are evidence that a colony resides in the house because these stages are less likely to disperse actively from house to house or from peridomestic sites to domiciliary areas. A house was 'invaded' when a single adult bug (or very few) was found in it.

2.3. Data analysis

The number of triatomine dejecta in sensor boxes or paper sheets in each 6 month survey and catch of triatomines for each calendar year (x) were transformed to $\log_{10}(x+1)$ to normalize the distributions. The ratio of triatomine dejecta in sensor boxes (y) to paper sheets (z) was calculated as $\log_{10}[(y+1)/(z+1)]$.

3. Results

To analyze the relative outcome of sampling methods, first we show the temporal pattern of infestation for the target species *T. infestans* separately from other triatomines.

3.1. Triatoma infestans

A total of 570 *T. infestans* were collected in domiciliary (50.2% of bugs) and peridomestic sites from 1993 to 1996. All three methods (flushing-out, house-dwellers and sensor boxes) recorded an increasing trend of infestation during this period (Fig. 1), but there were differences among methods in peak prevalence of infestation, catch and fluctuation patterns. Regarding the flushing-out method in bedroom areas, the first *T. infestans* was captured in 1994 (Fig. 1A) even though in other houses *T. infestans* eggs, exuviae or bugs had been captured by house-dwellers or in sensor boxes (Table 1, Fig. 1B). Other domiciliary arthropods were rare in 1993–1994, in contrast with observations made before deltamethrin spraying and from 1995 onwards. The prevalence of infestation and catch of *T. infestans* by flushing-out in peridomestic sites surpassed that in bedroom areas in all surveys for which data were available.

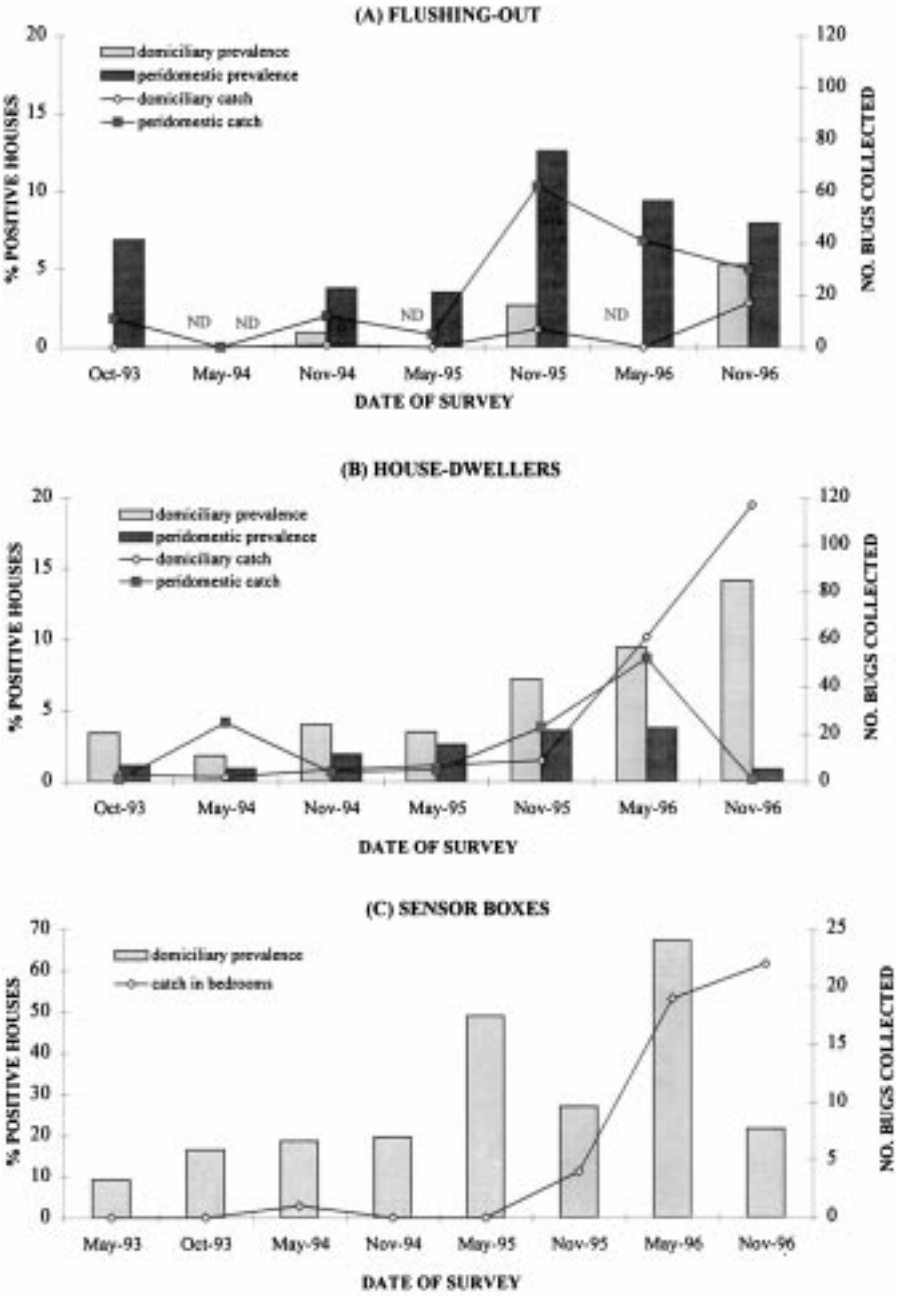

Fig. 1. Prevalence of infestation and total catch (all houses) of *T. infestans* in bedrooms and peridomestic sites by the flushing-out method (A) house-dwellers (B) and sensor boxes (C); Amamá and nearby villages, 1993–November 1996. Bars refer to the left axis labels; lines and points refer to the right axis labels. ND, no data.

Table 1
Detection of domestic infestations by triatomine bugs using 2–5 sensor boxes tacked to walls of bedroom areas; Amamá and close villages, December 1992–November 1996

Inspection date	No. houses positive[a]/no. inspected (%)	No. signs recorded				Mean no. of signs/house[b]
		Bugs	Eggs	Exuviae	Fecal smears	
May 1993	8/87 (9)	0	8	2	55	8.1
October 1993	14/85 (16)	0	0	0	20	1.4
May 1994	19/102 (20)	1	0	0	60	3.2
November 1994	20/102 (20)	0	0[c]	0	40	2.0
May 1995	49/100 (49)	0	1	1	194	4.0
November 1995	29/111 (26)	1[d]	17[d]	0	79	3.3
May 1996	69/98 (67)	19	6	16	591	9.6
November 1996	20/102 (20)	18[e]	15[e]	1	111	7.2

[a] Positive, any sign of infestation (triatomine fecal smears or *T. infestans* exuviae, eggs or bugs) in sensor boxes.

[b] Mean number of *T. infestans* eggs, exuviae, bugs or triatomine fecal smears per positive house.

[c] Excludes one finding of eggs from sylvatic triatomines concurrent with triatomine fecal smears in a sensor box.

[d] Excludes five findings of eggs, first-instar nymphs or adult sylvatic triatomines: four concurrent and one non-concurrent with triatomine fecal smears in sensor boxes.

[e] Excludes three findings of first-instar nymphs or eggs of a sylvatic triatomine: one concurrent and two non-concurrent with triatomine fecal smears in sensor boxes.

House-dwellers collections of *T. infestans* are shown in Fig. 1b. The median catch in bedrooms in any one survey ranged from one adult bug per house in 1994–1995 to 2–3 adult bugs in 1996 (overall mode, one bug). In peridomestic sites, dwellers usually collected one or two *T. infestans*, colonies with many bugs being found only in four occasions. Among households that did not capture triatomines, dwellers frequently reported the sighting of *T. guasayana* or *T. sordida* (according to their description) in bedrooms or peridomestic sites. The total number of households reporting positive sightings of triatomines without capture were 16 in October 1993, and for each successive 6 month period, 15 and 22 in 1994, six and 14 in 1995, and six and 24 in 1996.

Using sensor boxes, when the prevalence of houses with signs of domiciliary infestation was measured at the end of the warm season, in May, the prevalence rose from 9% in 1993 to 67% in 1996 (Table 1, Fig. 1C). However, when the prevalence of houses with signs of domiciliary infestation was measured after the cold season, the prevalence oscillated in the range from 16 to 26% over the same years, without a clear rising or falling trend. Typical triatomine fecal smears were the most common evidence of infestation, but dubious dejecta were almost as frequently found in each survey and correlated significantly, though weakly, with typical dejecta (e.g. for November 1995, $r = 0.21$, $df = 109$, $P < 0.05$). In decreasing order of importance, other signs of infestation recorded were *T. infestans* eggs, bugs (68% were nymphs), and exuviae. Eggs or first instar nymphs of an unidentified triatomine species and a male *T. guasayana* were also collected from the boxes, most of which also had concurrent triatomine fecal smears.

3.2. Sylvatic triatomines

From 1993 to 1996, the total capture of *T. guasayana* was 123 bugs in bedrooms and 463 bugs in peridomestic sites, whereas for *T. sordida* it was 15 bugs in bedrooms and 1065 in peridomestic sites. Using the flushing-out method in bedrooms, a total of ten adult *T. guasayana* in six houses and only one *T. sordida* were captured (Figs. 2A and 3A). During this period, the prevalence of peridomestic infestation rose from 1 to 23% for *T. guasayana*, and from 17 to 49% for *T. sordida*, whose numbers clearly exceeded those of *T. guasayana*. Only once was a *T. sordida* nymph collected in bedrooms.

House-dwellers very frequently collected adult *T. guasayana*, but only exceptionally *T. sordida* (Figs. 2B and 3B), while the bugs were attempting to bite them either in domestic or peridomestic sites. Exceptional collections of other triatomine species included one *Triatoma delpontei* Romaña and Abalos and one *Panstrongylus guentheri* Berg attracted to lights.

3.3. Comparison of methods

For flushing-out and house-dwellers' catches, we pooled individual house data for each method from October 1993 to November 1996 for those households with a complete data set. In bedrooms, dwellers captured *T. infestans* significantly more

frequently than skilled collectors using the flushing-out method, whereas in peridomestic sites the reverse pattern was recorded (Table 2).

In 1993 and 1994, 10 and 23% of paper sheets were lost as compared with 0 and 5% of sensor boxes, respectively. Less than 3% of both devices were damaged in any one survey. Table 3 shows the pooled results of all paper-sheets compared with those of all sensor boxes applied in each house after deltamethrin spraying in 1992. Sensor boxes were consistently more sensitive than paper-sheets in all surveys, even when the criterion of positivity for sensor boxes relied only on fecal smears (see footnote d of Table 3). Statistically significant differences between sensing devices were observed in May and November 1994 and May 1995. The log-transformed ratios of the number of triatomine fecal smears in sensor boxes to that on paper-sheets was taken as a measure of the relative sampling efficacy of the two

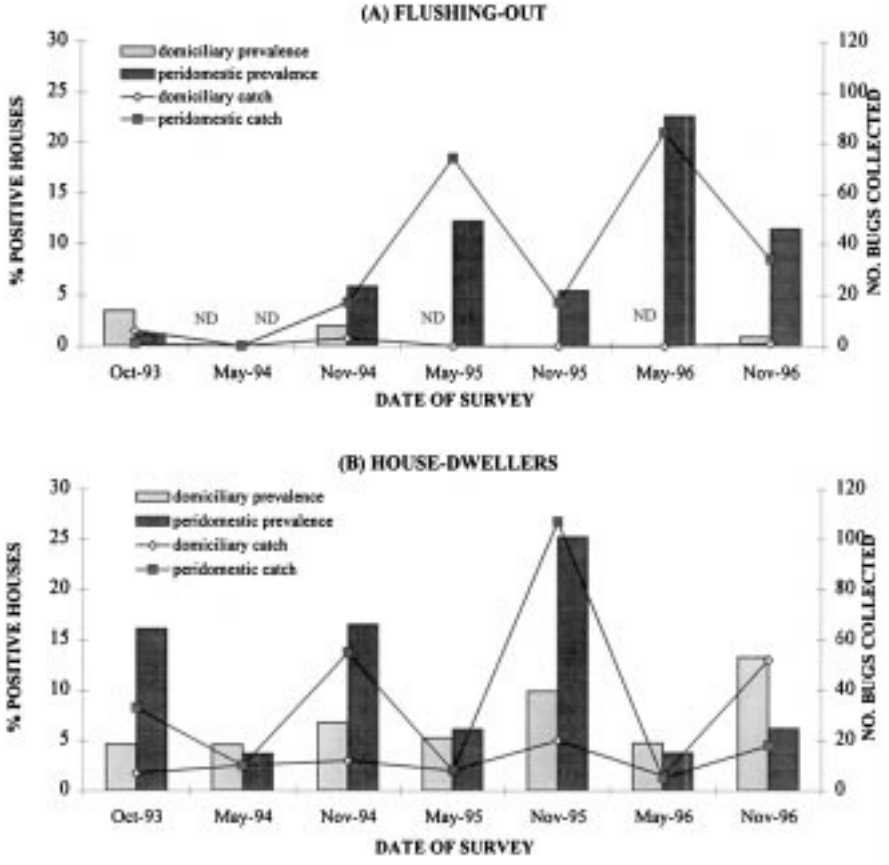

Fig. 2. Prevalence of infestation and total catch (all houses) of *T. guasayana* in bedrooms and peridomestic sites by the flushing-out method (A) and house-dwellers (B); Amamá and nearby villages, October 1993–November 1996. Bars refer to the left axis labels; lines and points refer to the right axis labels. ND, no data.

Table 2
Paired collections of *T. infestans* by the flushing-out method and house-dwellers' collections in domestic and peridomestic sites of 98 houses with a complete data set; Amamá and nearby villages, October 1993–November 1996

Collection site	No. positive			No. negative by both methods	McNemar's test
	By both methods	Only by flushing-out	Only by dwellers		
Bedrooms	5	3	21	69	$P<0.001$
Peridomicile	5	25	5	63	$P<0.001$

Table 3
Detection of domestic infestations by triatomines by paired sensor boxes and sheets of paper; Amamá and nearby villages, December 1992–May 1995. Data express the pooled results of all sensor boxes versus all paper-sheets in each house

Inspection date	No. positive[a]			No. negative by both methods	Geometric mean ratio of fecal smears (CI)[b]
	By both methods	Only by boxes	Only by paper sheets		
May 1993[c]	1	3[d]	1	42	–
October 1993[c]	0	5	0	40	–
May 1994	1	18[d]	0***	73	3.0 (2.4–3.8)
November 1994	2	18[e]	3*	79	3.2 (2.3–4.4)
May 1995	4	42	4***	37	2.0 (1.5–2.7)

[a] Positive, for sensor boxes means any type of evidence of infestation (triatomine fecal smears or *T. infestans* eggs, exuviae or bugs); for paper-sheets means the finding of triatomine fecal smears.

[b] Mean number of triatomine fecal smears in sensor boxes over paper sheets (transformed to $\log_{10}[(y+1)/(z+1)]$); CI, 95% confidence interval.

[c] Includes only houses from Amamá.

[d] One house positive by the finding of *T. infestans* eggs, exuviae or bugs but not triatomine fecal smears.

[e] Concurrent finding of eggs from a sylvatic triatomine and triatomine fecal smears in one house.

* $P<0.05$ by binomial or McNemar's test.

*** $P<0.001$ by binomial or McNemar's test.

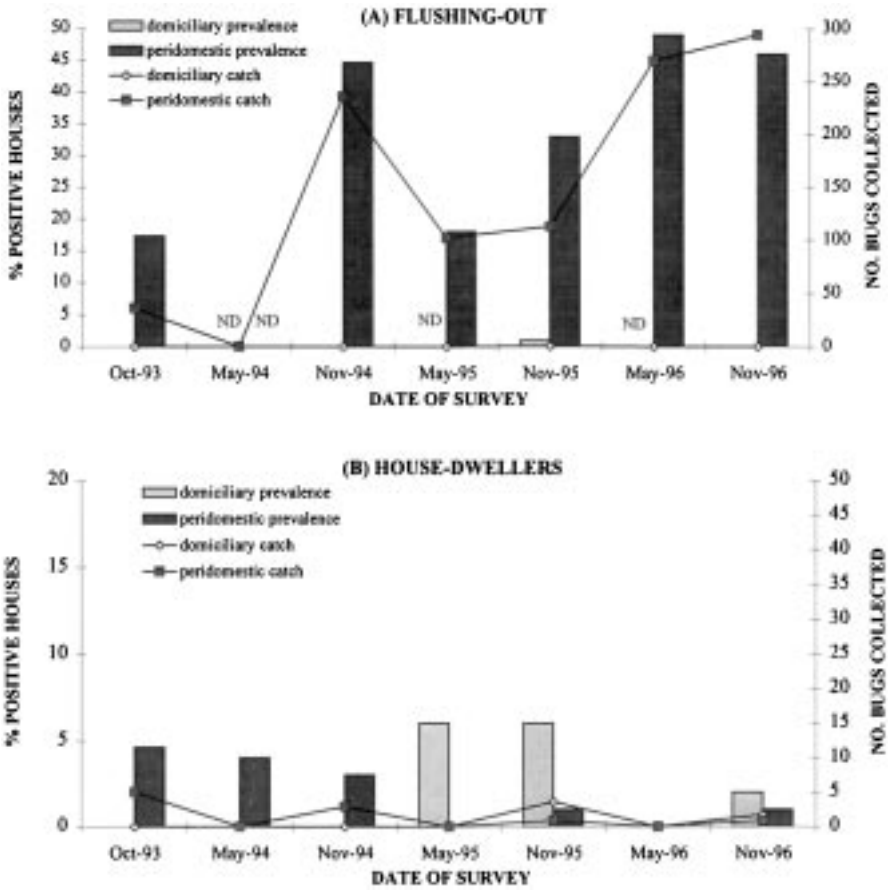

Fig. 3. Prevalence of infestation and total catch (all houses) of *T. sordida* in bedrooms and peridomestic sites by the flushing-out method (A) and house-dwellers (B); Amamá and nearby villages, October 1993–November 1996. Bars refer to the left axis labels; lines and points refer to the right axis labels. ND, no data.

methods (Table 3). In May 1994, the mean log ratio was 0.4824 (S.E. = 0.0484); thus, the geometric mean ratio was 3.0 smears in boxes per smear in the matched paper sheets. In November 1994 and May 1995, sensor boxes also recorded a significantly higher number of triatomine fecal smears than paper sheets.

Table 4 shows the relationship between the number of triatomine dejecta recorded by sensor boxes from November 1994 to November 1995 and the proportion of houses where triatomines were collected in bedrooms from November 1994 to November 1996. Among 30 houses with 1–40 triatomine fecal smears that were also assessed by knockdown collections in November 1995, *T. infestans* was collected inside bedrooms in 12 (40%) of the houses by any method in 1994–1995 ($12 = 3 + 5 + 4$ from column 3 of Table 4), and in five additional houses when the observation period was extended to November 1996 ($17 = 4 + 9 + 4$ from column 4

Table 4
Association between the frequency of triatomine dejecta in sensor boxes recorded from November 1994 to November 1995 and domiciliary findings of *T. infestans*, *T. guasayana* or *T. sordida* in the same period and during 1996; Amamá and nearby villages, November 1994–November 1996

Frequency of triatomine dejecta per house (range per box)	Number of houses	Cumulative number (%) of houses positive for				
		T. infestans in		*T. guasayana* or *T. sordida* in		
		1994–1995	1994–1996[a]	1994–1995	1994–1996	Overall
0	30	4 (13)	7 (23)	3 (10)	7 (24)	11 (35)
1–2 (0.2–0.7)[b]	11	3 (27)	4 (36)	4 (36)	4 (36)	7 (64)
3–9 (0.6–3)[b]	13	5 (38)	9 (69)	5 (38)	8 (62)	10 (77)
10–40 (2.4–20)[b]	6	4 (67)	4 (67)	4 (67)	4 (67)	5 (83)
Total	60	16 (27)	24 (40)	16 (27)	23 (38)	33 (54)

[a] The percentage of houses with *T. infestans* nymphs was 7, 9, 31 and 50% for increasing numbers of triatomine dejecta, respectively.
[b] Houses selected on the basis of having triatomine dejecta in sensor boxes from November 1994 to November 1995 that were also evaluated by knockdown in November 1995.

of Table 4). Therefore, *T. infestans* was detected in 57% (17/30) of dejecta-positive houses, but in only eight (27%) of these houses were nymphs collected. The proportion of houses where *T. infestans* was captured by any method in bedrooms from 1994 to 1996 increased from 23% when no dejecta were found in sensor boxes to 67–69% in houses with three or more dejecta. These values represent the predictive value of triatomine dejecta in sensor boxes over a defined time period. Similarly, the proportion of houses with *T. infestans* nymphs increased from 7 to 50% for increasing numbers of triatomine dejecta. The relationship between triatomine dejecta and the proportion of houses with *T. guasayana* or *T. sordida* collected in bedrooms was very close to that obtained with *T. infestans*. Overall, triatomine bugs were collected in bedroom areas of 73% (22/30) of houses with triatomine dejecta in sensor boxes. Extension of the follow-up to 1997 allowed seven new findings of *T. infestans*, five of which were in houses that previously had no dejecta, and only one catch of other triatomines.

To explain variations in the annual frequency of fecal smears in sensor boxes in the absence of established domiciliary infestations (i.e. colonizations), we used the catch of triatomines in a given collection site by a given method as an index of bug abundance or house invasion. For 85 houses with data for every year, the indices considered were: (a) the annual number of *T. infestans* collected by any method (sensor boxes, flushing-out or house-dwellers), considered separately for bedrooms and peridomestic sites; (b) the annual number of *T. guasayana* collected in domestic or peridomestic sites by any method; and (c) the annual number of *T. infestans* or *T. guasayana* collected by house-dwellers in domestic or peridomestic sites (i.e. mostly adult bugs that invaded by flight). None of these indices for every survey year correlated significantly with the frequency of fecal smears recorded by sensor boxes in 1993 (not shown in tables). The frequency of dejecta in 1994 correlated positively and significantly with future domiciliary *T. infestans* catches in 1995 ($r = 0.26$, $P < 0.05$), and with house-dwellers' catches of *T. infestans* or *T. guasayana* in 1995 ($r = 0.24$, $P < 0.05$), and 1996 ($r = 0.27$, $P < 0.05$). Triatomine fecal smears in 1995 correlated positively and significantly with concurrent *T. guasayana* catches ($r = 0.31$, $P < 0.01$), and dwellers' collections of *T. infestans* or *T. guasayana* in 1995 ($r = 0.31$, $P < 0.01$). A highly significant and positive correlation was observed between the frequency of dejecta in 1996 and current domiciliary *T. infestans* catch ($r = 0.61$, $P < 0.001$); significant correlations were also found for *T. guasayana* catches in 1995 ($r = 0.23$, $P < 0.05$), and dwellers' collections of both species in 1995 ($r = 0.25$, $P < 0.05$) and 1996 ($r = 0.31$, $P < 0.05$). *T. infestans* catches in peridomestic sites did not correlate with the frequency of dejecta in any survey.

Houses at which *T. infestans* bugs were collected in bedroom areas at least once from 1993 to May 1996 were scrutinized for concurrent or subsequent evidence of infestation with *T. infestans* (represented by the finding of adults or nymphs by any method) and colonization (at least one nymph) according to sampling method and type of evidence initially collected (Table 5). All houses were followed up for at least 1 year after initial detection (median, 1–1.5 years; range, 1–3.5 years). After the initial collection of *T. infestans* eggs, exuviae or bugs in sensor boxes, subse-

Table 5
Frequency of additional *T. infestans* (adults or nymphs) collections in domiciliary areas according to sampling method and type of evidence of domiciliary infestation initially collected; Amamá and nearby villages, December 1992–May 1997

Sampling method	Initial type of evidence	Number (%) of houses positive[a] for *T. infestans* in bedroom		Number of house-years of follow-up	Rate of further detection of bugs/year of follow-up (S.E.)[c]
		Initially[b]	Additionally		
Sensor boxes	Eggs	5	4 (80)	13	0.308 (0.304)
	Exuviae	6	3 (50)	10	0.300 (0.342)
	Bugs	10	7 (70)	12.5	0.560 (0.406)
Flushing-out in					
Bedroom areas	Bugs	3	3 (100)	5.5	0.546 (0.606)
Peridomestic sites	Bugs	28	12 (43)	57	0.211 (0.121)
House-dwellers[d]	Bugs	23	18 (78)	44.5	0.404 (0.187)

[a] Concurrent with (by other method) or after the initial finding of *T. infestans* by house-dwellers.
[b] Houses followed up for at least 1 year.
[c] Calculated as the number of houses additionally positive for *T. infestans* bugs by any method divided by the total number of years of follow-up for all houses with initial evidence by a given method. S.E., standard error.
[d] Collection in bedroom areas.

quent evidence of infestation was collected at 50–80% of houses depending on the type of evidence. Using the flushing-out method, additional domiciliary findings of *T. infestans* were made in all three houses initially positive in bedrooms (two of which were colonies), but only in 43% of houses with *T. infestans* caught initially in peridomestic sites (18% of colonies). Concurrent with or after the initial finding of *T. infestans* by house-dwellers in bedroom areas, further evidence of infestation was obtained at 18 (78%) houses; nearly half (56%) of these consisted of a single adult bug whereas nymphs were collected in only seven houses. To adjust for the fact that different houses were followed up for differing periods of time, the last column in Table 5 shows the rate of detection of further *T. infestans* bugs per house-year of follow-up (i.e. an incidence rate); this method assumes that the risk of occurrence is proportional to the length of observation, and that detection of further bugs is independent of time since initial discovery of infestation. When bugs were collected by sensor boxes or flushing-out in domiciliary areas, additional bugs were more likely to occur subsequently than when other type of evidence was initially detected.

We investigated whether the application of insecticide fumigant canisters in 1995–1996 or deltamethrin sprays in 1996 might have reduced the chance of detecting further evidence of domiciliary infestation by *T. infestans* in Table 5. For each and every class in Table 5, 2×2 contingency tables relating insecticide treatment and additional catch of *T. infestans* showed no significant or apparent trend towards reduction of detectability. House-dwellers collected additional domiciliary *T. infestans* more frequently in treated (14 of 16 houses) than in non-treated houses (4 of 7 houses), and so did flushing-out catches in peridomestic sites (8 of 12 houses versus 4 of 16 houses, respectively).

To establish the order of appearance of each type of domiciliary evidence of infestation, we selected houses at which one *T. infestans* was caught in bedroom areas by any method from 1993 to 1996 ($N = 52$) and ranked within each house the relative order of appearance of triatomine dejecta, exuviae, eggs and *T. infestans* bugs in sensor boxes, and *T. infestans* bugs collected by flushing-out and house-dwellers in bedroom areas. All types of evidence differed in their order of appearance (Friedman two-way ANOVA, $\chi^2 = 114.5$, 5 df, $P < 0.001$). Triatomine dejecta were the earliest signs (mean rank = 1.5, S.D. = 0.89), followed by bugs caught by dwellers (mean rank = 2.9, S.D. = 1.42) and bugs in sensor boxes (mean rank = 3.7, S.D. = 1.21). Start of counts in 1994 instead of 1993 did not affect the relative outcome.

4. Discussion

4.1. Method comparison

This may be the first published long-term study monitoring reinfestation by different triatomine species with multiple methods that specifically deals with the very low density populations that can occur after insecticide applications in a well-defined area. The results obtained differed according to the sampling method

for a given triatomine, site and date. For example, the prevalence and catch of *T. infestans* in domiciliary areas increasingly differed among methods over years, and peak catches of *T. guasayana* in peridomestic sites assessed by flushing-out and house-dwellers showed a clear lag. Such differences possibly arose because each method had a characteristic capture effort, sampled different fractions of the population (i.e. dispersive adults captured by house-dwellers), and type of evidence of infestation (i.e. fecal smears vs. bugs).

Timed manual capture with a flushing-out agent is a costly and time-consuming method not apt for community-based triatomine surveillance (Chuit et al., 1992). This method provides point indices of bug abundance biased toward large stages which lack sensitivity and precision (Schofield, 1978; Rabinovich et al., 1995). Moreover, the excito-repellent response of bugs to the flushing-out agent increases with environmental temperature and instar (Wood et al., 1993). In our study, the flushing-out method captured *T. infestans* less frequently than house-dwellers in bedroom areas, not in peridomestic sites, which may arise from the closer contact and concern of people with their habitations. For peridomestic areas, there is no current proven substitute for the flushing-out method.

House-dwellers' collections, not reports, provided one of the earliest evidence of infestation and thus proved to be an appropriate tool for community-based continuous monitoring of domiciliary triatomine populations. In addition to cost-related advantages, plastic bag collections put responsibility into the hands of the affected people and promoted community involvement with surveillance activities. As suggested by the high proportion of households that captured triatomines and stored them in plastic bags, acceptability and efficacy of this tool proved quite high. Its main disadvantage was lack of standardization and strong dependence on house-dwellers' motivation and capture effort, which may produce biased estimates. In our study, house-dwellers' performance should be envisaged in their long history of relationship with the research group. The capture of triatomines by house-dwellers was also found to be the most productive method in Brazil (García-Zapata and Marsden, 1993), although it is not clear which triatomine species was collected by the staff or house-dwellers in an area where several sylvatic triatomines invaded houses.

Dwellers' collections revealed the persisting house invasion and attack on humans of *T. guasayana*, but only rarely of *T. sordida*, in spite of the latter being more abundant. The invasion rate of *T. guasayana* or other triatomines to bedroom areas was most likely underestimated, as suggested by the large number of households that notified sightings without bug captures. The increase in the catch of *T. guasayana* by house-dwellers from 1993 to 1995 followed by a decrease in 1996 may be explained by: (i) progressive recovery of peridomestic *T. guasayana* populations after deltamethrin spraying in 1992; (ii) increasing stimulation of house-dwellers to collect triatomine bugs that invaded their living areas; and (iii) increasing awareness of local residents that *T. guasayana* was not the target vector species, which might have reduced their interest in catching this species alone. In support of this, dwellers collected more *T. infestans* in 1995–1996 and reported triatomine sightings without capture as frequently as in previous years. We did not perceive an increase in

deforestation or anthropogenic perturbation of surrounding sylvatic ecotopes that might have promoted increased dispersal of sylvatic triatomines. As the 1992–1997 control program was planned for *T. infestans*, less attention was given to other peridomestic species at baseline. Therefore, we do not have as good quality pre-intervention data for *T. guasayana* and *T. sordida* in peridomestic sites against which to compare the efficacy of insecticidal operations there.

Sensor boxes were significantly more sensitive than paper-sheets in detecting any sign of domiciliary triatomine infestation after spraying of residual insecticides. The higher sensitivity of sensor boxes relative to paper sheets is likely related to their greater surface and the type of refuge that they provide to triatomines, which may increase the probability that a bug leaves any sign. Feces may in turn attract more bugs (Lorenzo and Lazzari, 1996) or extend their residence period within the refuge and hence increase the likelihood of more signs being left. Sensor boxes were well accepted by house-dwellers in this and other study areas (Oscar Daniel Salomón, personal communication, 1996), thus contradicting speculations advanced by García-Zapata and Marsden (1993). Sensor boxes were also more sensitive than the flushing-out method, in agreement with previous studies (Chuit et al., 1992; Gürtler et al., 1995). A simile version of sensor boxes was judged to be less sensitive than the flushing-out method in a preliminary study (Oliveira Filho, 1997), but both methods were tried in two different groups of houses that might have differed in their exposure to reinfestation. The boxes revealed a rising trend of infestation with marked seasonal fluctuations and greater frequencies of signs in surveys carried out after the warm season (May), in which most bug activity is concentrated. Thus, the performance of the sensing devices is affected by environmental conditions.

During the first 3 years after deltamethrin spraying (1993–1995), most *T. infestans* and *T. guasayana* captured in bedrooms by any method were isolated adult bugs that most likely invaded the house from elsewhere but did not colonize it. Several isolated findings of *T. guasayana* or *T. sordida* nymphs in bedrooms were not followed by other signs of colonization. Of the 30 houses with triatomine feces in sensor boxes, only in 27% was evidence of domiciliary colonization obtained over 3 years. Considering the continual domestic invasion of *T. guasayana* followed by *T. infestans*, most triatomine fecal smears in sensing devices might be attributed to those house invasions without subsequent colonization. This is also supported by several findings of adult *T. guasayana* indoors and inside sensor boxes in concurrent association with recent fecal streaks, which were also observed in walls recently built, re-plastered or white-painted. In addition, the frequency of fecal smears in sensor boxes correlated significantly with the number of *T. guasayana* or *T. infestans* collected at each house; there is a noteworthy resemblance between the prevalence of infestation detected by sensor boxes (Fig. 1C) and the catch of *T. guasayana* by flushing-out in peridomestic sites (Fig. 2A). The relative contribution of both species to triatomine dejecta in sensing devices probably changed over time, as *T. infestans* populations increased in abundance and were more frequently collected in bedrooms than *T. guasayana* since late 1995 and thereafter.

Fecal smears may also lead to a ‘false positive’ (less specific) diagnosis of house infestation because other domestic arthropods colonize or invade the rural dwelling

and leave dejecta (e.g. cockroaches; Schenone et al., 1982). A reliable morphological differentiation between triatomine and other domestic arthropods' dejecta in vertical non-absorbent papers was considered possible (Schofield et al., 1986), but in practice many dubious cases appear (García-Zapata et al., 1985). The problem is even compounded because sensor boxes have many horizontal or inclined surfaces that do not allow fecal smears to streak as in non-absorbent paper-sheets. Such dubious diagnoses may increase when triatomine surveillance is in the hands of local residents, leading to unnecessary insecticide applications, increased expenses and adverse environmental impact.

4.2. Infestation, colonization and invasion

Infestation has usually been defined as the presence of any developmental stage of triatomines or their products in a specific habitat or sensing device (Gómez-Núñez, 1965; Schenone et al., 1979; Pinchin et al., 1981; García-Zapata et al., 1988; Wisnivesky-Colli et al., 1988; Chuit et al., 1992; Gürtler et al., 1994). Established infestations usually exhibit several triatomine bugs and nymphal instars, which is taken as conclusive evidence that the house has been colonized (Soler et al., 1969). Although most authors consider any sign and any number of signs as sufficient and equivalent evidence of infestation, in our study during the surveillance phase they were not. The finding of *T. infestans* bugs in sensor boxes or by flushing-out in bedroom areas and the frequency of triatomine dejecta over 1.5 years predicted a high chance of concurrent or future domiciliary collections. *T. infestans* eggs or exuviae in the boxes had a weaker predictive power, and peridomestic catches by flushing-out were the least predictive of all. The chance of a subsequent catch of *T. infestans* was not significantly affected by selective domiciliary applications of insecticides because: (i) most deltamethrin sprays followed the second bug capture; and (ii) fumigant canisters have a negligible residual effect and permit the survival of pre-existing eggs or triatomines that invade bedroom areas after treatment. However, repeated removal of bugs by house-dwellers or flushing-out might have reduced the colonization success of *T. infestans*. The impact of the control program on *T. infestans* will be treated in detail separately.

4.3. Consequences for vector detection and control

T. guasayana and *T. sordida* are not control targets in Argentina at present. None of these triatomines colonized domiciliary areas, even in the absence of *T. infestans*, a situation that does not appear to have changed in the last 50 years in Argentina (Abalos and Wydgozinsky, 1951; Wisnivesky-Colli et al., 1993). Therefore, the catch of isolated adult *T. guasayana* or *T. sordida* indoors does not warrant insecticidal sprays yet. The continual domiciliary invasion of *T. guasayana* without subsequent colonization poses problems for the decision whether to spray houses when based on the sole finding of triatomine feces in sensing devices. As there is no current means for identifying the origin of fecal smears to triatomine species, it would be desirable to develop molecular methods that may assist in the diagnosis

of bug species in the field, at least for research purposes. The lack of specificity of triatomine dejecta has received little attention, and may have lead some investigators to overestimate the prevalence of domiciliary reinfestation by *T. infestans*, as determined by feces in sensing devices, after application of insecticides. In our region, triatomine dejecta in sensing devices should be interpreted as evidence of triatomine activity in domestic and peridomestic areas rather than the widespread presence or colonization by *T. infestans*. Given the increasing number of reports on house invasion by different species of sylvatic triatomines in different countries, this issue may be quite general. Triatomine dejecta in sensing devices may be taken as suggestive or indicative, but not conclusive, evidence of house infestation unless accompanied by other signs such as exuviae, eggs or bugs of the target species. Consideration of the frequency of triatomine dejecta gave better clues to the actual status of house infestation.

The selection of an appropriate device for large-scale monitoring of domestic triatomine infestations must be framed in a cost-effectiveness analysis including social acceptability of the proposed tool, infestation levels and the objectives of vector vigilance (control or elimination). Sensor boxes have advantages over paper sheets in terms of sensitivity and durability, but the former are relatively expensive at present, which hinders its use at a large scale. Both sensing devices have problems related to the specificity of fecal smears because these may originate from other species of bugs or arthropods, but sensor boxes produce other direct and specific evidence of infestation, such as bugs, exuviae or eggs. In addition, house-dwellers' collections provided a means to certify which bug species invaded or colonized houses, while sensor boxes provided a more sensitive, permanent and standardized monitoring device, though less specific than capture by house-dwellers when the diagnosis is based on fecal smears. Their use in combination may help promote community involvement in surveillance activities and greatly enhance the capacity of early and continuous detection of reinfestations, a requisite for the elimination of *T. infestans* as it is currently being promoted under the Southern Cone Initiative (Schmunis et al., 1996). For such an objective, the simultaneous use of multiple sampling procedures is clearly indicated because no one method is 100% effective in detecting domestic infestations. As independent means to certify the elimination of *T. infestans* from a region, flushing-out and knockdown collections are additionally required.

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