

# Effects of chickens on the prevalence of infestation and population density of *Triatoma infestans* in rural houses of north-west Argentina

MARÍA C. CECERE,<sup>1</sup> RICARDO E. GÜRTLER,<sup>1,3</sup> ROBERTO CHUIT<sup>2</sup>

and JOEL E. COHEN<sup>3</sup> <sup>1</sup>Departamento de Ciencias Biológicas, Universidad de Buenos Aires, Argentina,

<sup>2</sup>Dirección de Epidemiología, Ministerio de Salud y Acción Social de la Nación, Buenos Aires, Argentina, and

<sup>3</sup>Laboratory of Populations, Rockefeller University, New York, U.S.A.

**Abstract.** Effects of the presence of chickens on population density of the bug *Triatoma infestans*, principal vector of Chagas disease, were investigated by standardized sampling (indoors and peridomestically) from sixty-eight houses of three rural communities in north-west Argentina, during March 1992.

The domiciliary density of *T. infestans* increased linearly with the percentage of bugs that fed on chickens, as identified by agar double-diffusion tests. Bug density was significantly higher in houses where hens usually nested indoors than in those where they did not, as determined by concurrent direct observations and interviews of householders. Multiple linear regression analysis of domiciliary bug density on (a) the total number of people, dogs and cats per house; (b) the percentage of domiciliary bugs that fed on chickens, or (c) the indoor-brooding habit of hens, showed the two variables (b, c) related to chickens as significant predictors in each regression model. Inclusion of both variables representing chickens increased the fit significantly. Addition of other potentially confounding factors (domestic insecticide use, type of roof and walls of house) did not affect the significant variables retained in the best-fitting regression model.

Peridomestic infestation was positively associated with the household number of fowls. Chickens were the main bloodmeal source of peridomestic *T. infestans* populations. Human-fed bugs were detected in peridomestic sites of sixteen houses, indicating active dispersal of adults and large nymphal instars from bedroom areas.

Exclusion of hens from domiciliary areas and promotion of chicken sheds, of an appropriate design that would not harbour bug populations, should limit the triatomine population growth rate and reduce the risk of infestation.

**Key words.** *Triatoma infestans*, *Trypanosoma cruzi*, Chagas disease, vector control, zoonophylaxis, host-feeding patterns, dispersal, Argentina.

## Introduction

The haematophagous bug *Triatoma infestans* (Klug) (Hemiptera: Reduviidae: Triatominae), the principal domestic vector of *Trypanosoma cruzi* (Protozoa: Kinetoplastida), feeds predominantly on humans, dogs and chickens (Minter, 1976a; Wisnivesky-Colli, 1987). In north-west Argentina the host-feeding pattern of domestic *T. infestans* varies seasonally, with higher frequencies

of feeding on chickens (*Gallus domesticus* L.) during spring and summer due to the presence of hens (female chickens) nesting indoors (Gürtler *et al.*, 1996). Minter (1976b) stated: 'It's not yet clear whether the presence of chickens in houses is beneficial (reduced overall *T. cruzi* bug infection rate, some predatory activity) or the reverse (support a larger total bug population)'. As yet there are no published data on the effects of the presence of chickens on the density of triatomine populations.

Host availability is considered to play a significant role in determining triatomine population density (Marsden *et al.*, 1982; Piesman *et al.*, 1983; Schofield, 1985; Gorla & Schofield, 1989). However, other factors related to features of house construction

Correspondence: Lic. Carla Cecere, Laboratorio de Ecología General, Departamento de Ciencias Biológicas, Ciudad Universitaria, (1428) Buenos Aires, Argentina.

affect bug density (Mott *et al.*, 1978; Schofield *et al.*, 1990). In a rural area of north-west Argentina where houses had never been sprayed with residual insecticides by official control services, the density of *T. infestans* in houses was significantly associated with the interaction between the type of thatch roof and the use of insecticides by inhabitants, the surface state of wall plaster, and the numbers of people and dogs sharing sleeping areas (Gürtler *et al.*, 1992). Although host-feeding data generally indicate the importance of chickens, to the best of our knowledge no field study has previously assessed the household availability of birds as a determinant of triatomine density.

As part of a wider project, the first objective of this study was to determine if the presence of hens nesting in domiciliary structures, i.e. including the bedroom(s), was positively associated with the density of *T. infestans* while adjusting statistically for effects of other factors known to be associated with bug density. Throughout this paper, we refer to *T. infestans* collected from people's bedroom areas as 'domiciliary' bugs. Second, we examine if the numbers of birds and other animals, kept outdoors in their own shelters and corrals, were related to the prevalence and density of peridomestic bug infestation. Third, we provide quantitative information about chicken-breeding practices relevant to vector population dynamics. In view of the strong link between the density of domiciliary triatomines and the risk to humans of acquiring *T. cruzi* infection (Piesman *et al.*, 1985), our results have obvious implications for vector control programmes. In a separate paper we deal with the effects of chickens on the transmission of *T. cruzi* (Gürtler *et al.*, 1997).

## Materials and Methods

**Study area.** The study was carried out in the three neighbouring rural villages of Amamá, Trinidad and Mercedes (27°S, 63°W), Province of Santiago del Estero, Argentina. The area and its history of infestation were described by Gürtler *et al.* (1992, 1994). Houses usually had two contiguous bedrooms and a front porch, comprising a 'domiciliary structure' with walls of sun-dried mud-bricks or sticks plastered with mud, and a thatched roof. Peridomestic structures included the kitchen, store-rooms, goat corrals and other possible refugia such as piled timber and waste materials within the area of human activity.

**Survey procedure.** In late March 1992, a total of seventy-one houses were visited and all resident family members and domestic birds and other animals were censused. The materials of roof and walls, the existence and state of wall plaster, and the domestic use of insecticides by householders were recorded at each house. Thatched roofs were categorized as made of 'simbol' (*Pennisetum* grass), 'jarilla' (*Larrea* shrub) or other brushwood materials, as described before (Gürtler *et al.*, 1992). Only three houses had a metal or brick roof, well-plastered walls and cement floors. Peridomestic structures were located in a sketchmap in relation to sleeping quarters; distance was measured by steps.

Householders were asked by one of us (M.C.C.) about places where birds usually nested, categorized as: (a) domiciliary, when hens or other birds nested in bedrooms, porch areas, external bedroom walls or in rooms adjacent to bedrooms, regardless of whether hens also nested in peridomestic areas; or (b) peridomestic, when hens nested exclusively outdoors. Direct observations

were made during vector collections in March 1992, October 1992 and 1993, and November 1994. Interviews were conducted in May 1993 and November 1994. A household was considered to have the habit of letting or putting hens to nest indoors when we directly observed them at least once, or when householders reported a positive habit in both interviews. Whenever there were discrepancies between resident's reports and direct observations (recorded at five of sixty-eight households), we gave priority to observations.

In November 1994, 105 families from the study area (including the adjacent villages of Pampa Pozo and Villa Matilde) were visited to collect additional information regarding: (a) months in which hens nested and the month when this was maximal; (b) place where hens, ducks and other birds brooded; (c) the number of fowls families had; (d) how many times hens and other fowls brooded per year, and (e) general practices of fowl breeding. Not all families could be interviewed, because elder family members were absent or did not give a definite answer on every item. The monthly percentage of households with hens nesting in any place was estimated as the number of households reporting a given month as a brooding month, divided by the total number of households giving an answer. The peak month with hens nesting in any place was estimated as the number of households reporting a given month or months as maxima, divided by the total number of households that gave an answer on this item.

**Vector studies.** Procedures for bug collection and analysis of results were as described by Gürtler *et al.* (1995). During 30 min per house, two expert technicians from the National Chagas Control Service searched all bedroom areas, household goods and beds for triatomine bugs, before and after repeated spraying of walls and roof with a dislodging spray of 0.2% tetramethrin (Icona, Argentina). Additional captures were done at peridomestic sites for 10 min per house. The density of *T. infestans* was estimated as the number of bugs captured in each house per person-hour, considering domiciliary and peridomestic catches separately. Bugs were identified to species, counted by instar and sex, and dissected for bloodmeal identification. Only a small sample of peridomestic bug collections was tested for bloodmeal identification.

The degree of chicken-vector contact was determined as the proportion of *T. infestans* that fed on birds, assuming that chickens are the only important type of avian host locally. Host-feeding patterns of domiciliary *T. infestans* in our study villages were reported by Gürtler *et al.* (1996). Briefly, bloodmeals were tested by agar double-diffusion tests using five specific antisera (human, dog, cat, chicken, goat/sheep) and rabbit serum as a negative control. The anti-chicken serum also detected sera from columbiforms (pigeons) and anseriforms (ducks). Bloodmeals that reacted against the anti-chicken serum were considered as feedings on chickens, because of the great numerical predominance of chickens (>95%) over other birds in household roosts (see results below), although some bloodmeals might have been on ducks.

**Data analysis.** Data on the domiciliary density of *T. infestans*, the total number of hosts (humans, dogs and cats) or fowls (chickens and ducks) per house ( $x$ ) were transformed to  $\log_{10}(x + 1)$ . The three houses with a metal or brick roof were excluded from the main analyses. The presence of chickens in bedroom areas was described by a dummy variable based on dweller's

reports and visual inspections during vector collection surveys; this variable took values of 0 when hens did not nest indoors, and 1 when they did. The domestic use of insecticides by housedwellers, the presence of cracks in bedroom walls, and a roof partly or totally made of 'simbol' were all treated as dummy variables. The condition that most favoured triatomine infestation (namely, no insecticide use, cracks in walls, and 'no simbol'; Gürtler *et al.*, 1992) took values of 1.

## Results

*T. infestans* were collected in domestic or peridomestic sites of sixty-six (93%) of seventy-one houses by manual timed searches. A total of 1429 bugs was collected from bedroom areas of fifty-five (77%) houses, and 472 bugs from peridomestic structures of thirty-five (49%) houses. The only other Triatominae found were a few specimens of two other species.

### Domiciliary infestations

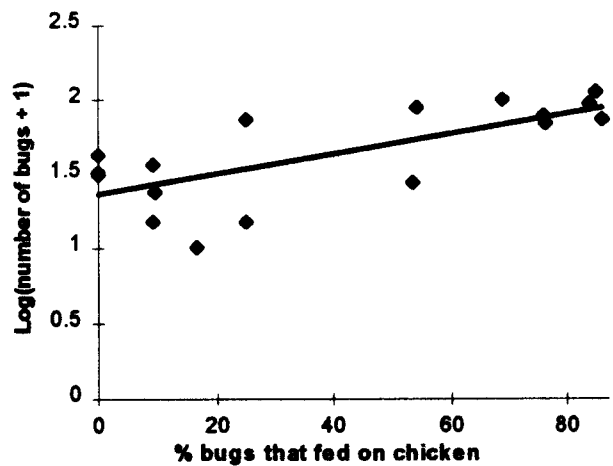
Indoor-nesting hens were found in 30% and 28% of houses in March 1992 and in November 1994, respectively. Residents reported that hens brooded indoors in 24% of houses in May 1993, and in 29% of houses in November 1994. Using the mixed classification criterion, we concluded that hens usually nested indoors in 44% of houses (Table 1). The proportion of houses with dense *T. infestans* populations (10 or more bugs/person-hour) was significantly and positively associated with the presence of hens nesting indoors. Similarly, median bug densities differed significantly between household classes. The prevalence of domiciliary infestation reached only a marginal level of significance in relation to the presence of hens indoors.

For forty-nine of fifty-six infested houses (87.5%), at least one *T. infestans* bloodmeal reacted against the antisera used. To obtain a statistically more reliable assessment of the degree of chicken-vector contact per household, we restricted attention to thirty-two houses with 5 or more *T. infestans* having a reactive bloodmeal. In these houses, the domiciliary density of *T. infestans*

**Table 1.** Prevalence of infestation and domiciliary density of *Triatoma infestans* (total number of bugs collected per person-hour) in relation to the presence of hens nesting indoors, as reported by house-dwellers or observed during vector surveys. Amamá, Trinidad and Mercedes; March 1992.

Presence of hens nesting indoors	No. of houses (%)			Density of <i>T. infestans</i>	
	Inspected	Infested <sup>a</sup>	≥ 10 bugs/person-hour <sup>b</sup>	Median <sup>c</sup>	Q1-Q3 <sup>d</sup>
Yes	30 (44)	27 (90)	18 (60)	18	5-62
No	38 (56)	28 (74)	12 (32)	4	0-15
Total	68	55	30		

<sup>a</sup> $\chi^2 = 2.9$ ,  $P = 0.089$ ;  $df = 1$ . <sup>b</sup> $\chi^2 = 5.5$ ,  $P = 0.019$ ;  $df = 1$ . <sup>c</sup>Kruskal-Wallis test:  $H = 8.49$ ,  $0.001 < P < 0.05$ ;  $N = 68$ . <sup>d</sup>Q1 and Q3, first and third quartile, respectively.



**Fig. 1.** Relationship between the log-transformed domiciliary number of *Triatoma infestans* collected per person-hour ( $y$ ) and percentage of bugs that fed on chickens ( $x$ ) in houses with hens nesting indoors ( $y = 1.3617 + 0.0069x$ ;  $r^2 = 0.513$ ;  $P = 0.001$ ;  $N = 17$ , with two points overlapping): Amamá, Trinidad and Mercedes, March 1992.

correlated positively and significantly with the percentage of bugs that fed on chickens ( $r = 0.40$ ;  $P = 0.024$ ). In houses where hens were reported or observed nesting indoors, bug density increased linearly and significantly with the percentage of bugs that fed on chickens (Fig. 1). In houses without hens nesting indoors, no significant relation between variables was observed ( $r^2 = 0.113$ ;  $P = 0.22$ ;  $N = 15$ ).

We tested whether factors usually associated with the domiciliary density of bugs could also be related to the presence of hens nesting indoors, and hence potentially confound that relationship. The total number of hosts (people, dogs and cats), but not of hens and ducks, was significantly and positively associated with the presence of hens nesting indoors ( $t = 2.11$ ;  $df = 66$ ;  $P = 0.04$ ). The presence of cracks in walls showed a borderline positive association ( $\chi^2 = 3.68$ ;  $df = 1$ ;  $P = 0.05$ ) with the presence of hens nesting indoors. Domestic use of insecticides ( $P = 0.4$ ) and type of thatch in roofs ( $P = 0.67$ ) were not significantly associated with the place where hens nested.

Multiple linear regression analysis was used to determine the effects on domiciliary bug density of (a) log-total number of hosts per house, (b) the percentage of domiciliary bugs that fed on chickens, and (c) the indoor-brooding habit of hens (Table 2). In sixty-eight houses the regression coefficient for the indoor habit of hens was highly significant, whereas that for total host numbers was not significantly different from 0. Similarly, in thirty-two houses with at least five reactive bugs, the regression coefficient for the percentage of domiciliary bugs that fed on chickens was highly significant, whereas that for total host numbers was not significantly different from 0. The estimated regression functions were significant but explained only 13-16% ( $r^2$ ) of changes in bug density. Inclusion of both the percentage of bugs that fed on chickens and the indoor-nesting habit of hens in the model increased the fit significantly ( $r^2 = 0.386$ ); significant predictors were the indoor nesting habit (with positive effects on bugs density) and total host numbers (with a marginal negative effect). To check if these results were modified by effects due to insecticide use, 'simbol' roof or cracked walls, terms for these variables were

**Table 2.** Multiple linear regression analysis of the  $\log_{10}$ -transformed domiciliary density of *Triatoma infestans* (number of bugs collected per person-hour, the dependent variable) on  $\log_{10}$ -total numbers of hosts (humans, dogs and cats) per house, the percentage of reactive bugs that fed on chickens (the chicken blood index), and/or the indoor-brooding habit of hens (independent variables); Amamá, Trinidad and Mercedes, March 1992.

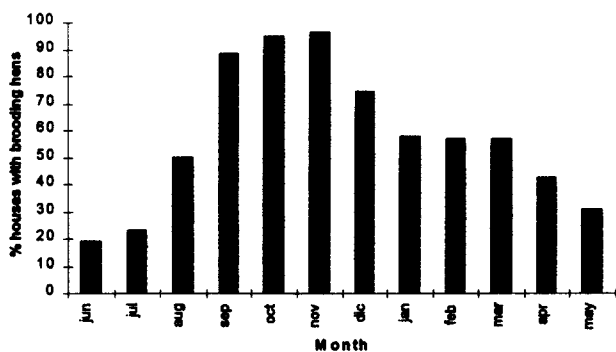
Dependent variable	Independent variable	Coef. <sup>a</sup>	SE <sup>b</sup>	$t$ <sup>c</sup>	$P$	$N$ <sup>d</sup>
Bug density	Intercept	0.9243	0.3042	3.04	0.003	
	No. hosts	-0.2130	0.3568	-0.60	0.553	
	Indoors hens	0.4720	0.1537	3.07	0.003	
	$r^2=0.127$	$F$ -ratio = 4.73 with 2 df		$P=0.012$	$N=68^d$	
Bug density	Intercept	1.4154	0.2585	5.48	<0.001	
	No. hosts	-0.1006	0.2643	-0.38	0.706	
	Chicken blood index	0.0047	0.0021	2.27	0.031	
	$r^2=0.164$	$F$ -ratio = 2.84 with 2 df		$P=0.075$	$N=32^d$	
Bug density	Intercept	0.6435	0.2365	6.95	<0.001	
	No. hosts	-0.5175	0.2651	-1.95	0.061	
	Indoors hens	0.3919	0.1232	3.18	0.004	
	Chicken blood index	0.0025	0.0019	1.33	0.196	
	$r^2=0.386$	$F$ -ratio = 5.86 with 2 df		$P=0.003$	$N=68^d$	

<sup>a</sup> y-intercept and regression coefficients. <sup>b</sup> Standard error. <sup>c</sup>  $t$  value. <sup>d</sup> Number of houses.

added to the best fitting regression model obtained (not shown); none of the added terms was significant ( $P > 0.1$ ) before or after a backward step-wise elimination procedure. The final model thus included a marginally significant effect of total hosts (negative) and a significant effect of the indoor-nesting habit of chickens (positive).

Among seventy-eight of 105 households providing definite answers in November 1994, 50–96% of responding households reported hens nesting from August to March, whereas only 19–42% had hens nesting from April to July (Fig. 2). This distribution was homogenous over communities. The peak month with hens nesting was November. Among fifty-five households that were interviewed in both March 1992 and November 1994 and gave a definite answer, only in four (7%) did the owners report a change in indoor-brooding habits.

In November 1994, 95% of 105 households owned chickens. The median number of hens per house was 10 (Q1=6; Q3=19),



**Fig. 2.** Monthly proportion of houses with hens nesting in any place: Amamá, Trinidad and Mercedes, November 1994.

and the median number of chicks up to 3 months old per house was 24 (Q1=13; Q3=40). Overall, hens brooded twice a year in nest-sites where they rested with eggs and then chicks until these were 1–3 months old. Thereafter they all began to roost in peridomestic trees. Only twenty-five (28%) of eighty-nine households interviewed on this matter raised ducks; in 13% (12/89) of houses ducks nested indoors. Ducks brooded once a year, and the median number of female ducks was 2 per duck-owning house (Q1 = 1; Q3 = 3). Turkeys and geese were less abundant than ducks and always nested peridomestically, farther away from bedrooms. No pigeons or passeriform birds were found to nest or roost in domiciliary situations.

#### Peridomestic infestations

Peridomestic infestation by *T. infestans* was significantly associated with the household number of birds (*c.* 95% chickens), but not with the number of other corral animals (*i.e.* sheep, goats, horses, pigs) (Table 3). Peridomestic bug density also showed an increasing trend with the number of birds but the association was not statistically significant.

Among 87 peridomestic *T. infestans* whose bloodmeals gave a positive reaction against at least one of the antisera used, chickens were identified as a bloodmeal source in the majority of bugs (70%), followed by humans (36%), dogs (16%) and cats or sheep/goat (2%). The sum of these percentages exceeds 100% because each bug may show several blood sources. Human blood-meals were detected in fourth- and fifth-instar nymphs as well as adult *T. infestans* collected from peridomestic sites of sixteen houses. Only in two of these houses did the residents report that people occasionally rested at night in the place where human-fed bugs were caught.

**Table 3.** Distribution of houses with peridomestic infestations by *Triatoma infestans*, and high density infestations, according to number of fowls (hens and ducks) or other corral animals. Amamá, Trinidad and Mercedes; March 1992.

Demographic factors	No. houses inspected	% infested	% houses with >6 bugs/person-hour
No. animals			
0–10	23	48 <sup>a</sup>	26 <sup>b</sup>
11–30	20	45	25
31–100	10	70	50
No. fowls			
0–10	11	36 <sup>c</sup>	27 <sup>d</sup>
11–30	19	47	32
31–100	19	79	42

<sup>a</sup> $\chi^2=1.82$ , 2 df,  $P=0.40$ . <sup>b</sup> $\chi^2=2.30$ , 2 df,  $P=0.32$ . <sup>c</sup> $\chi^2=6.37$ , 2 df,  $P=0.04$ . <sup>d</sup> $\chi^2=0.81$ , 2 df,  $P=0.67$ .

## Discussion

This is the first study to show that the domiciliary density of *T. infestans* was related positively and significantly to the presence of hens nesting indoors. The density of domiciliary *T. infestans* increased linearly with the percentage that fed on chickens. Peridomestic bug infestation was also related to the household number of fowls.

Exposure of chickens to domiciliary *T. infestans* was assessed independently by direct on-site observations, interviews of house-residents and vector host-feeding patterns. Direct observations during brief visits to houses have the limitation of being point assessments of a condition that varies seasonally on a rather fast time scale. However, observations confirmed the presence of brooding hens and ducks indoors, especially in houses whose inhabitants made a negative statement. Resident's reports were used as one source of information about presence of fowls indoors, but in a few cases this information proved to disagree with our actual observations. We believe that several rounds of observation and interviews, over a two-year period, provided a better assessment of the household's chicken breeding practices than single observations or responses obtained during the vector survey.

Agar double-diffusion tests can detect full bloodmeals taken up to 3 months previously (Wisniewsky-Colli, 1987), thus providing a period estimate of chicken exposure to domiciliary *T. infestans*. The percentage of chicken-fed bugs determined from late-March collections was probably an underestimate of the actual degree of chicken-vector contact in the peak nesting month (November), about 4 months before bugs were collected. This lag would have permitted new bugs, recruited during early to late spring (when there were more hens brooding), to reach larger stages (fourth and fifth instars and adults) which are easier to see and catch by hand (Rabinovich *et al.*, 1995) by the end of the summer. Hence the domiciliary density of *T. infestans* in March represents the increased vector population when bug-feeding on chickens declines at the end of summer, after the bird-nesting season. Surveys made in early spring, before the nesting season, may fail to detect the association between the density of bugs and their feeding on chickens.

The dominant effect of hens nesting indoors on the domiciliary density of *T. infestans* is clear from the multiple regression analyses, in which the effects of other hosts and characteristics of house construction were controlled statistically. People and dogs are more regular domiciliary hosts during the year, compared with the marked seasonal presence of hens. The proportion of domiciliary *T. infestans* feeding on dogs was found to be stable across seasons, whereas feeding on humans peaked in late winter, and feeding on chickens was minimal in winter (8%) and maximal (40–56%) in spring-summer (Gürtler *et al.*, 1996). Hence host-feeding patterns are consistent with the brooding curve shown in Fig. 2. The increased number of nesting hens during spring-summer, the extended period in which they nest in a constant location, and their widespread presence in domestic and peridomestic areas, all combine to boost the population growth rate of *T. infestans*.

Defensive grooming behaviour by vertebrate hosts has been proposed as a general regulatory mechanism for populations of domestic triatomine bugs (Schofield, 1985). More tolerant hosts would allow triatomines to take larger bloodmeals with smaller death risks for the bugs, reach the adult stage sooner and increase the total bug egg output. Chickens appeared as a preferred and tolerant host relative to their number (Gürtler *et al.*, 1997), allowing triatomines associated with them to achieve a higher basic reproductive rate. Although ducks frequently nest indoors, their relative contribution as a feeding source of *T. infestans* appears to be smaller than that of hens because, at least in our study area, ducks are much less abundant. Also, in experimental cages, ducks were less fed upon by *T. infestans*, and ducks were much more predatory than hens (Schweigmann *et al.*, 1995).

The proportion of domiciliary bugs that fed on chickens showed considerable variation among houses with indoor-nesting hens. This might be related to differing numbers of brooding hens and chicks, and to the bug stages tested for bloodmeals: cf. chicken-fed rates of 22% of first-second instars, 45–50% of adult bugs (Gürtler *et al.*, 1996). In other areas, the number of chickens is reported to vary on a weekly basis (Piesman *et al.*, 1983). Some *T. infestans* that reacted positively to chicken antiserum were collected from houses where hens were neither allowed nor observed to brood indoors. Presumably these bugs either fed on other birds (e.g. ducks), or on foraging chickens that visited the house temporarily, reinforcing the apparent importance of chickens as a favoured host. It seems less likely that blood-fed *T. infestans* would have dispersed from any other house or invaded from the peridomestic biotope, and we discount the possibility of 'false positive' reactions for the anti-chicken serum.

Birds, presumably chickens, were also the main bloodmeal source for peridomestic *T. infestans*, in accord with their observed association. During a 3-year follow-up after house-spraying with the residual pyrethroid insecticide deltamethrin, chickens were also the host most frequently associated with peridomestic *T. infestans* and other triatomines (Cecere *et al.*, 1996).

The frequent finding of human bloodmeals in adult and nymphal *T. infestans* collected from peridomestic sites of numerous houses could be due to their active dispersal from bedroom areas. However, during hot weather, local residents usually bring their beds outdoors to sleep under open sky, hence closer to infested peridomestic structures. Our host-feeding data impute a higher degree of interchange between domiciliary and peridomestic

*T. infestans* populations than ever reported before using this technique (cf. Wisnivesky-Colli, 1987).

As this study is not a randomized controlled trial, we cannot exclude the possibility that an unidentified third factor may be causally responsible for the observed association between the presence of chickens in the house and the density of *T. infestans*. We did allow for the statistical effects of well-known determinants of domiciliary infestation. For example, some householders were undoubtedly more tolerant of bugs and chickens in their bedrooms, thus encouraging the association. To confirm that the presence of chickens causes an increase in triatomine bug populations requires an experimental study in which chickens were removed from some randomly selected houses but not from others.

Our results have important consequences for vector control programmes in rural areas. Human behaviour certainly plays an important role in the epidemiology of Chagas disease (Miles, 1976). The simple act of letting hens brood indoors is related to increased triatomine densities. Restriction of brooding fowls to outdoor sites should decrease the risk of house colonization and limit the population growth rate of domiciliary triatomine populations. Additional measures should include the design of appropriate chicken sheds for nesting without becoming breeding sites for bug populations. Such sheds should be situated away from houses (Schofield *et al.*, 1990) to lower the force of house reinfestation following insecticide applications. Implementation of these measures, with community participation on a sustainable basis, would contribute towards integrated vector control.

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