Community Participation and Domiciliary Occurrence of Infected *Meccus longipennis* in Two Mexican Villages in Jalisco State

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Abstract. The entomological features of Chagas disease in two western Mexican villages were analyzed through triatomines collection by the inhabitants and active research in the peridomicile. The inhabitant collections have the following comparable characteristics: 1) *Meccus longipennis* was the dominant species (> 91%), 2) around 43% of the insects were collected indoors, 3) about 70% of triatomines were adults, 4) cumulated rates of infestation of the dwellings reached 40–50%, 5) the triatomine infection rate by *Trypanosoma cruzi* was > 50%, and 6) the indoor triatomines frequently feed on humans (range 38.5–56.2%). However, the collection was twice as abundant in the first village and the peridomicile infestation, evaluated by the active collection, reached up to 60% and only 4.9% in the other village. Furthermore, females predominated in the first village, whereas males in the other. The current results allow discussing the course of action to prevent Chagas disease in this region.

INTRODUCTION

The three principal domesticated vector species of Chagas disease, *Triatoma infestans* (Klug, 1834), *Rhodnius prolixus* (Stäl, 1859), and *Triatoma dimidiata* (Latreille, 1811), have received great attention over the last decades. Sub-regional vector control initiatives in the Southern Cone and Andean regions of South America, and in Central America, have undoubtedly diminished the transmission of *Trypanosoma cruzi,* the agent of Chagas disease, in countries where programs have been active for several years.[1] However, less attention has been given to other regions, including Mexico, where other triatomine species (i.e., sylvatic) are present, even though the seroprevalence and clinical data confirmed that Chagas disease was endemic in the majority of Mexican regions.[2] Moreover, between 1986 and 2006, the Mexican national epidemiologic surveillance system reported 1,814 cases of human chagas infection, having the majority of cases detected in the last 5 years (77%).† It is unclear whether the observed increase in Chagas disease incidence was caused by biotic and abiotic modifications of the environment, or whether the Public Health problem had not been adequately considered before.

In general terms, the transmission model linked to domestic vectors is coming out and is substituted by new features of vectorial transmission mainly associated with sylvatic triatomines.[3–6]

One exception is the insecticide resistance that has been recently documented in cases of the *T. infestans* domestic populations in Argentina.[7] Three domestic transmission scenarios can typically be observed: 1) re-infestation by sylvatic populations (same or different species) after vector control of domestic populations[7,8]; 2) expansion of human settlement in areas where environmental modifications favor the domestication of sylvatic autochthonous species[9]; and 3) low endemic regions that have not yet gained adequate attention, but where vectors are concurrently sylvatic and domestic.[10–13] The Mexican situation reflects the last two profiles. However, the study of emerging vector transmission systems is difficult because of the great diversity of triatomine species involved whose biological properties are poorly known. Biotic and abiotic modifications of the environment have become more and more important. The areas where sylvatic triatomines transmit disease caused by small-scale habitat colonization or temporary incursions are typically areas of low endemicity. Finally, low endemic areas may present specific challenges to seroprevalence surveys, diagnosis, and pathology. Human infection by sylvatic parasite strains needs to be better documented as unexpected clinical manifestations are possible.

In Mexico, at least 31 triatomine species have been described, 18 of them have been found naturally infected by *T. cruzi*.[14,15] All of these species are sylvatic, and except for *T. dimidiata,* they are only endemic to Mexico.[16,17] The most remarkable factor of these investigations with Mexican species is the high rate of infection by *T. cruzi*, also observed for most of the main South American vectors, such as *T. infestans* and *Triatoma brasiliensis* (Neiva, 1911).[11,16] Although several Mexican species have been found in human habitats, it remains difficult to measure the true role of each species in the transmission of disease as there exist few studies on the ecology, geographical distribution, behavior in the human habitat, behavior in the natural medium, and relationship with the prevalence of human infection in corresponding areas. Furthermore, regarding the Meccus species complex, which includes *Meccus longipennis,* the species involved in the current study, controversy exists regarding their taxonomic status as species or subspecies because of their close genetic relationships.[17,18]

The basic assumption of this work is that a better understanding of Chagas disease in Mexico should consider the zooanthroponotic systems (reservoir-vector-human), which occur in shared spaces. As analyses of entomological features on the community scale are undoubtedly primary to evaluate transmission risk, two rural communities in western Mexico, Los Guerrero (Jalisco) and Cacalután (Nayarit), were studied.

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The current study showed that *M. longipennis,* originally a sylvatic species, is the principal vector in these two communities, but shows different patterns of distribution and abundance. This implies a likelihood of different transmission risks and requires specific control strategies for each location.

MATERIALS AND METHODS

Study areas. The study was conducted in two villages. The village of Los Guerrero (20°26′56.4″N, 103°53′87.2″W, 1295 m a s) is a rural community of the San Martín de Hidalgo municipality located in the state of Jalisco, whose characteristics have been previously described.[19] Briefly, Los Guerrero is situated in a valley characterized by a deciduous seasonal forest (semi-arid region), which had been cleared to provide land for cultures around the village. Average temperatures are 20°C high and 28°C low, respectively. Annual rainfall averages range from 987.6 to 1,349 mm; the dry season extends from October until June. The village is composed of 314 dwellings, where 151 (48%) of them were closed because people were living and working in other places. The total surface of the village is 79.8 acres (323,000 m^2).

The second village, Cacalután (21°06′98.8″N, 104°15′69.7″W, 920 m a s), is located in the municipality of Ixtlán del Río, in the state of Nayarit. The size of the village is approximately 74 acres (300,000 m^2). Cacalután is located in a valley surrounded by mountains; the land is rough and steep, with a semi-dry climate. In winter temperatures range between 10 and 35°C, with an average of 13°C, and in summer between 20 and 40°C, with an average of 25°C and little precipitation. The population is dedicated to seasonal agriculture and cattle ranching. The village is composed of 134 dwellings, grouped along three principal streets, 30 (22%) of them were closed or abandoned. These villages have never been sprayed with insecticides before this study.

Perception of triatomines by the inhabitants. Adults of both sexes were questioned on their own observations about triatomines in their house (indoor) and in the peridomicile (outdoor) areas during the visit of their dwellings. In Los Guerrero, 86 persons were interviewed and 78 in Cacalután.

Active collection of triatomines in peridomiciles. At the beginning of the study, the villages were mapped, and all houses were drawn. One hundred occupied dwellings in Los Guerrero and 82 in Cacalután were randomly selected by an aleatory number assignment and visited during the study. The same team of well-trained health workers, assisted by an entomologist, searched vector insects manually with flashlights, with no insect repellent on them, during the day in the peridomestic area of Los Guerrero (July–August 2003) and of Cacalután (July 2005). A total of 369 sites in Los Guerrero and 300 sites in Cacalután were excavated: storage shelters, corrals, and chicken-coops were visited and the excavated sites consisted of piles of wood, rocks, brick, tile, or various goods and ends, which are in these structures and outdoors. All examined sites were numbered, characterized, and located on a map. Insects collected from each capture site were placed in separate labeled plastic flasks and transported to the laboratory for morphological identification. The mean time spent searching for insects at each dwelling was 1 hour and 15 min (± 40 min).

Collection of triatomines by the inhabitants. After the research was carried out by the team of professional agents, inhabitants were asked to collect the triatomines using plastic bags to preserve them and later in flasks. They were not asked to make excavations but to keep triatomines they found during their daily activities. Two flasks were distributed per family to separate the insects collected inside the house from those collected outside. Each month the flasks were gathered and transported to the laboratory by the team of professional agents. The collection by the inhabitants lasted 24 months from October 2005 through September 2007 in Los Guerrero where 126 families participated in the collection and from January 2005 to December 2006 in Cacalután (92 families).

Processing of triatomines. The identification of triatomines (adults and nymphs) was done according to the taxonomic keys.[14] Sex and stages of development were also determined. Feces from each bug, obtained by abdominal pressure, were mixed with phosphate-buffered saline (PBS), and examined for the presence of trypanosomatids by direct microscopic observation at 400× magnifications. Parts of the bugs were dissected under a safety hood: the terminal part of the abdomen was cut and the abdominal contents (blood meal and intestinal part) collected in a microtube. All samples were kept at –20°C for further processing. Classical entomological indicators, infestation, and colonization were calculated according to The World Health Organization (WHO) standards.[20]

Host feedings of triatomines collected indoors. The blood meal DNAs were extracted with the QIAamp DNA mini kit following the manufacturer's instructions (Qiagen, Courtaboeuf, France). The cytochrome b gene was then amplified with specific primers for the vertebrates from each DNA sample and heteroduplexes were produced with *Sigmodon mascotensis* amplified cytochrome b from a cloned gene in our laboratory as driver following the method previously described.[21] The heteroduplex products were formed by denaturizing at 100°C for 2.5 min of cytochrome b mixtures (V/V) of sample and driver and slow cooling of the denatured solution at room temperature. Finally, the comparison of the heteroduplex patterns was done with 10% acrylamide electrophoreses (29 acrylamide:1 bisacrylamide) in TBE buffer. Profiles were compared with different standards, including human. An absence of heteroduplex formation was equated to *Sigmodon* sp. blood meal after control of the profile using *Dasypus novemcinctus* as driver. Blood meal origin of part of the samples were also determined by direct sequencing of polymerase chain reaction (PCR) products or after cloning using the TOPO TA kit (Invitrogen, Cergy-Pontoise, France). The sequences were then blasted in gene banks.

RESULTS

Inhabitant experiences with triatomines. The inhabitants were questioned about their familiarity with triatomines. In the two villages, they were familiar with the well-known triatomines and could identify them by their local name of "chinche hocicona." In fact, 47% and 30% of the questioned people in Los Guerrero and Cacalután, respectively, reported seeing triatomes indoors, most frequently in their bedroom (61% and 50%). They also reported having seen bugs outdoors in the peridomiciliary area with comparable occurrence to indoors (50% in Los Guerrero, 38% in Cacalután). Furthermore, 20% and 29% of the families in the two villages were able to identify triatomine bites as causing a large and painful chagome.

Active manual search of triatomine in the peridomicile. The manual search of triatomines by the professional team showed that abundance was radically different between the

two villages. As previously reported in Los Guerrero, a total of 1,821 triatomine specimens were collected in 118 different sites, and the peridomestic infestation reached 60% among 100 visited units.[22] There, *M. longipennis* was the dominant species (93.2% of the adults) and *Triatoma barberi* (Usinger, 1939) the second most frequently observed (6.6%). Furthermore, a high colonization rate of both species was observed (93.3% and 75%, respectively). In Cacalután, only seven triatomines were caught in four sites located in four different peridomestic units (two *M. longipennis* adults and five nymphs (fifth stage) of the *Meccus* complex), and the infestation index of the peridomiciliar units was 4.9% among the 82 visited.

Collection of triatomines by the inhabitants during 24 months. Indoor and outdoor infestation patterns were followed by the longitudinal collection of triatomines by the inhabitants over 24 months (Table 1). In Los Guerrero, a total of 874 triatomines were collected, of which 43.6% were found indoors and 56.3% were found in peridomiciliar areas. The proportion of the different species calculated among adult specimens was as follows: *M. longipennis* (95.7%), *T. barberi* (2.5%), *Meccus pallidipennis* (Stål, 1945) (1.5%), and *Meccus picturata* (Usinger, 1939) (0.3%). A total of 269 nymphs belonging to the *Meccus* complex of species were captured (nymphs of Meccus species are not differentiated by morphology). The adult proportion reached 69.2% of the total population. In Cacalután, the inhabitants caught a total of 319 triatomines, of which 42.4% were indoors, and 57.6% outdoors. *Meccus longipennis* was the dominant species (91.7%), the secondary ones were *Triatoma recurva* (Stål, 1868) (2.5%), *M. picturata* (2.9%), and seven specimens remain to be determined (2.9%), but probably they belong to one species. A total of 78 nymphs were captured. Adult proportion reaches 75.5%. In Los Guerrero and in Cacalután no significant differences ($P > 0.05$) were found in the distribution of species and stages between indoor and outdoor collections (for the χ^2 analysis, *T. barberi*, *M. pallidipennis*, and *M. picturata* were grouped in Los Guerrero and similarly *T. recurva*, *M. picturata*, and *T.* sp. in Cacalután). Further comparison between the two villages shows significant differences of the species and stages distribution (Table 1); the number of collected nymphs was also significantly lower in Cacalután than in Los Guerrero, and the number of adult specimens of other species than *M. longipennis* was higher in Cacalután (Table 1). Moreover the triatomines were twice as abundant in Los Guerrero (12.2

triatomines/month/100 dwellings) compared with Cacalután (5.1 triatomines/month/100 dwellings).

Remarkably, the sex ratio of the triatomines collected indoors was significantly different between Los Guerrero and Cacalután, as shown in Figure 1 ($X^2 = 35$, degrees of freedom [df] = 1, $P < 10^{-4}$): whereas females predominate in Los Guerrero ($X^2 = 9.9$, df = 1, $P = 0.0019$), males were more abundant in Cacalután ($X^2 = 9.7$, df = 1, $P = 0.0018$). Moreover, these sex proportions were similar for the populations collected outdoors and indoors.

The seasonal examination of the indoor populations collected by the inhabitants show that they collected triatomines during the entire year in the two villages (Figure 2). The cumulated indoor infestation rates were also rather similar in the two villages, > 40% (Figure 3).

Parasite infection in triatomines collected by the inhabitants. The infection rates calculated from about 51% of *M. longipennis* adults and nymphs collected by the inhabitants in Los Guerrero and Cacalután, reached 55.1% and 49.4%, respectively (Table 2). Similar rates were observed for the triatomines collected indoors and outdoors and between sexes. The secondary species (*M. pallidepennis*, *M. picturata*, and *T. recurva*) were also found infected by *T. cruzi* but not the undetermined species (two *T.* sp. males analyzed).

Feeding blood meal origin of triatomines collected by the inhabitants. Blood meal origin was determined for 65 triatomines in Los Guerrero and 32 in Cacalután collected indoors (Table 3). Among these bugs, 38.5% and 56.2% in Los Guerrero and Cacalután, respectively, presented a heteroduplex profile corresponding to single or mixed human feedings. Remarkably, *Dasypus novemcinctus* blood meal origin was detected in 69.2% of the triatomines in Los Guerrero and in 53.1% in Cacalután; sequencing of two PCR products giving a *D. novemcinctus* heteroduplex pattern confirms the species (Y11832.1, 97% identity). Similarly, sequencing of PCR products confirmed the *Gallus gallus* (DQ512918.1, 99% identity) and *Sigmodon* sp. (AY041203.1, 89% identity) host origin identified by the heteroduplex assay. Two other PCR products (one from each village) with a heteroduplex multibanding were cloned; the heteroduplex profiles of 10 clones from each PCR sample were identical to standards: nine clones corresponded to *D. novemcinctus*, three to human, nine to *Sigmodon* sp., and one to *Mus musculus*. One triatomine contained four different blood meal hosts (Table 3).

TABLE 1
Triatomine species collected indoors and outdoors by the inhabitans in two Mexican villages during 24 months

| | Villages in the occidental part of Mexico | | | | | | χ^2 statistic† | | |
| | Los Guerrero | | | Cacalután | | | | | |
	Indoor	Outdoor	Unknown‡	Indoor	Outdoor	Unknown‡	X_2 value	df*	P value
M. longipennis§	259	315	5	76	103	42	0.97	1	> 0.05
Other species									
T. barberi	5	9	1	0	0	0	6.84	1	< 0.01
M. pallidipennis§	6	3	0	0	0	0			
M. picturata§	2	0	0	2	4	1			
T. recurva§	0	0	0	3	1	2			
T. sp.§	0	0	0	2	4	1			
Nymphs of *Meccus* complex	103	157	9	26	36	16	4.53	1	< 0.05
Total	375	484	15	109	148	62	10.11	2	< 0.01

* df = degrees of freedom.
† Significant differences were evaluated between Los Guerrero and Cacalutan raw data.
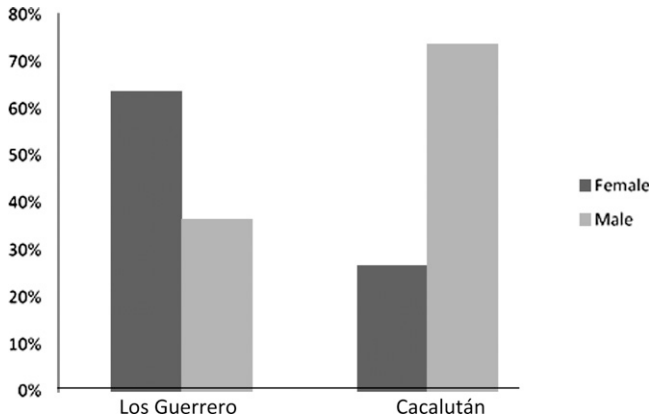‡ The triatomines were collected indoors or outdoors.
§ Adults specimens.

FIGURE 1. Histograms presenting the sex distribution of *Meccus longipennis* specimens collected indoors by the inhabitants of Los Guerrero and Cacalután villages, Mexico, over 2 years.

DISCUSSION

The earlier entomological works pointed out the large geographical distribution of various species of the *Meccus* complex: *M. longipennis*, *Meccus mazzottii* (Usinger, 1899), *M. pallidipennis*, *Meccus phyllosoma* (Burmeister, 1835), and *M. picturata* in Mexico, but as yet, basic entomological studies aimed at determining the microdistribution of the vectors, the population density, and the transmission risk are scarce.[23–27] Previous entomological data of the triatomine species belonging to this complex at the community scale have informed epidemiological trends; the peridomestic area can be heavily infested with one of these species in some villages but not in
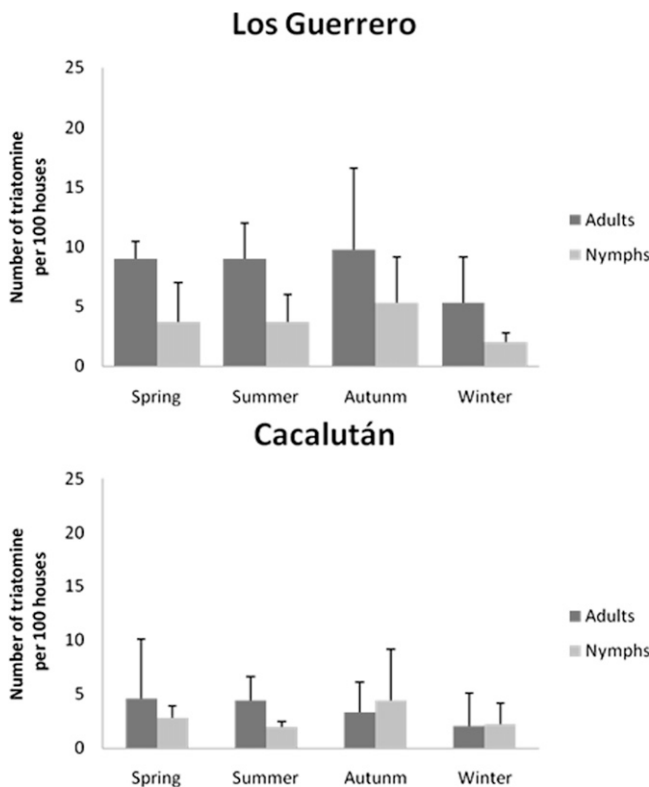

FIGURE 2. Seasonal variation of total numbers of triatomines harvested indoors by the inhabitants in Los Guerrero and Cacalután villages per 100 houses.
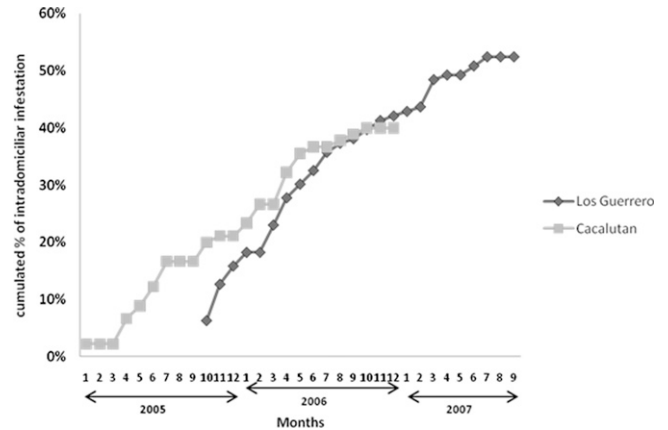

FIGURE 3. Cumulative rates of indoor infestation in Los Guerrero and Cacalután villages, Mexico, based on triatomine collection by the inhabitants per month, over 2 years.

others, whereas intra-domicile infestation is generally low or undetectable by active search during the day.[11,28]

The current results show clearly that in two villages located in the western part of México, there is a year-round indoor risk of Chagas disease transmission, because a high proportion of the insects collected in the house by the inhabitants displayed a high rate of infection by the parasite, and these insects frequently feed on humans. However, the abundance of the vectors was different between the villages, which lead to differential exposures and associated transmission risks. The analysis of the insect life stages suggests low indoor colonization as a high proportion of adults (> 70%) was observed in both villages. Furthermore, the inhabitants seldom collected several insects at the same time in their houses. Moreover, several blood meals taken from non-domestic animals were identified, showing that these insects had invaded domicile. These data suggest that triatomines collected indoors came from peridomestic and/or sylvatic areas.

In Los Guerrero, the triatomines that penetrate the houses could originate from peridomestic areas, which are heavily infested as previously reported.[22] In this work we have reported a high number of sites with only one or two insects (adult and

TABLE 2
Infection rates by *Trypanosoma cruzi* of *Meccus longipennis* adults and nymphs of Meccus complex collected by the inhabitants*

	Indoors			Outdoors			Total % positive
	N	Positive	% Positive	N	Positive	% Positive	
Los Guerrero							
N1 + N2	0	0	–	1	0	–	–
N3	8	0	0.0	6	3	50.0	21.4
N4	20	7	35.0	24	6	25.0	29.5
N5	44	22	50.0	55	32	58.2	54.5
F	95	55	57.9	88	55	62.5	60.1
M	62	33	53.2	83	55	66.3	60.7
Total	229	117	51.1	257	151	58.8	55.1
Cacalután							
N1 + N2	1	0	–	0	0	–	–
N3	0	0	–	2	0	–	–
N4	11	3	27.3	6	2	33.3	29.4
N5	8	4	50.0	18	5	27.8	34.6
F	13	9	69.2	17	7	41.2	53.3
M	30	17	56.7	48	29	60.4	59.0
Total	63	33	52.4	91	43	47.3	49.4

*N1 to N5 = nymphs; F = adult female; M = adult male; N = total number of processed insects.

	Blood meal origin	Villages		
		Los Guerrero	Cacalután	Total
Single blood meal	Human	11	13	24
	Dasypus novemcinctus	32	12	44
	Sigmodon sp.	4	2	6
	Gallus gallus	1	0	1
	Human + *D. novemcinctus*	9	4	13
	Human + *Sigmodon* sp.	1	0	1
	Human + other	3	0	3
Several blood meals	*D. novemcinctus* + *Sigmodon* sp.	1	0	1
	D. novemcinctus + other	2	0	2
	Human + *D. novemcinctus* + *Sigmodon* sp.*	1	0	1
	Human + *D. novemcinctus* + *Sigmodon* sp. + *Mus musculus**		1	1

* Origin of blood meals was determined by cloning and sequencing.

nymphs), which indicates intense dispersive activity. Several field observations and experiments show that the dispersal capacity of triatomines by walking is somewhat important. For example, artificial hen houses placed in tropical Petén forests of Guatemala are first infected with fifth-instar nymphs before they are infected by adults of the species *T. dimidiata*.[28] Thus, walking incursion of nymphs and adults from the peridomestic sites to the houses must be considered. However, the assumption of incursion of sylvatic triatomines into the house is not excluded because it was observed wild mammal feeding origins in several triatomines were collected indoors. Indeed, in Los Guerrero 48% of the dwellings are closed because people were living and working in other places, these houses have peridomiciles that are not maintained for months and can act as refuges for wild mammals, thereby increasing colonization by triatomines.[19] Consequently, some triatomines found in the houses can be from these peridomestic-sylvatic areas.

The scenario in Cacalután could be different from Los Guerrero. In Cacalutan the peridomicile colonization by *M. longipennis* was extremely lower, because the active research for triatomines in 82 peridomiciles allowed collecting only seven triatomines, the infestation rate was 4.9%. In these conditions it is difficult to believe that triatomines entering the home are from triatomine colonies located in the peridomicile. Most remarkable was the high proportion of *M. longipennis* males collected by the inhabitants of Cacalután. This high proportion of males could explain the very low colonization load of the peridomicile, whereas the abundance of peridomestic structures (permanent or built structures such as storage shelters, animal shelters, corrals, chicken-coops, and temporary structures, like piles of wood, brick, tile, or various goods and ends), and domestic animals would suggest a favorable situation for colonization, such as in the village of Los Guerrero.

The analysis of the blood meals also showed that a high proportion of the triatomines found in houses of Cacalután were carrying wild mammal feeding origins. In addition, several inhabitants testified to see triatomines flying in the street during the evening, which indicates active dispersion of triatomines in this area. These data make it possible to hypothesize that in Cacalután, the infestation by *M. longipennis* would originate from a wild environment rather than from the peridomi-

ciles. Likewise, the secondary species *T. recurva,* regarded as a sylvatic species, is also caught by the inhabitants indoors and outdoors.

Our study and others clearly show that in low endemic regions, longitudinal monitoring of the triatomine infestation by the inhabitants themselves made it possible to more accurately describe the risk patterns when compared with transversal active research.[29,30] In the current work, several secondary species were collected only by the inhabitants (indoors and outdoors) and were infected by *T. cruzi*. Moreover the longitudinal collection by the inhabitants shows that, even if the peridomicile present a very low infestation (active collection in Cacalután); a risk of transmission exists because infected indoor triatomes are collected. The proposed index of triatomine abundance can be used to compare different regions. As an example, in the city of Merida, Yucatán, México, only 0.7 triatomines/month/100 dwellings were found, a significant smaller number than those observed in the villages examined during this study (Los Guerrero, 12.2 triatomines/month/100 dwellings; Cacalután, 5.1 triatomines/month/100 dwellings).[29]

In Yucatán, another region of México where the *T. dimidiata* species is the principal vector, a seasonal indoor incursion model occurs; a mathematical model predicted that the majority of the domestic population is comprised of immigrants and genetic population studies found a low genetic differentiation between domestic triatomines and wild populations.[13,31] In the current region, the incursion of triatomines indoors was only slightly reduced during the coldest months, but the research in the sylvatic environment must be performed to clearly assess the origin of the populations that penetrate the dwellings using population genetics.

The vector systems related to the wild triatomine species are various and complex. They involve many species (about 30) whose wild habitat is terrestrial or arboricolous. Their distribution spreads over large geographic areas characterized by a wide variety of landscapes. In the current study, which focuses on the analysis of villages, we found that two different vector dynamic models of the same species could operate. In addition, we did not gather information or observe any factors that suggested an emerging process of transmission in any of the villages. Traditional control approaches (e.g., household insecticide spraying) are unlikely to be effective against vectors that have an incursion behavior. Novel strategies are urgently needed, and their development crucially depends on innovative research.

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