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Risk Factors Associated with Triatomines and Its Infection with *Trypanosoma cruzi* in Rural Communities from the Southern Region of the State of Mexico, Mexico

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Abstract. *Trypanosoma cruzi* prevalence in triatomines and risk factors associated to the presence of the insect were studied in 990 rural houses in the southern region of the State of Mexico, Mexico. In each house, triatomines were collected, and information related to house construction material was obtained. *T. cruzi* infection was diagnosed in all triatomines. A primary screening was performed using 2×2 contingency tables of exposure variables. All variables with $P \leq 0.20$ were analyzed by logistic regression. Triatomines ($N = 125$) were collected from 822 houses and analyzed for *T. cruzi* infection. *Triatoma pallidipennis* (97.4%) and *Triatoma dimidiata* (2.6%) were identified in 52.1% of the localities and in 6.1% of the houses. Infection was found in 28.0% of triatomines, from which 28.9% were nymphs. Factors associated with triatomine infestation were flooring construction material (dirt floor: odds ratio [OR], 10.05; 95% confidence interval [CI], 5.31–18.04; $P = 0.0001$), house rooms (at least three rooms: OR, 2.04; 95% CI, 1.07–3.86; $P = 0.028$), and ceiling construction material (cardboard lamina tile: OR, 6.84; 95% CI, 1.49–31.31; $P = 0.013$). This study shows *T. cruzi* circulation in triatomines in the area of study, and because triatomines are adapted for living and reproducing in the domestic environment, there is a potential risk of Chagas disease transmission to humans. Also, we can conclude that the construction materials and house inhabitants are risk factors of triatomines infestation.

INTRODUCTION

Chagas disease is a zoonosis caused by *Trypanosoma cruzi*; it is transmitted by hematophagous triatomines, arthropods from the Reduvidae hemiptera family known as kissing bugs.[1] *T. cruzi* can be found in the triatomine's feces and is transmitted when vector feces containing metacyclic trypomastigotes is inoculated into the insect's bite site through scratching, skin abrasions, or even through some mucous membranes.[2] It can also be transmitted by blood transfusion[3,4] and congenital infection[5,6] or the ingestion of infected food.[7]

In Mexico, 28 species of hematophagous triatomines have been reported, from which 23 are endemic in some specific regions and the remaining 4 are distributed throughout most of the Chagas-endemic regions of the country.[8–11] Considering the vectorial transmission capacity and widespread distribution in Mexico, the most important species are *Triatoma barberi*, *Triatoma dimidiata*,[12] *Triatoma phyllosoma, Triatoma longipennis, Triatoma mazzottii*,[12,13] *Triatoma pallidipennis, Triatoma picturata*,[12,14] *Triatoma mexicana,*[15] and *Triatoma gerstaeckeri*.[11] They can be found in altitudes ranging from sea level to 2,500 m in different ecosystems, such as deserts, forests, and mangals. Nevertheless, the most affected zones are those with warm and humid weather.[15,16]

About 18 million people in Latin America are estimated to be infected with *T. cruzi,* and it is believed that it is the main cause of morbidity and death in endemic areas.[17] Based on the seroepidemiologic surveys made in rural zones of endemic regions, it has been estimated that, in Mexico, there are three million people infected with *T. cruzi*.[9,18]

National morbidity statistics in 1999 still did not include the State of Mexico as an endemic area of transmission of Chagas disease.[18,19] However, in 1998, a few positive cases were identified in people living in the State of Mexico who received medical attention at the State of Hidalgo.[20] In 2000, immunofluorescence and hemagglutination screening tests were performed by the State Blood Transfusion Center from the Health Institute of the State of Mexico and found seven *T. cruzi* seropositive cases.[21] Recent studies showed, on one hand, that *Triatoma pallidipennis* is present in 24.4% of the localities of the southern region of the state of Mexico and that 11% of the triatomines were infected with *T. cruzi,*[22] and a different study reported a seroprevalence of 7.1% in humans and 21.0% in dogs, from the same region, observing a direct seropositive correlation between humans and dogs.[23]

Some of the risk factors associated with Chagas disease include living in geographic areas with warm and humid weather, poor hygiene, living conditions related to poverty, and the presence of animals living inside the houses.[24–26] Because the Chagas disease dynamics has not been fully characterized in this putative endemic area of the southern region of the State of Mexico, the aim of this study was to identify the main species of triatomines infesting the dwellings in this region, to determine the prevalence of triatomines infected with the *T. cruzi,* and to find the risk factors associated to the presence of kissing bugs within the dwellings.

MATERIALS AND METHODS

Area of study. This study was conducted in the Tejupilco sanitary jurisdiction, which includes Temascaltepec, Tejupilco, Luvianos, Amatepec, and Tlatlaya municipalities (Figure 1). The sanitary jurisdiction has a total population of 186,457 inhabitants. The altitude of the study area ranges from 500 to 1,200 m, with sub-humid warm weather all year long and rainfalls ranging from 800 to 1,600 mm.[27] The main economic activities in the region are agriculture and livestock farming. More than 50% of the population has a low socioeconomic status. Most of the rural houses are built with adobe brick walls, cardboard roofing, and dirt flooring, with no electricity or drainage.[26]

Study description. A sample of 822 houses belonging to 84 human settlements from four municipalities with an estimate of 186,271 inhabitants was studied. Sample size was estimated considering a *T. cruzi* prevalence of 8.0%,[23] with a 95%

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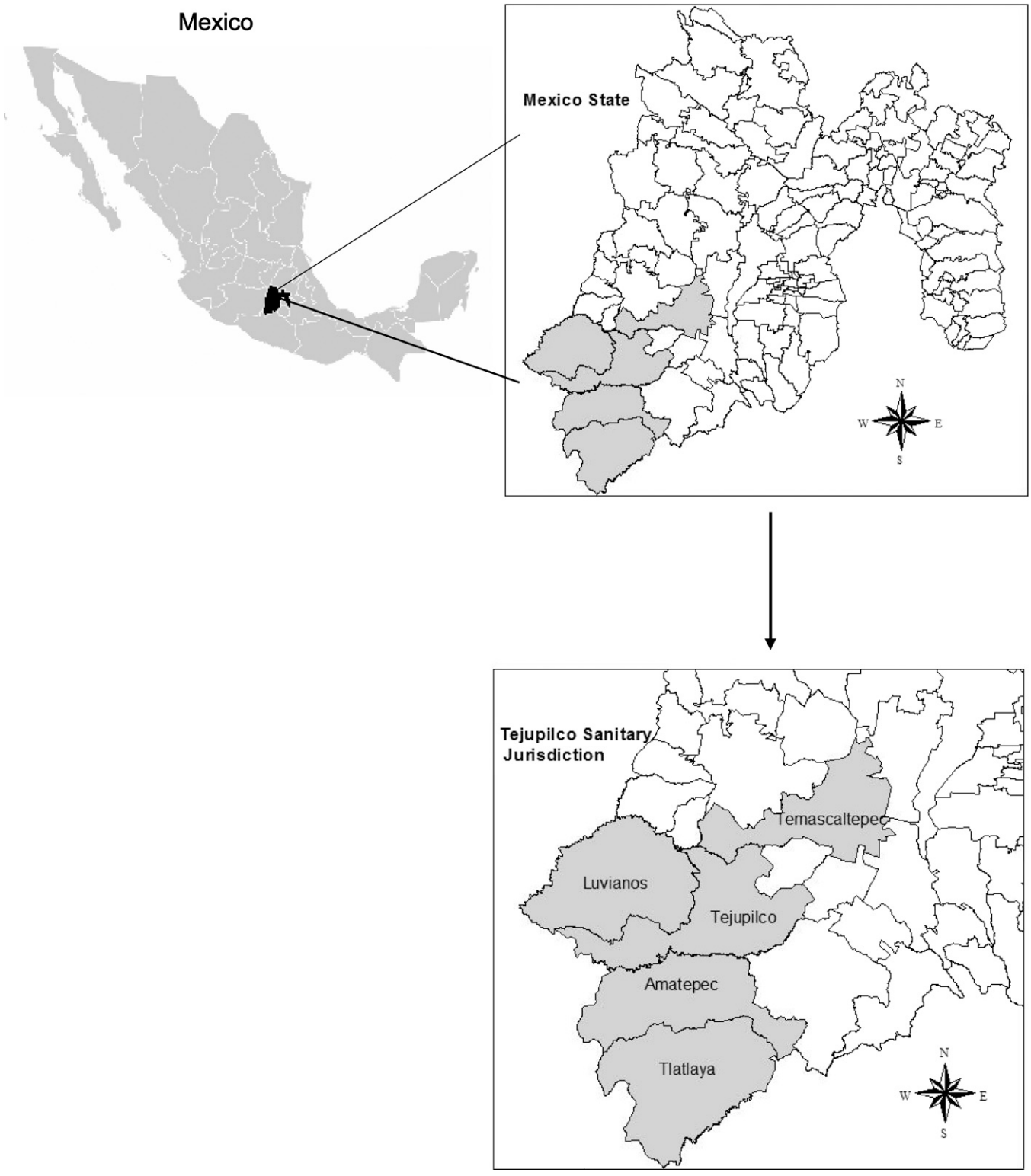

FIGURE 1. Tejupilco sanitary jurisdiction. Temascaltepec, Tejupilco, Luvianos, Amatepec, and Tlatlaya municipalities of Mexico State, Mexico.

confidence interval (CI). Dwelling sampling was made with a systematic aleatory method, according to the registers of the National Institute for Statistics, Geography, and Informatics from Mexico for the municipalities mentioned above.[28]

Triatomines sampling. The entomologic exploration was performed indoors and outdoors of the dwellings. The purpose of the exploration was to find triatomines, and the search was performed according with the national official methodology for the surveillance, prevention, and control of vector-transmitted diseases.[29] Inside the houses, a triatomine search mainly focused on frames, furniture, wall cracks, floors, and roofs. On the outside, a search was conducted mainly in piles of wood,

TABLE 1

Independent variables evaluated as possible risk factors associated to triatomine presence in the house of the municipalities of Tejupilco sanitary jurisdiction, Mexico State, Mexico

Variable	Description	Term
Houses' inhabitants	Number of inhabitants	1–2 inhabitants ≥ 3 inhabitants
Houses' rooms	Number of rooms	1–2 rooms ≥ 3 rooms
Flooring construction material	Number of houses with different flooring construction material	Dirt Concrete
Walls construction material	Number of houses with different walling construction material	Wood Adobe bricks Bricks
Ceiling construction material	Number of houses with different ceiling construction material	Concrete Curved lamina tiles Cardboard tiles

stones, construction supplies, wooden fences, wall slits, roofs, and floors. An entomologic brigade was composed of two people who conducted the search for triatomines inside and outside the house in a clockwise direction from the front door of the house. The search time was 30 minutes. The specimen collector used specialized clothing, safety boots, and leather gloves, and triatomines were taken with entomologic flexible forceps. Once the entomologic exploration was conducted, triatomines found were placed in plastic bottles. The bottles were tagged with the following data: family information, address, community name, municipality, collection date and specific site, and the name of the collecting person. The triatomine survey was performed with the help of the inhabitants of houses.

Triatomine samples were identified by community name and by collection date; insects were examined with a stereoscopic microscope for taxonomic identification (subfamily, genus, and species) according to the classification proposed by Lent and Wygodzinsky.[30]

The triatomine developmental stages of collected specimens were registered (egg, nymph I, nymph II, nymph III, nymph IV, adult). Also, the triatomine collection sites were registered (inside the house and outside the house: within 2 m of periphery).

***Trypanosoma cruzi* identification.** Triatomines feces were taken from the intestine by abdominal pressure, placed on a slide, and diluted with 50 µL of phosphate-buffered saline (PBS; pH 7.0). Smears were made and dried at room temperature; 100 µL of absolute methanol was added and dried at room temperature. Subsequently, samples were stained with 300 µL of 70% Giemsa stain.[29] Smears were examined with a light microscope at ×40 and classified as negative, slight, moderate, or severe infection (absence of parasites, 1–5, 6–20, or > 21 epimastigotes or trypomastigotes, respectively).[11]

Survey for risk factors. During the triatomine sampling, a survey was conducted with the objective of identifying the risk factors associated with triatomine presence in the houses. A set of questions was asked to the head of each house and included in the study according to the national official methodology for the surveillance, prevention, and control of vector-transmitted diseases.[29] Five independent variables were studied: number of bedrooms and inhabitants and house building materials (flooring construction material, wall construction material, ceiling construction material; Table 1).

Statistical analysis. A primary screening (univariate analysis) was performed using 2×2 contingency tables of exposure variables. All variables with $P < 0.20$ were analyzed by logistic regression (multivariate analysis), using Stata Statistical Software, version 2006 (Stata Corporation, College Station, TX), which provides exact regression estimates, 95% CIs, odds ratios (ORs: a measure of association that quantifies the relationship between the exposure variables and outcomes), and P values. The logistic regression was used to control possible confounding variables.[31–33] All triatomines and houses characteristics were considered independent variables (Table 1), whereas triatomine presence within the dwellings was considered the dependent variable.

RESULTS

The number of villages and houses visited and infested with triatomines per municipality is presented in Table 2. One hundred twenty-five triatomine specimens were captured in 52.1% of the visited communities and on 6.1% of the houses. Triatomines (91.3%) were mainly captured within houses: 28% were positive for *T. cruzi* and 28.94% were infected nymphs. Two species of *Triatoma* were identified: *T. pallidipennis* (97.4%) and *T. dimidiata* (2.6%). Triatomines were found within the five municipalities studied in which at least one infected triatomine was detected. Municipalities with the highest *T. cruzi* dispersion in triatomines were Luvianos and Tejupilco; the former was the only municipality where two *Triatoma* species were found (Table 2).

In the univariate analysis used to identify factors associated with triatomines, all exposure variables were identified with $P \leq 0.20$ (flooring, ceiling and wall construction materials, and inhabitants and rooms in houses). The logistic regression showed a positive association ($P < 0.05$) with flooring construction material (dirt floor: OR, 10.05; 95% CI: 5.31–18.04; $P = 0.0001$), house rooms (at least three rooms: OR, 2.04; 95% CI, 1.07–3.86; $P = 0.028$), and ceiling construction material (cardboard lamina tile: OR, 6.84; 95% CI, 1.49–31.31; $P = 0.013$; Table 3).

TABLE 2

Triatomines species with *T. cruzi* prevalence on municipalities of the south of Mexico State, Mexico

Municipality	Villages visited	Percent of the total	Houses visited	Percent of the total	Villages positive to triatomines	Percent	Villages positive for *T. cruzi*	Percent of triatomine infected	Number of triatomines found per species
Amatepec	28	23.9	166	16.7	3	2.5	1	33.3	*pallidipennis* (3)
Luvianos	31	26.4	238	24.0	25	21.3	5	20.0	*pallidipennis* (23) *dimidiata* (2)
Tejupilco	28	23.9	83	8.3	21	17.9	10	47.6	*pallidipennis* (21)
Tlatlaya	24	20.5	203	20.5	10	8.5	3	30	*pallidipennis* (10)
Temascaltepec	6	5.1	300	30.3	2	1.7	2	100	*pallidipennis* (2)
Total	117	100	990	100	61	52.1	21	34.42	*pallidipennis* (59) *dimidiata* (2)

TABLE 3
Risk factors associated with the triatomines in the houses of the south of the Mexico State, Mexico

Variable	Total	Positive	Frequency (%)	OR	95% CI	*P* value
Flooring construction material						
Concrete	744	13	1.74			
Dirt	246	48	19.51	10.05	5.31–19.04	0.0001*
House inhabitants						
1–2	525	45	3.44			
≥ 3	465	16	8.57	2.04	1.07–3.86	0.028†
House rooms						
≥ 3	190	9	4.73			
1–2	800	52	6.50	1.00	0.046–2.14	1.000‡
Walls construction material						
Adobe brick	58	1	1.72			
Wood	23	1	4.34	0.41	0.02–7.47	0.551‡
Adobe	909	59	6.49	3.81	0.48–30.81	0.203‡
Ceiling construction material						
Concrete	181	2	1.10			
Curve lamina tile	672	35	5.20	2.59	0.59–11.31	0.204‡
Cardboard tile	137	24	17.51	6.84	1.49–31.31	0.013†

* Highly significant.
† Significant.
‡ Not significant.

DISCUSSION

It has been reported that 18 of the 28 species of the *Triatoma* genus, present in Mexico, are naturally infected with *T. cruzi*.[6,11,14,18] According to the adaptation capacity of Triatomine species to live in human houses, they can be collected inside human dwellings and from peridomestic and sylvatic areas.[9,13] Findings in this study confirmed previous reports that show that *T. pallidipennis* is an endemic species from the southern region of the State of Mexico.[16] This triatomine is well adapted to the domestic cycle, because 91.3% of the specimens found were captured within houses, and from these, 28.94% were nymphs, meaning that reproductive activity is occurring within the dwellings. These findings are epidemiologically relevant because triatomine reproduction indoors is an important element in the transmission dynamics of Chagas disease, considering the increment in the number of insects within the house and the time they spend in it.

Most of the triatomines found in this study were *T. pallidipennis* (97.4%). This type of triatomine has been identified in 10 states of the country[34] and has been shown to be one of the most important vectors for Chagas disease in Mexico.[1] The remaining 2.6% of the insects were classified as *T. dimidiata*. A similar proportion of this species has been reported for the other 14 states of the Mexican Republic.[11] This low proportion of *T. dimidiata* is not surprising, because the search was mainly performed indoors, and this vector inhabits mainly diverse outdoor ecotopes including piled up rocks, bat caves, and tree holes occupied by mammals or birds.[35,36] These insects most likely are accidentally introduced to houses through firewood or other materials that farmers take into their homes.[35] Nevertheless, even though *T. dimidiata* was found in low numbers within houses, it should be kept in mind that this triatomine is one of the most important vectors of Chagas disease, and therefore, more studies should be conducted to understand its role in *T. cruzi* transmission in human Chagas disease in the State of Mexico.

In this study, 28.94% of the triatomines were infected with *T. cruzi,* and from these, 28.94% were nymphs. Nymph infection prevalence was found to be higher than in the State of Morelos, Mexico,[35] where 16.4% of infected nymphs was reported. These findings suggest that triatomines are actively reproducing and feeding from mammals inhabiting the house, increasing the infection possibilities of animals and humans within the dwellings.

In this study, we reported that dirt flooring (OR, 10.5) and cardboard tile (OR, 2.59) were associated with a higher probability of triatomine prevalence. Our results confirmed previous reports of these risk factors,[26] where the association between housing building materials and triatomine proliferation was described. In our study, triatomines were also found in a low proportion in houses made out of concrete and curved lamina tile, because roof fissures constitute a risk factor for triatomine proliferation.[37–40] This condition should be kept in mind by the sanitary authorities when implementing vector control programs.

As far as the number of people per house, more triatomines were found in houses with at least three inhabitants (OR, 2.04), which suggests that triatomines have a certain predilection for houses that are more heavily populated.[34] In conclusion, this study showed that a relatively large proportion of houses in the area under study were infested with triatomines infected with *T. cruzi*, the causal agent of Chagas disease, and that *T. pallidipenis* has adapted to the domestic life cycle in this geographic region; therefore, there is a potential high risk of Chagas disease transmission to humans. Also, we conclude that the construction materials and house inhabitants are risk factors for triatomine infestation.

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