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BRIEF COMMUNICATION

CHICKEN COOPS, *Triatoma dimidiata* INFESTATION AND ITS INFECTION WITH *Trypanosoma cruzi* IN A RURAL VILLAGE OF YUCATAN, MEXICO

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SUMMARY

This study longitudinally investigated the association between *Triatoma dimidiata* infestation, triatomine infection with *Trypanosoma cruzi* and household/backyard environmental characteristics in 101 homesteads in Molas and Yucatan, Mexico, between November 2009 (rainy season) and May 2010 (dry season). Logistic regression models tested the associations between insect infestation/infection and potential household-level risk factors. A total of 200 *T. dimidiata* were collected from 35.6% of the homesteads, mostly (73%) from the peridomicile. Of all the insects collected, 48% were infected with *T. cruzi*. Infected insects were collected in 31.6% of the homesteads (54.1% and 45.9% intra- and peridomiciliary, respectively). Approximately 30% of all triatomines collected were found in chicken coops. The presence of a chicken coop in the backyard of a homestead was significantly associated with both the odds of finding *T. dimidiata* (OR = 4.10, CI 95% = 1.61-10.43, $p = 0.003$) and the presence of triatomines infected with *T. cruzi* (OR = 3.37, CI 95% = 1.36-8.33, $p = 0.006$). The results of this study emphasize the relevance of chicken coops as a putative source of *T. dimidiata* populations and a potential risk for *T. cruzi* transmission.

KEYWORDS: Peridomicile; *Triatoma dimidiata; Trypanosoma cruzi;* Chagas disease.

In the Mexican state of Yucatan, Chagas disease is an endemic zoonosis transmitted domestically by *Triatoma dimidiata* Latreille 1811 (Hemiptera: Reduviidae), the only locally proven vector. *T. dimidiata* can be collected in domestic, peridomestic and sylvatic habitats of Yucatan. House infestation is described as seasonal, occurring mainly due to the dispersal of adult insects from peridomestic and sylvatic habitats during the late dry season with non-apparent or limited colonization[2,5].

Few studies in Yucatan have examined the importance of household and backyard characteristics in the prevalence of triatomine infestations and their infection rates with *T. cruzi* in and around houses. GUZMÁN-MARÍN *et al*.[8] reported that household triatomine infestation in rural communities was associated with the type/quality of housing, e.g. construction with natural materials, thatched roofs, unplastered walls or walls with adobe plastering and the lack of cemented floors. However, other studies have reported that the location of a house within a community (especially if located on the periphery) is a significant risk factor for infestation and the invasion of dispersing adult insects[2,5,13]. REYES-NOVELO *et al.*[14] showed that *T. dimidiata* did colonize animal shelters (e. g. chicken and dove coops, dog houses and opossum nests). More recently, DUMONTEIL *et al.*[5] quantified that the number of dogs in a house and keeping chickens in a corral were strong determinants for house infestation in rural communities. Such findings from Yucatan agree with reports of house infestation and colonization by *T. dimidiata* in Guatemala[11]. This study confirms the significance of the peridomicile environment, and particularly of chicken coops, as a source of *T. dimidiata* populations and a potential risk factor for *T. cruzi* transmission in a rural village in Yucatan, Mexico.

Fieldwork was carried out between November 2009 and May 2010 in a sample of 101 homesteads (each homestead including the house and all peridomestic structures found in the front and backyard) from Molas, a rural village located in the Southeast of Mexico (20° 48'58'' N and 089° 37'54' W). The community has a population of 2,014 inhabitants, living in 553 houses and surrounded by a subtropical deciduous forest within the Cuxtal ecological reserve. Altitude is 10 m. a. s. l. Climate is characterized by an average annual temperature of 25.9 °C, with an annual rainfall of 800-1000 mm, occurring mainly between June and November. Molas is located within the highest risk area for Chagas disease in the state of Yucatan[2,3].

Homestead infestation with triatomines was evaluated through: i) active collections both intra- and peridomiciliary and; ii) householders'

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participatory collections within the houses. Two cross-sectional timed manual active searches for triatomines (described by Gürtler *et al*. 1999) were performed, one during the 2009 rainy season (November) and the other one during the 2010 dry season (May).

Collections were performed inside houses (interdomiciliary) and in front/backyards (peridomiciliary) by teams of two trained research personnel (30 min in each ecotope to complete one man/hour/homestead between 8:00 a.m. and 1:00 p.m.). Intradomiciliary searches included inside walls, the base of the roof and furniture. Peridomiciliary searches focused on animal housing, rock and woodpiles, tree trunks, and any other potential triatomine refuges. In addition, householders were invited to take part in a six-month participatory vector surveillance strategy[4] between December and March, 2010.

Trypanosoma cruzi DNA extraction from individual triatomines and PCR amplification of *T. cruzi* kinetoplast DNA were performed as described by REYES-NOVELO *et al*.[14] based on the EDWARDS *et al*.[6] and MOSER *et al*.[12] protocols. The primers used were TcZ1: 5'-CGAGCTCTTGCCCACACGGGTGCT-3'- and TcZ2 5'-CCTCCAAGCAGCGGATAGTTCAGG-3'. Amplification was performed in a Techne TC132 (Barloworld Scientific LTD, Staffordshire, UK) thermal cycler. A 188bp fragment identified the presence of *T. cruzi* DNA following the electrophoresis of a percentage of PCR product in a 2% agarose-TBE stained with ethidium bromide (10 μg/mL) and further documentation in an EDAS 290 gel documentation system (Kodak, Rochester, USA). Local strains of *T. cruzi* were used as positive controls, whereas the whole mixture minus DNA was used as a negative control.

A household survey was performed to investigate a range of household/backyard characteristics previously reported as significant in the infestation of *T. dimidiata* and other triatomines[1,10,15] - type/material of the house (roof, walls, floor); use of window screening; presence of rubbish, rock/wood piles, stone walls, abandoned lots on the sides; the presence of domestic animals e. g. dogs, cats, poultry, horses, sheep, cattle, and the presence of animal housing structures (organized by species) e. g. chicken coops, pig corrals, house stables and kennels.

Using a Fisher's exact test, statistical analyses compared the sex and stage of development of *T. dimidiata* between locations (intra- and peridomicile). Comparisons of infection by sex between seasons were not performed because of the low number of insects collected. Tests were carried out to study the association between triatomine infestation (positive homesteads) and *T. cruzi* infection with household-level potential risk factors. Variables of interest were analyzed using χ^2 to reduce the model, by comparing percentages in contingency tables. Those with $p < 0.25$ were included in a logistic regression analysis. Adjusted Odds Ratio and Confidence Intervals ($\alpha = 0.05$) were calculated with SPSS® (v17.0).

A total of 200 *T. dimidiata* specimens were collected from 35.6% (36/101) homesteads throughout the study period (Table 1). Overall, a greater number of adults were collected than nymphs ($p < 0.05$), with a higher male:female abundance ratio ($p < 0.05$) between ecotopes (Table 1). The majority of specimens (73%) - both adults and nymphs - were collected in the peridomicile environment; nevertheless, 22.5% of the adults and 4.5% of the nymphs collected were reported to have been found intradomiciliary.

Collection methods were complementary. Active collections yielded more specimens (65%) than participatory collections. 130 *T. dimidiata* specimens were captured by active collection, mostly peridomiciliary (97.7%), with a sample composed by adults and nymphs in a similar ratio. 70 specimens of *T. dimidiata* were captured through householders' collections, mostly reported as intradomiciliary (74.3%) and consisting mostly of adult triatomines (77%).

Overall, 48% (96/200) of the *T. dimidiata* specimens collected tested positive for *T. cruzi* (Table 1) and were found in 31.6% (32/101) of homesteads. The infected specimens were mostly adults ($p < 0.05$); but the proportion of nymphs infected was high (37.5%). Slightly more infected *T. dimidiata* were found intradomiciliary (54.1%) than in the peridomicile environment (45.9%). Infection prevalence detection was higher in participatory collections (59/70) than in active collections (37/130) ($p < 0.05$).

Table 1

Triatoma dimidiata infestation (by stage of development, sex and location) and infection with *Trypanosoma cruzi* in homesteads in the rural community of Molas, Yucatan, Mexico

	Total (%)	Nymphs (%)	Adults (%)	♂ (%)	♀ (%)
Infestation					
Intradomiciliary	54 (27.0)	9 (16.7)	45 (83.3)	19 (35.2)	26 (48.1)
Peridomiciliary	146 (73.0)	79 (54.1)	67 (45.9)	42 (28.8)	25 (17.1)
Total	200 (100)	88 (44.0)	112 (56.0)*	61 (30.5)	51 (25.5)*
Infection					
Intradomiciliary	52(54.1)	9 (17.3)	43 (82.7)	18 (34.6)	25 (48.1)
Peridomiciliary	44 (45.9)	27 (61.4)	17 (38.6)	8 (18.2)	9 (20.4)
Total	96 (100)	36 (37.5)	60 (62.5)*	26 (27.1)	34 (35.4)

* Significant statistical difference in the frequencies of developmental stage and sex between locations, as given by Fisher's exact test ($p < 0.05$). Statistical tests regarding infestation and infection were performed separately.

Table 2
Active collection and *Trypanosoma cruzi* infection of *Triatoma dimidiata* from peridomicilary chicken coops in homesteads in the rural community of Molas, Yucatan, Mexico

	Total	Nymphs (%)	Adults (%)	♂ (%)	♀ (%)
1st active collection Rainy season					
Infestation	41	23 (56.1)	18 (43.9)	15 (36.6)*	3 (7.3)
Infection	20	19 (95)*	1 (5)	0 (0)	1 (5)
2nd active collection Dry season					
Infestation	26	13 (50.0)	13 (50.0)	5 (19.2)	8 (30.8)
Infection	10	4 (40)	6 (60)	3 (30)	3 (30)

*Significant statistical difference in frequencies of developmental stage and sex between seasons, as given by Fisher's exact test ($p < 0.05$). Statistical tests regarding infestation and infection were performed separately.

Three housing/backyard characteristics initially had $p < 0.25$: the presence of a chicken coop in the backyard, the type of walls in the house and wood storage; but only the presence of a chicken coop was significantly and positively associated with the presence of *T. dimidiata* (OR = 4.10, $p = 0.003$; 95% CI = 1.61-10.43) in the final model. The presence of a chicken coop was also positively associated with *T. dimidiata* infected with *T. cruzi* (OR = 3.37, $p = 0.006$; 95% CI = 1.36-8.33).

Fifty-four homesteads were found in the area with at least one chicken coop. The general structure of local chicken coops consists of cages of 1.30 - 1.50m in height, square in shape, and with sides of 2 - 3m in length. Coops are built on a 4-log base, one on each corner, holding a roof made of either zinc or cardboard and surrounded by a chicken wire fence. The ground is commonly covered with compacted dirt and small stones. Approximately 26% (14/54) of the chicken coops had *T. dimidiata,* and 64.3% (9/14) had triatomines infected with *T. cruzi*. Of all peridomestic triatomines, 45.9% (67/146) were collected from chicken coops, with nymphs and adults found in a similar ratio (36:31, respectively. 44.8% of all specimens collected from chicken coops were infected (30/67), with a higher percentage of infected nymphs (76.7% compared to 23.3% in adults).

Triatomine specimens collected from chicken coops were obtained exclusively by active collection and were found on the floor and under stones. During the sectional-active collection in the rainy season, 9.3% (5/54) of homesteads with chicken coops were positive for triatomines. During the second active collection in the following dry season, 16.7% (9/54) of homesteads with chicken coops were positive for triatomines (the majority identified positive for the first time and only two were consistent from the collection four months earlier (Table 2).

Chicken coops are known to play an important role in the maintenance of *T. dimidiata* populations, both as a refuge for invading insects[11,16] and as a primary source of blood for triatomines[9]. All coops that tested positive in Molas had chickens, except for one, where chickens were removed two weeks before the survey. Among the total homesteads sampled in the locality, only two other sites were found to be used by triatomines as refuges: a rabbit hutch and a pile of rocks and wood. Although the rabbit hutch had a large population of triatomines, this type of refuge was not as commonly found in the peridomiciles as chicken coops. Piles of rocks and wood were quite common, but only one was found to be infested with triatomines.

While the debate concerning whether house infestation by triatomines is influenced by the peridomicile and/or the sylvatic habitats continues[4,13,14], the results of this study expose the significance of chicken coops located in the peridomicile as a potential source of *T. dimidiata* populations. Preceding studies in Yucatan report that *T. dimidiata* infestations occur seasonally but transiently, i.e. with a limited capacity for colonizing households in Yucatan[2,7]. These findings indicate the existence of peri- and intradomiciliary infestation and the high prevalence of infected triatomines not only during the dry season, but also in the rainy season. Colonization in houses (based on the collection of nymphs) is indeed uncommon during the rainy season, but increases during the dry season.

This study shows that chicken coops are a risk factor for insect infestation and parasite infection. Nonetheless these findings should be re-evaluated in other communities infested by *T. dimidiata*.

RESUMEN

Gallineros, la infestación por *Triatoma dimidiata* y su infección con *Trypanosoma cruzi* en una localidad rural de Yucatán, México

Investigamos longitudinalmente la asociación entre la infestación por *Triatoma dimidiata*, su infección con *Trypanosoma cruzi* y las características ambientales de los domicilios/peridomicilios en 101 viviendas de Molas, Yucatán, México entre Noviembre de 2009 (temporada lluviosa) y Mayo de 2010 (temporada seca). Mediante modelos de regresión logística se probaron asociaciones entre la infestación/infección de *T. dimidiata* y factores de riesgo potenciales a nivel de las viviendas. Se colectó un total de 200 individuos de *T. dimidiata* en el 35.6% de las viviendas, mayormente del peridomicilio (73%). De todos los triatominos colectados el 48% se encontraron infectados con *T. cruzi.* Los triatomas infectados fueron colectados en el 31.6% de las viviendas (54.1% y 45.9% en intra y peridomicilio

respectivamente). Aproximadamente el 30% de todos los triatominos colectados, fueron encontrados en gallineros. La presencia de un gallinero en el peridomicilio de una vivienda se asoció significativamante tanto con las posibilidades de encontrar *T. dimidiata* (OR = 4.10, CI 95% = 1.61-10.43, $p = 0.003$) como con la presencia de triatominos infectados con *T. cruzi* (OR = 3.37, CI 95% = 1.36-8.33, $p = 0.006$). Los resultados de este estudio enfatizan la relevancia de los gallineros como fuente putativa de poblaciones de *T. dimidiata* y como una fuente potencial de riesgo de transmisión de *T. cruzi*.

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