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Major Article

Toxicological profile of deltamethrin in *Triatoma brasiliensis* (Hemiptera: Reduviidae) in State of Ceará, Northeastern Brazil

***Grasielle Caldas D'Ávila Pessoa*[1], *Nathália Abreu Borges Trevizani*[1], Letícia Sena Dias[1], *Claudia Mendonça de Bezerra*[2] , *Bernardino Vaz de Melo*[3] *and Liléia Diotaiuti*[1]**

[1]. Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, MG. [2]. Secretaria de Saúde do Estado do Ceará, Fortaleza, CE. [3]. Secretaria de Saúde do Estado de Minas Gerais, Belo Horizonte, MG.

ABSTRACT

Introduction: *Triatoma brasiliensis* is the species of greatest epidemiological relevance in the semi-arid region of Brazil. This species is predominantly found in domestic environments, and it has the ability to build large colonies with high levels of natural infection via *Trypanosoma cruzi*. Thus, *T. brasiliensis* is one of the most efficient transmitters of Chagas disease (CD) to humans. Despite household spraying with residual insecticides, many areas report persistent reinfestations for reasons that remain poorly understood. Therefore, this study sought to characterize the toxicological profile of deltamethrin in *T. brasiliensis* from areas with persistent reinfestation in State of Ceará, Brazil. **Methods:** The susceptibility reference lineage (SRL) was derived from Umari. Serial dilutions of deltamethrin were prepared and applied to the dorsal abdomen of first instar nymphs. The control group received only pure acetone. Mortality was evaluated after 72h. Qualitative tests assessed mortality in response to a diagnostic dose of $1xLD_{99}$ (0.851 nanograms of active ingredient per treated nymph) of the SRL. **Results:** The susceptibility profile characterization of the *T. brasiliensis* populations revealed 50% resistance ratios (RR_{50}) that ranged from 0.32 to 1.21. The percentage of mortality in response to the diagnostic dose was 100%. **Conclusions:** We demonstrated that *T. brasiliensis* was highly susceptible to deltamethrin. The control difficulties found might be related to the recolonization of the triatomines originating from neighboring environments and the possible operational failures related to the process of spraying that enabled specimens less susceptible to deltamethrin to survive.

Keywords: Triatominae. *Triatoma brasiliensis.* Insecticide resistance. Deltamethrin.

INTRODUCTION

Chagas disease[1] (CD) affects approximately 1.8 to 2.5 million people in Brazil. This disease is endemic to much of the country and targets entire populations with poor access to basic public health services (sanitation, housing, health, and so on). After many years of effort and investment in housing improvements and chemical control, home triatomine levels have been significantly reduced in general, especially those of *Triatoma infestans*. However, studies of existing autochthonous species show that the effective entomological surveillance led by endemic/health agents, in partnership with the general population, must be conducted to maintain this result.

In many regions of Brazil, humans are in close contact with triatomines because of the proximity and overlap of domestic and wild environments, which often occurs in the Brazilian semi-arid region. The species of greatest epidemiological relevance in this region is *Triatoma brasiliensis*. Studies conducted in Bahia[2], Piauí[3] and Ceará[4] have indicated that *T. brasiliensis* is the most common species in domestic environments, occasionally forming colonies of more than 100 specimens[5] and having high levels of natural infection via *Trypanosoma cruzi*; thus, this species is one of the most efficient transmitters of CD to humans. Domestic infestation occurs more commonly during the dry season, and triatomines are usually attracted to light[6].

In the wild, *T. brasiliensis* is associated with the Caatinga (a semi-arid scrub forest in northeastern Brazil) and is usually found in dens of various rodents[7]. Valença-Barbosa et al.[8] recently examined the *T. brasiliensis* colonization of the *Pilosocereus gounellei* cactus, popularly known as *xique-xique* in the Valley of Jaguaribe, Northeastern Brazil. The proximity of wild and domestic environments enables the dispersion of insects to housing units[9]. In peridomiciles, *T. brasiliensis* shows a preference for animal shelters built with bricks and covered with tiles[5]. Rodrigues et al.[10] have noted a strong tendency for *T. brasiliensis* to be located in poultry houses and near bird resting areas, especially in the lower, drier and warmer portions of walls.

Address to: Dra. Liléia Diotaiuti. Lab. de Triatomíneos e Epidemiologia da Doença de Chagas/CPqRR/FIOCRUZ. Av. Augusto de Lima 1715, Barro Preto, 30190-002 Belo Horizonte, MG, Brasil.
Phone: 55 31 3349-7762; **Fax:** 55 31 3295-3115
e-mail: diotaiuti@cpqrr.fiocruz.br
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The proximity of wild and domestic environments in the northeastern semi-arid region enables an intense flow of *T. brasiliensis* between these environments. As such, reinfestation processes are frequently reported[11] [12]. In this sense, the use of residual insecticides is extremely relevant to prevent new specimens from invading housing units. However, despite systematic and continuous spraying activities in accordance with the recommendations of the Ministry of Health[13], controlling triatomines has been difficult in many areas. Diotaiuti et al.[12] examined households in Independência four months after deltamethrin spraying and found triatomines of all development phases in 9.7% of the sampled units. This finding suggests that triatomine recolonization from neighboring locations occurs via specimens surviving the spraying process. This result might be related to operational failures in the spraying activity, *T. brasiliensis* resistance to the insecticides used for vector control, or both.

To better understand the factors that hinder the control of *T. brasiliensis* in the households of Ceará, Brazil, this study characterized the toxicological profile of deltamethrin pyrethroid in *T. brasiliensis* populatio across six municipalities using laboratory bioassays.

METHODS

The specimens for this study were manually collected without using a dislodging agent from peridomiciles in endemic areas of Ceará where the Chagas Disease Control Program had performed continuous and systematic applications of insecticides with residual action over the last 30 years: Jaguaruana (04° 50' 02" S 37° 46' 51" W), Quixeré (05° 04' 26" S 37° 59' 20" W), Jaguaribe (05° 53' 27" S 38° 37' 19" W), Independência (05° 23' 45" S 40° 18' 32" W), Ipaporanga (04° 54' 00" S 40° 45' 32" W), and Tauá (06° 00' 10" S 40° 17' 34" W). Dr. Marcos Obara kindly provided a susceptibility reference lineage (SRL). This colony originated from the insectary of the Laboratory of Parasitology in the Department of Biological Sciences at *Universidade Estadual Paulista Júlio de Mesquita Filho/UNESP*. The insectary was started in 1984.

Bioassays were performed using the methods of Pessoa[14] and the World Health Organization (WHO)[15]. Serial dilutions (0.1 - 8.0ng/µL) of deltamethrin (98.2% purity, Bayer: São Paulo, Brazil) were prepared and applied to the abdomen of the first instar nymphs from the F1 generation (five days old, fasting weight 1.2 ± 0.2mg) using a Hamilton micro-syringe (0.5µL per insect). The insecticide was diluted in acetone. At least six doses surrounding the lethal dose of 50% (LD_{50}) producing mortality rates between 10% and 90% were administered. Acetone alone was applied to the insects in the control group. Three replicates of ten nymphs were performed for each dose. Mortality was recorded at 72h. The mortality criterion was the inability of the nymphs to walk out of a filter paper disc (7cm in diameter[15]).

The mortality data were analyzed using Basic Probit Analysis[16] software to estimate slope and the LD_{50} in nanograms of active ingredient per treated nymph (ng a.i./nymph). Fifty percent resistance ratios (RR_{50}) were calculated by dividing the LD_{50} of each field population by its corresponding SRL. A susceptibility status classification was performed according to the Pan American Health Organization (PAHO)[17].

After setting the base susceptibility line of the *T. brasiliensis* reference population, 30 nymphs from all of the field samples received a diagnostic dose of $1xLD_{99}$ (0.851ng a.i./nymph) based on the SRL. The survival of at least two insects in three replicates was interpreted as an indicator of resistance[15].

RESULTS

The susceptibility reference lineage presented an LD_{50} of 0.293 ng a.i./nymph treated. The susceptibility profile characterization of the *T. brasiliensis* samples revealed RR_{50} values ranging from 0.32 to 1.21. The mortality percentage in response to the diagnostic dose for all samples was 100%. Only six samples from *Jaguaruana* (Cipriano Lopes), *Independência* (Pajeú), *Jaguaribe* (Serrote), *Quixeré* (Boqueirão), *Ipaporanga* (Lagoa de Dentro, Casa 6) presented a slope less than the SRL, revealing higher frequencies of individuals with resistant alleles **(Table 1)**.

DISCUSSION

The *virtual* control of *T. infestans* (a native species of Bolivia, introduced in the late 18th century in Brazil)[18], accompanied by the *International Elimination of the Transmission of Chagas Disease Certificate* in 2006[19], strengthened the spurious idea that CD had been eliminated in Brazil and disregarded the epidemiological importance of native triatominic species in the Northeast region, such as *T. brasiliensis*. The significance of these triatomines with regard to maintaining the cycle of *T. cruzi* near human homes should not be neglected, hence the importance of control activities, epidemiological surveillance and health awareness. Despite systematic and continuous spraying activity, reinfestation has often been reported because of operational failures, resistance to the insecticides used in vector control strategies, or both.

Sonoda et al.[20] found low levels of *T. brasiliensis* resistance to deltamethrin in populations in Tauá, Ceará. Intradomicile populations (*Cachoeira dos Pedrosas* RR_{50}=1.16, *Cachoeira do Júlio* RR_{50}=1.74, *Dormideira* RR_{50}=1.79 and *Mutuca* RR_{50}=1.58), peridomicile populations (*Cachoeira dos Pedrosas* RR_{50}=1.26, *Morada Nova do Thomas* RR_{50}=1.74 and *Mutuca* RR_{50}=1.58) and wild populations (*Cachoeira dos Pedrosas* RR_{50}=1.0) were assessed, and all were categorized as highly susceptible to the tested insecticide.

Obara[21] characterized the toxicological profile of 16 populations of *T. brasiliensis* with regard to deltamethrin in the States of Paraíba, Pernambuco, Piauí and Rio Grande do Norte. All of these populations presented low RR_{50} values ranging from 1.0 to 2.17. Field trials to assess the effect of WG250 deltamethrin at 25 ng a.i./nymph on different substrates (as recommended by the Ministry of Health) have confirmed the susceptibility of all populations, with 100% mortality rates for all trials.

TABLE 1 - Toxicity of topically applied deltamethrin to *Triatoma brasiliensis* first instars of an SRL and peridomestic samples collected from State of Ceará, Brazil.

Population: Municipality; Location	Number*	LD_{50} (95% CIs)	RR_{50}	Slope (SD)	Diagnostic dose (% mortality)
Umari – SRL	360	0.293 (0.263 – 0.325)	-	3.959 ± 0.537	-
Jaguaruana; Cipriano Lopes	330	0.093 (0.075 – 0.114)	0.32	2.358 ± 0.376	100.0
Quixeré; Boqueirão do Adauto	330	0.112 (0.09 – 0.126)	0.38	5.096 ± 0.855	100.0
Jaguaruana; João Duarte	330	0.126 (0.104 – 0.151)	0.43	2.933 ± 0.501	100.0
Jaguaribe; Alto Grande	330	0.176 (0.154 – 0.207)	0.60	3.871 ± 0.656	100.0
Independência; Cachoeira do Lopes	300	0.188 (0.148 – 0.231)	0.64	3.527 ± 0.610	100.0
Ipaporanga; Serrinha	270	0.221 (0.118 – 0.255)	0.75	4.427 ± 1.896	100.0
Independência; Pajeú	240	0.220 (0.119 – 0.290)	0.75	2.421 ± 0.567	100.0
Tauá; Barra	330	0.233 (0.199 – 0.269)	0.80	3.430 ± 0.460	100.0
Jaguaribe; Serrote	300	0.241 (0.192 – 0.299)	0.82	2.421 ± 0.511	100.0
Quixeré; Boqueirão	300	0.274 (0.226 – 0.350)	0.93	2.512 ± 0.410	100.0
Ipaporanga; Alegre – Casa 6	240	0.275 (0.141 – 0.412)	0.94	1.422 ± 0.480	100.0
Ipaporanga: Lagoa de Dentro	270	0.299 (0.257 – 0.356)	1.02	2.884 ± 0.432	100.0
Tauá; Viração	270	0.348 (0.318 – 0.381)	1.19	5.043 ± 0.673	100.0
Ipaporanga; Alegre – Casa 5	270	0.355 (0.310 – 0.410)	1.21	4.053 ± 0.578	100.0
Jaguaruana; Currais do Felipe	240	0.354 (0.303 – 0.561)	1.21	3.063 ± 0.529	100.0

SRL: susceptibility reference lineage; **LD_{50}:** 50% lethal dose; **95% CIs:** 95% confidence intervals; **RR_{50}:** 50% resistance ratio; **SD:** standard deviation. * number of triatomines used.

This work assessed 15 populations of *T. brasiliensis* from Ceará and identified low RR_{50} values (0.32-1.21). According to the WHO (1994), all populations are susceptible to deltamethrin. In the present study, the qualitative assays confirmed this susceptibility and showed 100% mortality rates for all of the samples in response to diagnostic dose. Importantly, populations from different locations within the same municipality presented different toxicological profiles. This finding suggests that the selection process for pesticides can occur independently, even in neighboring houses[22,23].

The existence of populations with RR_{50}<1.0 (i.e., those more susceptible than the susceptibility reference strain used) suggests that the SRL already had resistant alleles when it was collected in the field in 1984 and that they remain to the present day. A similar finding was reported for *Triatoma sordida*[23,24], which led us to reflect on the weak criteria recommended by the PAHO[17] for the choice of SRLs: over five generations in the laboratory without insecticide contact, external material contribution, or originating from places that have never been treated with insecticide.

Of the 15 populations studied, 9 presented slopes equal to or greater than the SRL, suggesting little intrapopulational heterogeneity. This finding corroborates molecular studies showing that genetic diversity is lower in areas with chemical treatment than untreated areas[25,26,27]. However, the other 6 populations showed lower slopes than the SRL, indicating greater heterogeneity most likely because of the high dispersal rates of the *T. brasiliensis* specimens in Ceará that invade houses by flying[12]. This fact shows the significant recolonization potential of artificial environments from wild and peridomicile sources. Importantly, one cannot exclude the possible development of resistance among populations with lower slopes than that of the SRL when pressured by insecticides. This possibility justifies the need to monitor timeline susceptibility changes.

The results obtained from qualitative and quantitative bioassays did not prove that *T. brasiliensis* is resistant to deltamethrin. Despite successive insecticide spraying, the persistence of triatomines might be associated with their behavior. Peridomicile (and even intradomicile) infestations present a wide range of environments, corresponding to an infinite number of hiding spots. Spraying these environments requires extensive work; unstacking all of the material accumulated inside is often impossible for field agents. Consequently, triatomines (eggs, nymphs and adults) might remain in the area after insecticide application, having no or limited contact with the chemical, thereby encouraging the selection of those that are least susceptible and enabling their survival[28,29]. Moreover, the high temperatures and ultraviolet (UV) radiation of northeastern Brazil can degrade pyrethroids faster, thereby reducing their duration, availability on treated surfaces, and their consequent triatomicide action.

Importantly, the small changes in susceptibility found in this study are unlikely to have contributed to the emergence of CD cases in the study area; however, they serve as a warning to improve the quality and methodological rigor of the chemical control activities associated with the spatial reordering of households to avoid refractory *T. brasiliensis*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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