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Major Article

Susceptibility characterization of residual Brazilian populations of *Triatoma infestans* Klug, 1834 (Hemiptera: Reduviidae) to deltamethrin pyrethroid

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ABSTRACT

Introduction: Despite years of efforts towards the elimination of *Triatoma infestans* in Brazil, residual foci still persist in some areas of the States of Bahia and Rio Grande do Sul. The persistence of these *T. infestans* populations in the country has two different origins of equal concern: operational failures or insecticide resistance. Thus, the objective of this study was to characterize the susceptibility profile of the residual Brazilian populations of *T. infestans* to deltamethrin. **Methods:** The susceptibility reference lineage was derived from Cipein/Argentina. The populations studied were manually collected using a dislodging agent in peridomiciles in the States of Bahia (Novo Horizonte) and of Rio Grande do Sul (Santa Rosa and Doutor Maurício Cardoso). Serial dilutions of deltamethrin were prepared and applied at the dorsal abdomen of first instar nymphs. The control group received only pure acetone. Mortality was evaluated after 72h. Qualitative tests assessed the mortality of a diagnostic dose of $1xLD_{99}$ (2.76ng a.i./nymph) determined for the susceptibility reference lineage. **Results:** The susceptibility profile characterization of the *T. infestans* populations revealed an RR_{50} ranging from 1.73 to 3.26. The mortality percentage in response to a diagnostic dose was 100%. The results obtained in the quantitative and qualitative assays corresponded for all populations. **Conclusions:** The results of this study indicate that the persistence of residual foci of *T. infestans* in Bahia and Rio Grande do Sul is not related to insecticide resistance but may be associated with operational failures. In Rio Grande do Sul, we must consider the possibility of continuous reinfestation by Argentinian individuals, which justifies active and efficient epidemiological surveillance.

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

INTRODUCTION

Triatoma infestans is not a native species of Brazil and therefore must be eradicated. *T. infestans* was most likely introduced to Brazil from the south, reaching São Paulo and other southern states in the late 18th century, during the expansion of coffee cultivation in Brazil. The patterns of human migration and rural settlement and associated ecological changes appear to have favored the triatomine, allowing it to colonize poor rural dwellings over a wide area[1]. The first prophylactic attempts at triatomine control in Brazil date to the 1920s, but only with the advent of synthetic insecticides, particularly pyrethroids, was progress in vector control achieved. In 1977, control actions were systematized and structured in national programs [Brazilian Chagas Disease Programme (BCDP)] in which priority was given to the control of the vector across all endemic areas. Most of the efforts to combat *T. infestans* have focused on regions with a predominance of species with greater ecological valence, such as *Panstrongylus megistus, Triatoma brasiliensis, Triatoma pseudomaculata,* and *Triatoma sordida*[2].

The initial dispersion of *Triatoma infestans* in Brazil (1975/1983) corresponded to 711 counties in 12 states. In 1983, when preliminary entomological evaluations were concluded, 162,136 specimens of *T. infestans* were captured, representing 13.5% of all triatomines captured, regardless of species. In 1997, the dispersion corresponded to 105 counties in 7 states, including new counties that were created since 1983. A total of 1,080 specimens of

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Triatoma infestans specimens were captured between 1983 and 1997, corresponding to only 0.5% of all specimens collected. Thus, there was a 99.3% reduction in the number of *T. infestans* specimens captured between 1983 and 1997[3].

In the State of Minas Gerais at the beginning of the 1980s, Ministry of Health data indicated that *T. infestans* represented 64.6% of triatomines captured by BCDP, followed by *T. sordida* and *P. megistus*. In 1989, after nine years of spraying, there was a 68% reduction in the number of *T. infestans* captured and an increase in the number of *T. sordida*, followed by *P. megistus*[4]. In that same period, São Paulo achieved a virtual elimination of *T. infestans* and vector transmission associated with this species[1].

In 1991, Argentina, Bolivia, Brazil, Paraguay and Uruguay, supported by the Pan American Health Organization (PAHO), created an intergovernmental commission known as the Southern Cone Initiative to, among other objectives, eradicate house infestation by *T. infestans*[5]. Between 1980 and 2000, there was a progressive elimination of *T. infestans* in several Brazilian states, in addition to a significant reduction in triatomine intradomicile infestation rates across the country. Thus, in 2006, Brazil received the *International Elimination of Transmission of Chagas' Disease Certificate* from PAHO[6]. However, for unknown reasons, residual foci of *T. infestans* remain in Bahia and Rio Grande do Sul.

The persistence of triatomine populations in spite of control activities may be related to two distinct scenarios that are equally worrying. The first scenario, in which operational failures complicate vector control, indicates an urgent need for improved training of managers and public health officials. The second scenario, in which ineffective control is due to intrinsic characteristics of triatomines that confer insecticide resistance[7] [8] [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19] [20] [21], requires a better understanding of the biological, biochemical, molecular and behavioral aspects of these characteristics. In Rio Grande do Sul, the possibility of reinfestation by specimens from Argentinian areas is also relevant.

Thus, to better understand the factors that complicate the control of *T. infestans* populations in Brazil, this study characterized the toxicological profile of these populations to deltamethrin pyrethroid in six different municipalities using laboratory bioassays.

METHODS

The populations studied were manually collected using a dislodging agent from peridomiciles in the States of Bahia (Novo Horizonte: 12° 48' 28" S 42° 10' 04" O) and Rio Grande do Sul (Santa Rosa: 27° 52' 15" S 54° 28' 51" O and Doutor Maurício Cardoso: 27° 30' 21" S 54° 21' 39" O), in which the Chagas Disease Control Program[22] conducted continuous and systematic applications of insecticides with residual action in the previous 30 years **(Table 1).**

A susceptibility reference lineage (SRL) was used; breeding of the SRL began in 2005 at the insectary of the *Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas* (LATEC) from *Centro de Investigaciones de Plagas e Insecticidas* (CIPEIN), in accordance with the criteria adopted by PAHO[23].

Bioassays were performed according the methods of Pessoa[24] and World Health Organization (WHO)[25]. Serial dilutions (0.1 - 8.0ng/µL) of deltamethrin (98.2% purity, Bayer: São Paulo, SP - Brazil) were prepared and applied to the abdomen of first instar nymphs from the F1 generation (five days of age, fasting weight 1.2 ± 0.2mg) using a Hamilton micro-syringe (0.5µL per insect). Acetone alone was applied to the insects in the control groups. At least six doses encompassing the lethal dose 50% (LD_{50}) and producing between 10 and 90% mortality were administered. Three replicates of ten nymphs were conducted for each dose at different times. Mortality was recorded at 72h. The criterion for mortality was the inability of a nymph to walk out of a filter paper disc 7cm in diameter.

The mortality data were analyzed using Basic Probit Analysis[26] software to estimate the LD_{50} expressed in nanograms of active ingredient per treated nymph (a.i./nymph) and the slope. The 50% resistance ratios (RR_{50}) were calculated by dividing the LD_{50} of each field population by that of the corresponding SRL. The susceptibility status classification was performed according to PAHO[23].

After establishing the baseline susceptibility of the *T. infestans* reference population, 30 nymphs from each peridomestic population were subjected to a diagnostic dose of $1xLD_{99}$ (2.76ng a.i./nymph.) based on the SRL. The survival of at least two insects among three replicates was interpreted as a resistance indicator[25].

TABLE 1 - Samples of the peridomestic populations of *Triatoma sordida*, geographical origin, capture site (ecotope), and the number of insects captured in each stage of development.

Site of collection	Municipality/location	Capture site (ecotope)	Number of insects collected			
			NI - NIV	NV	Ad♀	Ad♂
Bahia	Novo Horizonte/Queimados VI	Chicken coop	18	2	-	-
	Doutor Maurício Cardoso/Lageado do Cafuru	Hangar (roof)	16	8	7	3
Rio Grande do Sul	Santa Rosa/Linha Treze de Maio (DU 100)	Pig corral with chickens	-	14	17	7
	Santa Rosa/Linha Treze de Maio (DU 75)	Cow corral with chickens	30	25	8	7
	Santa Rosa/Linha Treze de Maio (DU 73)	Cow corral whit chickens	100	176	70	82

NI: First instar nymph; NIV: fourth instar nymph; NV: fifty instar nymph; N: nymphs; Ad: adults; ♀: females; ♂: males. DU: domiciliary unit.

RESULTS

The susceptibility reference lineage exhibited an LD_{50} of 0.415ng a.i./nymph treated. The susceptibility profile characterization of the *T. infestans* populations revealed RR_{50} values ranging from 1.73 to 3.26. All populations presented slopes equal to or higher than the slope of the SRL, revealing a lower heterogeneity. The mortality percentage in response to a diagnostic dose in all populations was 100% **(Table 2).**

DISCUSSION

In Southern Bolivia and Northern Argentina, the reinfestation of households treated with insecticides is a serious concern and occurs particularly rapidly in the region of Gran Chaco[26] [27] [28], due to the high levels of insecticide resistance in domestic[29] [30] and wild populations[31] [32] [33], among other factors. The different pyrethroid susceptibility profiles observed in this area are associated with changes in one or more toxicokinetic/toxicodynamic processes during the insect-insecticide interaction, highlighting the changes in the chemical active penetration site, the increase in its enzymatic detoxification rate and mutations in the sodium channel[10] [13] [17] [20] [21] [32].

Regarding Brazilian populations of *T. infestans*, Vassena et al.[7] first characterized the susceptibility of a population from Rio Grande do Sul to deltamethrin (RR_{50} 7.0), betacypermethrin (RR_{50} 0.92), betacyfluthrin (RR_{50} 3.6), lambdacyhalothrin (RR_{50} 1.75) and cypermethrin (RR_{50} 3.35). Laboratory bioassays identified resistance only to deltamethrin, which involves mixed-function oxidases. The authors associated this resistance with the intensive use of pyrethroids (deltamethrin and cypermethrin) for triatomine control after 1982, when Lindane was discouraged[33] [34].

Sonoda et al.[15] characterized the toxicological profile of the *T. infestans* populations originating from four municipalities subjected to successive spraying with pyrethroids in Rio Grande do Sul (Doutor Maurício Cardoso RR_{50} 0.72, Guarani das Missões RR_{50} 0.57, Mato Queimado RR_{50} 1.47, and Três de Maio RR_{50} 1.86). The studied populations had very low resistance ratios, indicating a lack of deltamethrin resistance. The populations studied by Vassena et al.[7] and Sonoda et al.[15] originated from different locations, which may explain the discrepancies in the results of these studies.

In this work, the results of quantitative bioassays of the Novo Horizonte population in Bahia indicated an RR_{50} of 1.74. For the Rio Grande do Sul populations, the RR_{50} ranged from 1.73 to 3.26. In both cases, in accordance with the PAHO[23], all populations presented RR<5 and were thus classified as susceptible to the insecticide evaluated. The qualitative tests demonstrated 100% mortality in all populations of *T. infestans* in response to the diagnostic dose, further confirming the susceptibility to deltamethrin[25]. Our results and those of Sonoda et al.[15] demonstrate that the populations of *T. infestans* in the municipalities of Doutor Maurício Cardoso and Santa Rosa are susceptible.

The slope for all populations studied was equal to or greater than that for the SRL, suggesting a small degree of intrapopulational heterogeneity and a reduced possibility of change in the toxicological profile before the selection pressure with continued use of insecticides. This result may be related to the structuring of the triatominic populations as small groups with limited dispersal and reduced genetic flow, as observed in some Triatominae species. In addition, molecular studies have demonstrated that genetic diversity is significantly lower in chemically treated areas than in untreated areas, indicating active chemical pressure on populations[19] [35] [36] [37].

Notably, the susceptibility profile observed for these populations of *T. infestans* in response to deltamethrin cannot be extended to other insecticides. The same triatomine population may present different susceptibility profiles to different chemical classes of insecticides[10] [12] [13] [20] [33] [37] [38] [39] and to chemicals belonging to the same class[7] [10] [12].

In addition, the different toxicological profiles of the two populations of *T. infestans* from Santa Rosa (Linha Treze de Maio), in which insects were collected in different households, suggest that the selection process for insecticide resistance can occur independently, even in neighboring houses[39] [40]. This study indicates the

TABLE 2 - The toxicity of topically applied deltamethrin on *Triatoma infestans* first instars of a susceptible reference lineage and peridomestic populations collected from States of Bahia and Rio Grande do Sul, Brazil.

Population: municipality/location-State	LD_{50} (95% CI)	RR_{50}	Slope	Diagnostic dose (% mortality)
CIPEIN (SRL)	0,415 (0,345 – 0,497)	-	2,825 +/- 0,363	-
Novo Horizonte/Queimados VI - BA	0,726 (0,602 – 0,859)	1.74	2,766 +/- 0,410	100.0
Doutor Maurício Cardoso/Lageado do Cafuru - RS	0,741 (0,629 – 0,868)	1.76	2,762 +/- 0,432	100.0
Santa Rosa/Linha Treze de Maio (DU 100) - RS	0,733 (0,446 – 0,985)	1.73	2,162 +/- 0,591	100.0
Santa Rosa/Linha Treze de Maio (DU 75) - RS	0,863 (0,766 – 0,962)	2.05	4,033 +/- 0,449	100.0
Santa Rosa/Linha Treze de Maio (DU 73) - RS	1,372 (1,156 – 1,696)	3.26	3,378 +/- 0,670	100.0

DU: domiciliary unit; LD_{50}: 50% lethal dose; 95% CI: 95% confidence interval; RR_{50}: 50% resistance ratio; CIPEIN: *Centro de Investigaciones de Plagas e Insecticidas*; SRL: susceptible reference lineage; BA: State of Bahia; RS: State of Rio Grande do Sul.

inadequacy of transposing results from one population to another, even for populations that are geographically close. Amelloti et al.[41] demonstrated that a single female, when kept isolated throughout her life, has the potential to generate more resistant offspring when she is young and more susceptible offspring when she is older. This result indicates the complexity of genetic variability at the individual level and its potential impact in the population context. This variability emphasizes the need for caution when defining sample size in studies of resistance in triatomines to ensure that the sample is truly representative of the study population.

Thus, the results of this study indicate that the persistence of residual foci of *T. infestans* in Bahia and Rio Grande do Sul is not related to insecticide resistance but more likely to operational failures in vector control strategies, such as 1) a lack of insecticide efficiency due to poor-quality active ingredients and/or an inadequate formulation; 2) operational failures related to the lack of training of health agents[42]; 3) environmental conditions, mainly within peridomiciles, that accelerate degradation of the insecticide[43] [44]; 4) an effect of peridomicile characteristics on the behavior of *T. infestans*; and 5) spraying cycle discontinuity due to administrative and budgetary concerns[45] [46] [47]. These factors, individually or in combination, expose the triatomine to sublethal doses that select for the least susceptible insects.

Finally, the artificial environment colonization capacity and the transmission potential of *Trypanosoma cruzi* by *T. infestans* emphasize the importance of systematic, continuous chemical control activities and guaranteed operational quality as well as health awareness activities. These activities should focus of the reorganization of household units to avoid refractory colonization by triatomines. The geographical proximity of Southern Brazil to Argentina and Bolivia allows *T. infestans* to flow between these regions, justifying active and efficient entomological and epidemiological surveillance to control the identified targets as early as possible.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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