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Deltamethrin pyrethroid susceptibility characterization of *Triatoma sordida* Stål, 1859 (Hemiptera: Reduviidae) populations in the Northern Region of Minas Gerais, Brazil

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ABSTRACT

Introduction: *Triatoma sordida* is the most captured Triatomine species in the Brazilian artificial environment. In 2008, the discovery of three Triatomine populations with altered susceptibilities to deltamethrin highlighted the importance of investigating the genetic potential for resistance in triatomines. The purpose of this study was to characterize the susceptibility to deltamethrin of peridomestic *T. sordida* populations in Minas Gerais, Brazil. **Methods:** A susceptibility reference lineage derived from Uberaba, Minas Gerais, Brazil was used. 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} of the susceptibility reference lineage. **Results:** Susceptibility profile characterization of *T. sordida* populations revealed resistance ratios (RR_{50}s) ranging from 0.42 to 3.94. The percentage mortality in response to the diagnostic dose varied from 70% to 100%. A comparison of the results obtained in the quantitative and qualitative assays demonstrated a lack of correspondence for some populations. **Conclusions:** We demonstrated that only *T. sordida* populations that present a RR_{50}>1.0 have altered susceptibility, and the execution of simultaneous field and laboratory tests is required to understand the actual effect of vector control. A possible cause of the observed resistance ratios might be the continuous use of pyrethroids in Brazil since the 1980s.

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

INTRODUCTION

The Triatominae subfamily comprises vectors for *Trypanosoma cruzi*[1], whose importance in human parasite transmission, within the classic epidemiological model, is associated to the colonization capacity of the domicile[2]. *Triatoma sordida* is the most captured Triatomine species in the Brazilian artificial environment. It is a local native species and has been very difficult to eradicate. *T. sordida* is frequently found in peridomiciliary areas in association with hens and low rates of infection by *T. cruzi*. Its marked ornithophily makes it a vector with less epidemiological importance than *Panstrongylus megistus* or *Triatoma brasiliensi*[3].

According to a survey performed in 1940 in Minas Gerais, *Panstrongylus megistus* represented 79.8% of the captured triatomines, *Triatoma infestans* represented 9.3%, and *T. sordida* represented 9.7%[4]. Through control activities in 1989, *Triatoma infestans* was nearly eliminated in Minas Gerais. At that time, the number of captured *T. sordida* specimens demonstrated a marked increase[5]. In 2006, Brazil was awarded the *International Certification of Transmission Elimination of the Chagas Disease by Triatoma infestans*[6]. Considering the diversity of the Brazilian triatomine fauna with native species of attested epidemiological importance, epidemiological surveillance assumed a critical role.

It is possible to conclude that triatomine chemical control has been feasible and efficient when performed with technical, methodological and operational accuracy. Contradicting the expectations of some researchers, in 2008, three *T. sordida* populations with altered susceptibilities to deltamethrin were found in Minas Gerais[7]. This fact highlighted the necessity of developing studies concerning the genetic potential of triatomines for resistance to insecticides with the purposes of determining the occurrence frequency of this phenomenon, identifying the affected areas and identifying the relevant mechanisms.

The purpose of this work was to characterize the deltamethrin susceptibility of peridomestic *T. sordida* populations in the northern region of Minas Gerais, Brazil.

METHODS

The study populations were manually collected, without using a dislodging agent, from peridomiciles in endemic areas of the northern region of Minas Gerais (Cônego Marinho, Montalvânia, Monte Azul e Porteirinha) in which the Chagas

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Disease Control Program performed continuous and systematic applications of insecticides with residual action in the last 30 years.

A susceptibility reference lineage (SRL) was used; breeding of the SRL started in 1992 at the insectary of the *Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas* (LATEC) from Uberaba/MG in accordance with the criteria adopted by the Panamerican Health Organization (PAHO)[8].

Bioassays were performed according to the methods of Pessoa[7]. Serial dilutions (0.01 - 4.0ng/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 (0.2µL per insect). The insecticide was diluted in acetone. At least six doses surrounding the lethal dose of 50% (LD_{50}) and producing between 10 and 90% mortality were administered. Acetone alone was applied to the insects in the control group. Three replicates of ten nymphs were conducted for each dose. The 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[9].

The mortality data were analyzed using Basic Probit Analysis[10]software, estimating the LD_{50} the active ingredient per treated nymph (a.i./nymph) as well as the slope in nanograms. Fifty percent resistance ratios (RR_{50}) were calculated by dividing the LD_{50} of each field population by the corresponding SRL. The susceptibility status classification was performed according to Zerba and Picollo[11] and PAHO[8].

After setting the base susceptibility line of the *T. sordida* reference population, 30 nymphs from each field population were subjected to a diagnostic dose of $1xLD_{99}$ based on the SRL. The survival of at least two insects in three replicates, was interpreted as a resistance indicator[11].

RESULTS

The susceptibility reference lineage presented an LD_{50} and LD_{95} of 0.064 and 0.255ng a.i./nymph treated, respectively. The susceptibility profile characterization of *T. sordida* populations revealed RR_{50} values ranging from 0.42 to 3.4. Only four populations from Cônego Marinho/Cruz dos Araújos e Sapé, Montalvânia/Quilômetro and Porteirinha/Furado da Onça presented slopes lower than the slope of the SRL, revealing higher frequencies of individuals with resistant alleles. The mortality percentage in response to the diagnostic dose ranged from 70% to 100% **(Table 1)**.

DISCUSSION

A growing number of reports of triatomine populations with increased resistance ratios have had a major effect, stimulating scientists and sanitarians to search for new options for vector

TABLE 1 - Toxicity of topically applied deltamethrin to *Triatoma sordida* first instars of a susceptible reference lineage (SRL) and peridomestic populations collected in Minas Gerais, Brazil.

Population: municipality/location	LD_{50} (95% CI)	RR_{50}	Slope	Diagnostic dose (% mortality)
Uberaba/Uberaba (SRL)	0.065 (0.052 - 0.077)	1.00	2.766 +/- 0.410	-
Cônego Marinho/Cruz dos Araújos	0.046 (0.036 - 0.058)	0.72	1.990 +/- 0.252	100.0
Cônego Marinho/Sapé	0.077 (0.060 - 0.097)	1.72	1.905 +/- 0.269	100.0
Cônego Marinho/Cabeceira do Cônego Marinho	0.173 (0.143 - 0.208)	2.66	2.271 +/- 0.278	83.3
Montalvânia/Batedeira	0.027 (0.022 - 0.031)	0.42	2.609 +/- 0.307	100.0
Montalvânia/Quilômetro	0.079 (0.062 - 0.101)	1.22	1.721 +/- 0.217	100.0
Montalvânia/Gergelim	0.116 (0.095 - 0.139)	1.79	2.422 +/- 0.316	96.6
Montalvânia/Vereda	0.131 (0.108 - 0.158)	2.02	2.169 +/- 0.235	90.0
Monte Azul/Perneta	0.034 (0.022 - 0.041)	0.53	2.368 +/- 0.299	100.0
Monte Azul/Porteiras	0.085 (0.070 - 0.100)	1.30	2.615 +/- 0.326	100.0
Monte Azul/Landinho	0.103 (0.083 - 0.131)	1.63	2.252 +/- 0.350	100.0
Monte Azul/Canabrava	0.171 (0.142 - 0.204)	2.63	2.711 +/- 0.398	93.3
Monte Azul/Brejinho	0.256 (0.210 - 0.310)	3.94	2.357 +/- 0.320	70.0
Porteirinha/Furado da Onça	0.068 (0.053 - 0.085)	1.05	1.872 +/- 0,213	100.0
Porteirinha/Curral Velho	0.133 (0.107 - 0.163)	2.05	2.126 +/- 0.302	93.3
Porteirinha/Cova da Mandioca	0.196 (0.158 - 0.245)	3.01	2.037 +/- 0.242	73.3

LD_{50}: 50% lethal dose; **95% CI:** 95% confidence interval; **RR_{50}:** 50% resistance ratio.

control. Accordingly, studies concerning susceptibility to insecticides have taken priority in the field of Chagas disease. There are two major problems in the interpretation of the obtained results: the selection of the susceptibility reference population and the actual meaning of the resistance ratios for field vector control.

Considering that resistance ratio (RR) is calculated from SRL lethal doses, its choice directly interferes in the results obtained and consequently, in future developments to be adopted by the managers involved with Triatominae control in the field. According to Obara et al.[12], there are no records of Brazilian *T. sordida* populations resistant to deltamethrin. A susceptibility study of 11 populations from the center-west region of Brazil revealed RR_{50} values ranging from 1.05 to 1.48. The LD_{50} estimated for the reference population from the municipality of Cordeiros (State of Bahia) was 0.585ng a.i./nymph. Redefining the RR_{50} for the populations of Obara et al.[12] using the LD_{50} from the SRL adopted in this work, the RR_{50} values range from 9.9 to 13.3, which results in all of the populations being designated as resistant and suggests that the insecticide used in the field should be changed. Additionally, the reference population could be considered resistant.

These results emphasize in a practical manner that the utilization of different SRLs hinders comparisons and the interpretation of RRs, highlighting the importance of adopting a single population of each species for all groups investigating the susceptibility of triatomines to insecticides. In this context, another concern involves the maintenance of this SRL in the laboratory *ad infinitum*, considering the difficulties of keeping viable colonies for extended periods inside an insectary without a supply of external materials.

Another issue focuses on the concept of the susceptibility reference lineage – a population with more than five generations in the laboratory, without contact with insecticide or supply of external material and collected in locations where there was no treatment with insecticide[8]. To comply with these criteria, this study selected a lineage from Uberaba, State of Minas Gerais, Brazil. However, three field populations were found to be more susceptible than the SRL (RR_{50} <1.0).

The SRL used in this study was established in 1992 in an insectary without contact with insecticide and without a supply of external materials. Since the 1970s, the region of Uberaba has been subjected to intense environmental degradation because of the implementation of pasture areas intended for livestock. Additionally, in the past, the same area was subjected to strong pressure from insecticides for triatomine and anopheline control (organochlorine and organophosphate insecticides). In this context, the discovery of populations more susceptible than the SRL suggests that this population already harbored resistant alleles when it was collected in the field and that these alleles have been retained.

The existence of 11 populations with slopes equal to or higher than the slope of the SRL suggests a small degree of heterogeneity among the populations for the assessed characteristic. Populations from different localities within the same municipality, although geographically close to each other, presented distinct RR_{50}s: Cônego Marinho (0.42 to 2.66), Montalvânia (0.42 to 2.02), Monte Azul (0.53 to 3.91) and Porteirinha (1.05 to 3.01). A possible explanation for the small intrapopulational heterogeneity observed and the different interpopulational susceptibility profiles within a single municipality is the distribution into small groups with limited dispersion and reduced genetic flow, as observed in some Triatominae species. Considering this possibility, a population in a certain location might undergo a selection process from insecticides regardless of what happens to triatomines in neighboring locations.

The small intrapopulational heterogeneity inferred from the slope analysis for the majority of the populations corresponds to molecular studies demonstrating that in areas with chemical treatment, the genetic diversity is lower than in non-treated areas[13-15]. Studies using allozymes of *T. sordida* from three different ecotopes in Minas Gerais revealed low levels of genetic variation in peridomestic populations[16]. For populations with slopes lower than that of the SRL, the possibility of resistance evolving because of insecticide exposure could not be discounted, which justifies the need for follow-up regarding susceptibility changes over time.

There are two interpretations for the meaning of RR_{50} values. According to Zerba and Picollo[10], populations with RR >2 are considered resistant, whereas according to the PAHO[8], only populations with RR >5 receive this classification. This criterion should be better defined. The RR_{50} values observed in this work range from 0.42 to 3.4. We affirm that only the *T. sordida* populations that presented an RR_{50} >1.0 have altered susceptibility. Simultaneous field and laboratory tests are required to understand the actual effect of vector control.

In bioassays, the diagnostic dose mortality ranged from 70 to 100%. Using the criterion proposed by the World Health Organization (WHO)[9], populations with mortality lower than 96.7% must be considered to be resistant. A comparison of the response-dose results obtained in this work with the diagnostic-dose results revealed a lack of correspondence for some populations, i.e., possible resistance detected via DD was not confirmed by the RR_{50} value. A possible explanation for this discrepancy could be the reduced sampling number used in the qualitative tests, which might not represent the characteristics of the population. Amelotti et al.[17] studied a population of *T. infestans* from Argentina and assessed the susceptibility to deltamethrin of nymphs generated by females maintained individually throughout their lifecycle. While they were young, the females generated more resistant breeds; as they aged, the females generated more susceptible offspring. These results demonstrate how complex the issue of genetic variability is at the individual level, allowing us to reflect on the effect of such variation in the context of the population.

A possible cause of the resistance ratios observed in this work could be attributed to the continuous use of pyrethroids in Brazil since the 1980s, as described by Vassena et al.[18] in studies performed with *T. infestans* populations from Rio Grande do Sul (RR_{50} 3.6 to 7.0). Pressure continues from insecticides used for multiple purposes (agricultural, domestic), even overlapping with control programs for other vectors. The northern region of Minas Gerais, where the populations studied were collected, was subjected to intense environmental changes in the 1970s to implement cotton cultivation, supporting the possibility of significant insecticide pressure from agriculture[19].

Additionally, the persistence of *T. sordida,* predominantly in the peridomicile environment, despite successive insecticide treatments over time might be related to behavioral aspects of this triatomine. The peridomicile presents a wide variety of ecotopes corresponding to an infinite number of hidden loci associated with different sources of food for *T. sordida*, such as chickens, dogs, pigs, and cats. Spraying the peridomicile is exhausting work in which unstacking all of the material accrued is operationally impossible for the field agent in charge. After insecticide application, eggs and nymphs could remain nearly free of contact with active chemicals and/or could be in contact with sublethal doses, thus selecting for the specimens less susceptible to the chemicals and allowing for the survival of those insects in such ecotopes[19,20].

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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