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Major Article

Variability of susceptibility to deltamethrin in peridomestic *Triatoma sordida* from Triângulo Mineiro, State of Minas Gerais, Brazil

***Grasielle Caldas DÁvila Pessoa*[1], *Tamara Rodrigues Martins dos Santos*[1], *Gabriela Chadid Salazar*[1], *Letícia Sena Dias*[1], *Bernardino Vaz de Mello*[2], *Marcela Lencine Ferraz*[2] *and Liléia Diotaiuti*[1]**

[1]. Laboratório de Referência em Triatomíneos e Epidemiologia da Doença de Chagas, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil. [2]. Secretaria de Saúde do Estado de Minas Gerais, Governo do Estado de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

ABSTRACT

Introduction: Despite chemical and physical vector control strategies, persistent infestations of *Triatoma sordida* have been reported in a large part of Minas Gerais, Brazil, and the cause for this is little investigated. We aimed to characterize the deltamethrin toxicological profile in peridomestic *T. sordida* populations from Triângulo Mineiro area of Minas Gerais. **Methods:** Susceptibility to deltamethrin was assessed in seventeen peridomestic *T. sordida* populations. Serial dilutions of deltamethrin in acetone (0.2μL) were topically applied on the first instar nymphs (F1; five days old; fasting weight, 1.2 ± 0.2mg). Dose response results were analyzed using Probit software, and the lethal doses, slope and resistance ratios were determined. Qualitative tests were also performed. **Results:** The deltamethrin susceptibility profile of *T. sordida* populations revealed resistance ratios ranging from 0.84 to 2.8. The percentage mortality in response to a diagnostic dose was 100.0% in all populations. **Conclusions:** From our results, the lack of resistance to insecticides but persistent *T. sordida* infestations in the Triângulo Mineiro area may be because of: 1) environmental degradation facilitating dispersion of *T. sordida*, allowing colonization in artificial ecotopes; 2) operational failures; and 3) complexity of the peridomicile in the study area.These variables are being investigated.

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

INTRODUCTION

Triângulo Mineiro, State of Minas Gerais, Brazil, is an old colonization area, cleared by pioneers at the end of the past century during their search for gold and precious stones. The region took on great economic importance with the introduction of the Zebu cattle, which also resulted in a drastic reduction of the savanna areas, by about 70%[1]. Agriculture became important only in the beginning of the 1970s, with the development of techniques that promoted soybean culture in the savanna. By the end of the 1980s, the region was characterized by a small strip of land, with a small population that owned large properties. Currently, this region is the center for large agroindustrial projects, such as livestock, represented by large cattle-producers, the main economic activity of the region[2].

Correponding author: Dra. Liléia Diotaiuti. Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas/CPqRR/FIOCRUZ. Av. Augusto de Lima 1715, Barro Preto, 30190-002 Belo Horizonte, Minas Gerais, Brasil.
Phone: 55 31 3349-7762; **Fax:** 55 31 3295-3115
e-mail: diotaiuti@cpqrr.fiocruz.br
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The Triângulo Mineiro region also pioneered in prophylactic campaigns against Chagas disease (CD). The National Program of Chagas Disease Control (NPCDC) was observed with regularity from 1975 to 1988. In 1982, the chlorinated insecticide hexachlorobenzene (HCB), used in field control strategies, was replaced with pyrethroids (deltamethrin)[3]. By using this insecticide, *Triatoma infestans* was eradicated from the area. Presently, the prevalent species are *Triatoma sordida*, *Panstrongylus megistus*, and *Rhodnius neglectus*. Data for 2010-2015, from the Health Secretariat of the State of Minas Gerais, revealed that *T. sordida* was the most captured triatomine (22,337 specimens), representing 92.3% of sampled triatomines, followed by *P. megistus* (4.5%), *R. neglectus* (1.2%), and other (2.0%) species (ML Ferraz: Personal Communication, 2015).

Triatoma sordida is an endemic triatomine of the cerrado, living mainly under the bark of trees that are preserved during the conversion of forests to fields and pastures. Because of its habitat, which sustains few vertebrates, it frequently encounters long periods without feeding. A recent ecological niche modeling study revealed the possibility that the *T. sordida* distribution area could be larger than that initially known, i.e., this triatomine could also inhabit other biomes such as Caatinga and Pantanal[4]. Such biological characteristics, including their ability to resist fasting, ease of adapting to different hosts, and relative mobility (mainly the adults) facilitate dispersion and

colonization in artificial ecotopes[5]. This, *T. sordida* can be considered a semidomicile species, given the frequency with which it has been found in peridomicile and intradomicile environments[6]. Currently, four Triatominae species deserve special attention in *Trypanosoma cruzi* transmission to man: *Triatoma brasiliensis*, *P. megistus*, *Triatoma pseudomaculata*, and *T. sordida*[7].

Although CD is endemic to a large part of Minas Gerais, significant reductions in the occurrence of new cases has been noted in recent decades, possibly due to 1) the chemical activities of the National Program of Chagas Disease Control, 2) epidemiological vigilance employed in municipalities, and 3) socio-economic factors of rural areas, such as rural exodus, income and habitation improvement, electricity, and access to education and health services, among others[8]. However, despite these efforts, reports show persistent *T. sordida* infestation in Minas Gerais, and the causes are little investigated[9]. This could slowly and progressively cause the resurgence of this endemic disease mainly in poverty-stricken areas and less politically represented regions, such as the Triângulo Mineiro.

To verify the involvement of insecticide resistance in the difficulty of vector control strategies in the field, we characterized the toxicological profile for deltamethrin in peridomestic *T. sordida* populations from Triângulo Mineiro that has persistent infestation.

METHODS

Triatoma sordida populations

Twenty populations were manually collected in the peridomiciles of eight endemic areas of Triângulo Mineiro (Carneirinho, Frutal, Gurinhatã, Iturama, Limeiro do Oeste, São Francisco de Sales, Tupaciquara, and União de Minas), where the NPCDC was employed for the past 30 years, with continuous and systematic applications of insecticides with residual action **(Figure 1)**. No dislodging agent was used in the collection of the triatomines.

Insecticide

Deltamethrin (pyrethroid) technical grade ([(*S*)-α-cyano-(3-phenoxyphenyl)methyl] (1*R*-3*R*)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate [99.1%; Bayer, Brazil]), a standard insecticide commonly used in resistance studies of triatomine, was used. The choice of insecticide was based on its chemical properties and mode of action.

Deltamethrin and alphacypermethrin, the insecticides used by the Brazilian government for Triatominae control, are pyrethroid insecticides. Several authors have demonstrated that the same triatominic population exhibits different

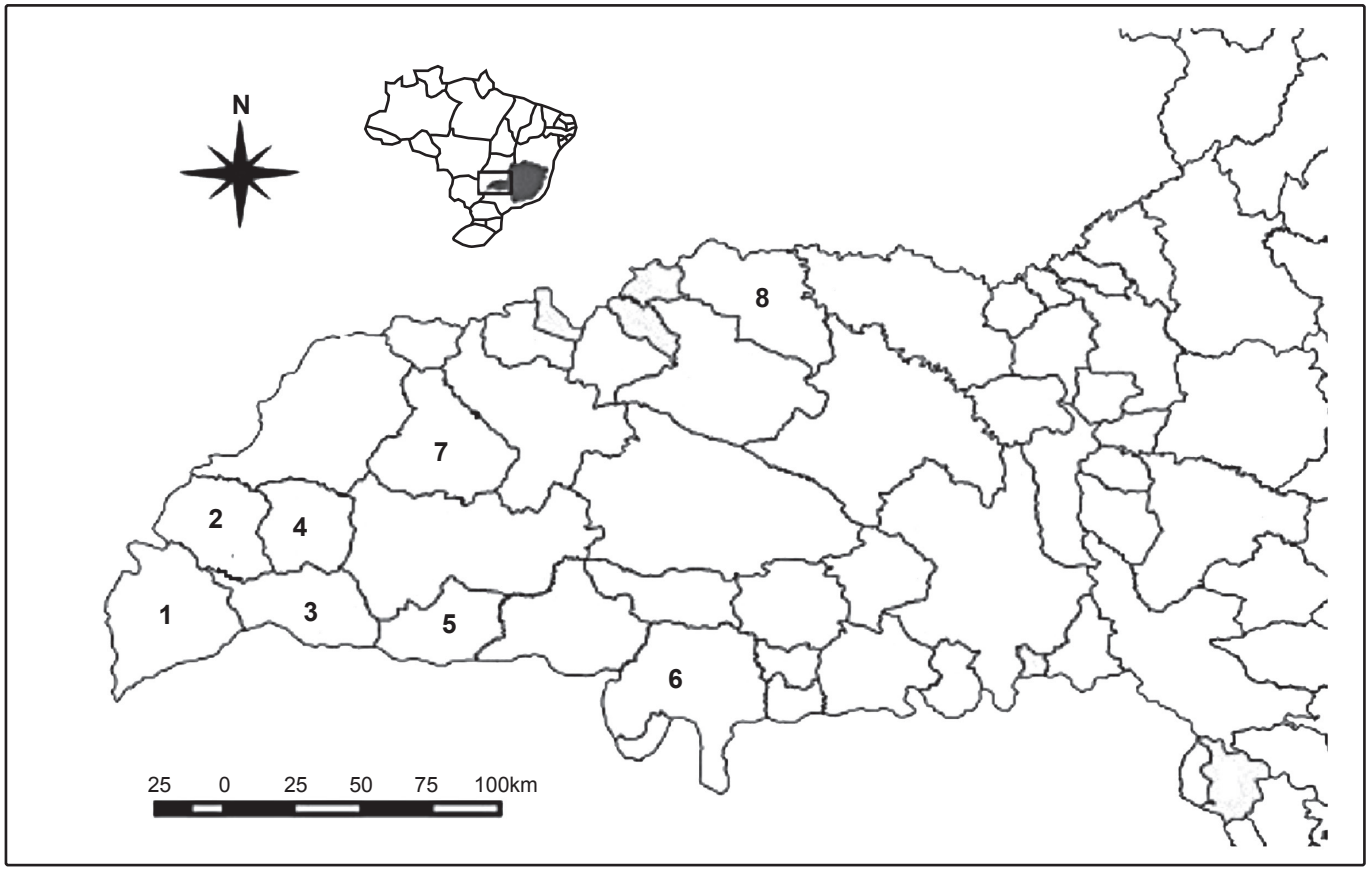

FIGURE 1 - Map of the Brazilian State of Minas Gerais showing municipalities from which *Triatoma sordida* were obtained: 1: Carneirinho; 2: Limeira do Oeste; 3: Iturama; 4: União de Minas; 5: São Francisco de Sales; 6. Frutal; 7: Gurinhatã and 8: Tupaciguara.

toxicological profiles when exposed to the same chemical class of insecticide[10]. We emphasize that laboratory studies are only indicative of resistance in the field, and they cannot suggest another pyrethroid as a replacement. In case of indication of resistance, field trials must be carried out by using the insecticide (formulated product) used by the Ministry of Health.

Bioassays

The susceptibility reference lineage (SRL) of *T. sordida* was obtained from Uberaba (19° 44′ 52″ S 47° 55′ 55″ O), where it has been maintained in the laboratory for more 20 years, without contact with insecticide or inclusion of external material[11].

Serial dilutions of deltamethrin in acetone were prepared. For each concentration, three repetitions were carried out with ten first instar nymphs of the F1 generation (5 days-old, fasting, weight 1.2 ± 0.2mg). The treatment consisted of the application of 0.2µL of insecticide dilution on the dorsal abdomen by using a Hamilton microsyringe mounted on a repeating dispenser, according to the procedures of the World Health Organization[12] and Pessoa[13]. For each populations, a minimum of eight doses of insecticide active ingredient (a.i.) ranging from 0.01 to 4.0ng to cause mortalities between >0% to <100% of the individuals, were applied per insect. Acetone was applied to the control group. Mortality was assessed 72h after application, and it was determined by the inability or lack of coordination of the nymphs to move from the center to the edge of a filter paper (7cm diameter). Other signs such as paralysis and lack of response to external stimuli were also considered. During and after the experiment, the insects were maintained under controlled conditions of temperature and humidity (25°C ± 1°C; 60% ± 10% RH).

Diagnostic dose

After setting the base susceptibility dose for the *T. sordida* reference population, 30 nymphs from each peridomestic populations were subjected to a diagnostic dose of 1× LD_{99} (0.45ng a.i./nymph.) based on the SRL. The survival of at least two insects in three replicates was interpreted as a resistance indicator[12][13].

Data analysis

Data from the dose-response tests of each population were analyzed using the Probit program[14] Thus, we estimated the slope and lethal doses required to kill 50% of the treated individual (LD_{50}), as well as the resistance ratio 50% (RR_{50}), which was calculated by dividing the LD_{50} of each field population by the LD_{50} of the SRL.

Ethical considerations

This study was approved by the Animal Ethics Committee of Fundação Oswaldo Cruz (number 29/14-1).

RESULTS

The SRL showed an LD_{50} of 0.064ng a.i./nymph. The susceptibility profile characterization of *T. sordida* populations revealed RR_{50} values ranging from 0.84 to 2.8. All populations, except that from União de Minas (Três Barras), showed slopes lower or equal to that of the SRL. We noted 100% mortality in all populations in response to the diagnostic dose **(Table 1)**.

DISCUSSION

Despite reports of persistent *T. sordida* infestation, only two studies have determined the susceptibility of Brazilian populations of this triatomine to insecticides[13][15]. Pessoa[13] showed the deltamethrin toxicological profile in eleven peridomestic *T. sordida* populations collected from areas with reports of persistent infestation, in the North of Minas Gerais. The resistance ratios (RR_{50} 2.6-6.8) in that study were the highest for *T. sordida*. In accordance with the Pan-American Health Organization (PAHO)[11], five (45.0%) populations with RR_{50} > 5 were considered resistant to deltamethrin. Following this, Obara et al.[15] determined the toxicological profile for eleven *T. sordida* populations from Goiás, Mato Grosso, and Mato Grosso do Sul, showing discrete alterations of susceptibility to deltamethrin (RR_{50} 1.19-2.36). All populations were classified as susceptible to the tested insecticide.

The susceptibility profile of *T. sordida* in our study is similar to that by Obara et al.[15] but unlike that by Pessoa et al.[13]. However, the different toxicological profiles of deltamethrin in *T. sordida* populations from distinct locations in the same municipality (e.g., Frutal/Guilherme F. Correa and Frutal/Aureliano N. Cruz), showed the complexity of the resistance phenotype, at both macro- and microgeographical levels, as observed by Pessoa et al.[16] in their studies of Brazilian *T. infestans* populations from Rio Grande do Sul. They observed that deltamethrin does not present homogenous effects over different populations from the same locality, suggesting independent selection pressure. Such results prove that the results of one population cannot be transposed to another, even if these populations are geographically close.

Of 18 populations, 17 (95%) presented a slope equal or less than the SRL, suggesting a small degree of intrapopulational heterogeneity and reduced possibility of change in the toxicological profile before selection pressure occurs with continued use of insecticides. This could be because of the triatomine population structure with small groups of limited dispersal and reduced genetic flow. In addition, molecular studies have shown that genetic diversity is significantly lower in chemically treated areas than in untreated areas, indicating active chemical pressure on populations[17].

According to Pan American Health Organization **(**PAHO)[11], the alteration of susceptibility observed in the *T. sordida* populations in this study is by individual variations among triatomines. Therefore, the field chemical control activities with the insecticide currently in use may be continued but resistance must be monitored.

Despite insecticide spraying difficulties and low permanence in the peridomicile, the lack of resistance and only one annual cycle of *T. sordida*, which means slow reconstruction of the original population, suggest that one annual spraying is sufficient for the control of this triatomine[18]. Thus, the persistent *T. sordida* infestation in Triângulo Mineiro may be because of the following: 1) environmental degradation that facilitate the dispersion of *T. sordida*, allowing colonization in artificial ecotopes[5][19][20]; 2) operational failures[9]; and 3) complexity of the peridomicile in the Triângulo Mineiro region[18].

TABLE 1 - Toxicological profile for deltamethrin in peridomestic *Triatoma sordida* from the Triângulo Mineiro area, State of Minas Gerais, Brazil.

Population: municipality/location	LD_{50} (95% CI)	RR_{50}	Slope ± SD	Diagnostic dose (% mortality)
Uberaba-SRL	0.064 (0.052-0.077)	-	2.766 ± 0.410	100.0
Carneirinho/Barra da Olaria	0.071 (0.052-0.095)	0.84	1,6621 ± 0.320	100.0
Limeira do Oeste/Alfredo S. Maia II	0.182 (0.146-0.230)	2.80	1.754 ± 0.216	100.0
Iturama/Bebedouro	0.054 (0.037-0.077)	0.84	1.662 ± 0.319	100.0
União de Minas/Cabeceira Três Barras	0.068 (0.059-0.078)	1.05	4,526 ± 0.725	100.0
São Francisco de Sales/João Minaré	0.056 (0.045-0.068)	0.86	2.710 ± 0.437	100.0
Frutal/Guilherme F. Correa	0.084 (0.064-0.104)	1.31	2.125 ± 0.307	100.0
Frutal/Matão	0.129 (0.103-0.164)	1.99	1.796 ± 0.238	100.0
Frutal/São Mateus	0.136 (0.107-0.172)	2.10	1.675 ± 0.205	100.0
Frutal/Buriti	0.138 (0.111-0.175)	2.13	1.875 ± 0.245	100.0
Frutal/João Correa	0.144 (0.118-0.176)	2.22	2.239 ± 0.309	100.0
Frutal/Aureliano N. Cruz	0.163 (0.136-0.194)	2.51	2.401 ± 0.283	100.0
Gurinhatã/Alceu Mendes	0.139 (0.114-0.169)	2.15	2.287 ± 0.311	100.0
Gurinhatã/Vicente Coelho	0.147 (0.121-0.176)	2.27	2.287 ± 0.275	100.0
Gurinhatã/Cachoeirão	0.150 (0.125-0.181)	2.32	2.454 ± 0.322	100.0
Gurinhatã/Cor da Mata	0.219 (0.183-0.264)	3.38	2.445 ± 0.344	100.0
Tupaciquara/Adenigê Rosa	0.086 (0.071-0.104)	1.33	2,892 ± 0.492	100.0
Tupaciquara/Pé de Serra	0.071 (0.054-0.089)	1.09	2.504 ± 0.573	100.0

LD_{50}: lethal dose 50%; **95%CI**: confidence interval 95%; **RR_{50}**: resistance ratio 50%; **SD**: standard deviation; **SRL:** susceptibility reference lineage.

Regarding environmental degradation, studies have shown that profound modifications to the natural environment of the Triângulo Mineiro region to promote agriculture and increase livestock caused displacement or disappearance of the refuges and natural food sources of *T. sordida*, which then sought these in the alternative artificial environment in order to survive. It seems the change in vegetable coverage, hair least to some extent, provide *T. sordida* dispersion. High infestation in houses closest to the wild environment suggests recolonization of triatomines (nymphs to adults) in the artificial environment from their natural ecotopes[5][19][20], contributing to the persistent infestation in the region.

Control and operational failures[11] can contribute to the persistence of *T. sordida* in the Triângulo Mineiro area. Such failures may be because of lack of insecticide efficacy owing to poor quality of the active ingredient and/or inadequate formulation. Operational failures due to insecticide dilution errors, inadequate application, and spraying machine problems. Discontinuity of spraying cycles due to administrative, budgetary, and logistical reasons must also be considered. We recommend ensuring 1) the use of good quality and effective formulations of insecticides, proven by control programs of triatomines and 2) good quality work by thorough spraying of endemic agents proven in the vector control of Chagas disease. Currently, in Brazil, triatomines control is maintained using alphacypermethrin. Similar to other pyrethroids, the lethal dose of this insecticide leaves little residual effect and does not remain on the treated surfaces, but the recommended doses produce an initial high impact on the populations. The environmental conditions that promote the persistence of triatomines are ultraviolet radiation, rain, alkaline pH of the insecticide formulation, characteristics of the substrate on which the insecticide is applied, and/or other favorable physical conditions favorable to permanence of triatomine (e.g. complexity of peridomestic structures)[21].

Finally, the persistent *T. sordida* infestation in the study area can be related to the complexity of the peridomicile. The Triângulo Mineiro area has a complex peridomicile, with high triatomine densities (about 13 triatomines per peridomicile). The large variety of ecotopes in these peridomiciles makes insecticide spraying an exhaustive job, which requires shifting of materials accumulated in these ecotypes, presenting an operationally impossible situation for the responsible field agent. Consequently, *T. sordida* (eggs, nymphs, and adults) persist even after application of the insecticide, deep in piles of firewood, on the roofs of barns, and in a variety of other nearly inaccessible hiding places, free from contact with the active ingredient and/or in contact with sub-lethal doses, favoring their multiplication in these ecotopes[18].

Considering that *T. sordida* is a native species of Brazil, and may not be eradicable, we recommended the following: 1) development and fortification of effective epidemiological surveillance, entomological surveillance, and environmental surveillance in the Triangulo Mineiro area and 2) investigation of possible operational failures that could compromise the effectiveness of actions in the field, in order to prevent intradomiciliary colonization of *T. sordida* and possible transmission of *T. cruzi* to human beings.

The *T. sordida* populations from Triângulo Mineiro region showed high susceptibility to the tested insecticide. Low resistance ratios (RR_{50} 0.84-2.8) and 100.0% mortality were noted in all populations in response to diagnostic dose were observed. The persistent *T. sordida* infestation in the Triângulo Mineiro may be caused by 1) environmental degradation associated with the facilitated dispersion of *T. sordida*, allowing colonization in artificial ecotopes by triatomines originating from wild environments; 2) operational failures; and 3) complexity of the peridomicile in the Triângulo Mineiro area.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Machado ABM, Drummond GM, Paglia AP. Livro vermelho das espécies ameaçadas de extinção da fauna de Minas Gerais. Belo Horizonte: Fundação Biodiversitas; 1998.
2. Soares JM, Costa FF, Santos CR. Manejo de irrigação em fruteiras. *In*: Anais do 27° Congresso Brasileiro de Engenharia Agrícola. Poços de Caldas: Sociedade Brasileira de Engenharia Agrícola; 1998. p. 281-309.
3. Gurgel-Gonçalves R, Galvão C, Costa J, Peterson AT. Geographic distribution of Chagas disease vectors in Brazil based on ecological niche modeling. J Trop Med 2012; 2012b:1-15.
4. Silveira AC. Controle da Transmissão Vetorial da Doença de Chagas no Brasil. Taller sobre Evaluación de Efecto Insectica sobre Triatomínos. Acta Toxicol Argent 1994; 2:29-58.
5. Forattini OW, Ferreira OA, Rocha e Silva EO, Rabello EX. Aspectos ecológicos da Tripanossomíase americana. XIV. Persistência e potencial de domiciliação de populações triatomínicas silvestres em região de intensa atividade agropecuária. Rev Saude Publica 1979; 13:123-146.
6. Coura JR. O falso dilema sobre a luta antivetorial e as perspectivas de controle da Doença de Chagas no Brasil. Cad Saude Publica 1993; 9:514-518.
7. Ministério da Saúde. Informações técnicas sobre a Doença de Chagas e seus vetores no Brasil referente ao ano de 2014. Brasília: Ministério da da Saúde; 2014. (Accessed 2015 May 10). Available at: www.portalsaude.gov.br
8. Dias JCP. Doença de Chagas: sucessos e desafios. Cad Saude Publica 2006; 22:2020-2021.
9. Pessoa GCD. Perfil da suscetibilidade a deltametrina em populações de *Triatoma sordida* (Hemiptera: Reduviidae) do Estado de Minas Gerais procedentes de áreas com infestação persistente. 2012. 179p. (Doctor´s Thesis). Universidade Federal de Minas Gerais; 2012 Belo Horizonte.
10. Vassena CV, Picollo MI, Zerba EN. Insecticida resistance in Brazilian *Triatoma infestans* and Venezuelan *Rhodnius prolixus*. Med Vet Entomol 2000; 14:51-55.
11. Organización Panamericana de la Salud (OPAS). II Reunion técnica latinoamericana de monitoreo de resistência a insecticidas em triatominos vectores de Chagas, OPS. Panamá: OPAS; 2005.
12. World Health Organization (WHO). Taller sobre la evaluación de efecto insecticida sobre triatominos. Workshop on the insecticide effect evaluation in triatominos. Acta Toxicol Argentina 1994; 2:29-33.
13. Pessoa GCD, Pinheiro LC, Ferraz ML, Mello BV, Diotaiuti L. Standartization of laboratory bioassays for the study of *Triatoma sordida* susceptibility to pyrethroid insecticides. Parasit Vectors 2015; 8:109-114.
14. Raymond M, Prato G, Ratsira D. PROBIT Analysis of mortality assays displaying quantal response, version 3.3. St. Georges dÓrques, France: Praxéme Sarl; 1993.
15. Obara MT, Otrera VCG, Gonçalves RG, Santos JP, Santalucia M, Rosa JA, et al. Monitoramento da suscetibilidade de populações de *Triatoma sordida* Stål, 1859 (Hemiptera: Reduviidae) ao inseticida deltametrina, na região Centro-Oeste do Brasil. Rev Soc Bras Med Trop 2011; 44:206-212.
16. Pessoa GCD, Rosa AC, Bedin C, Wilhelms T, Mello F, Coutinho HS, et al. Susceptibility characterization of residual Brazilian populations of *Triatoma infestans* Klug, 1834 (Hemiptera: Reduviidae) to deltamethrin pyrethroid. Rev Soc Bras Med Trop 2015; 48:157-161.
17. Perez de Rosas AR, Segura EL, Fichera L, Garcia BA. Macrogeographic and microgeographic genetic strutucture of the Chagas disease vector *Triatoma infestans* (Hemiptera: Reduviidae) from Caramarca, Argentina. Genetica 2008; 133:247-260.
18. Diotaiuti L, Azeredo BVM, Busek SCU, Fernandes AJ. Controle do *Triatoma sordida* no Peridomicílio Rural do Município de Porteirinha, Minas Gerais, Brasil. Rev Pan Salud Publica 1998, 3:21-25.
19. Forattini OP, Ferreira OA, Rocha e Silva EO, Rabello EX, Santos JLF. Aspectos ecológicos da tripanossomíase Americana. II. Distribuição e dispersão local de triatomíneos em ecótopos naturais e artificiais. Rev Saude Publica 1971; 5:163-191.
20. Forattini OP, Rocha e Silva EO, Ferreira AO, Rabello EX, Patolli DGB. Aspectos ecológicos da tripanossomíase Americana. III. Dispersão local de triatomíneos, com especial referência ao *Triatoma sordida*. Rev Saude Publica 1971; 5:193-205.
21. Santos MST, Areas MAA, Reyes FGR. Piretróides – uma visão geral. Alim Nutr 2007; 18:339-349.