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Secondary Kill Effect of Deltamethrin on *Triatoma infestans*

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Abstract

Control of the Chagas disease vector, *Triatoma infestans*, relies on the application of pyrethroid insecticides, especially deltamethrin. We performed laboratory studies to determine whether a *T. infestans* nymph that comes into contact with a deltamethrin-treated surface horizontally transfers the insecticide to subsequent triatomines. We found that a triatomine that walks on a deltamethrin-treated surface for a short period of time has the ability to transport the insecticide in concentrations sufficient to kill other triatomines with which it comes into contact. The effect was limited to high-density environments, and mortality as a result of secondary exposure was greater among second-instar nymphs compared with fifth-instar nymphs. Our results suggest that deltamethrin could be killing triatomines through both direct and indirect contact, although it remains unclear whether the phenomenon occurs in natural conditions.

Keywords

secondary exposure; *Triatoma infestans*; horizontal transfer; Chagas disease; delta-methrin

In Latin America, ~100 million people are at risk for acquiring American trypanosomiasis (Chagas disease), and an estimated 8–14 million people are currently infected (Ribeiro et al. 2009). A total of 10–30% of these individuals will develop heart and digestive conditions, which are often fatal if not treated (Bern et al. 2007). Vector control for Chagas disease has relied heavily on indoor residual spraying of pyrethroid insecticides. Whereas insecticide application campaigns have successfully reduced the incidence of Chagas disease, the disease still accounts for the greatest burden, measured in disability adjusted life years, among all neglected tropical diseases in the Americas (Hotez et al. 2008).

Horizontal transfer of insecticides can be very effective for the control of disease vectors and other unwanted insects, such as cockroaches, ants, mosquitoes, fleas, and termites (Hackler et al. 1998). Horizontal transfer of insecticide occurs when an individual insect is able to transfer insecticide back to its colony or nest, thereby exposing other insects (Buczkowski and Schal 2001). One way insects are secondarily exposed to insecticides is through contact with feces or sputum of an insect that has fed on an insecticide-laced bait

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and carried it back to its colony. (Miller and Peters 1999, Wang et al. 2008). Insecticide can also be horizontally transferred mechanically, as is the case of the mosquito larvicide pyriproxyfen (Itoh 1993, 1994; Dell Chism and Apperson 2003; Sihuincha et al. 2005; Devine et al. 2009).

Research shows that under ideal conditions, many species of triatomines will form large, dense colonies (Gajate et al. 2001, WHO 2002b, Abad-Franch et al. 2005). Triatomines typically emerge from their colonies at night to feed and return to the colony before dawn (Lorenzo and Lazzari 1998). Their refuges are typically dark, confined, and narrow (Lazzari and Lorenzo 2009). Such behaviors make insecticides that work through secondary exposure a promising strategy to control triatomines (Lazzari and Lorenzo 2009).

Among the triatomine species, *Triatoma infestans* is one of the most competent vectors of *Trypanosoma cruzi* (Cerisola et al. 1971, Zeledon 1974). Historically found in Bolivia, Brazil, Argentina, Peru, Chile, Uruguay, and Paraguay, the vector may have been responsible for as much as half of the Chagas disease cases reported worldwide (Panzera et al. 2004). In this study, we assess whether a single *T. infestans* that is exposed to a deltamethrin-covered surface has the potential to transfer the insecticide to subsequent triatomines it contacts, decreasing their survival.

Materials and Methods

Setting

This study was conducted under laboratory conditions between May 2009 and January 2010 at the Arequipa Chagas Control Laboratory in Arequipa, Peru. Insects used in this study were raised in a large triatomine colony. Insects in the colony originated from Arequipa, Peru. The insects were a mix of sub-populations collected from across the city, all from areas that had not received insecticide treatment at the time of collection. Insects were kept under ambient temperatures (16–24 °C) and humidity (12–47%) during the experiments.

Exposure of Triatomines to Insecticide

Deltamethrin-suspended concentrate from Bayer was diluted in water and applied, using a Hudson X-Pert compression sprayer, on a brick surface at a target dose rate of 25 mg/m². The insecticide was allowed to dry for 24 h under ambient conditions. A second identical brick surface was left untreated. Eight fifth-instar *T. infestans* nymphs were placed on the deltamethrin-treated brick surface. An additional eight fifth-instar *T. infestans* nymphs were placed on the untreated brick. In each treatment group, four of the nymphs were allowed to walk on the brick surface for 10 min, and four of the nymphs for 60 min. A plastic ring, 10 cm in diameter and 3 cm high, was used to confine the triatomines to their respective brick surfaces.

Horizontal Transfer Experiments

To mimic high-density colony conditions, 40 unexposed second-instar *T. infestans* nymphs were placed in four plastic cups (10 per cup). An additional 40 unexposed fifth-instar *T. infestans* nymphs were placed in four separate plastic cups (10 per cup). All of the plastic cups were identical and measured 5 cm in height and 7 cm in diameter. To determine whether deltamethrin is horizontally transferred among individuals, two fifth-instar nymphs exposed to the deltamethrin-treated brick surface for 60 min and two that were exposed for 10 min were randomly selected. One of the nymphs from each exposure time was placed in a plastic cup with 10 unexposed second-instar nymphs and one from each exposure time with 10 unexposed fifth-instar nymphs. This same procedure was repeated for control conditions in which fifth-instar nymphs were exposed to the untreated brick surface. The

number of dead triatomines was noted daily for 18 d. Dead triatomines were not removed from the plastic cups. This procedure was repeated three times for each of the treatments.

A low-density colony was created by placing 10 insects in a plastic container measuring 30 × 38 × 53 cm (height × length × width). A large brick measuring 8 × 13 × 21 cm (height × length × width) was included in the container. Experiments were performed as described above for the high-density colonies. The experiments in low-density colony were only performed once.

Data Analysis

Fisher exact test was used to assess differences in the number of dead triatomines between the two treatment groups at the endpoint of the study (day 18). The numbers of insects killed were compared by stage, exposure time, and colony density, using a Student *t* test. A Kaplan-Meier survival function was used to determine the probability of survival, and a log-rank test was used to compare the probability of survival between groups. The difference in the probability of survival between the two treatment groups during the 18 d of observation was compared by stage, exposure time, and density. All analyses were completed using STATA Version 10.0 SE (StataCorp 2007).

Results

Table 1 shows the mortality of *T. infestans* secondarily exposed to deltamethrin at two exposure times, across high- and low-density settings. In high-density conditions, six of 30 (20%) secondarily exposed second-instar nymphs in the 10-min exposure group died as compared with zero in the control group ($P < 0.03$). Among second-instar nymphs in the 60-min exposure group, 10 of 30 (33%) secondarily exposed nymphs died, which was significantly more than one of 30 in the control group ($P < 0.01$). No excess mortality occurred among secondarily exposed fifth-instar nymphs in the 10-min exposure group. However, 17 of 30 (57%) fifth instars in the 60-min exposure group died as compared with zero in the control group ($P < 0.01$). In low-density conditions, no difference was observed between the mortality of the secondarily exposed insects and the controls, regardless of exposure time or life stage.

Kaplan-Meier survival curves reveal a clear difference between experimental and control colonies in high-density conditions (Fig. 1). The probability of survival of the second-instar nymphs that were secondarily exposed to deltamethrin in both the 10- and 60-min exposure groups was significantly lower than the probability of survival of insects in their control groups ($\chi^2 = 6.56$, $df = 1$, $P < 0.01$, and $\chi^2 = 8.87$, $df = 1$, $P < 0.01$, respectively). Among the fifth instars, only the 60-min exposure group experienced a significantly lower probability of survival than the unexposed comparison group ($\chi^2 = 23.99$, $df = 1$, $P < 0.01$). Although the probability of survival of second-instar nymphs in high-density conditions was not significantly different for those nymphs in the 10- and 60-min exposure groups ($\chi^2 = 1.03$, $df = 1$, $P < 0.31$), among fifth-instar nymphs, the probability of survival was lower for nymphs in the 60-min exposure group as compared with the 10-min exposure group ($\chi^2 = 23.99$, $df = 1$, $P < 0.01$).

Discussion

This study demonstrates that a triatomine that walks on a deltamethrin-treated surface for short periods of time has the ability to transport the insecticide in concentrations sufficient to kill other triatomines with which it comes into contact. Both the stage of the secondarily exposed insect and the density conditions of the colony modify the secondary kill effect.

Our finding that second-instar triatomines succumbed more easily to secondary exposure to delta-methrin is probably the result of a simple dose effect. Older triatomines (adults and fifth instars) have been previously shown to be much more resistant to the effects of insecticide, presumably because of their larger size relative to the insecticide dose (Reyes et al. 2007). We also observed that the duration of exposure had a larger effect on the survival of fifth-instar recipient insects as opposed to the smaller second-instar recipient insects. Longer exposure times may have allowed for a greater amount of insecticide to accumulate on the exposed insects, and in some cases reach doses sufficient to kill the fifth instars.

We observed secondary mortality only in high-density experimental colonies, where insects were forced to come into direct contact with each other. Density-dependent effects of horizontal transfer of insecticide have been demonstrated in other insects. Chloe and Rust (2008) demonstrated that when an exposed dead ant was placed in the nest, more ants picked up lethal doses of insecticide than they would if placed outside the nest. The authors attributed their findings to the crowding in the nest that led to higher contact rates with the exposed ant; a similar process may have occurred with triatomines in the high-density colonies presented in this work.

Certain aspects of *T. infestans* behavior are well documented, such as feeding, resting, and circadian rhythms (Lazzari and Lorenzo 2009). However, very little is known about how much contact *T. infestans* have with each other under natural conditions. Triatomine density and contact rates may be affected by the number and size of refuge sites in households (Cecere et al. 1998, 2003), as well as the number and species of hosts in the household (Cohen and Gurtler 2001, Levy et al. 2006, McGwire et al. 2006). Additional research describing colony development, contact rates, the conditions in which contact occurs, and the frequency and duration of contact is needed to better evaluate the role of horizontal transfer of deltamethrin on *T. infestans* mortality.

Our study had a number of limitations. We used only fifth- and second-instar nymphs; we could not assess the effect of secondary contact in adult insects, where it may be the most important. Our system was experimental and only very roughly mimicked the habitat of triatomines in households. We were also unable to discriminate between two possible modes of secondary killing of deltamethrin, as follows: through contact with the exoskeletons of other insects, or through contact with their feces.

Triatomine reinfestation after insecticide control campaigns continues to pose a challenge, particularly as triatomines are expanding their range to densely populated periurban and urban areas (Albarracin-Veizaga et al. 1999, Vallve et al. 1999, Gajate et al. 2001, Carrasco et al. 2005, Ramsey et al. 2005, Zeledon et al. 2005, Levy et al. 2006, Bowman et al. 2008). Return of the insect provides both justification and impetus to find alternative methods to effectively control triatomines. Slow-acting, nonrepellent, or attractant insecticides that are designed to work through secondary exposure are ideally suited for social insects that forage regularly and return to a shared habitat, such as *T. infestans* and other species of triatomine bugs (Abad-Franch et al. 2005). A better understanding of triatomine colony dynamics and the mechanisms by which insecticide control works on colonies may facilitate the development of new control strategies that could work alongside community residual insecticide application.

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References Cited

- Abad-Franch F, Palomeque F, Aguilar H, Miles M. Field ecology of sylvatic *Rhodnius* populations (Heteroptera, Triatominae): risk factors for palm tree infestation in western Ecuador. *Trop Med Int Health*. 2005; 10:1258–1266. [PubMed: 16359406]
- Albarracin-Veizaga H, deCarvalho ME, Nascimento EM, Rodrigues VL, Casanova C, Barata J. Chagas disease in an area of recent occupation in Cochabamba, Bolivia. *Rev Saude Publica*. 1999; 33:230–236. [PubMed: 10456995]
- Bern C, Montgomery S, Herwaldt BL, Rassi AJ, Marin-Neto JA, Dantas RO, Maguire JH, Acquatella HA, Morillo C, Kirchhoff LV, Gilman RH, Reyes PA, Salvatella R, Moore AC. Evaluation and treatment of chagas disease in the United States: a systematic review. *J Am Med Assoc*. 2007; 298:2171–2181.
- Bowman NM, Kawai V, Levy MZ, Carpio del Cornejo JC, Cabrera L, Delgado F, Malaga F, Benzaquen E, Pinedo V, Staurer F, Seitz A. Chagas disease transmission in periurban communities of Arequipa, Peru. *Clin Infect Dis*. 2008; 46:1822–1828. [PubMed: 18462104]
- Buczowski G, Schal C. Method of insecticide delivery affects horizontal transfer of Fipronil in the German cockroach (Dictyoptera: Blattellidae). *J Econ Entomol*. 2001; 94:680–685. [PubMed: 11425023]
- Carrasco HJ, Torrellas A, Garcia C, Segoia M, Feliciangeli M. Risk of *Trypanosoma cruzi* I (Kinetoplastida: Trypanosomatidae) transmission by *Panstrongylus geniculatus* (Hemiptera: Reduviidae) in Caracas (Metropolitan District) and neighboring states, Venezuela. *Int J Parasitol*. 2005; 35:1379–1384. [PubMed: 16019006]
- Cecere, MC.; Gurtler, RE.; Chuit, R.; Cohen, JE. Bull. Vol. 76. WHO; 1998. Factors limiting the domestic density of *Triatoma infestans* in north-west Argentina: a longitudinal study; p. 373-384.
- Cecere MC, Canale DM, Gurtler RE. Effects of refuge availability on the population dynamics of *Triatoma infestans* in central Argentina. *J Appl Ecol*. 2003; 40:742–756.
- Cerisola JA, Rohwedder RW, Del Prado CE. Rendimiento del xenodiagnostico en la infeccion chagastica cronica humana utilizando ninfas de diferentes especies de triatomos. *Bol Chil Parasitol*. 1971; 26:57–58. [PubMed: 5002112]
- Chloe DH, Rust MK. Horizontal transfer of insecticides in laboratory colonies of the Argentine ant (Hymenoptera: Formicidae). *J Econ Entomol*. 2008; 101:1397–1405. [PubMed: 18767753]
- Cohen JE, Gurtler RE. Modeling household transmission of American trypanosomiasis. *Science*. 2001; 293:694–689. [PubMed: 11474111]
- Dell Chism DH, Apperson CS. Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. *Med Vet Entomol*. 2003; 17:211–220. [PubMed: 12823839]
- Devine GJ, Perea EZ, Killeen GF, Stancil JD, Clark SJ, Morrison AC. Using adult mosquitoes to transfer insecticides to transfer *Aedes aegypti* larval habitats. *Proc Natl Acad Sci USA*. 2009; 106:11530–11534. [PubMed: 19561295]
- Gajate P, Pietrokovsky S, Abramo Orrego L, Perez O, Monte A, Belmonte J, Wisnivesky-Colli C. *Triatoma infestans* in greater Buenos Aires, Argentina. *Mem Inst Oswaldo Cruz*. 2001; 96:474–477.
- Hackler, R.; Johnson, G.; Owen, J. U.S.P. a. T. Office. United States Patent Full-Text and Image Database. Dow AgroSciences LLC; Indianapolis, IN: 1998. Benzoylphenylurea insecticides and methods of using them to control cockroaches, ants, fleas, and termites.
- Hotez PJ, Bottazzi ME, Franco-Paredes C, Ault SK, Periago MR. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. *PLoS Negl Trop Dis*. 2008; 2:e300. [PubMed: 18820747]
- Itoh T. Control of DF/DHF vector, *Aedes* mosquito, with insecticides. *Trop Med*. 1993; 35:259–267.

- Itoh T. Utilization of bloodfed female of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator, pyriproxyfen to larval habitats. *J Am Mosq Control Assoc.* 1994; 10:344–347. [PubMed: 7807075]
- Lazzari CR, Lorenzo MG. Exploiting triatomine behaviour: alternative perspectives for their control. *Mem Inst Oswaldo Cruz.* 2009; 104:65–70. [PubMed: 19753460]
- Levy MZ, Bowman NM, Kawai V, Waller LA, Cornejo del Carpio JG, Cordova Benzaquen E, Gilman RH, Bern C. Periurban *Trypanosoma cruzi*-infected *Triatoma infestans*, Arequipa, Peru. *Emerg Infect Dis.* 2006; 12:1345–1352. [PubMed: 17073082]
- Lorenzo MG, Lazzari CR. Activity pattern in relation to refuge exploitation and feeding in *Triatoma infestans* (Hemiptera Reduviidae). *Acta Trop.* 1998; 70:163–170. [PubMed: 9698262]
- McGwire K, Segura EL, Scavuzzo M, Gómez A, Lamfri M. Spatial pattern of reinfestation by *Triatoma infestans* in Chancaní, Argentina. *J Vector Ecol.* 2006; 31:17–28. [PubMed: 16859086]
- Miller, P.; Peters, B. In: Robinson, WH.; Rettich, F.; Rambo, GW., editors. Performance of goliath cockroach gel against German cockroach (Blattodea: Blattellidae) and a mixed population of American cockroach and Australian cockroach (Blattodea: Blattidae) in the field; Proceedings, 3rd International Conference on Urban Pests; 1999; Prague: Grafickezavodny Hronov; 1999. p. 153-159.
- Panzer F, Dujardin JP, Nicolini P, Caraccio MN, Rose V, Tellez T, Bermudez H, Bargues MD, Mas-Coma S, O'Connor JE, Perez R. Genomic changes of Chagas disease vector, South America. *Emerg Infect Dis.* 2004; 10:438–446. [PubMed: 15109410]
- Ramsey JM, Alvear AL, Ordonez R, Munoz G, Garcia A, Lopez R, Leyva R. Risk factors associated with house infestation by the Chagas disease vector *Triatoma pallidipennis* Cuernavaca metropolitan area, Mexico. *Med Vet Entomol.* 2005; 19:219–228. [PubMed: 15958028]
- Reyes M, Angulo VM, Sandoval CM. Toxic effect of beta-cipermethrin, deltamethrin and fenitrothion in colonies of *Triatoma dimidiata* (Latreille, 1811) and *Triatoma maculata* (Erichson, 1848) (Hemiptera, Reduviidae). *Biomedica.* 2007; 27:75–82. [PubMed: 18154247]
- Ribeiro I, Sevcik AM, Alves F, Diap G, Don R, Harhay MO, Chang S, Pecoul B. New improved treatments for Chagas disease: from the R & D pipeline to patients. *PLoS Negl Trop Dis.* 2009; 3:e484. [PubMed: 19582163]
- Sihuíncha M, Zamora-Perea E, Orellana-Rios W, Stancil JD, Lopez-Sifuentes V, Vidal-Ore C, Devine GJ. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. *J Med Entomol.* 2005; 42:620–630. [PubMed: 16119551]
- StataCorp. Stata Statistical Software: Release 10 computer program, version. StataCorp; College Station, TX: 2007.
- Vallve SL, Rojo H, Wisnivesky-Colli C. Urban ecology of *Triatoma infestans* in San Juan, Argentina. *Mem Inst Oswaldo Cruz.* 1999; 91:405–408. [PubMed: 9070399]
- Wang, C.; Yang, X.; Aboul-El-Nour, MA.; Bennett, GW. Factors affecting secondary kill of the German cockroach by gel baits. Proceedings, 6th International Conference on Urban Entomology; 13–16 July 2008; Budapest, Hungary. Budapest, Hungary: OOK-Press Kft; 2008. p. 153-159.
- [WHO] World Health Organization. Control of Chagas Disease, WHO Technical Report Series 905. World Health Organization; Geneva, Switzerland: 2002b.
- Zeledon, R. Epidemiology, modes of transmission and reservoir hosts of Chagas' disease. In: Amsterdam, L., editor. Trypanosomiasis and Leishmaniasis with Special Reference to Chagas' Disease. Elsevier; New York, NY: 1974.
- Zeledon R, Calvo N, Montenegro VM, Lorosa ES, Arevalo C. A survey on *Triatoma dimidiata* in an urban area of the province of Heredia. *Costa Rica Mem Inst Oswaldo Cruz.* 2005; 100:507–512.

Fig. 1.

Panels A and B show Kaplan-Meier survival curves for *T. infestans* in colonies into which a single fifth-instar individual, exposed to deltamethrin insecticide, had been introduced (solid line) and control colonies into which a single fifth-instar individual, not exposed to deltamethrin, had been introduced (dotted line).

Table 1

Mortality of *T. infestans* nymphs secondarily exposed to deltamethrin at two exposure times, across high and low densities

Life stage	Density	n	Mortality by exposure time no. (%)					
			10 min			60 min		
			Experimental	Control	P value	Experimental	Control	P value
Second	High	30	6 (20) *	0 (0)	0.01	10 (33) **	1 (3)	0.01
Second	Low	10	0 (0)	0 (0)	N/A	2 (20)	0 (0)	0.15
Fifth	High	30	0 (0)	1 (3)	0.32	17 (57) **	0 (0)	0.01
Fifth	Low	10	0 (0)	0 (0)	N/A	2 (20)	0 (0)	0.15

Fisher's exact test.

***, $P < 0.01$;

* $P < 0.05$;

N/A, not available because there were no failures.