Expression of Insecticide Resistance in Immature Life Stages of *Triatoma infestans* (Hemiptera: Reduviidae)

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J. Med. Entomol. 50(4): 816–818 (2013); DOI: http://dx.doi.org/10.1603/ME12116

ABSTRACT The aim of the current study was to investigate the susceptibility to the insecticide deltamethrin and the expression of resistance to this insecticide in developing eggs and neonate nymphs of *Triatoma infestans* from two areas of Argentina (Campo Largo) and Bolivia (Entre Ríos), where resistance to this pyrethroid is suspected. Both nymphal populations showed resistance to deltamethrin, with lower resistance ratio for Entre Ríos (173×) than Campo Largo (1108×). Efficacy of deltamethrin on 4-, 7-, and 12-d-old eggs for both field populations were significantly lower than efficacy on eggs of the susceptible strain. This is the first documented evidence of the expression of pyrethroid resistance during the embryonic development of Chagas Disease vectors.

KEY WORDS *Triatoma infestans*, resistance, pyrethroid, insecticide

The kissing bug *Triatoma infestans* Klug is the main vector of the parasite *Trypanosoma cruzi*, the causative agent of Chagas disease or American trypanosomiasis. Since the 1980s, pyrethroid insecticides have been the main products used to control domestic populations of this vector. However, resistance to deltamethrin and other pyrethroids associated with ineffective field treatments has been reported among areas of the Gran Chaco of Argentina and Bolivia (Picollo et al. 2005, Toloza et al. 2008, Gemio et al. 2010, Germano et al. 2010, Lardeaux et al. 2010). Initial studies demonstrated highly deltamethrin-resistant populations exist in areas neighboring Salvador Mazza (Argentina) and Yacuiba (Bolivia), with resistant ratios (RR) 133× and 154×, respectively (Picollo et al. 2005, Santo Orihuela et al. 2008). Later, higher levels of resistance to deltamethrin (RR from 247× to 541×) were reported for other field populations from the south of Bolivia (Tarija Department), demonstrating that resistant area occurred to a greater extent than expected previously (Toloza et al. 2008).

Recently, toxicological studies demonstrated important differences in the expression of resistance in eggs of these pyrethroid-resistant populations (Toloza et al. 2008). In fact, eggs in northern Argentina expressed high levels of deltamethrin resistance (RR Salvador Mazza: 114.3×), whereas eggs in southern Bolivia were susceptible to deltamethrin (RR Yacuiba: 0.56×). These authors were focused on the measurement of susceptibility in older eggs (12 d old) according to previous studies on the effects of insecticides on *T. infestans* eggs (Villar et al. 1980, Visciarelli et al. 2011).

The present report analyzes the expression of insecticide resistance in developing eggs of *T. infestans.* Insect embryogenesis involves numerous morphological, biochemical, and physiological events that affect the toxicity of insecticide, and probably the expression of the lower susceptibility to an insecticide. For example, the resistance may not be expressed in early embryonic eggs because the target of insecticide is not present at this stage (Picollo et al. 1979). The aim of the current study was to investigate the susceptibility to insecticides and expression of deltamethrin resistance in developing eggs and neonate nymphs of *T. infestans* field populations from two areas with resistance in Argentina and Bolivia.

Materials and Methods

Insects. Insecticide-susceptible *T. infestans* were obtained from descendents of insects from Coordinación Nacional de Control de Vectores (Punilla, Córdoba), where they were reared for 6 yr. They were reared in our laboratory for 3 yr under standard conditions of controlled temperature (28°C), humidity (50–70%), and a photoperiod of (12:12 (L:D)h), and were weekly fed on pigeons. Field *T. infestans* were collected in 2009 from infested houses in northern Argentina (Campo Largo, 22° 0′19.32″ S, 63° 56′2.24″ W) and southern Bolivia (Entre Ríos, 21° 17′44.07″ S, 63° 56′54.36″ W), where vector control campaigns based on pyrethroid insecticides were considered ineffective by the Ministries of Health of Argentina and Bolivia. Captured insects and their offspring were raised under standard conditions, and the second generation of captured insects was used for susceptibility bioassays.

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0022-2585/13/0816–0818$04.00/0 © 2013 Entomological Society of America

Table 1. Toxicity of topically applied deltamethrin to 3-d-old first-instar nymphs and ovicidal activity of topically applied deltamethrin to *Triatoma infestans* 4-, 7-, and 12-d-old eggs of a susceptible reference colony and two insecticide-resistant populations from Argentina and Bolivia

Population	Age	*n*	LD_{50} (ng/i) (CL)	Slope	RR (CL)
Reference	3-d nymph	100	0.20 (0.14–0.28)	1.4 ± 0.37	—
Campo Largo	3 d	150	228 (46–1022)	0.8 ± 0.07	1,108 (685–1,790)
Entre Ríos	3 d	130	36 (21–66)	0.7 ± 0.06	173 (149–230)
Reference	4-d egg	90	0.50 (0.32–0.77)	1.56 ± 0.98	—
	7 d	147	0.42 (0.25–0.68)	1.18 ± 0.12	—
	12 d	187	1.55 (1.16–2.18)	1.99 ± 0.69	—
Campo Largo	4 d	138	576 (397–936)	2.87 ± 0.17	1,144 (609–2,150)
	7 d	227	501 (394–646)	2.02 ± 0.15	1,193 (687–2,070)
	12 d	251	1,277 (1,013–1,650)	2.27 ± 0.30	822 (558–1211)
Entre Ríos	4 d	142	10.63 (1–21)	0.92 ± 0.15	21 (9–47)
	7 d	90	6 (2–12)	0.544 ± 0.09	15 (6–37)
	12 d	90	61 (20–207)	0.77 ± 0.08	39 (23–63)

Chemicals. Technical grade deltamethrin (99.0%) was obtained from Dr Ehrenstorfer, Augsburg, Germany. Analytical grade acetone was purchased from J.T. Baker (Estado de México, México).

Bioassays

Nymphs. First-instars nymph (35 d old; mean weight 1.3 ± 0.2 mg) starved since eclosion were selected for toxicity tests by using the methods of the World Health Organization protocol (WHO 1994). Treatment consisted of topical application on the dorsal abdomen. Final concentrations ranged from 0.0001 to 15 mg/ml of deltamethrin in acetone. At least four different concentrations that gave between 10 and 90% mortality were used. These concentrations plus a control were assayed for each population in three replications containing at least 10 first instars per dose. After 24 h, mortality rates of controls and treated insects were recorded as indicated by immobility according to the WHO protocol (WHO 1994).

Eggs. For toxicity studies, eggs were collected from adult rearing boxes and stored in petri dishes. Eggs of 4, 7, and 12 d were selected based on the external morphological characteristics as described by Picollo et al. (1979). Briefly, 4-d-old eggs were white with a mild depression in the center. Seven-day-old eggs were slightly more orange, with the posterior section clearer and two reddish eyespots; 12-d-old eggs were orange with dark eye spots. Groups of at least 10 eggs per concentration were fixed to a microscope slide by double-sided adhesive tape. Treatment of individual eggs was performed by topical application to the operculum with 0.2 μl of insecticide diluted in acetone, by using a 10-μl Hamilton syringe, at the same rate of concentrations and application method as used with nymphs.

After topical application, eggs were incubated in a rearing cabinet (FOC-225E, Velp Scientifica, Milan, Italy) at 28°C, 50% RH, and a photoperiod of 12:12 (L:D) h. At these laboratory conditions, the estimated hatching of control eggs is 15 d. So mortality data were assessed at 14, 11, and 6 d after topical application to 4-, 7-, and 12-d-old eggs, respectively.

Statistical Analysis. Mortality data were corrected using Abbott formula before analysis (Abbott 1925). Dose–mortality data for eggs and first instar nymphs of each *T. infestans* population were subject to probit analysis (Litchfield and Wilcoxon 1949) by using the Polo PC program (LeOra Software 1987) to estimate the median dose required to kill 50% of the eggs or nymphs (LD_{50}). Differences in LD (lethal dose) were determined via the lack of overlap between 95% CL (confidence level). RRs and 95% CLs were calculated as described by Robertson and Preisler (1992) by comparison with the susceptible reference strain.

Results

Susceptibility varied greatly, with LD_{50} values ranging from 0.2 nanogram per insect (ng/i) in the reference population to 228 ng/i in Campo Largo (Table 1). Both field populations showed resistance to deltamethrin; resistant ratio for the strain from Entre Ríos was lower than the strain from Campo Largo.

At all stages of development, the efficacy of deltamethrin on eggs of both field populations was significantly lower than its efficacy on eggs of the susceptible strain.

Moreover, the level of susceptibility was significantly lower in eggs from Campo Largo than Entre Ríos. In Campo Largo and Entre Rios, there was not significant difference between the susceptibility levels estimated on 4- and 7-d-old eggs, but lower susceptibility was assessed in 12-d-old eggs. Similarly, 4- and 7-d-old eggs of Entre Ríos showed similar level of deltamethrin susceptibility, but it was significantly lower in 12-d-old eggs.

Discussion

The present laboratory tests on first instar nymphs of *T. infestans* demonstrated that the field populations from northern Argentina (Campo Largo) and southern Bolivia (Entre Ríos) have developed high resistance to deltamethrin insecticide. The resistant level for the Argentinean population was significantly higher than that of the Bolivian one. These results are consistent with previous reports that demonstrated the emergence of insecticide resistance associated with ineffective field treatments in *T. infestans* of this endemic area of the Gran Chaco ecoregion (Picollo et al. 2005, Santo Orihuela et al. 2008, Germano et al. 2010, Lardeux et al. 2010).

Our bioassays with eggs demonstrated that resistance to both deltamethrin in both populations was also expressed in eggs. This is the first documented evidence of the expression of pyrethroid resistance during the early embryonic development of *T. infestans.*

Few previous studies have reported the expression of insecticide resistance in insect eggs, and these studies were conducted only on late development eggs. For example, Toloza et al. (2008) using 12-d-old eggs, studied the patterns of pyrethroid resistance in nymphs and eggs of *T. infestans* from Argentina and Bolivia. These authors concluded that the expression of resistance in late development eggs varied between populations and that the pyrethroid resistance diagnosed in first nymphs was not indicative of resistance in the egg stage.

Our study also showed that deltamethrin resistance is expressed throughout embryonic development, although the toxic effect of deltamethrin was different at different stages. For the reference and both resistant populations, the susceptibility of early eggs was similar to that of intermediate eggs, but lower in 12-d-old eggs. A decrease in susceptibility to organophosphorus insecticides was also reported for later embryogenesis in *T. infestans* (Zerba and Picollo 1987). These authors reported the detection of nerve cells and cholinergic system in 4-d-old embryos of *T. infestans* and concluded that the greater tolerance of late embryos was related to the presence of the embryonic cuticle that must be crossed by the insecticide to reach the target. It has been assessed in other insects (e.g., *Locusta migratoria*) that the last embryonic cuticle is the one of the first instar larva (Klowden 2007). In *T. infestans*, topical application of radioactive parathion in 11-d-old eggs showed that 70% of the radioactivity was between the chorion and the embryonic cuticle in 12-d-old eggs (Picollo and Zerba 1987). This double barrier may justify the greater tolerance of the oldest eggs.

Acknowledgments

We thank Drs Cynthia Spillmann, Mario Zaidenberg, Raúl Stariolo, and technicians of the National Vector Control Coordination of Argentina. Tamara Chavez and Mirko Rojas of Ministry of Health of Bolivia supported us in field sampling. This investigation received financial support from the UNICEF/UNDP/World Bank/World Health Organization Special Programmer for Research and Training in Tropical Diseases (TDR) project ID A80360, and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina.

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Received 18 May 2012; accepted 26 March 2013.