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Microgeographical study of insecticide resistance in *Triatoma infestans* from Argentina

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ABSTRACT

Chagas disease is a chronic parasitic infection restricted to America where it is currently estimated that 90 million people are at risk of acquiring the infection. Chemical control with pyrethroid insecticides has been effective to reduce disease transmission in several areas of the Southern Cone, although insecticide resistance has evolved and diminished the campaigns' results. Considering previous reports on the different levels of resistance between *Triatoma infestans* from different geographical areas, the objective of this work was to determine if *T. infestans* populations are toxicologically structured within localities. Response to the insecticide was measured and compared between houses of two Argentine localities. Different toxicity of deltamethrin was detected between dwellings of Chaco province, accounting for both susceptible and resistant houses within the same locality. However no difference was found among houses of Salta province. The results obtained in this work suggest that geographical structure is present not only at the between localities level, but also at the microgeograhical level.

1. Introduction

Chagas disease is a chronic parasitic infection restricted to America where it is currently estimated that 15 million people carry the disease, and 90 million people are at risk of acquiring the infection (Rodrigues Coura and Dias, 2009). The disease is caused by the protozoa *Trypanosoma cruzi*, which is transmitted to human through the feces of infected blood-sucking insects belonging to the subfamily Triatominae (Heteroptera: Reduviidae). *Triatoma infestans* (Klug, 1834) is the triatomine species responsible for most cases of Chagas disease in the continent (Dias and Schofield, 1999). Because no treatment is available for the chronic forms of the disease, control campaigns reply primarily on vector insect control (WHO, 2006). Chemical control, historically based on the use of organochlorine, organophosphate, carbamate and pyrethroid insecticides, led to the reduction of *T. infestans* distribution and consequent interruption of disease transmission in several areas of the Southern Cone (Dias et al., 2002; WHO, 2006; Zerba, 1999). However the insecticide resistance evolved in *T. infestans* populations. Resistance to deltamethrin was detected in South America since 1990s where low, medium and high resistance levels have been reported (Germano et al., 2010a, 2012; Picollo et al., 2005; Santo Orihuela et al., 2008; Vassena et al., 2000). Moreover, high resistance levels correlated with field control failures were reported for the Argentine provinces of Salta and Chaco (Carvajal et al., 2012; Picollo et al., 2005). After a decade of studies, the resistance to insecticides in *T. infestans* is evidenced as a complex problem: the resistance evolved in different areas of the geographic distribution of the species, different resistance profiles were found in different areas, and different resistance mechanisms were described (i.e. enhanced metabolism, modified site of action, reduced penetration) (Fabro et al., 2012; Germano et al., 2010a, 2012; Pedrini et al., 2009; Picollo et al., 2005; Santo Orihuela et al., 2008; Toloza et al., 2008). Considering the high levels of structuring of *T. infestans* populations that have been found in the past (Marcet et al., 2008; Pérez de Rosas et al., 2007, 2008), we evaluate the toxicological response to deltamethrin in insects from individual houses of the same locality and we demonstrate that some houses host resistant insects while other houses host susceptible insects.

2. Materials and methods

2.1. Study design and insect rearing

Insects were collected at La Esperanza, Province of Chaco, Argentina and Acambuco, Province of Salta, Argentina, two rural areas where infestation after chemical treatment with deltamethrin (25 mg/m^2) had been previously reported (Germano et al., 2012, 2013). Last chemical treatment in these areas had taken

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Table 1
Survivorship to discriminant dose and resistance levels (LDR) to deltamethrin in *T. infestans* from individual dwellings from Acambuco, Salta, Argentina.

House code	Dwelling structure[a]	Latitude/longitude	Survivorship to DD (%) (*n*)[b]	LD_{50} (ng/i) (95% CL) (*n*)[b]	Slope ± SE[c]	LDR (95% CL)[c]	Toxicological status[d]
Susceptible reference[e]	–	–	0 (30)	0.13 (0.11–0.15) (125)	2.15 ± 0.70	–	–
Q2 Locality reference[f]	–	–	–	6.4 (4.58–8.92) (169)	1.7 ± 0.21	32.5 (31.8–44.6)	R
A1	P	22°09′19″S 63°54′49″W	70 (34)	4.87 (1.99–11.27) (140)	1.85 ± 0.30	37.51 (25.89–54.35)	R
A2	P	22°20′11″S 63°50′35″W	62 (39)	2.59 (1.94–3.43) (135)	2.14 ± 0.32	19.90 (14.57–27.18)	R
A3	P	22°20′11″S 63°50′35″W	45 (40)	2.16 (1.22–3.26) (139)	1.28 ± 0.25	16.71 (10.35–26.94)	R

[a] Site of insect collection within houses, namely domestic (D) or peridomestic (P) structures.
[b] Number of insects used for bioassays (*n*).
[c] Slope and lethal dose ratios (LDR) with respective standar error (SE) and 95% confidence limits (CL).
[d] Toxicological status expressed as resistant (R) vs susceptible (S) to deltamethrin.
[e] Data from Picollo et al. (2005).
[f] Data from Germano et al. (2012).

place between 1 and 5 years before insect collection, which took place between November 2009 and March 2010 at Acambuco and from August to December 2011 at La Esperanza. A susceptible strain raised at the laboratory since 1975 was used as a reference, as its response to deltamethrin had been repeatedly verified with susceptible field strains (Germano et al., 2010b; Picollo et al., 1976; Toloza et al., 2008).

Every house was numbered and georeferenced. Both the house code and the structure from which the individuals were collected were recorded for insect rearing and further analysis. All of the captured insects from Acambuco were found at the peridomestic structures, namely chicken coops, pig and goat corrals. In the case of La Esperanza, infestation was generally higher and in addition to the houses that presented peridomestic infestation, some presented domiciliary infestation.

The search of insects was conducted in all of the houses from both localities. Insects were collected using timed manual collections, such that two trained workers did the search during 30 min, one in the domestic area, and the other in the peridomestic areas. Approximately 30% of the houses presented *T. infestans* infestation, although only those houses in which 10 or more adults were found were included in this assay, since a sufficient number of insects for bioassays were required. Considering that bioassays were conducted on the F1 (see below), a second requirement for house inclusion was that at least three adult females were available for reproduction.

Individuals were captured from infested dwellings using manual forceps and with the use of 0.2% tetramethrin as a dislodging agent (Icona SA, Buenos Aires, Argentina). Captured insects and their offspring were reared at the laboratory maintaining the house population independence, under controlled temperature (26 ± 1 °C), humidity (50–70%) and photoperiod (12:12 L:D). A pigeon was weekly provided as a blood meal source (WHO, 1994).

2.2. Bioassays

Tests to determine insecticide susceptibility were done on the first generation obtained from the field collected insects, according to the World Health Organization protocol (WHO, 1994). The tests were conducted by topical application on *T. infestans* first instars (5–7-d-old, mean weight 1.3 ± 0.2 mg) starved since eclosion. Each insect was treated with 0.2 μl of deltamethrin diluted in acetone, applied on the dorsal abdomen using a 10 μl Hamilton syringe provided with a repeating dispenser. Survivorship to a discriminant dose was used as a reference for insecticide response. This dose was previously established as twice the minimum dose which causes total mortality on the reference strain, and its value is 2 nanograms per insect (ng/i). For dose–mortality assays at least four doses in a range that produced between 10 and 90% mortality were tested, and each dose was replicated at least three times. The concentrations ranged from 0.02 to 500 ng/i of deltamethrin, and 10 insects were used for each replicate. Topical application with acetone was conducted for controls. After treatment, insects were held at the rearing laboratory conditions for 24 h, when mortality was recorded. The criterion for mortality was the inability to walk from the center to the border of an 11 cm diameter filter paper disk, meaning that only those nymphs which were able to reach the paper border, with or without mechanical stimulation, were considered alive.

2.3. Chemicals

Technical grade deltamethrin (99.0%) used for bioassay was obtained from Ehrestorfer, Germany. The analytical grade acetone used for dilutions was purchased from J.T. Baker, Mexico.

2.4. Data analysis

Mortality data were corrected using Abbott's formula (Abbott, 1925). Dose–mortality data were subjected to probit regression analysis (Litchfield and Wilcoxon, 1949) to estimate the lethal dose to kill 50% of the population (LD_{50}) by using POLO PC (LeOra, 1987). Lethal dose ratio (LDR) and 95% confidence limits (CL) were calculated as described by (Robertson et al., 2007). Studied populations were considered resistant if the LDR 95% confidence limits did not include the number one. Fisher's exact test was used to evaluate the association between the toxicological profile (resistant vs susceptible) and the original location of the insect within dwellings (domestic or peridomestic structures).

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