

Residual effect of a micro-encapsulated formulation of organophosphates and piriproxifen on the mortality of deltamethrin resistant *Triatoma infestans* populations in rural houses of the Bolivian Chaco region

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The Bolivian Chaco is part of the endemic region of Chagas disease and an area where pyrethroid resistant Triatoma infestans (Hemiptera: Reduviidae) populations has been reported. The World Health Organization identified these resistant populations as an important focus for research. The objective of this study was to evaluate the residual effect of a micro-encapsulated formulation containing organophosphate active ingredients and a juvenile hormone analogue (Inesfly 5A IGR) on the mortality of T. infestans. Studies took place in rural houses of the Bolivian Chaco that were treated up to 34 months before and evaluated the susceptibility to pyrethroids of the offspring of field collected insects. Thirty houses were randomly selected within three communities to carry out wall bio-assays with T. infestans nymphs. Mortality was recorded 24, 48 and 72 h after wall contact. Eggs laid by females collected in the area were used to obtain first-instar nymphs and carry out pyrethroid susceptibility tests. The wall bio-assays showed that the micro-encapsulated insecticide eliminates T. infestans populations and produces detectable mortality of insects exposed to walls treated 34 months prior to the tests. The discriminant dose of deltamethrin (0.01 mg/mL) showed 65% nymph survival, whereas at the highest tested dose (1.0 mg/mL) 14% of the nymphs survived. These results show that Inesfly 5A IGR is an appropriate tool for the elimination of intradomestic and peridomestic populations of T. infestans resistant to pyrethroids.

Key words: Chagas disease - vector control - Triatominae - *Triatoma infestans* - insecticide resistance

Triatoma infestans (Hemiptera: Reduviidae) is the main vector of *Trypanosoma cruzi* (the etiologic agent of Chagas disease) in the Gran Chaco region of South America. Chagas disease is the most important parasitic disease in Latin America, the most frequent cause of myocardial pathologies and one of the most important neglected diseases identified by the World Health Organization (WHO). In the case of Bolivia, *T. cruzi* infection prevalence is among the highest in Latin America, ranging from 26-71% in children between 1-6 years of age and from 71-95.3% in the adult population (Jannin & Salvatella 2006).

Due to the absence of a vaccine and the limited therapeutic response of currently available drugs, the options to reduce the burden of the disease depend on vector control using residual insecticides, house improvement and health education (Schofield 1994, WHO 2002). Since the 1980s, triatomine control has been primarily based on the use of pyrethroid residual insecticides that commonly have deltamethrin, lambda-cyhalothrin and cyper-

methrin as active ingredients (Morel 1999). Pyrethroids are currently the main vector control tool because of their high efficacy, efficiency and residual activity, together with low risk of environmental contamination (Pinchin et al. 1980, Zerba et al. 1997, Gürtler et al. 2004, Cecere et al. 2006). After the remarkable success of the Southern Cone Initiative against Chagas disease vectors, which interrupted the vectorial transmission of *T. cruzi* in Uruguay, Chile, Brazil and several regions of Argentina and Paraguay, studies carried out during the last decade showed that vector control interventions in the Gran Chaco region had a lower performance compared with vector control interventions outside of the region (Schofield et al. 2006). The main difference is given by the presence of abundant populations of *T. infestans* living in the peridomestic structures of the Gran Chaco rural houses. In those habitats, the pyrethroid formulations showed lower efficacy, leaving residual populations that after 1-3 years reconstituted the original populations and eventually reinstalled the vectorial transmission of *T. cruzi* (Gürtler 2009). The recommended strategy for these places of repeating an insecticide application every six months is frequently not met for a number of reasons, with infrastructure, material and human resources availability and operational problems amongst the main problems.

Chagas, malaria and dengue are all endemic to the Chaco region of Bolivia. Due to this, the region has received vector control interventions based on dichlorodiphenyltrichloroethane (DDT), dieldrin and hexachloro-

Financial support: CONICET, Inesfly SL
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Received 22 April 2010
Accepted 6 July 2010

cyclohexane (HCH) since 1959, followed by pyrethroid treatments in the 1990s. More recently, the discovery of pyrethroid resistant *T. infestans* populations in the north of Argentina and southeastern Bolivia (Picollo et al. 2005, Catalá et al. 2007) led national control programs of both countries to use carbamate and organophosphate (OP) insecticides in several localities. The situation was identified as an important focus for research, because of its potential impact on the control of Chagas disease vectors (WHO 2005).

Since 1988, south and southwestern regions of the Bolivian Chaco have benefited from house improvement programs led by non-governmental organizations (NGO), oil companies and governmental agencies. Additionally, a series of field tests using a micro-encapsulated formulation of OP insecticides (developed by Inesfly, Spain) were carried out in the region of Camiri (Santa Cruz de la Sierra). The evaluation of several groups of houses by the departmental vector control programme showed a remarkable decrease in *T. infestans* prevalence among the treated communities (Dias & Jemmio 2008). The success of these interventions was highly appreciated by the homeowners and other communities of Guaraní, in Santa Cruz and Tarija departments asked for the treatment of their new houses with the Inesfly paint.

The effect of the Inesfly 5A IGR micro-encapsulated formulation, together with the Inesfly 5A IGR NG formulation based on pyrethroid active ingredients was experimentally studied under natural climatic conditions (Amelotti et al. 2009). Using different material supports, the authors reported 84% and 98% mortality of fourth-instar nymphs of *T. infestans* 18 months after application of the micro-encapsulated formulation.

The objectives of this study were (i) to evaluate the residual effect of Inesfly 5A IGR on the mortality of *T. infestans* in rural houses of the Bolivian Chaco and (ii) to evaluate the susceptibility to pyrethroids of the offspring of field collected *T. infestans* specimens. Although the treatment of houses was not planned for the present objective, the existing situation was used to design an observational study based on the time since the houses received the treatment with Inesfly 5A IGR.

MATERIALS AND METHODS

Study area - The study was carried out in October 2009 in houses of the Guaraní people in Ivamirapinta, municipality of Gutierrez (19°47'45.65"S, 63°34'24.79"W) and Pampa Redonda, municipality of Lagunillas (19°45'58.92"S, 63°33'41.26"W) of the Santa Cruz de la Sierra and in Zapaterambia, municipality of Entre Ríos (21°11'49.75"S, 63°47'21.69"W) of the Department of Tarija, towards the southeast of Bolivia (Fig. 1).

The area was sprayed with DDT and dieldrin by the National Malaria Eradication Service in the late 1950s and early 1960s. In the 1980s, the area was sprayed with HCH and during the 1990s with pyrethroids (lambdacyhalothrin WP 12.5% and deltamethrin SC 2.5 and 5%). In 2002 alphacypermethrin SC 20% was used. In areas with known resistance to pyrethroid, bendiocarb WP 80% was used during 2008.

Ivamirapinta has 194 houses belonging to Guaraní people, some of them recently built with adobe walls and very good adobe plastering that were treated with Inesfly 5A IGR by professional technicians up to 30 days before this study (September 2009). A few houses were relatively old, with adobe walls of poorer quality than the newer ones, including many cracks in the adobe plastering. These old houses were treated with the micro-encapsulated formulation in the same way as the new houses. Peridomestic structures were infrequent in this locality. All pets (dogs, cats) and domestic animals for food production (goats, pigs, fowls) were in general kept on the common grounds surrounding the houses of the community. The only observed peridomestic structures were grain deposits ("troja") and a few chicken coops. House treatment activity in this locality was carried out during this study and a number of houses were not treated at the time.

Pampa Redonda has 36 houses occupied by a mix of Criollo and Guaraní ethnic groups. House plastering was improved and painted with Inesfly 5A IGR between December 2006-July 2007 (between 27-34 months before the present study was carried out) by community volunteers under professional technical supervision. The community keeps free range domestic animals (chickens, goats, pigs) without enclosures.

Zapaterambia has 40 relatively new houses, with good adobe walls and well finished adobe plastering. Zapaterambia is a Guaraní community close to an oil company that has a neighboring drilling field. The oil company, the local government and a group of NGOs developed a community based project to build new houses in Zapaterambia, including treatment with the Inesfly micro-encapsulated formulation applied by professional technicians between 3-4 months before the present study. No peridomestic structures for domestic animal enclosure were present.

The product - Inesfly 5A IGR is a micro-encapsulated formulation containing OP active ingredients (chlorpyrifos 1.5% and diazinon 1.5%) and an insect growth

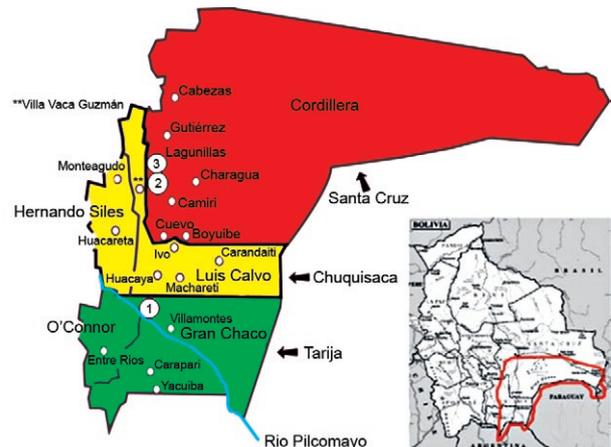


Fig. 1: map of the study area. Zapaterambia (1) (municipality of Entre Ríos) is located in the province of Gran Chaco (Tarija Department). Ivamirapinta (2) (municipality of Gutierrez) and Pampa Redonda (3) (municipality of Lagunillas) are located in the province of Cordillera (Santa Cruz de la Sierra Department).

regulator (pyriproxifen 0.063%) that mimics the action of a juvenile hormone. The formulation has an aqueous base with the active ingredients encapsulated within micro-spheres of vinyl VeoVa polymer chains. The active ingredients are slowly and progressively liberated, a process that extends the insecticide residual activity of the formulation, as shown by Amelotti et al. (2009). More details about Inesfly 5A IGR, such as physical properties, advantages over conventional insecticides and insecticidal paints, are detailed in Herrero (2009).

Each house was painted with the micro-encapsulated formulation using four-gallons Jacto HD400 hand held sprayers, with JD12P cone nozzles (delivering 0.162 gallons per minute at 15-65 psi). An average of 1000 cc of formulation was used to cover 10 square meters of internal walls and roofs, external walls and all peridomestic structures, where present. The product was applied as normal vinyl paint and presented no problems to obtain complete and smooth wall coverage. No particular treatment was used to treat wall or roof cracks (either adobe, wood logs or wooden sticks). After the intervention with the micro-encapsulated formulation, no additional vector control interventions were carried out in the three communities.

Wall bio-assays - This test was carried out to estimate *T. infestans* mortality produced by Inesfly 5A IGR applied at different times. Insects used in the bio-assays were fourth and fifth *T. infestans* nymphs collected in Ivamirapinta during the four days prior to the wall bio-assays. They were collected in peridomestic structures of Ivamirapinta houses that had not been treated with the insecticide formulation.

Ten houses were randomly selected within each of the three communities. One bedroom was randomly selected in each house to carry out the wall bio-assays. Two Petri dishes on different bedroom walls, containing 10 fourth and/or fifth-instar nymphs of *T. infestans* were attached to the walls, as described by Schofield (2001). The Petri dishes kept the insects in direct contact with the walls and remained attached between 12-16 h. After the contact period expired, Petri dishes were collected. Petri dishes with the same number of insects were allocated to the control group and were not attached to walls. All Petri dishes were kept at room temperature within an untreated house acting as a field laboratory. Nymph mortality was recorded 24, 48 and 72 h after wall contact.

Laboratory test of T. infestans susceptibility to pyrethroids - Eggs laid by *T. infestans* adult females collected in the peridomestic structures of Ivamirapinta were used to carry out pyrethroid susceptibility tests. The eggs were incubated in a rearing cabinet at a temperature of $28^{\circ} \pm 1^{\circ}\text{C}$, relative humidity of $50 \pm 2\%$ and a photoperiod of 12:12 h (L:D). *T. infestans* first-instar nymphs, 5-7 days of age, mean weight 1.3 ± 0.2 mg and unfed, were selected for pyrethroid susceptibility tests according to the WHO protocol (1994). The treatment consisted of topical application on the dorsal abdomen with 0.2 μL of technical grade deltamethrin[®] (Bayer), diluted in acetone at different concentrations (0.01, 0.1, 0.5 and 1 mg/mL), using a 10 μL Hamilton syringe. Nymphs of the control group were treated with 0.2 μL acetone alone. Three replicates, each with 10 first-instar nymphs, were assigned to each dose and the control groups. After 72 h, the mortality rates of control and treated insects were recorded according to the WHO protocol (1994).

Data analysis - Kruskal Wallis ANOVA was used to compare the nymph mortality between communities. An analysis of mortality variability was carried out using the frequency distribution of mortality between communities.

Ethics - The study received authorization from the Santa Cruz de la Sierra Ministry of Health. House owners received a complete explanation about the objective of the study, procedures for the wall bio-assays and safety matters. House owners gave verbal individual consent to participate in the study.

RESULTS

Wall bio-assays - Wall bio-assays showed that the OP and juvenile hormone analogue microencapsulated insecticides produced detectable mortality of insects exposed to walls treated up to 34 months prior to the study. Mortality showed no significant difference (96.5 and 81% in Ivamirapinta and Zapaterambia, respectively) up to six months post treatment, but had decreased after 34 months to 22% (in Pampa Redonda). All insects in the control group were alive after 72 h (Table).

The nymph mortality variability was different among bio-assays performed in houses with different post-application times of the formulation. Nymph mortality in houses treated one month before the bio-assay (Ivamirapinta)

TABLE
Mortality of fourth and fifth-instar nymphs of *Triatoma infestans* exposed to house walls treated with the micro-encapsulated formulation Inesfly 5A IGR

Locality	Time after insecticide application (months)	Replicates (houses) n	Exposed <i>T. infestans</i> n	<i>T. infestans</i> mortality % (CI 95)
Ivamirapinta	0-1	10	200	96.5 (92.9-98.6)
Zapaterambia	2-6	11	220	82.0 (76.1-86.7)
Pampa Redonda	27-34	10	200	22.0 (16.5-28.1)

exposure time varied between 12-16 h. CI 95: confidence interval for 95% significance.

was higher than 75% in 95% of the replicates, whereas 75% of the replicates in houses treated 34 months before the bio-assay (Pampa Redonda) showed 30% or less mortality. Nymph mortality in domiciles treated six months before the bio-assay (Zapaterambia) was high on average, although both replicates in one of the houses showed very low mortality (1 dead nymph out of 20).

Deltamethrin susceptibility tests - The topical application of deltamethrin in increasing doses showed that, even at the highest concentration, there were surviving nymphs after 72 h. The discriminant dose of deltamethrin (0.01 mg/mL) and the highest tested dose (1.0 mg/mL) showed 65 and 86% nymph mortality, respectively (Fig. 2).

DISCUSSION

Results shown by this study indicate that the micro-encapsulated insecticide formulation Inesfly 5A IGR produces a detectable effect on the mortality of *T. infestans*, even in a locality where the compound had been applied 34 months before the present assay was performed. The measured residual effect is several times longer than the reported residual effect of suspension concentrate formulations under similar environmental conditions against *T. infestans* (Gürtler et al. 2004, Rojas de Arias et al. 2004). The wall bio-assays gave similar results to the experimental ones carried out on different substrate materials (wood, cement and adobe) reported by Amelotti et al. (2009) and confirmed previous reports (Dias & Jemmio 2008). A similar micro-encapsulated formulation, using chlorpyrifos as active ingredient, produced a similar long lasting effect on *Anopheles gambiae* mortality when applied to experimental huts and bed nets (N'Guessan et al. 2010).

Houses in the studied communities are annually evaluated for infestation by *T. infestans* by field teams from the vector control programme of Santa Cruz de la Sierra and Tarija. Unpublished reports of these evalua-

tions indicate that none of the houses in the three studied communities were found infested after treatment in the intra or peridomestic structures.

The absence of house infestation by *T. infestans* is remarkable, especially in Pampa Redonda, where houses were treated 34 months prior to the present study. This case is remarkable because it is the first community that, although surrounded by nearby (untreated) localities with persistent *T. infestans* populations within houses, remains without detectable house infestation 34 months post treatment, according to the vector control programme of Santa Cruz de la Sierra. The wall bio-assays showed that the micro-encapsulated formulation produced 22% mortality of exposed *T. infestans* in Pampa Redonda 34 months post treatment. This implies that during the previous period, the formulation probably killed all triatomines in Pampa Redonda, leaving no residual population to repopulate, as is often the case in many localities throughout Argentina, Bolivia and Paraguay (Gorla et al. 2009, Gürtler 2009). Considering these results, Pampa Redonda is free of triatomines unless a reintroduction occurs in the future.

The other remarkable aspect of this vector control intervention is that *T. infestans* populations of the Ivamirapinta region have shown high resistance to deltamethrin in the susceptibility tests carried out in this study. Resistance to pyrethroid was detected in northern Argentina in 2001 and several studies have reported pyrethroid resistance in *T. infestans* populations since then, especially in the Bolivian Chaco (Picollo et al. 2005, Catalá et al. 2007). Resistance to insecticides in triatomines is not completely understood. In several cases, the phenomenon seems nearer to insecticide tolerance than insecticide resistance, in other words, not strictly a consequence of the repetitive use of insecticides in a particular area. Pyrethroid resistance in triatomines shows a patchy spatial distribution, not necessarily associated with the intensity of pyrethroid use for triatomine control, as shown by Sonoda et al. (2009). Although there are no previous reports concerning *T. infestans* pyrethroid resistant populations in the studied region, this work indicates that it is effectively a deltamethrin resistant area. The long residual effect of the micro-encapsulated formulation against deltamethrin resistant *T. infestans* populations makes the studied product a useful candidate for the elimination of intradomestic and peridomestic populations of *T. infestans* in the area.

House owners of the studied communities showed satisfaction with the micro-encapsulated formulation, either because no triatomines, house flies, mosquitoes and spiders were present in the houses during a long period (more than 2 years according to some members of the communities) and/or because it gave the houses an aesthetic aspect that pleased the community (Dias & Jemmio 2008).

ACKNOWLEDGEMENTS

To the Chagas Programme of Santa Cruz de la Sierra and to Jorge Llanos Espejo and Eddy Franz Espejo Lazarte, for the field support.

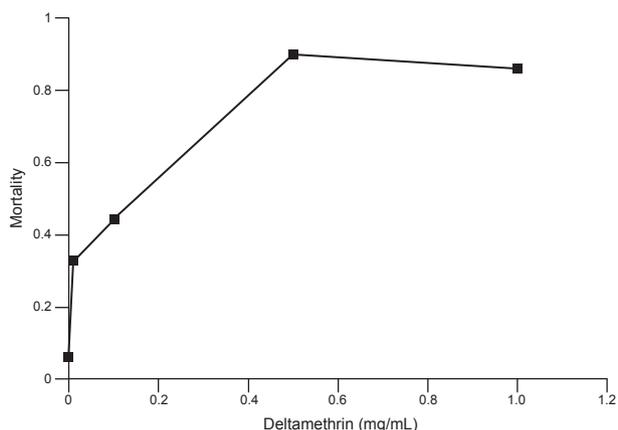


Fig. 2: survival of first instar nymphs of *Triatoma infestans* collected in Ivamirapinta (Santa Cruz de la Sierra, Bolivia), after 72 h of topical application of increasing concentrations of deltamethrin [0.01 mg/mL is the susceptible discriminant doses according to World Health Organization (1994)]. Each concentration is plotted with mean and standard deviation.

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