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Triatomicidal effect of new spot-on formulations applied to poultry in semi-field conditions

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Abstract Chagas disease is an endemic disease affecting ten million people in the American continent. Produced by a parasite transmitted by triatomine insects, the main actions for reducing the incidence of this disease are focused on the control of insect vectors. This type of control has produced highly effective results within rural homes, but not in peridomestic areas (kitchens, warehouses, hen houses and other buildings not attached to the houses). The object of the present study was to assess the triatomicidal effect of new spot-on formulations developed by our laboratory in a semi-rural environment. The active ingredients of the formulations were βcypermethrin, pyriproxyfen, or β -cypermethrin + pyriproxyfen. All formulations were applied to hens and tested in miniature replicas of rural households where experimental populations of Triatoma infestans, the main vector of Chagas disease in Argentina, had been previously released. The experimental populations exposed to formulations containing β -cypermethrin or β cypermethrin + pyriproxyfen were noticeably reduced compared to non-treated control groups. However, no differences were observed between the effects produced by β -cypermethrin alone and β-cypermethrin + pyriproxyfen. Pyriproxyfen alone

Introduction

Chagas disease, considered the most severe parasitic disease of the American continent (World Bank 1993), affects around ten million people while another 100 million people live in endemic areas and are in risk of infection (Organización Panamericana de la Salud 2006). Chagas disease is

produced no significant reduction in the experimental

populations of T. infestans. These results suggest that

spot-on application of β-cypermethrin could be a useful

complementary tool for controlling triatomine insects in

the peridomestic areas of rural homes.

around ten million people while another 100 million people live in endemic areas and are in risk of infection (Organización Panamericana de la Salud 2006). Chagas disease is produced by *Trypanosoma cruzi*, a protozoan transmitted by blood transfusions or congenitally (Schofield 1994). Chagas vector insects are haematophagous bugs of the Triatominae subfamily.

There is no vaccine against Chagas disease, and the available drugs do not fulfil the requirements to be considered "good medicines" according to the World Health Organization (Rodrigues Coura 2009). Actions for reducing the incidence of the disease are mainly focused on the chemical control of insect vectors and preventing infected persons from donating blood (Zerba 1989; Moncayo 1999). International initiatives launched in South America towards the end of the last century produced a considerable reduction in Chagas disease incidence, and its transmission was in fact interrupted in Uruguay, Chile and Brazil (Dias et al. 2002; Schofield et al. 2006). However, the disease is still a serious problem in Bolivia, Paraguay and some provinces of Argentina.

In rural areas, the group of rooms and galleries covered by the same roof is considered the house (Cécere et al. 1996). The peridomestic area includes other non-attached buildings (kitchens, tool sheds, hen houses and goat pens).

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D. Canale Coordinación Nacional de Control de Vectores, Córdoba, Argentina *Triatoma infestans*, the main vector of Chagas disease in Argentina and limiting countries, has adapted to living in both rural houses and the surrounding peridomestic area (Canale and Carcavallo 1985).

As previously demonstrated, the use of pyrethroid insecticides is the best way of eliminating *T. infestans* populations within houses (Zerba 1999a, b; Gürtler et al. 2007; Vázquez-Prokopec et al. 2009). However, the treatment of peridomestic areas is usually less successful (Abad-Franch et al. 2010). Good insecticide coverage is difficult to attain in some peridomestic structures due to the building materials used (branches, for example); treated surfaces are more easily covered by dust and animal excrement, and the insecticides are directly exposed to environmental factors that accelerate their degradation (mainly sunlight).

Additionally, peridomestic areas generally host larger-sized populations of *T. infestans* and can provide refuge for the insects that survive house treatments (Cécere et al. 2004; Porcasi et al. 2007). This increases the risk of house infection when control activities are interrupted (Cécere et al. 1996, 2004). Even a low number of peridomestic insects are enough for the transmission of *T. cruzi* to resurface (Gürtler et al. 2007). Therefore, the treatment of peridomestic areas is an unavoidable activity in any program for controlling *T. infestans*.

As peridomiciliary populations of *T. infestans* feed on the blood of the dogs and poultry they live with, several studies have recently assessed the application of insecticides on these animals. Exposure to dogs using collars treated with deltamethrin reduced blood intake (Reithinger et al. 2005) and annihilated experimental populations of *T. infestans* under semi-field conditions (Reithinger et al. 2006). High mortality and a reduction in blood intake and nymph moulting rate were also observed in *T. infestans* bugs fed on hens treated with pour-on cypermethrin under laboratory conditions (Amelotti et al. 2009).

Our laboratory recently developed a spot-on formulation constituted by a polyvinyl acetate, turpentine, soya oil methyl ester matrix and chlorpyrifos insecticide (20 %), which showed very good performance for controlling bovine ectoparasites (Juan et al. 2010). Taking into account the fact that poultry is the main source of food for this insect (Cécere et al. 1996, 1997), we studied the effect of hens treated with spot-on β-cypermethrin, pyriproxyfen or β-cypermethrin + pyriproxyfen on exposed *T. infestans* nymphs and adults. β-Cypermethrin is a pyrethroid insecticide widely used in Argentina for controlling *T. infestans* (Zerba et al. 1997; Wood et al. 1999; Zerba 1999a, b). Pyriproxyfen is an insect growth regulator that mimics the activity of the juvenile hormone, and is recommended for controlling different species of Homoptera, Diptera and Coleoptera (Thacker 2002). It is commercialised alone or in combination with other insecticides as collars or spot-on formulations for controlling lice in cats and dogs (Rust 2005; Dryden et al. 2011).

Materials and methods

Biological material

We used *T. infestans* individuals from a susceptible colony maintained by the Servicio Nacional de Chagas de Argentina (Santa María de Punilla, provincia de Córdoba) at 26–28 °C and 50–70 % relative humidity. The insects were starved for 15 days before starting the experiments.

Insecticide formulations were applied to commercial semi-heavy laying hens with an average weight of 3 kg each, fed on balanced layer food and drinking water. During the experiment, hens were housed in wooden roofed 150×150 -cm pens.

Activities involving animals were performed according to a protocol approved by an ad hoc Committee from the Centro de Investigaciones de Plagas e Insecticidas (CIPEIN-UNIDEF/CONICET).

Chemicals

We used β-cypermethrin (99.4 %) donated by Chemotecnica S. A. (Spegazzini, Argentina) and pyriproxyfen (97.8 %; China Kelinon Agrochemical Co., Ltd.; Shanghai, China).

The matrix of the experimental formulations was composed of Elvax® 220 ethylene vinyl acetate resin in the form of pellets (DuPont Packaging and Industrial Polymers, Buenos Aires, Argentina), soya oil methyl ester (Ferar Química, Buenos Aires, Argentina) and turpentine (Química Oeste, Buenos Aires, Argentina).

The concentrations of the active ingredients used in the formulations for the hen treatments were β -cypermethrin (10 % w/v), pyriproxyfen (3 % w/v) and β -cypermethrin (10 % w/v) + pyriproxyfen (3 % w/v).

Experimental huts

Experiments were carried out in experimental huts built specifically for this purpose in a 35-m² terrain belonging to the Estación de Campo del Programa Nacional Argentino de Control de Vectores de la Provincia de Córdoba (31°28′S, 64°45′W). Before building the hen huts, the area was weeded and levelled.

Huts of $1 \times 1 \times 1$ m were built by layering adobe bricks (Fig. 1). An opening of 1 m (high) \times 30 cm (width) was left on one of the sides. The roof was built using *Larrea divaricata* bush branches layered on 1.3×1.3 -m wooden frames. A black plastic cover was placed on top of the branches to protect the hut from the weather. Earth was layered on top to keep the plastic in place and to maintain a comfortable temperature inside.

To prevent insects from escaping, each hut was surrounded by a "cage" built with a Teflon mosquito-type



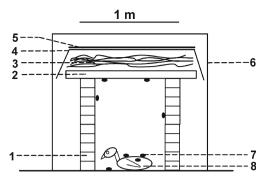


Fig. 1 Experimental hut used in bioassays. *1*, Adobe brick; *2*, wooden frame; *3*, *Larrea divaricata* bush branches; *4*, black plastic cover; *5*, earth; *6*, Teflon mosquito-type netting held by an aluminium structure; *7*, triatomine; *8*, hen

netting held by an aluminium structure. A guillotine door was placed on one of the sides for putting in and taking out the hens. The cage bases were buried 8 cm underneath the ground, and earth was layered around it to prevent insects escaping.

Hen treatment

Formulations were applied using 2.5-ml Prexajet® syringes without needles (Pi-Ro S.A., Buenos Aires, Argentina). Each hen was treated with 1.5-ml formulation applied on the skin as follows: 0.5 ml in the dorsal area of the neck and 0.5 ml in each axilla. In the first experiment, hens were randomly distributed into three groups and subjected to the following treatments: Group A, β-cypermethrin (10 %) formulation; Group B, β-cypermethrin (10 %) + pyriproxyfen (3 %) formulation; Group C no treatment (control). In other experiments, each hen was treated with 1.5 or 3 ml of pyriproxyfen (3 %). Hens belonging to each group were identified with coloured tags on a leg. Finally, each group of hens was placed in a different hen hut to avoid any possible contamination between them. Hen houses were separated by a distance of 10 m.

Bioassays

In the first experiment, nine experimental huts were used, three for each treatment and three for the control. At 07:00 p.m. of day 1, 30 *T. infestans* fifth instar nymphs were places in each hut. Twenty-four hours later, the treated and control hens were placed in the respective huts and removed after 12 h. From then on, the hens were placed back into the huts for 12 h every 3 days until the end of the experiment. The last day, each hut was completely dismantled to collect all the eggs and insects it contained. The number of eggs and insects in each stage were then counted, and the weight of the latter was determined using a digital scale SF-700 Sinoscale (Jiang Su, China). This

experiment began on February 3 and ended on April 27, 2011. During this period, the temperature in the area where the experiment was performed varied between 6 °C, 7 °C and 33.8 °C (mean, 20.4 ± 0.3); the mean RH was 64.9 ± 1.4 %; and 232.7 mm of rain fell.

In other experiments, with formulations containing only pyriproxyfen as active ingredient, six experimental huts were used: three for the treatment (1.5 or 3 ml of formulation/hen) and three for the respective control. These experiments began on October 27, 2011 and ended on January 3, 2012. During this period, the temperature in the area where the experiments were performed varied between 33 °C and 19 °C (mean, 25.1 ± 3.4); the mean RH was 60.2 ± 13.4 %; and 432.7 mm of rain fell.

Weather information was provided by the Servicio Meteorológico Nacional from Argentina.

Statistical analysis

All the results were analysed with one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test when significant differences were detected.

Results

In the first experiment, populations of *T. infestans* were periodically exposed during 84 days to hens treated with a formulation containing β -cypermethrin (10 %) alone, β -cypermethrin (10 %) + pyriproxyfen or non-treated hens (control). At the end of the experiment, the average size of the experimental populations varied significantly according to the treatment (ANOVA, P=0.002; Fig. 2). The average population size in control huts was 30 individuals. The average size of populations exposed to β -cypermethrin (10 %) alone was 12 individuals, significantly less than the controls (Fisher's LSD, P=0.001). The average size for the population exposed to the β -cypermethrin

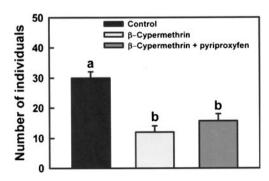


Fig. 2 Size of experimental populations of *Triatoma* infestans exposed to hens treated with spot-on β-cypermethrin, β-cypermethrin + pyriproxyfen or untreated (controls). *Vertical lines* are SE. *Different letters* indicate significant differences (Fisher's LSD; P < 0.05)



(10 %)+pyriproxyfen formulation was 15.7 individuals, also significantly less than the controls (Fisher's LSD, P=0.003). There was no significant difference between both formulations (Fisher's LSD, P=0.28).

Figure 3 shows the composition per stage for each of the experimental populations. The average number of fifth instar nymphs were 14.3 (control), 8.7 (β -cypermethrin) and 11.7 (β -cypermethrin + pyriproxyfen). There were no significant differences between these values (ANOVA, P=0.06).

The average number of adults varied significantly according to the treatment (ANOVA, P<0.001). An average of 13 adults was found in the control populations, while 3.3 adults were found in the population treated with β -cypermethrin alone and 3.7 in the population treated with β -cypermethrin + pyriproxyfen. These last two values were significantly different from the controls (Fisher's LSD, P<0.001), but not significantly different between each other (Fisher's LSD, P=0.81).

The average weight of nymphs and adults also varied significantly (ANOVA, P<0.01; Fig. 4). The final average weights of fifth instar nymphs were 0.35 mg (control), 0.20 mg (β -cypermethrin alone) and 0.25 mg (β -cypermethrin + pyriproxyfen). Significant differences were observed between the control and treated populations (Fisher's LSD, P<0.001), but no significant differences were found between the treatments.

The average weights of adults were also significantly different (ANOVA, P<0.001): 0.35 mg (control), 0.20 mg (β -cypermethrin alone) and 0.22 mg (β -cypermethrin + pyriproxyfen). Differences between the control and treated populations were significant (Fisher's LSD, P<0.001), but no significant differences were observed between the treatments (Fisher's LSD, P=0.57).

Figure 5 shows the average number of eggs laid by the experimental populations. Controls laid 47.7 eggs and populations exposed to β -cypermethrin 1.3 eggs. The difference

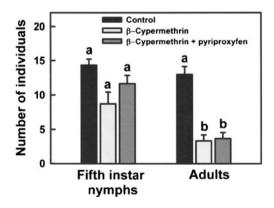


Fig. 3 Number of fifth instar nymphs and adults of *Triatoma* infestans exposed to hens treated with spot-on β-cypermethrin, β-cypermethrin + pyriproxyfen or untreated (controls). *Vertical lines* are SE. In each group of bars, *different letters* indicate significant differences (Fisher's LSD; P<0.05)

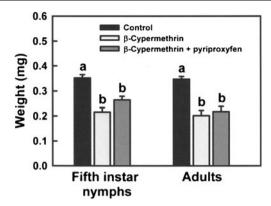


Fig. 4 Weight of fifth instar nymphs and adults of *Triatoma* infestans exposed to hens treated with spot-on β-cypermethrin, β-cypermethrin + pyriproxyfen or untreated (controls). *Vertical lines* are SE. In each group of bars, *different letters* indicate significant differences (Fisher's LSD; P<0.05)

between both these values was significant (ANOVA, P= 0.04). Populations exposed to β -cypermethrin + pyriproxyfen did not lay eggs and hence were not included in the ANOVA.

As the inclusion of pyriproxyfen in the spot-on formulation with β -cypermethrin showed no improvement in the control performance when compared to the formulation with β -cypermethrin alone, semi-field trials using only pyriproxyfen as the active ingredient were performed.

Populations of *T. infestans* were periodically exposed during 70 days to hens treated with 1.5 ml of a formulation containing pyriproxyfen (3 %) or non-treated hens (control). Later, a similar experience was performed but with hens treated with 3 ml of a formulation containing pyriproxyfen (3 %) or non-treated hens (control).

Figure 6 shows the number of fifth instar nymphs and adult insects for each of the experimental populations treated with pyriproxyfen formulations. The average number of fifth instar nymphs and adults showed no significant differences between the control and treated populations (ANOVA, P=0.422 and P=0.854,

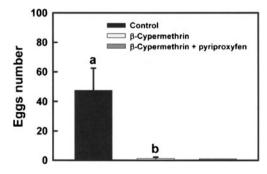
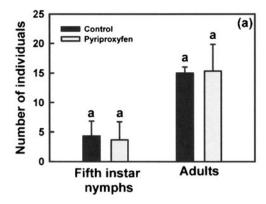


Fig. 5 Number of eggs laid by *Triatoma* infestans exposed to hens treated with spot-on β-cypermethrin, β-cypermethrin + pyriproxyfen or untreated (controls). *Vertical lines* are SE. *Different letters* indicate significant differences (Fisher's LSD; P<0.05)





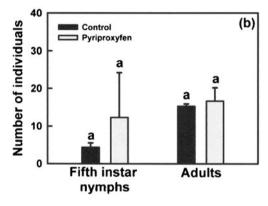


Fig. 6 Number of fifth instar nymphs and adults of *Triatoma* infestans exposed to hens treated with 1.5 ml (a) or 3 ml (b) of spot-on pyriproxyfen. *Vertical lines* are SE. In each group of bars, *same letters* indicate no significant differences (Fisher's LSD; *P*>0.05)

respectively). The average weights of fifth instar nymphs and adults varied in a similar way in control and treated populations (results are not shown).

Figure 7 shows the average number of eggs laid by the experimental populations exposed to hens treated with pyriproxyfen formulations. The difference between the number of eggs laid by the populations treated with both doses of pyriproxyfen and the control were not significant (ANOVA, P=0.316).

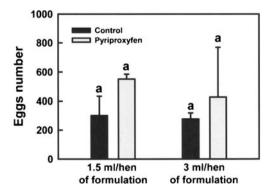


Fig. 7 Number of eggs laid by *Triatoma* infestans exposed to hens treated with spot-on pyriproxyfen. *Vertical lines* are SE. *Same letters* indicate no significant differences (Fisher's LSD; *P*>0.05)

Discussion

There are various commercially available spot-on formulations for controlling ectoparasites in domestic animals (Kahn et al. 2005). Apart from the active ingredient, these products contain a solvent and a dispersal agent. A highly viscous spot-on formulation was developed by our laboratory, whose active ingredient is the organophosphate insecticide chlorpyrifos. Evaluated in semi-field and field experiments on cattle, this product proved to be as effective as ear tags for controlling *Haematobia irritans* flies (Juan et al. 2010). In the present study, we assessed the triatomicidal effect of two formulations based on the same matrix used for the studies on *H. irritans*, but using β -cypermethrin (10 %) or β -cypermethrin (10 %) and pyriproxyfen (3 %) as active ingredients. These new formulations were applied to hens to evaluate their potential as triatomicidal agents.

The active ingredients contained in spot-on formulations spread over the skin of treated animals or penetrate it entering the organism. In the latter case, they act systemically as they are directed against haematophagous ectoparasites. In mammals, the bioavailability of active ingredients is affected by its dissolution in the subcutaneous fatty layer covering the animal's body and by its accumulation in pilosebaceous units followed by a subsequent sustained release (Brayden 2003; Kahn et al. 2005). Comparatively, hens have a much thinner and drier skin (Young 1977). They also lack sweat glands, but have a uropygial gland near the base of their tail that produces oily secretions. Hens distribute these secretions over their feathers by rubbing their beak and head against the gland and then rubbing the accumulated oil over the rest of their body.

In laboratory experiments, Amelotti et al. (2009, 2010) exposed *T. infestans* nymphs to hens treated with pour-on cypermethrin. Experimental populations showed a higher mortality rate and lower blood intake and nymph moulting rates compared to the controls.

In the present study, the total *T. infestans* population size, following 84 days of periodical exposure to hens treated with the formulations containing β -cypermethrin alone or β-cypermethrin + pyriproxyfen, was significantly less than in the control groups. Moreover, populations exposed to treated hens had less weight gain, reduced moulting rates and laid less eggs than the controls. Analysing the insect stages separately, we found that the number of fifth instar nymphs was similar in all the populations, but the number of adults was considerably less in the populations exposed to the formulations. This could be due to a differential toxicity of pyrethroids, with fifth instar nymphs being more tolerant to the insecticide than adults. Likewise, it has previously been observed that treated nymphs showing no signs of intoxication are then affected and die after moulting to adults (Zerba et al. 1989).

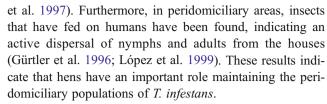


Taking into account that weight gain, moulting and egg production in triatomines critically depend on blood intake (Montenegro 1989; Galvao et al. 1995); the observed effects could be attributed to the fact that the insects that survived the exposure to treated hens fed less than the controls. This effect could be due to the repellent or antifeedant effects of β-cypermethrin. Fabrics treated with β-cypermethrin produced a strong repellent effect in first instar T. infestans nymphs (Wood et al. 1999). The only evidence of a possible antifeedant effect produced by pyrethroids in T. infestans was described by Amelotti et al. (2009, 2010), who showed a lower rate of blood intake in the insect populations exposed to hens treated with cypermethrin. Reports on other insects have also shown that exposure to cypermethrin decreased food intake in the Lepidoptera Heliothis punctigera (Bayley and Comery 1987), the Coleoptera Pieris brassicae (Tan 1981), Epilachna varivestis (Dobrin and Hammond 1985) and *Phaedon cochleariae* (Hajjar and Ford 1989), and the Hymenoptera Pimpla turoniellae (Sak et al. 2006).

The addition of pyriproxyfen to the studied formulation was intended to test the hypothesis that the presence of this insect growth regulator would increase the effects of βcypermethrin. As no differences were observed between the effect on *T. infestans* populations exposed to hens treated with β -cypermethrin alone or β -cypermethrin + pyriproxyfen, the effect of pyriproxyfen alone was also evaluated under the same conditions. The results of the experimental populations of fifth instar nymphs and adults *T. infestans* exposed to hens treated with the spot-on formulation of pyriproxyfen in the semi-field trials showed that it had no significant effect on the moulting of exposed fifth instar nymphs nor on the number of eggs laid by adult insects. This was an unexpected result because pyriproxyfen is a potent juvenile hormone mimic affecting hormonal balance in different insect species; it produces a strong suppression of embryogenesis, metamorphosis and adult formation (Ishaaya and Horowitz 1998).

An analysis of the intestinal content of 720 insects in central Argentina (province of Córdoba) revealed that 48.9 % had fed on dog blood and 34.8 % on hen blood, while only 11.9 % had ingested human blood (Wisnivesky-Colli et al. 1982). Another study carried out in northeast Argentina on 1,964 insects found that dogs were the main source of non-human food towards the end of winter (39 %), while hens were the main source at the end of summer (54 %; Gürtler et al. 1996). Similarly, a study carried out in 25 peridomestic structures of the central endemic area of Argentina (provinces of Santiago del Estero and Córdoba) showed that 87 % of the insects collected had fed on hens (López et al. 1999).

There is evidence that the domestic density of *T. infestans* increases linearly with the percentage of insects feeding on hens, and in peridomiciliary areas, the degree of infestation is positively correlated with the amount of poultry (Cécere



Our results suggest that the application of spot-on formulation containing β -cypermethrin might be an interesting complementary tool to other recommended actions for controlling triatomines in the peridomiciliary areas of rural homes. These actions must include environmental modifications for reducing the number of insect refuges, the use of insecticides formulations on poultry houses that are less degradable by environmental factors, pens and other peridomiciliary constructions and early detection of infestation hot spots (Cécere et al. 1996).

It is important to evaluate the β -cypermethrin spot-on formulation in field conditions to evaluate whether the present study is valid under real conditions with an interaction between domestic animals and T. *infestans*. Another important issue to assess is the toxicological risk for humans using hens, or their eggs, that have been treated with this formulation for food. Pyriproxyfen and β -cypermethrin are safe active ingredients for veterinary use (Pesticide Properties Data 2011a, b). Both compounds were studied on domestic animals treatments (Pap et al. 1997; Stanneck et al. 2003); however, neither the experimental animals nor their products (eggs and milk) were used as human and/or animal feeds.

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