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Effects of topical application of fipronil spot-on on dogs against the Chagas disease vector *Triatoma infestans*

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Summary

We assessed the insecticidal effects of fipronil spot-on applied to experimental dogs on the blood-feeding success and other vital parameters of the *Trypanosoma cruzi* vector *Triatoma infestans*. In the first trial, the cumulative mortality of 30 third or fourth instar nymphs exposed to eight fipronil-treated dogs differed significantly from those exposed to untreated dogs at 1 week post-treatment, but not at baseline or at 2–6 weeks post-treatment. In the second trial, the effects of multiple exposures to fipronil-treated dogs on bug population dynamics were assessed. A population of 80–84 bugs of various life stages were allowed to colonize eight closed experimental huts, and then exposed twice weekly to control or treated dogs over a period of 110 days and censused at monthly intervals. Throughout the trial, multiple exposure to fipronil did not significantly affect bug population size, fecundity, hatching, molting, survival, blood-feeding success and degree of engorgement. Only when engorgement was taken to include only fully fed bugs, did fipronil significantly reduce their degree of engorgement relative to bugs exposed to control dogs. We conclude that at tested dosages fipronil spot-on would have little effect in controlling (peri)domestic *Tri. infestans* or protecting dogs from contact with the bugs.

Keywords

Chagas disease; *Triatoma infestans*; Insecticides; Fipronil; Dogs; Disease vectors

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1. Introduction

Prevention of transmission of *Trypanosoma cruzi*, the causal agent of Chagas disease, has relied on residual spraying of households with insecticides to eliminate triatomine bugs and on screening of blood donors. A series of intergovernmental initiatives dramatically reduced the prevalence and incidence of *Tryp. cruzi* infections in a number of Latin American countries.¹ Nevertheless, some endemic regions (e.g. Gran Chaco, Argentina) are currently experiencing renewed transmission to humans 3–5 years after cessation of control activities as recurrent domestic recolonization by triatomine bugs occurs.² For such areas, simple, safe and effective control tools that can be used by the affected communities for the extended surveillance phase, after insecticide spraying campaigns have been completed, are urgently required.³

Domestic dogs, cats, goats and chickens play a key role in the eco-epidemiology of Chagas disease as frequent sources of blood meal for triatomine bugs, and dogs and cats are major domestic reservoirs of *Tryp. cruzi*.^{2,4,5} Mathematical modeling predicts that elimination of infected dogs from a household with infected people could be sufficient to almost extinguish transmission of *Tryp. cruzi*, barring reintroduction of infected dogs or bugs.⁶ For practical reasons and due to lack of adequate tools, Chagas disease control programs have not considered targeting infected domestic animal reservoirs through culling or treatment with the available anti-*Tryp. cruzi* drugs. Yet, treating domestic dogs with deltamethrin-impregnated collars significantly reduced the feeding success of *Triatoma infestans* and eliminated infestations in closed experimental huts.^{7,8} The recent emergence of pyrethroid resistance in *Tri. infestans* in northern Argentina and Bolivia,⁹ combined with the low effectiveness of residual spraying with pyrethroid insecticides in peridomestic structures,¹⁰ promoted the search for cost-effective alternative tactics. One possible approach is the application of powder or systemic formulations to the domestic animals themselves (xenointoxication), which then would act as baited lethal traps.¹¹

One candidate for xenointoxication is fipronil, a phenylpyrazole insecticide that is highly effective against a broad range of hematophagous arthropods. Fipronil exerts no adverse effects on dogs, cats, goats and people at the recommended low doses, but shows low toxicity to waterfowl and is highly toxic to fish, lizards and gallinaceous birds.¹² Fipronil has a slow lethal activity on triatomine bugs as a spray formulation, and residual effects on mud blocks may persist for up to 3 months post-treatment.¹³ A topical formulation of 1% fipronil pour-on in a single dose at 1 mg/kg on a dog, a cat, and a chicken against six third-instar nymphs of *Tri. infestans* exposed to each host was estimated to be 100% and 89% effective at 7 and 30 days post-treatment, respectively.¹⁴ In the same study, a topical application of fipronil pour-on on four dogs, one cat and two chickens residing in an infested rural house was considered to have reduced peridomestic bug densities by 65% (relative to pre-treatment bug densities) at 30 days post-treatment at the end of fall (i.e. June 2003).¹⁴ Both studies have some caveats because they (i) were based on a before-and-after study design, (ii) had a very small sample size, (iii) lacked replicates and (iv) did not include negative controls (i.e. untreated infested compounds). Despite the concerns expressed and limited evidence,¹⁴ provincial vector control programs in Salta and Santiago del Estero (Argentina) started to treat dogs with fipronil pour-on with the expectation of eliminating (peri)domestic bug infestations. In this study we assessed the effects of fipronil spot-on on the vital parameters of *Tri. infestans* when the bugs were exposed once or repeatedly to the dogs.

2. Materials and methods

2.1. Test insecticide

The test insecticide was fipronil spot-on (Frontline Plus, 10% (w/v) fipronil, 9% (*S*)-methopren; Merial Argentina, Martínez, Buenos Aires, Argentina; lot 003/05; manufactured May 2005, expiry date May 2007). Fipronil spot-on for dogs also includes the insect growth regulator methoprene, which interferes with development in insects, including triatomine bugs.¹⁵ According to the manufacturer, a topical application of fipronil is effective against ticks and fleas for up to one month post-application. Fipronil was administered with a pipette provided by the manufacturer (up to 10 kg of body weight, pipette size was 0.67 ml; 10–20 kg, 1.34 ml) by parting the dogs' hair on the back midline following manufacturer's instructions.

2.2. Study site and protocol

The trials were carried out in the field station run by the Argentinian National Vector Control Program in Punilla, Province of Córdoba (31°14'S, 64°28'W) between 24 August and 5 December 2006. Study location, design of experimental huts and experimental set-up have been comprehensively described previously.^{7,8,16} A total of 25 small experimental huts (80 cm × 80 cm × 80 cm with a 40 cm-wide entrance) simulating typical mud-and-thatch houses are kept for research purposes in a 50 m² fenced compound under natural climatic conditions. Hut internal structure is such that bugs cannot escape when released inside the huts. Weather conditions during each bioassay were measured using data loggers (HOBO H08; Onset, Bourne, MA, USA).

Eight adult mongrel dogs (6–18 kg) were used in the trial. All dogs had been exposed to *Tri. infestans* and had worn deltamethrin-impregnated collars for a 3-month period up to September 2005.⁸ When *Tri. infestans* bugs were exposed to dogs that previously wore insecticide-impregnated collars in early and mid-2006, they engorged and survived normally, thereby indicating that no insecticide residual effects persisted. Treated and untreated dogs were kept in separate kennels made of chicken wire and a roof, approximately 10 m apart within a fenced compound, and fed twice daily. For the multiple-exposure test (see below), each animal was stationed individually inside a specific experimental hut twice a week, and then released every morning into its specific area within the compound, with no restrictions regarding exposure to rain or movement. The bugs used in this study had been reared on chickens and had been starved for 2–3 weeks prior exposure to the dogs.

Two trials were conducted. The first (single-exposure) trial included a single application of fipronil spot-on to five randomly chosen dogs at day 0 (treated group) and no application of fipronil to three randomly chosen dogs (controls). Each dog was exposed to groups of 30 *Tri. infestans* (15 third-instars and 15 fourth-instars) just before treatment and at weekly intervals for a total of 6 weeks post-treatment. All dogs were exposed to bugs at the same time points to adjust for any background changes in bug feeding and survival over time. The bugs were allocated in groups of 10 in plastic boxes (6 cm in diameter and 8 cm long) applied directly on the fur of each dog for 30 min. After exposure, the average degree of engorgement of each group was checked; a few times the degree achieved was less than medium fed, and therefore a new exposure on the dog's belly followed. The study bugs were then kept in the insectary at 27 °C and 50% relative humidity without subsequent blood-feeding, and their survival was monitored at weekly intervals over 8 weeks.

The second (multiple-exposure) trial aimed at measuring the effects of a single topical application of fipronil spot-on on the vital parameters of *Tri. infestans* populations exposed repeatedly to a fixed dog in a fixed closed hut every 3–4 nights⁸. For this trial we used the

same three untreated control dogs and the five treated dogs as in the single-exposure trial. Each bug population in a hut was founded by 80–84 color-marked *Tri. infestans* (at least 20 females, 20 males, 20 fourth- and 20 fifth-instar nymphs in each hut). In total, 240 and 405 bugs were released into the experimental huts housing control and treated dogs, respectively. Huts were dismantled approximately at monthly intervals and bugs censused as described previously.¹⁶ At every census, founder bugs that were not found alive or dead or had not molted to the next instar (as indicated by the occurrence of unmarked fifth-instars or adults or marked exuviae) were considered 'lost' (i.e. absence of a bug cadaver) given the closed hut set-up. All live bugs and unhatched eggs were returned to their source experimental hut two days after bug collection.

The nutritional status of live bugs was assessed semi-qualitatively by a subjective classification of the size of the bug abdomen; bugs were scored as not fed, little-fed, medium-fed, and fully fed.^{8,17} The proportion of blood-fed bugs is defined as the sum of bugs little-fed, medium-fed and fully fed relative to the total number of bugs examined for nutritional status. The proportion of engorged bugs is the number of medium-fed or fully fed bugs relative to the total number of blood-fed bugs. The fecundity rate at time t (i.e. the observed number of eggs at time t per live female-day between time t and $t + 1$) was calculated as the total number of all eggs collected (hatched or unhatched) at time t relative to the product of the mean number of live female bugs between time t and $t + 1$ and the time period (in days) between two successive censuses. For the first census and for the newly emerged females, we discounted fecundity estimates by 25% to allow for the pre-reproductive period after adult emergence.¹⁶ Hatching rates at time t were calculated relative to total numbers of eggs laid at $t - 1$. In view of the results at the third census, the trial was terminated at 110 days post-treatment of dogs.

2.3. Statistical analysis

Generalized linear models in Stata 9.0 (Stata Corp., College Station, TX, USA) were used to test for significant ($P < 0.05$) effects of fipronil on bug feeding success, engorgement, hatching, molting and survival in comparison to negative controls. We used analysis of deviance, specifying binomial errors, of the log odds that the bugs' vital event was reduced as a result of fipronil.⁸ Poisson regressions were used to test for effects of fipronil on fecundity (adjusted for the mean number of females present between t and $t - 1$), and bug abundance by stage after pooling first to third-instar nymphs. All analyses were clustered by dog and provided robust standard errors. Odds ratios (OR) for logistic regressions or incidence rate ratios (IRR) for Poisson regressions were adjusted for time effects and the interaction between treatment and bug life stage. A survival analysis based on count-time was carried out to estimate whether the survival curves of bugs exposed to fipronil-treated or control dogs differed.

3. Results

3.1. Single exposure trial

In the single-exposure trial, a total of 1680 bugs was allowed to feed on the dogs. Prior to topical treatment with fipronil (i.e. at time/day 0), cumulative mean bug mortality over the subsequent 8-week observation period did not differ significantly ($P = 0.25$) between treated (4.0%) and control dogs (4.4%). Cumulative mean bug mortality after exposure differed significantly only at 1 week after treatment ($P = 0.015$), when mortality increased to 18.7% for fipronil-treated dogs but remained at 4.4% for control dogs (Figure 1). Bugs exposed to treated dogs at 2 weeks post-treatment succeeded in molting. No dog showed visible side-effects from treatment with fipronil spot-on.

3.2. Multiple exposure trial

In the multiple-exposure trial (Figure 2), the time trends of mean bug abundance by life stage in huts housing treated and control dogs were nearly indistinguishable (excluding eggs, IRR = 0.94; 95% CI 0.85–1.03). Mean ambient temperature at sunset was 21.4 °C (SD 4.7 °C) and gradually increased from 18.7 °C (SD 3.6 °C) to 23.9 °C (SD 4.3 °C). All populations of *Tri. infestans* increased in numbers at a very similar rate. The mean total production of eggs per hut with a treated dog increased sharply from 10 to 827 eggs between 30 and 110 days post-treatment, respectively, and from 7 to 751 eggs per hut with a control dog (Figure 2).

The mean fecundity of *Tri. infestans* was not significantly different in huts with treated and control dogs (Figure 3) (IRR = 1.42; 95% CI 0.47–4.30). In control huts, mean fecundity rates increased from 4.9 (SD 4.9) at 30 days post-treatment to 107.1 (SD 8.6) eggs per 100 live female-days at 110 days post-treatment, and in huts housing fipronil-treated dogs mean fecundity rates increased from 7.1 (SD 3.8) to 119.0 (SD 48.0) eggs per 100 live female-days, respectively. Hatching rates were not affected by treatment with fipronil spot-on (OR = 1.22; 95% CI 0.37–3.99), nor was the proportion of unhatched eggs in a poor state (i.e. black color) (OR = 1.05; 95% CI 0.89–1.24).

The mean survival of founder bugs (all stages pooled) exposed to fipronil-treated dogs decreased to 85%, 74% and 61% at 30, 63 and 110 days post-treatment, respectively (solid lines, Figure 4) and was not significantly different from that of founder bugs exposed to control dogs (87%, 78% and 61%, respectively; dashed lines in Figure 4) ($\chi^2 = 1.04$, d.f. = 1, $P = 0.31$).

Stage-specific vital rates throughout the observation period for the founder *Tri. infestans* bugs exposed to fipronil-treated and control dogs are shown in Table 1. Similar percentages of bugs were retrieved dead in huts with treated (13.1%) or control (11.3%) dogs (OR = 0.81; 95% CI 0.27–2.44). Loss rates were slightly higher in bugs exposed to treated dogs, though not significantly so when adjusted for time effects and the interaction between treatment and life stage (OR = 1.52; 95% CI 0.86–2.67). The loss rates of female or male bugs were marginally or significantly lower than for fourth-instar nymphs. The total loss rates (i.e. death plus loss) in treated (39.3%) and control groups (38.3%) were not significantly different (OR = 1.35; 95% CI 0.71–2.57), nor were the molting rates of fourth- or fifth-instar nymphs exposed to treated (7.9%) and control (10.0%) dogs (OR = 0.93; 95% CI 0.41–2.10).

The overall percentage of blood-fed founder bugs was very high during the entire trial, and did not differ significantly between treated (95.9%, $n = 870$) and control huts (98.3%, $n = 539$ bugs examined) when adjusted for life stage and time effects (OR = 0.40; 95% CI 0.12–1.31). Similarly, the mean degree of engorgement (i.e. medium-fed plus fully fed) of bugs on treated (77.7%, $n = 834$) and control dogs (78.7%, $n = 530$) was not significantly different (OR = 1.00; 95% CI 0.54–1.86). There was a marked individual host effect on the degree of engorgement: treated dog 2 (T2) consistently allowed all bug stages to feed less. When engorgement was taken to include only fully fed bugs, fipronil significantly reduced their degree of engorgement relative to control bugs throughout the study period when adjusted for significant life stage effects (OR = 0.58; 95% CI 0.35–0.94; $P < 0.001$).

4. Discussion

Our study demonstrates that, unlike treatment for ticks and fleas, topical treatment of dogs with fipronil spot-on exerted only slight and short-term lethal effects after a single feeding on fipronil-treated dogs, and no detectable reduction of bug abundance and vital parameters

in the experimental hut trial, despite repeated bug exposure to the treated dogs. These results contrast with our earlier studies in which bug populations continuously exposed to dogs wearing deltamethrin-impregnated collars had reduced survival, fecundity and degree of engorgement leading to the extinction of treated bug populations.⁸

Our conclusions on the effects of fipronil on *Tri. infestans* differ from those reported in a previous study.¹⁴ In addition to major differences in study design, sample size, duration of observation period and bug resistance to pyrethroids, Gentile et al.¹⁴ used 1% fipronil pour-on at an intended dose of 1 mg/kg whereas we used 10% (w/v) fipronil spot-on at a dose of approximately 6.7 mg/kg at the upper limit of each dog weight class. Although both products were marketed by the same manufacturer, we used Frontline Plus (which also contained methoprene and is licensed for application on dogs and other small domestic animals in Argentina), whereas fipronil pour-on was Ectoline. Nonetheless, the applied dose in our spot-on study was several times higher than in the pour-on trial, and the pipette provided to apply the insecticide on dogs served as an insurance against overdosing. In single-exposure tests, both studies detected significant, though not strong lethal effects of fipronil early after treatment (<7 days). Bug mortality was very fast and reached 100% in the pour-on bioassay whereas cumulative mean bug mortality only peaked at 18.7% at 1 week post-exposure in the fipronil spot-on bioassay. At about 30 days post-exposure to fipronil, excess mortality only was revealed in the pour-on bioassay. Census counts of bugs provided much more accurate assessments of the effects of insecticides on bug populations in the experimental hut trial than the semi-quantitative estimates of bug abundance derived from timed manual searches, which lack precision and sensitivity.¹⁸ Moreover, the apparent reduction of *Tri. infestans* abundance in peridomestic sites after topical application of fipronil pour-on to domestic animals at the end of fall¹⁴ may be explained by the combined effects of bug removal before treatment and the temperature-related decline of bug abundance and other demographic parameters occurring from late summer to winter in the absence of any insecticide treatment.¹⁶

The appeal of topical lotions is that they are easy to use and can be safely implemented by the community; unlike collars, wear-and-tear is not a concern, and the improvement in animal health after ectoparasite control is fully evident to the villagers.¹⁹ The relative advantages of insecticide collars are that their presence on dogs is proof that the intervention is being applied during a control campaign, and for some collars, the insecticide is continuously discharged over a period of several months. The implementation of any topical insecticide treatment or collars is likely to have the consent of affected rural villagers. Cost-effectiveness issues need also to be considered.

In conclusion, at doses recommended for triatomine bugs, our study demonstrates that fipronil has only a limited and transient effect on bug feeding and dynamics and, thus, should not be used to control (peri)domestic *Tri. infestans* in vector control programs or to protect dogs from contact with triatomine bugs. Resistance to fipronil in *Tri. infestans* from Bolivia was recently reported.²⁰ Because residual spraying of pyrethroid insecticides rarely eliminates peridomestic triatomine populations in semiarid areas,²¹ unless higher doses are used,¹⁶ finding simpler and cheaper alternatives, such as collars or topical lotions for application to domestic animal hosts, is imperative given the difficulty of sustaining recurrent spraying campaigns.

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Figure 1. Eight-week cumulative mean mortality of third- or fourth-instar nymphs of *Triatoma infestans* exposed once to fipronil-treated or control dogs at different time periods after treatment. Thirty bugs were initially exposed to a given dog at each time point.

Figure 2.
Mean abundance of *Triatoma infestans* by stage in huts housing fipronil-treated or untreated control dogs over a 110-day observation period.

Figure 3. Fecundity of *Triatoma infestans* in huts housing fipronil-treated or untreated control dogs over a 110-day observation period. C1–C3: control dogs (dashed lines); T1–T5: treated dogs (solid lines).

Figure 4. Survival of *Triatoma infestans* (all stages pooled) in huts housing fipronil-treated or untreated control dogs over a 110-day observation period. C1–C3: control dogs (dashed lines); T1–T5: treated dogs (solid lines).

Stage-specific mean vital rates (death, loss, death or loss, and molts) of *Triatoma infestans* bugs throughout the observation period

Table 1

Stage	No. of bugs released		% bugs dead ^a		% bugs lost ^a		% bugs dead or lost ^a		% molted ^a	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Fourth instar	100	60	9.0	11.7	50.0	40.0	59.0	51.7	28.0	31.7
Fifth instar	103	60	9.7	8.3	21.4	20.0	31.1	28.3	3.9	8.3
Male adults	101	60	17.8	16.7	14.9	18.3	32.7	35.0		
Female adults	101	60	15.8	8.3	18.8	31.7	34.7	40.0		
Total	405	240	13.1	11.3	44.2	27.5	39.3	38.8	7.9	10.0

^a Percentages were calculated relative to the total number of bugs initially released into each group of experimental huts at time 0.