Effects of fipronil on dogs over Triatoma infestans, *the main vector of* Trypanosoma cruzi, *causative agent of Chagas disease*

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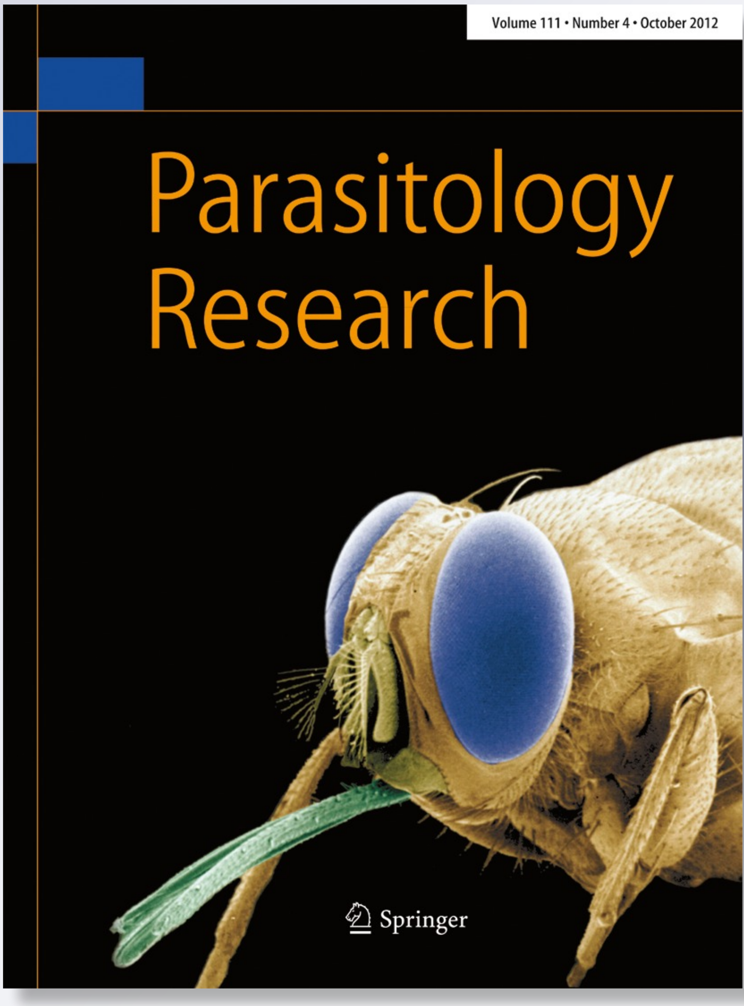

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ORIGINAL PAPER

Effects of fipronil on dogs over *Triatoma infestans*, the main vector of *Trypanosoma cruzi*, causative agent of Chagas disease

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Abstract Chagas disease is the most important endemic disease in Latin America, mainly transmitted by *Triatoma infestans* in the Southern Cone countries of South America. Dogs are one of the main domestic reservoirs of *Trypanosoma cruzi,* the etiological agent of Chagas disease. The presence of dogs in rural households of endemic areas significantly increases the likelihood of the vectorial transmission of the parasite. We studied the mortality and blood intake of *T. infestans* exposed to dogs treated with different doses and formulations of fipronil. Two doses, two formulations, and different distances to the application point of fipronil were compared. Third instar nymphs of *T. infestans* were fed at different time intervals after the insecticide application up to 45 days post-application. No significant difference was found between the blood intake of nymphs fed on control and treated dogs with different doses and formulations ($p>0.05$). The spray formulation showed lower effect and persistence than the spot-on formulation. The mortality rate caused by the spot-on formulation in the 26.8-mg active ingredient (a.i.)/kg dose was higher (48 %) than with the13.4-mg a.i./kg dose (25 %), 24 h after the insecticide application. The effect was highly heterogeneous among replicates of the same treatment. The mortality rate of nymphs fed over the point of the insecticide application was higher than the mortality of nymphs fed over places 12 cm apart from the fipronil application point, suggesting that the distribution of fipronil over the dog body is lower than the needed one to obtain a persistent triatomicide effect.

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Introduction

Triatoma infestans (Hemiptera: Reduviidae) is one of the main vector species of *Trypanosoma cruzi,* the etiological agent of Chagas disease, the most important parasitic disease of the Americas (Hotez et al. 2008). Vector control programs using the traditional spraying of intra and peridomestic structures of rural houses of the endemic regions within the South American Gran Chaco region have not been as successful as programs outside the Gran Chaco region using pyrethroids. Traditional spraying is highly effective inside domiciles, but it frequently leaves a number of residual peridomestic vector populations, in the southern part of the Chaco Region (Porcasi et al. 2006, 2007). These residual populations eventually recolonize the domestic rooms and reestablish the domestic transmission cycle of *T. cruzi*. Domestic dogs, cats, goats, and chickens play a key role in the eco-epidemiology of Chagas disease as frequent sources of blood meals for triatomine bugs. Dogs are the main domestic reservoirs of *T. cruzi* because of their high infection rate and high parasitemia, as several studies have shown (Minter 1976; Diosque et al. 2004; Cardinal et al. 2006; Crisante et al. 2006; Gürtler et al. 2007; Pineda et al. 2010). *T. infestans* prefers to feed on dogs (Gürtler et al. 2009a). Infected dogs are more infectious than infected children and adults (Gürtler et al. 2005), and the average number of dogs per household was positively associated with the triatomine population size (Gürtler et al. 1992). A mathematical model predicted that elimination of infected dogs from a household with infected people could be sufficient to nearly extinguish the transmission of *T. cruzi* (Cohen and Gürtler 2001).

Different control methods have been considered to reduce the importance of dogs in the *T. cruzi* domestic transmission cycle. A rubber collar with 40 mg/g deltamethrin on dogs reduced the feeding success and the survival of *T. infestans* (Reithinger et al. 2005, 2006). Gürtler et al. (2009b) assessed the insecticidal effects of fipronil spot-on at a dose of 13.4 mg active ingredient (a.i.)/kg of body weight (a.i./kg, from now on) on experimental dogs and did not observe significant effects comparing treated and control groups on the size and survival of the bugs population.

Early studies on spot-on insecticides indicated that solvents may play a role in the distribution of the active ingredient and may therefore influence the efficacy of the insecticide (Endris et al. 2003). A higher number of ticks were observed on the legs of treated dogs compared with the rest of the body (Otranto et al. 2005). The authors explained this result as the consequence of a possible uneven distribution of the active ingredient over the body surface after the topical application. It is known that topical insecticide doses normally used according to the weight of the treated animal do not always reflect the amount of active ingredient that would be present per unit area of the animal (Metzger and Rust 2002).

The objective of this study was to evaluate different doses and formulations of commercially available fipronil registered for veterinary uses in Argentina when applied to dogs under field conditions on mortality and blood intake of *T. infestans.* The exposure of *T. infestans* to treated animals at different post-application intervals and at different distances from the insecticide application site was studied to reveal the residual activity and dispersion of the insecticide.

Materials and methods

The trials were carried out between July 2008 and March 2011, in Bañado de Los Pantanos village (28 °27′S, 66 °46′ W), located in the Arauco Department (La Rioja, Argentina). This village was chosen because it is located within the endemic region for *T. infestans* and because of the high dog density per household. The adult mongrel dogs used in this study belonged to rural residents. The dogs'owners were contacted through the local sanitary agent. Houses of families with three or more dogs with similar characteristics were chosen for the study. The owners were invited to participate in the trial after receiving a complete explanation of the intended study. Each owner authorized the participation of their dogs.

Before the trial, each dog was weighed, dewormed (two doses of albendazole and praziquantel), analyzed by xenodiagnosis, and randomly assigned to a treatment. The dogs remained in their corresponding home and each owner took care and fed their own dogs following his normal routine. Each owner received the same amount of dogs' food to feed the dogs during the assays. A file with a picture and individual characteristics of each dog was built to monitor the dog's weight and aspect during the assay. When the trial was finished, dogs of the control group received the corresponding dose of insecticide.

Selected dogs were adults, short haired, responded to the owner call, and were classified as “regular” according to their External Clinical Aspect (sensu Petersen et al. 2001). The average weight of selected dogs was 10.75±4.7 kg (range 5–19). Six females and nine males were selected. Sex was not considered in the experimental design, but females with nursing pups or pregnant were not included as experimental units.

Insects used in this study were third instar nymphs (N3) of *T. infestans* provided by the breeding facility of the Coordinación Nacional de Control de Vectores in Punilla (Córdoba, Argentina). The bugs were bred under controlled conditions and fed on chickens (*Gallus* sp.). The N3 used in the experiments had 15 days post-molting on average, unfed, and kept under controlled temperature (26–28 °C) and humidity (50–70 % RH) in appropriately labeled plastic jars. Each test of insecticide effectiveness was replicated using three independent groups of 10 N3 that fed over the individually identified dog. During the feeding procedure, dogs were immobilized with the owner help. Nymphs were allowed to feed during daytime for 15 min on the dog abdomen using a plastic container covered by a nylon mesh. During the feeding process, the nymphs walked on the nylon mesh in partial contact with the dog's skin. The environmental temperature during feeding varied between 25–29 °C. After feeding, nymphs were kept in appropriately labeled plastic jars under the above mentioned controlled conditions. Mortality was recorded weekly, and blood intake was calculated as the difference of the nymph group weight before and after each feeding, divided by the number of nymphs in each jar. The nymphs were weighed as a group and not individually to avoid excessive manipulation that could artificially increase the mortality rate. Insects were considered dead when they showed no movement. Two formulations of fipronil (spot-on and spray) combined with three doses were included in the experimental design as described below. Only registered fipronil formulations, commercially available for veterinary use in Argentina were evaluated.

Fipronil spot-on formulation, doses comparison

The assay was carried out during July and August 2008 to compare the effect of different fipronil doses spot-on 10 % (Frontline®, Merial, Buenos Aires, Argentina). Fifteen dogs were used. A complete randomized block design with three treatments was used. Each block had three dogs that lived in one house: one received a fipronil quantity according to the company recommendation based on body weight (13.4 mg a.i./kg), another dog received double the recommended quantity (26.8 mg a.i./kg), and a third dog did not receive the insecticide. The company recommended application point is over the dog's neck. For this study, the fipronil was applied covering the dog's abdomen, in the same place where the nymphs were fed. The residual effect of the insecticide was measured using four independent groups of nymphs fed 1, 20, 35, and 50 days after the application of the insecticide. At each time interval, three groups with 10 third instar nymphs each were fed on an individually identified dog, totaling 450 third instar nymphs (30 nymphs×5 dogs×3 treatment) on each feeding occasion.

Fipronil spray formulation, doses comparison

The assay was carried out during February 2010. Because of the high variability of the mortality among the replicates recorded on the previous treatment, a formulation that covered the whole body of the dog was evaluated. The purpose was to compare the insecticide effect of different doses of fipronil spray 0.25 % (Frontline®, Merial, Buenos Aires, Argentina). Fifteen dogs, different from the previous assay, were used. A complete randomized block design with three treatments was used. Each block had three dogs living in the same house: one dog received fipronil according to the company recommendation based on body weight (13.4 mg a.i./kg), another dog received twice the recommended doses (26.8 mg a.i. /kg), and the third dog did not receive the insecticide. Each dog was completely covered with the insecticide solution. The residual effect of the insecticide was measured using two independent groups of nymphs fed at 1 and 15 days after the application of the insecticide. At each time interval, three groups with 10 third instar nymphs each were fed on individually identified dog, totaling 450 third instar nymphs (30 nymphs×5 dogs×3 treatment) per experimental group on each feeding occasion.

Fipronil spot-on formulation, effect of distance to the application point

The assay was carried out during February and March 2011. Because of the high variability on the mortality among nymphs recorded within previous treatments, *T. infestans* mortality fed at different distances from the fipronil spot-on application location was evaluated to estimate the insecticide dispersion on the body of the treated dog. Thirteen dogs were used. Ten dogs received double the recommended doses of fipronil according to body weight (26.8 mg a.i. / kg) and the other three dogs did not receive the insecticide. Fipronil was applied along line on the right side of the dog's abdomen. The insecticide effect was measured using a container with three compartments. Ten third instar nymphs were placed in each of the three compartments. The container (12 cm in length) was placed perpendicular to the insecticide application line. Each compartment was coded. Compartment number 1 was placed over the insecticide application line and the compartment number 3 was placed towards the middle of dog's abdomen, the furthest site to the insecticide application point. The residual effect of the insecticide was measured using four independent groups of 390 N3 fed at 1, 15, 30, and 45 days after the application of the fipronil spot-on. When all the nymphs fed on a dog showed no mortality, the dog was not used again, and it was assumed that the insecticide was degraded and the mortality would continue to be zero.

Data analysis

Mortality data of the randomized blocks design were analyzed using a Friedman test. A correlation analysis between blood intake and mortality of each sub-sample on each date was made. The rest of the comparisons were analyzed with parametric ANOVA when variance heterogeneity was rejected (Levene test). Cases with heterogeneous variances were analyzed with the Kruskal–Wallis test. All statistical calculations were carried out with Infostat 2011 (Infostat Group 2011). The 95 % confidence intervals (95 % CI) were estimated for mortality rates.

Results

All dogs were negative to *T. cruzi* in the xenodiagnostic test. Dogs' weight did not change during the assay. No difference between dogs' weight exposed to different treatments was found ($p>0.05$).

Comparison of fipronil spot-on doses

After 24 h of fipronil application, nymphs exposed to dog treated with double doses of fipronil showed higher mortality (average mortality 48 %, range 93–3 %) than the nymphs fed on dogs treated with the recommended fipronil dose (average mortality 25 %, range 65–3 %). Both treated groups showed higher mortality than nymphs exposed to control dogs ($p<0.01$). Both treatments showed high variability between replicates (dogs). A high variability was observed on the mortality within the sample replicates (groups of nymphs fed on the same dog at the same date), with mortality rate that varied between 90 and 0 %. Nymphs fed 20, 35, and 50 days after fipronil application showed a decrease in mortality and did not show difference in mortality between treated and control groups. On days 35 and 50 after the insecticide application, none of the nymphs in the control and treated groups died. Blood intake did not show difference between the groups of nymphs exposed to treated or control dogs ($p>0.05$) (Fig. 1).

Comparison of fipronil spray doses

Groups of nymphs fed 24 h after fipronil application showed higher mortality when exposed to a treated dog (with both doses of fipronil) than nymphs exposed to control dogs ($p<0.01$). The mortality rate in the two treated groups was 50±14.5 % and in the control group 8.9±10.2 %. The average blood intake per individual in all groups was 7.35±3 mg (range 3.3–17.6 mg), and no difference was found between treated or control groups ($p>0.05$). The spray formulation was rejected by the dogs, as after the spray application some went to a puddle and others took a dust bath. This assay was finished because of the problems observed during the insecticide application and the low insecticide effect observed.

Effects of feeding at varying distances from the fipronil spot-on application point

Mortality of nymphs fed on treated dogs was higher than nymphs fed on control dogs until 15 days post fipronil application ($p<0.05$). The mortality of control groups on all dates was 0.8±2.8 % (Fig. 2). Nymphs fed 30 and 45 days after fipronil application showed a decrease in mortality, and no difference in mortality was found between treated and control groups. After 24 h of the fipronil application, nymphs fed further away from the application point showed lower mortality [66 % (95 % CI, 43–89 %)] than nymphs fed on the application point [92.3 % (95 % CI, 89–100 %)] (Table 1). After 15 days of fipronil application, mortality of nymphs fed directly over the application point showed significantly higher mortality [81.3 % (95 % CI, 71–90 %)] than nymphs fed further away [50.6 % (95 % CI, 29–69 %)]. No difference in mortality was observed among nymphs at different distances from the application point

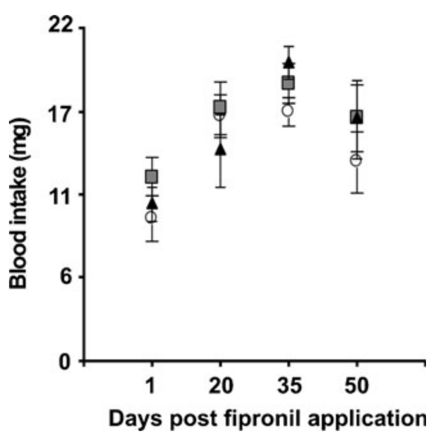

Fig. 1 Blood intake (mg) of third instar *T. infestans* nymphs fed on dogs treated with different doses of fipronil spot-on at different intervals after the insecticide application. *Gray squares*: recommended doses (13.4 mg active ingredient/kg); *Black triangles*: double doses (26.8 mg active ingredient /kg); *White circles*: without insecticide (control group). *Lines over the bars* are standard deviations

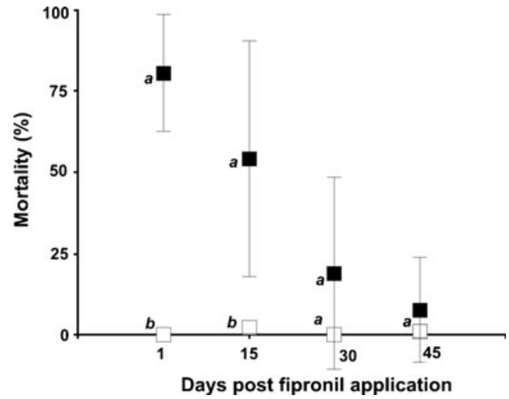

Fig. 2 Mean mortality rates [%] and standard deviations of independent groups of 30 third instar *T. infestans* nymphs fed on dogs treated with different doses of fipronil spot-on at different intervals after the insecticide application. *Black squares*: double doses (26.8 mg active ingredient/kg); *White squares*: without insecticide (control group). *Different letters* indicate groups differing significantly at $p<0.05$. *Lines over the bars* are standard deviations

Table 1 Mortality of third instar *T. infestans* nymphs fed on dogs treated with double doses of fipronil spot-on (26.8 mg active ingredient/Kg) at different intervals after the insecticide application

Days post fipronil application	Distance to application point	N	Average mortality (%)	Standard deviation	Min	Max
1	1	10	92.3a	12.3	70	100
	2	10	84.0ab	20.1	50	100
	3	10	66.0b	33.4	20	100
15	1	8	81.3a	34.4	20	100
	2	8	56.3b	41.7	10	100
	3	8	45.0b	35.9	10	100
30	1	10	31.0a	36.0	0	100
	2	10	17.0a	34.7	0	100
	3	10	9.0a	25.1	0	80
45	1	6	20.0a	30.3	0	80
	2	6	11.7a	19.4	0	50
	3	6	6.7a	12.1	0	30

Codes of distance to application point as follows: 1, fed directly over the insecticide application point; 3, fed 12 cm from the application point; 2, fed at an intermediate distance. Different letters indicate groups differing significantly at $p<0.05$

after 30 days of the fipronil application (Table 1). Blood intake did not differ ($p>0.05$) between the groups of nymphs exposed to treated and control dogs at different dates after the insecticide application. The average blood intake on each feeding occasion in all groups was 14.7± 7.8 mg (range 0.3 –31 mg)/nymph. The correlation between the blood intake and mortality in each sub-sample was not significant ($p>0.05$).

Discussion

The three assays reported here complement the published information about the fipronil effect on *T. infestans.* The recommended doses showed a small effect and a short persistence over mortality. This finding coincides with the data reported by Gürtler et al. (2009b). They observed no detectable reduction of bug abundance in an experimental trial using dogs kept in huts, despite of repeated bug exposure to the treated dogs with the recommended doses of fipronil. Results obtained in the present study suggest that the low effect observed by Gürtler et al. (2009b) (significant mortality up to 7 days after the insecticide application) was most likely a consequence of a low doses used and inappropriate application point of the fipronil (because of the low dispersion ability of the formulation).

The double doses of fipronil spot-on evaluated in the present study increased the persistence effect, although the residuality did not reach the effects observed by other authors using other formulation. Bioassays carried out using different surfaces treated with fipronil wettable powder formulation (Rojas de Arias and Fournet 2002) showed 50 % mortality of *T. infestans* fifth instar nymphs exposed up to 90 days post fipronil application (190.6 mg/m^2). These studies showed that the fipronil active ingredient has higher triatomicide effect when it is used as wettable powder formulations.

Studies by other authors showed that deltamethrin dispersion attains the necessary concentration to kill ticks at different distance to the application point until 28 days post insecticide application (Lüssenhop et al. 2011). Dogs treated with different fipronil spot-on formulations were protected from ticks re-infestations with an efficacy of >90 % until 5 weeks (Bonneau et al. 2010). Our study showed that the fipronil spot-on formulation is not able to maintain a significant residuality to kill *T. infestans* nymphs fed on different parts of a dog body after 24 h of the fipronil application. This finding suggests that hematophagous arthropods living over the host body are more vulnerable to spot-on formulations than *T. infestans*, making occasional contact with the host. The dispersion rate of the active ingredient through the dog skin is very low, and causes the significant variability in nymph mortality after feeding at different distances of the fipronil application point (up to 12 cm) observed during the present study. A similar result was observed in studies using spot-on formulations for the control of *Haematobia irritans* on cattle (Juan et al. 2010). The variability recorded among dogs exposed to same treatment was a consequence of the different behavior of individual dogs. The used fipronil spray had low active ingredient concentration (0.25 %), so a high liquid volume was necessary to obtain the appropriate dose, causing spillovers from the dog's hair. The spray formulation was specially rejected by the dogs. All these factors contributed to the low triatomicide effect and the high variability observed among the replicates of the formulations used in the present study.

Fipronil did not show a knock-down effect as the one produced by pyrethroids (Alzogaray and Zerba 1997; Amelotti et al. 2010). The mortality of nymphs exposed to fipronil was not immediate; the intoxication symptoms appear after 24 or 48 h of the contact with the treated skin surface.

The blood intake was not different between nymphs fed on control and fipronil treated dogs, suggesting that fipronil does not produce repellence in *T. infestans* and that it does not affect the normal bug feeding the way the pyrethroids produce (Reithinger et al. 2005, 2006; Amelotti et al. 2009, 2010).

This study shows that fipronil spray is worse than fipronil spot-on formulation, and that the spot-on formulation is not able to produce a significant effect over the mortality of *T. infestans* nymphs, at least with the currently available commercial formulations. A change of the fipronil spot-on formulation towards an improvement of the dispersion ability of the active ingredient would increase the triatomicide effect.

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