Veterinary Parasitology 268 (2019) 98–104

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

An oral dose of Fluralaner administered to dogs kills pyrethroid-resistant and susceptible Chagas disease vectors for at least four months

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ARTICLE INFO

Keywords:
Triatomine bugs
Xenointoxication
Pyrethroid resistance
Fluralaner
Integrated vector control management

ABSTRACT

New vector control tools that can fit into a broader integrated vector management strategy are notably lacking. We conducted a seven-month randomized trial to assess the efficacy of a single oral dose of Fluralaner (Bravecto®) administered to dogs on the blood-feeding success, engorgement levels and mortality of pyrethroid-resistant and -susceptible *Triatoma infestans* third- and fifth-instar nymphs. The trial included 10 Fluralaner-treated and 10 placebo-treated (control) outbred healthy dogs residing in rural houses of the Argentine Chaco. Most (92.7%) of the 3017 triatomines exposed were able to blood-feed. Generalized linear models showed that blood-feeding success was not significantly modified by Fluralaner treatment, time posttreatment and their interaction. However, pyrethroid-susceptible fifth instars blood-fed significantly more frequently than susceptible third instars, and no significant differences were observed between the latter and resistant fifth instars. Engorgement levels were not significantly modified by Fluralaner treatment, time posttreatment and their interaction. Nearly all the triatomines that blood-fed on treated dogs up to 60 days posttreatment (DPT) died within 24 h regardless of pyrethroid susceptibility status combined with bug stage. Cumulative bug mortality over 4 days postexposure remained high over 90–120 DPT (70–81% in susceptible third and fifth instars, and 47–49% in resistant fifth instars), and was virtually nil at 210 DPT. Triatomines that fed on control dogs suffered marginal mortality (0–4%) except at 4 and 30 DPT. Fluralaner and xenointoxication are eligible for Phase III efficacy trials alone or combined with other methods in the frame of an integrated vector management strategy in areas with or without pyrethroid resistance.

1. Introduction

Triatomine bugs are the vectors of the protozoan *Trypanosoma cruzi*, the etiologic agent of Chagas disease, which affects 6–7 million people in the Americas and is a leading cause of disability and reduced life expectancy (Hotez et al., 2014; WHO, 2017). Other transmission routes (blood-borne, vertical) follow in relevance depending on specific historical details and type of setting. House spraying with pyrethroid insecticides has been the main or only tactic employed to prevent vector-borne transmission of *T. cruzi* since the mid-1980s (Rozendaal, 1997; Gorla and Hashimoto, 2017). Although pyrethroids are considerably more effective at lower doses, longer lasting and safer than organochlorine and organophosphate insecticides, their residual effects in outdoor structures housing domestic animals are much more limited than indoors (Gürtler et al., 2004; Cecere et al., 2006, 2013). In consequence, while a well-conducted triatomine control campaign classically suppresses virtually every treated domestic infestation, peridomestic foci persist and eventually these triatomines re-invade human habitations and reestablish parasite transmission if adequate surveillance measures are not in place (Gürtler et al., 2007; Gurevitz et al., 2013). This description applies to the Gran Chaco ecoregion shared by Argentina, Bolivia and Paraguay, and to other regions threatened by the house invasion of various species of triatomines occupying nearby sylvatic habitats (Guhl et al., 2009; Waleckx et al., 2015).

This scenario turned more complex with the emergence of pyrethroid resistance in the main vector *Triatoma infestans* in northwestern Argentina by the year 2000 (Picollo et al., 2005; Zaidenberg, 2012) and throughout Bolivia (Lardeux et al., 2010; Gomez et al., 2016; Echeverria et al., 2018). These findings were soon followed by the discovery of additional resistant foci in distant districts, including

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https://doi.org/10.1016/j.vetpar.2019.03.005
Received 9 February 2019; Received in revised form 15 March 2019; Accepted 16 March 2019

Chaco province in northeastern Argentina, where moderate levels of pyrethroid resistance unexpectedly caused vector control failures (Gurevitz et al., 2012) and reached peak values (Mougabure-Cueto and Picollo, 2015; Fronza et al., 2016). In the absence of better alternatives, vector control programs in the affected region returned to the application of organophosphate insecticides (fenitrothion, malathion) and carbamates, which were effective against pyrethroid-resistant triatomines. Their less favorable safety profiles, limited substrate-dependent residual activity (Germano et al., 2014a; Mougabure-Cueto and Picollo, 2015) and low acceptability both among householders and spray crews frequently hindered their re-application (Zaidenberg, 2012; Gurevitz et al., 2013; Germano et al., 2014a). Other alternative (bio)insecticides against triatomines were either less cost-effective or have not been licensed for domestic or public health use yet (Rojas de Arias and Fournet, 2002; Alarico et al., 2010; Carvajal et al., 2014; Forlani et al., 2015).

Domestic dogs, cats, chickens and goats are frequent bloodmeal sources of triatomine bugs of various species, especially of *T. infestans* (Gürtler et al., 1997, 2014; Rabinovich et al., 2011). Moreover, dogs and cats are important domestic reservoir hosts of *T. cruzi* and a risk factor for vector and human infection (Cohen and Gürtler, 2001; Gürtler and Cardinal, 2015). Dogs may be targets of xenointoxication, a targeted vector control strategy involving the application of pesticides on chickens, dogs or goats to suppress triatomine infestations (Schofield, 2000). Several "pour on" or "spot-on" insecticides (fipronil, pyrethroids, imidacloprip, pyriproxyfen) and deltamethrin-impregnated dog collars were effective against triatomines (Rojas de Arias et al., 2003; Reithinger et al., 2006; Gürtler et al., 2009b; Juan et al., 2013; Carvajal et al., 2014; Amelotti et al., 2012), though for more limited time periods than residual house spraying with pyrethroids (e.g., Rojas de Arias et al., 2003, 2004). In consequence, none of these alternatives progressed to larger-scale efficacy trials.

Fluralaner (Bravecto®), a non-competitive gamma-aminobutyric acid and L-glutamate receptor antagonist in the novel isoxazoline compound class, is a potent ectoparasiticide registered for use against tick, flea and mite infestations of dogs and poultry (Rohdich et al., 2014; Thomas et al., 2017). It shows significant selectivity for arthropod rather than for mammalian neurones (Gassel et al., 2014). Oral administration of a single dose of Fluralaner to three dogs housed in experimental kennels killed all the second- or third-instar nymphs of *T. infestans* that fed on them over a 30 min exposure period up to 51 days posttreatment (DPT), whereas virtually all the bugs exposed to one control dog survived the full observation period (Loza et al., 2017). Triatomine feeding success on Fluralaner-treated dogs was significantly greater than for other systemic insecticides administered to the dogs (Loza et al., 2017). Whether Fluralaner affects pyrethroid-resistant triatomines and harder-to-kill late stages with greater reproductive value (i.e., fifth-instar nymphs), and the maximum duration of treatment effects have not been established.

Here, we report the outcomes of a seven-month long randomized trial conducted in rural houses of the Argentine Chaco to assess the efficacy of a single oral dose of Fluralaner administered to outbred healthy dogs on the blood-feeding success, engorgement levels and mortality of pyrethroid-resistant and susceptible *T. infestans*. Given the continuing scarcity of insecticides for public health use (Hemingway et al., 2006), finding effective, longer-lasting and safe vector control tools that may cope with the increasing problem of pyrethroid-resistant triatomines and contribute to integrated vector management is a key issue (WHO, 2012).

2. Materials and methods

2.1. Study area

Fieldwork was conducted in rural villages located in the neighboring municipalities of Pampa del Indio (Campo Alemany, Ex-Parque, Las Muñecas, and Santos Lugares, centered at 25°55′S 56° 58′W) and Juan José Castelli (Campo Alto, at 25° 56′ 48″S 60° 37′ 12″W) in Chaco Province, Argentina. Both areas share similar characteristics (Gurevitz et al., 2011, 2012). The mean temperature over the study period was 20 °C, with absolute maxima reaching 28.1 °C and minima 11.6 °C at the closest weather station located in Presidencia Roque Saenz Peña (Chaco) whereas the mean daily rainfall averaged 0.36 mm.

2.2. Study design

A randomized, placebo-controlled efficacy trial was conducted at 10 randomly selected dog-owning households between March and October 2018. The sampling frame came from a house infestation survey that covered the study villages in early March 2018. Dog owners were explained the ectoparasitic effects of Fluralaner and signed an informed consent form to participate in the trial. They were requested information on each dog's name, age, sex, origin, feeding habits, resting site, vaccination status, and any recent insecticide or deworming treatment.

The trial included 10 treated dogs and 10 control dogs. Dogs in an apparently healthy status, > 2 months of age, and > 2 kg body weight were eligible for the trial; exclusion criteria were pregnancy, aggressiveness, and having recently been treated with insecticide. We randomly selected two eligible dogs at each of the study houses; one was randomly assigned to treatment with a single dose of Fluralaner (Bravecto®, MSD Animal Health, Vicente López, Argentina) according to manufacturer instructions (25–56 mg/kg), administered with soft cheese to facilitate its uptake and increase bioavailability (Walther et al., 2014). The second dog was treated with a single dose of placebo (Antiparasitario Total Nort, containing the anthelminthics febantel, praziquantel and pyrantelpamoate, Laboratorios Nort, Moreno, Argentina), which do not affect triatomines. Each study dog was photographed for subsequent re-identification. The recruited dogs had a mean reported age of 2.5 yr (SD, 3; range, 0.2–11 yr). Dog body size was classified as small (< 10 kg, 10%), medium (10–25 kg, 80%) or large (> 25 kg, 10%) by visual inspection, and body weight estimated accordingly. There were no significant differences in the ages of control (mean ± SD, 2 ± 1.4 yr) and treated (3 ± 3.1 yr) dogs (*t*-test, P > 0.2).

2.3. Ethical approval

This study complied with guidelines on research and biological testing activities involving live vertebrate animals issued by the Institutional Animal Care and Use Committee at the Faculty of Exact and Natural Sciences of the University of Buenos Aires, which is based on the International Guiding Principles for Biomedical Research Involvity of Buenos Aires, which is based on the International Guiding Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences. The research program was supervised by Comité de Etica en Investigación Clínica (Protocol No. TW-01-004).

2.4. Assessment of treatment effects

We assessed the effects of Fluralaner on triatomines by means of bioassays conducted at 0, 4, 30, 60, 90, 120, and 210 DPT. On each occasion the study dogs were evaluated clinically, and their owners asked for any treatment-related adverse effect (e.g., diarrhea, vomit, convulsions) or any other health-related event. All the *T. infestans* exposed to the dogs had been raised on chickens at the insectary run by the National Chagas Control Program (Punilla, Córdoba, Argentina), and were free from infection with *T. cruzi* and *Blastocrithidia triatomae*. The pyrethroid-susceptible triatomine colonies came from Córdoba and Santiago del Estero provinces, Argentina, whereas the pyrethroid-resistant colony was from Mataral (Cochabamba, Bolivia) (Germano et al., 2014b). Prior to exposure to the dogs, bugs had been fasted for 2–3 weeks.

Each study dog was exposed to 3 groups of triatomines held in wooden boxes (6 cm in diameter and 8 cm long) covered by a double tulle mesh secured with rubber bands. The boxes were applied directly on the inguinal section of each dog for 20 min before treatment; at approximately monthly intervals up to 120 DPT, and additionally at 210 DPT. Each dog was exposed to 10 pyrethroid-susceptible third-instar and 10 fifth-instar nymphs, and to 3–8 pyrethroid-resistant fifth instars (depending on availability) at each test time. All dogs were exposed at the same time points to adjust for any background changes in bug feeding and survival over time. When treated and control dogs of a given house showed no significant difference on bug mortality, they were not included in any subsequent assessment. Thus, 10 houses were surveyed at 0, 4, 30, 60, 90 DPT, 9 houses at 120 DPT, and 8 houses at 210 DPT. The degree of engorgement of each fifth-instar insect was assessed visually against a flashlight at the end of each day's fieldwork and classified as unfed, little fed, medium fed, and fully fed as described (Ceballos et al., 2005). "Fed" bugs comprised the total number of little fed, medium fed, and fully fed bugs. The exposed bugs were then kept in the insectary at approximately 27 °C and 50% relative humidity, and their survival monitored every 24 h over the first four days post-exposure to the study dogs. Moribund bugs (defined as those with impaired mobility) were pooled with dead bugs for mortality analysis.

To test for any lagged treatment effect on blood-feeding success, all triatomines that survived exposure at 90 DPT were offered an artificial blood meal (heparine-treated rabbit blood, 0.65 ml of heparine every 50 ml of blood) 14 days later in a laboratory setting as described (Cardinal et al., 2008).

3. Data analysis

The effects of Fluralaner on blood-feeding success, engorgement of fifth-instar nymphs (taken as those fully or medium fed relative to the total number exposed, including those unfed) and bug mortality, relative to placebo-treated controls, were tested using generalized linear models with a logit link and binomial family, with data clustered by dog and robust standard errors. Odds ratios (OR) and 95% confidence intervals (CI) for Fluralaner effects on the response variables were adjusted for time posttreatment, susceptibility status combined with bug stage, and the interaction between treatment and other predictors for all bugs exposed to the dogs (i.e., intention-to-treat analysis). A similar analysis restricted to triatomines that actually blood-fed returned the same qualitative results, which are not shown. Analyses were carried out for the time series spanning from 0 to 120 DPT since the time point at 210 DPT virtually displayed no difference between treatment and control groups. All statistical analyses were conducted in Stata 15.1 (Stata Corp., College Station, TX, USA).

4. Results

Dog-owners reported the drugs were well tolerated by all study dogs. In total over a seven-month period, 1496 and 1520 triatomines were exposed and allowed to feed on control and treated dogs, respectively (Table 1). Most (92.7%) of them were able to blood-feed. Multiple logistic regression analysis showed that blood-feeding success was not significantly modified by Fluralaner treatment, time post-treatment and their interaction (Table 2). However, pyrethroid-susceptible fifth-instar nymphs had significantly greater feeding success than susceptible third instars, whereas there were no significant differences between the latter and resistant fifth instars.

Most of the susceptible fifth-instar nymphs were either fully (58–59%) or medium fed (20–27%), and few were little fed (10–15%) or unfed (5–7%) regardless of treatment group (Fig. 1). Pyrethroid-resistant fifth instars showed significantly lower engorgement levels than susceptible fifth instars (OR = 0.61, CI = 0.40–0.92), with no significant treatment effects on engorgement (OR = 1.36, CI = 0.69–2.67).

Nearly all triatomines exposed to Fluralaner-treated dogs over 4–60 DPT died within 24 h postexposure regardless of pyrethroid susceptibility status and stage, whereas all bugs exposed to control dogs survived over this period except at 4 and 30 DPT (range, 10–26%) (Table 1). After exposure to Fluralaner-treated dogs at 90–120 DPT, cumulative bug mortality declined to 70–81% (susceptible third or fifth instars) and 47–49% (resistant fifth instars), and then reached

Table 1
Blood-feeding success and cumulative mortality (over 4 days postexposure) of *T. infestans* exposed to dogs treated with a single oral dose of Fluralaner or placebo according to pyrethroid susceptibility status combined with bug stage, and time posttreatment (in days, DPT).

Susceptibility/stage	DPT	Control				Treated			
		Nº exposed	Nº fed (%)	Nº dead (%)		Nº exposed	Nº fed (%)	Nº dead (%)	
				Total	Fed only			Total	Fed only
Susceptible third instars	0	100	96 (96)	3 (3)	0 (0)	100	97 (97)	1 (1)	0 (0)
	4	100	95 (95)	22 (22)	20 (21)	100	97 (97)	99 (99)	97 (100)
	30	100	91 (91)	20 (20)	16 (18)	100	95 (95)	98 (98)	95 (100)
	60	100	90 (90)	7 (7)	0 (0)	100	92 (92)	94 (94)	92 (100)
	90	90	74 (82)	3 (3)	0 (0)	90	85 (94)	72 (80)	69 (81)
	120	80	67 (84)	4 (5)	0 (0)	80	71 (89)	57 (71)	55 (77)
	210	60	44 (73)	14 (23)	1 (2)	70	57 (81)	9 (13)	1 (2)
Susceptible fifth instars	0	100	89 (89)	9 (9)	1 (1)	100	93 (93)	5 (5)	1 (1)
	4	100	96 (96)	26 (26)	24 (25)	100	98 (98)	99 (99)	98 (100)
	30	100	92 (92)	19 (19)	19 (21)	100	97 (97)	96 (96)	96 (99)
	60	100	96 (96)	0 (0)	0 (0)	100	97 (97)	97 (97)	96 (99)
	90	90	84 (93)	1 (1)	0 (0)	90	82 (91)	66 (73)	65 (79)
	120	80	79 (99)	1 (1)	0 (0)	80	79 (99)	55 (69)	55 (70)
	210	60	52 (87)	1 (2)	0 (0)	69	64 (93)	1 (1)	1 (2)
Resistant fifth instars	0	0	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)
	4	80	72 (90)	17 (21)	15 (21)	80	70 (88)	70 (88)	69 (99)
	30	44	42 (95)	5 (11)	4 (10)	48	44 (92)	45 (94)	44 (100)
	60	30	29 (97)	0 (0)	0 (0)	30	28 (93)	28 (93)	28 (100)
	90	18	18 (100)	0 (0)	0 (0)	15	15 (100)	7 (47)	7 (47)
	120	40	39 (98)	1 (3)	0 (0)	40	39 (98)	19 (48)	19 (49)
	210	24	24 (100)	1 (4)	1 (4)	28	27 (96)	0 (0)	0 (0)

Table 2
Random-intercept multiple logistic regression models of *T. infestans* blood-feeding success and cumulative mortality (over 4 days postexposure) on Fluralaner treatment of dogs, time posttreatment (in days), pyrethroid susceptibility status combined with bug stage, and their interactions.

Response variables	Explanatory variables	Levels in model	Odds ratio	95% confidence interval		*P* value
Blood-feeding success	Fluralaner	Yes	1.73	0.89	3.37	0.11
	Time posttreatment		1.00	0.99	1.00	0.45
	Fluralaner*Time posttreatment		1.00	0.99	1.01	0.83
	Susceptibility status/stage[b]	Susceptible fifth instars	1.75	1.20	2.55	0.00
		Resistant fifth instars	1.85	0.51	6.63	0.34
	Susceptibility status/stage*Fluralaner	Susceptible fifth instars*Yes	0.80	0.46	1.38	0.42
		Resistant fifth instars*Yes	0.38	0.09	1.57	0.19
Mortality	Fluralaner	Yes	9.65	2.82	32.96	0.00
	Time posttreatment[a]		0.98	0.97	0.99	0.00
	Fluralaner*Time posttreatment		1.02	1.01	1.04	0.00
	Susceptibility status/stage[b]	Susceptible fifth instars	0.94	0.77	1.14	0.55
		Resistant fifth instars	1.03	0.63	1.67	0.91
	Susceptibility status/stage*Fluralaner	Susceptible fifth instars*Yes	1.03	0.82	1.30	0.78
		Resistant fifth instars*Yes	1.34	0.63	2.83	0.44

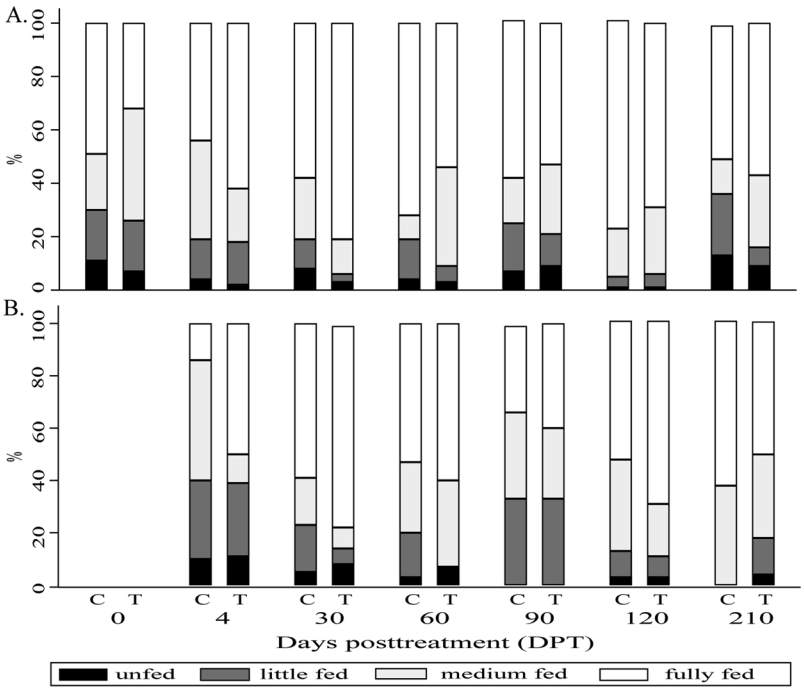

Fig. 1. Degree of blood engorgement of fifth-instar nymphs of *T. infestans* exposed to dogs treated with a single oral dose of Fluralaner (T) or placebo (C) according to pyrethroid susceptibility status (A, susceptible; B, resistant) and time posttreatment.

minimum values (0–2%) at 210 DPT (Table 1). Bugs exposed to control dogs displayed no mortality during 90–120 DPT, and reached 0–4% at 210 DPT. Multiple logistic regression analysis of cumulative bug mortality revealed highly significant effects of Fluralaner, time posttreatment and their interaction, and no significant effects of susceptibility status combined with bug stage (Table 2).

Bug mortality peaked within 24 h of exposure to treated dogs over 4 and 210 DPT (Fig. 2). Cumulative bug mortality rates increased from 39% to 70–80% between 24 and 96 h postexposure to treated dogs over 90–120 DPT in susceptible third instar bugs; from 28% to 73% in susceptible fifth instars, and from 13% to 48% in resistant fifth instars. Triatomines that survived four days after exposure to treated dogs at 90 DPT and were offered an artificial blood meal two weeks later had a significantly lower blood-feeding success (76%) than the bugs that had been exposed to control dogs (95%) and were similarly offered an artificial blood meal (OR = 0.14, CI = 0.04–0.49).

5. Discussion

Our study documents that Fluralaner induced very large mortality on *T. infestans* exposed to treated dogs over at least 120 DPT under field conditions, which nearly doubles (2.4×) the reported maximum duration of lethal activity in early-stage triatomines with an undefined pyrethroid-susceptibility status (Loza et al., 2017). Time to death postexposure was within 24 h up to 60 DPT and gradually increased to 48–96 h thereafter, which are well below the time period needed for development of *T. cruzi* infective stages in *T. infestans* after feeding on infected animals (de Souza, 2000). However, triatomines that survived beyond four days postexposure at 90 DPT had reduced chances of subsequently obtaining an artificial bloodmeal relative to bugs exposed to control dogs. Treatment affected similarly pyrethroid-resistant and -susceptible triatomines, as expected from the different mechanisms of action of pyrethroids and isoxazolines (Gassel et al., 2014; Mougabure-

Cueto and Picollo, 2015), or early and late-stage nymphs. The latter have submaximal reproductive value and require larger insecticide doses to die (Zerba et al., 1987).

The estimated residual lethal activity of Fluralaner on *T. infestans* exceeds substantially the best performances of other xenointoxicants such as deltamethrin-impregnated dog collars (Reithinger et al., 2006),"spot-on" fipronil or imidacloprip and "pour-on" pyrethroids (Gürtler et al., 2009a,b; Amelotti et al., 2012; Carvajal et al., 2014). Having at least four months of residual lethal activity, Fluralaner is very close to experimental and field estimates of residual house spraying with pyrethroids, which ranged from weeks to three months depending on type of setting, substrate and surface (Rojas de Arias et al., 2003, 2004; Gürtler et al., 2004), making it a competitive alternative in terms of this metric.

Fluralaner did not modify significantly the blood-feeding success and degree of engorgement of resistant and susceptible triatomines relative to the levels they achieved on control dogs at any time post-treatment, in agreement with previous results (Loza et al., 2017), thereby facilitating the uptake of a systemic insecticide. Most triatomines engorged fully or substantially within 20 min of exposure, including the large fifth-instar nymphs. Moreover, pyrethroid-resistant and susceptible fifth instars succeeded in blood-feeding with similar frequencies over time posttreatment but the former engorged significantly less than susceptible fifth instars, as in carefully designed experiments using resistant and susceptible *T. infestans* originated elsewhere (Lobbia et al., 2018).

The major advantages of the oral formulation of Fluralaner (i.e., commercially available, ease of administration for mass use, long lasting, good safety profile, and control of ecto- and endo-parasites), weighed against its substantial cost (Loza et al., 2017; Travi, 2019), need to be compared to the cost-effectiveness of the reference treatment (i.e., house spraying with insecticide in the hands of vector control personnel) in real-life contexts (e.g., Vázquez-Prokopec et al., 2009). It is yet unclear whether a single or more rounds of Fluralaner treatment are needed to suppress house infestations with *T. infestans*, and whether treatment would be equally effective in domestic or peridomestic premises of various types.

In real-life contexts, the effectiveness of Fluralaner on reducing or suppressing house infestation and *T. cruzi* transmission will depend on the actual exposure of treated dogs, alternative host availability (humans, chickens, and untreated dogs), the fraction of triatomines feeding on treated dogs, and daily blood-feeding rates. The fraction of domestic *T. infestans* with a blood meal on dogs ranged from > 20% to 65% in nearly half of the studies conducted in rural villages throughout the vector's range, and the more the domestic bugs fed on dogs the less they fed on humans (Fig. 3 in Gürtler et al., 1997). On average, dogs were seven times more often preferred to chickens when offered simultaneously in closed experimental huts, and the average interval between blood meals averaged 4–7 days in mid-spring (Gürtler et al., 2009a,b, 2014). These results, taken together with a mathematical model of vector-borne transmission (Cohen and Gürtler, 2001) and the high *T. cruzi* infectiousness of dogs (Enriquez et al., 2014), predict that dog treatment with Fluralaner will decrease the relative abundance and fraction of infected triatomines, and hence, will reduce the risk of human infection with *T. cruzi*. In contrast, neither dog-vector contact rates nor the probability of dog infection given a potentially infective contact with an infected bug are expected to vary with Fluralaner treatment. Householders will play a key role in the implementation of such control strategy; they should be adequately informed of its benefits and limitations, and participate both in the implementation phase and outcome evaluation.

We conclude that Fluralaner and xenointoxication are eligible for Phase III efficacy trials alone or combined with other methods (i.e., insecticide spraying or housing modifications) in the frame of an integrated vector management strategy in areas with or without pyrethroid resistance.

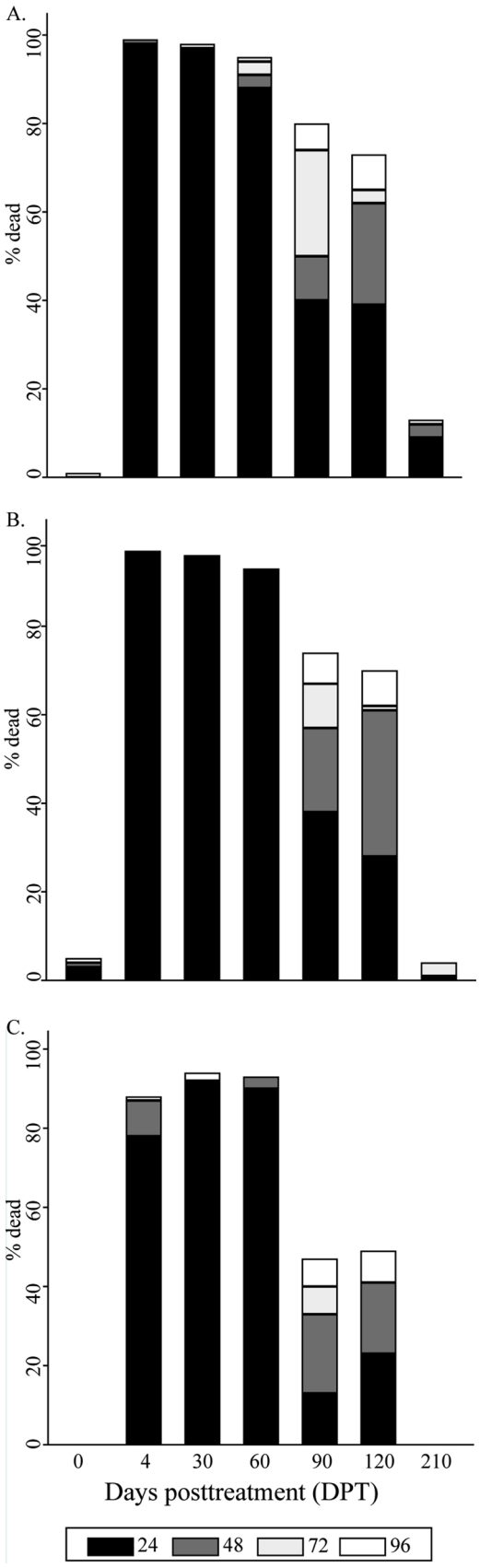

Fig. 2. Cumulative mortality (over four days postexposure) of *T. infestans* that had been exposed to dogs treated with a single oral dose of Fluralaner or placebo over time posttreatment according to pyrethroid susceptibility status combined with bug stage (A, susceptible third instars; B, susceptible fifth instars, and C, resistant fifth instars).

Financial support

This project was supported by grants awarded by the Fundación Bunge and Born, University of Buenos Aires, and PICT 2015-2921. Alejandra Alvedro was supported by a scholarship from Fondo Nacional de Ciencia y Técnica (FONCYT). The funders had no role in study design, data collection and analysis, decision to publish and preparation of the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Consent for publication

Not applicable.

Author contribution

All the authors have contributed substantially to this study. MAL, MVC, GFE, MSG, REG conceived the study. All authors read and approved the final manuscript.

Declarations of interest

None.

Animal welfare

Animal experimentation was conducted following the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences.

CRediT authorship contribution statement

M.A. Laiño: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - review & editing. **M.V. Cardinal:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing - review & editing. **G.F. Enriquez:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing - review & editing. **A. Alvedro:** Investigation, Methodology. **M.S. Gaspe:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing - review & editing. **R.E. Gürtler:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Validation, Writing - original draft, Writing - review & editing.

Acknowledgments

We thank Raúl Stariolo and Julián Alvarado-Otegui for their valuable assistance.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2019.03.005.

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