Prevention of Flea Egg Development in a Simulated Home Environment by Frontline® Gold (fipronil, (S)-methoprene, pyriproxyfen) Applied Topically to Cats

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ABSTRACT

A single topical application of a combination of fipronil, (S)-methoprene, pyriproxyfen (Frontline® Gold, Merial, now part of Boehringer Ingelheim) on cats significantly prevented flea egg development (Ctenocephalides felis) in a simulated home environment for 15 weeks. Six healthy male cats were treated and then split in two groups of 3, and each group was housed in separate, identical rooms. For the duration of the study, the cats had free access to three sites in rooms that were covered by a carpet to allow contact with the animals' fur during resting. A portion of the original carpet was retained for use as control carpet. In order to assess the prevention of flea egg development, carpet discs of 48 mm in diameter were sampled on a weekly basis from each carpet, i.e., 6 discs/week, for 15 weeks, and placed into vials with 60 C. felis eggs and flea growth medium and were then incubated for 35 to 37 days. In parallel, six identical discs of carpets without any contact with treated cats were used as untreated controls. During the 15 weeks of the study, an average of 41.0 newly emerged adult fleas (36.5 – 45.3) were recovered weekly from the control carpets, and an average of 2.6 fleas (0.7 – 5.7) were recovered from the carpets in contact with the treated cats. Significantly fewer adults fleas were recovered from the carpets in contact with FRONTLINE Gold treated cats than the control carpets at all time-points ($p<0.005$). A single administration of FRONTLINE Gold administered topically to cats reduced

flea egg development by an average of 93.6% for 15 weeks in this simulated environment.

INTRODUCTION

Control of dog and cat infestation by fleas is currently based on an approach that combines killing adult fleas and preventing of development of immature stages (eggs, larvae, and pupae) in the environment.[1,2] Several products include long-acting insecticides in association with Insect Growth Regulators (IGRs),[3,4,5,6] killing adult fleas and affecting flea eggs in utero or on the skin before falling off in the environment. The combination of fipronil, (S)-methoprene, and pyriproxyfen (FRONTLINE® Gold, Merial, now part of Boehringer Ingelheim) was developed as an insecticide/acaricide to kill fleas and ticks, with the two IGRs that potentiate the inhibition of the different developmental stages of fleas.[7] When topically applied, the IGRs are durable and lipophilic, so they tend to diffuse into the natural oils on the skin surface, resulting in continuous presence of the active molecules on the skin and hair coat.[8, 9] This explains the topical activity and the long-lasting effects. It is probable that some of these IGRs are deposited in the pet's environment through normal shedding of hair and skin debris, or mechanically by rubbing on surfaces (like bedding carpet). Besides the primary mode of action of topical IGRs, via direct contact with adult fleas and flea eggs on the animal, a secondary benefit could be activity via contact with flea eggs and larvae that are present in the environment. This could be an important pathway for IGRs, as cats typically frequent the same locations or sleeping areas (resting sites),[10,11] so these are likely the places of flea development. FRONTLINE Gold spot-on solution for cats is a combination of fipronil (9.80% w/w), (S)-methoprene (11.80% w/w) and pyriproxyfen (0.25% w/w) that kills fleas and prevents flea infestations, and kills ticks and controls chewing lice infestations. A single topical application provides immediate and sustained insecticidal efficacy against adult fleas, flea eggs, and flea larvae for up to 6 weeks, and regular use of Frontline Gold may reduce flea infestations in the home.[12]

Figure 1.

The aim of the present study was to evaluate the inhibition of development of fleas eggs inoculated into carpet that was exposed to cats that received one application of FRONTLINE Gold and to assess the duration of this inhibition.

MATERIALS AND METHODS

This study was designed in accordance with the VICH guidelines for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats[13] and the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP).[14]

It complied with Good Clinical Practices as described in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products, VICH Guideline 9.[15] This study was conducted at Ecole Nationale Vétérinaire de Toulouse,[23] chemin des Capelles 31076 Toulouse, France.

Animals

Six Shorthair, male, laboratory-reared, cats, weighing 4.2 – 7.2kg and aged of 1.2 - 1.3 years, were selected for inclusion in this study based on good health and social

Figure 2.

behavior. Cats were not previously treated with a long acting acaricide/insecticide (either topical or systemic) during the previous 3 months or with any compounds containing Insect Growth Regulators (IGRs) within 6 months prior to initiating the study. Treated cats were group housed in two identical controlled rooms (dimensions: 1.7m x 3.5m), with three cats per room. From acclimation to the end of the in-life phase, cats had free access to three areas covered by a synthetic fiber carpet (two shelves and a small cat box) to allow contact of the animal's fur with the carpet while resting (Figure 1). A total of six carpet pieces were installed in the cat rooms, three per room. Two carpets (90 x 25 cm) were placed on the upper shelves in the lower two cages and one carpet (30 x 40cm) was placed in the small cat house in each room. The same pieces of carpet remained in the rooms at all times, and were returned to their defined positions after samples were taken (Study Design). All carpet pieces were from the same roll of new carpet and were used directly from the roll. The animals were observed once daily for general health from the start of acclimation to the end of the in-life phase (Day 105). Cats were fed ad libitum with standard commercially available food. Potable water was available ad libitum using drinking bowls.

All animals were managed similarly with due regard for their well-being and were handled in compliance with the Boehringer Ingelheim Animal Health Ethics Committee and local applicable animal welfare regulations and requirements.

Study Design

Animals were acclimatized to the study facility for 7 days prior to treatment. A physical examination performed by a veterinarian during the acclimation confirmed that they were clinically healthy.

A European laboratory bred strain of Ctenophalides felis (routinely fed on cats) was used to produce the eggs for inoculation of the carpet discs.

On Day 0, each of the six cats received a single 0.5 mL topical application of FRONTLINE Gold (fipronil (9.80% w/w), (S)-methoprene (11.80% w/w), and pyriproxyfen (0.25% w/w)). The treatment was applied as directed on the label, in a single spot by parting the hair the cat's back and applying the formulation directly onto the skin, between the base of the skull and the shoulder blades. Following treatment, cats were kept individually in cages in each room for 6 hours, and observed to any adverse reactions hourly for 4 hours. Once this period was finished, the cats were allowed to move freely in their respective cat rooms.

On Days -1, 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 97, and 105 a standard disc of 48 mm in diameter was cut in a random manner from each carpet using a cutting-punch (i.e., six carpet discs per week, one disc from each the six individual carpets in contact with the treated cats). Using a different cutting-punch, six carpet discs were taken from an identical control carpet stored in a separate area of the building from the cat rooms. All carpet pieces used in this study originated from the same roll of new carpet. Each sampled carpet disc was placed into a covered Petri dish (65 x 50 mm diameter), and 0.75g of flea rearing/growth medium (blood meal, yeast, hops, barley, etc.) were added. Each preparation was infested with 60 C. felis eggs from a local flea breeding colony and incubated at

Table 1.*Individual flea counts obtained from carpet discs after incubation. A: Fleas obtained from Carpet discs in contact with treated cats; B= Fleas obtained from Control carpet. 60 flea eggs were deposited on each carpet disc at each time-point.*

	Day		Week															Mean
Carpet ID	-1	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
A1	47	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	2	0.3
A2	42	12	1	16	8	1	0	0	3	0	1	10	3	0	1	1	10	4.2
A3	44	2	0	2	0	1	0	0	0	0	0	0	2	0	0	0	1	0.5
A4	42	12	1	0	2	0	1	0	0	2	5	0	1	4	0	9	8	2.8
A5	48	8	16	14	4	2	5	2	8	5	1	4	8	10	7	18	6	7.4
A6	39	0	2	1	2	1	0	1	0	0	0	0	2	0	0	0	0	0.6
B1	45	38	46	45	42	36	45	48	33	48	33	36	44	37	37	43	39	40.6
B2	42	41	48	41	37	43	40	46	42	39	38	35	39	32	41	45	44	40.7
B3	41	50	39	44	45	37	45	50	44	43	39	38	38	43	36	38	36	41.6
B4	38	44	37	47	36	46	32	44	39	42	43	42	45	33	38	42	34	40.3
B5	45	40	51	43	42	44	42	46	43	48	34	37	48	41	37	36	39	41.9
B6	49	46	40	48	45	42	35	38	34	40	32	38	45	46	40	43	40	40.8

27°C/70-85% humidity. Each preparation was identified with study number, date, and code number (Figure 2). To avoid cross-contamination, the control discs and the discs from the cat rooms were maintained in two different preparation rooms in the laboratory.

Emerged adult fleas in each Petri dish were counted following 35 to 37 days of incubation. The personnel in charge of assessing the number of emerged adult fleas were blinded as to the origin of the 12 carpet discs that were assessed each week.

STATISTICAL ANALYSIS

The primary endpoint for treatment effectiveness was the percentage inhibition of adult flea emergence. Percent efficacy of inhibition of flea emergence from the exposed carpets with respect to the control carpets was calculated at each post-treatment time point using the formula:

Efficacy (%) = 100 x (ac – at) / ac, where

ac = Mean number of emerged adult fleas from the six control carpets discs on each assessment week, and

at = Mean number of emerged adult fleas from the six carpets discs sampled from the cat rooms on each assessment week.

The primary efficacy calculations were based on arithmetic mean values with geometric mean values considered secondary. Differences in adult emergence between the cat room carpets and the control carpets were statistically analyzed by means of a non-parametric Mann-Whitney U test on untransformed data.

In order to assess the variability of flea emergence from each carpet piece, non-parametric Kruskal-Wallis tests were performed to compare the flea counts in all carpet samples during 15 weeks (i.e. 16 sampling).

RESULTS

No adverse reactions or health problems were observed in any cat throughout the study, indicating that treatment was safe and well tolerated.

The study was considered valid as the control carpets demonstrated adequate flea emergence rate during the whole study duration, which ranged from 60.8% to 75.6% of eggs developing to adults by 36 (+ 1) days

post inoculation. Moreover, the six carpet discs sampled on Day -1 in the cat room confirmed that cats had not been treated with any acaricide/insecticide compound with a mean emerged flea count of 43.7 vs. 43.3 on the control samples.

Individual carpet sample flea counts are presented in Table 1, and adult emergence inhibition is summarized in Table 2.

By Day 1 after application of FRONTLINE Gold, only 9.4% of the eggs deposited on the six discs originated from the carpets in contact with the treated cats developed into adults (mean flea count = 5.7). A mean of 43.2 adult fleas was counted in the six control discs. Therefore, the reduction of emergence was already 86.9% at Day 1. The prevention of new flea emergence remained between 86.9% and 98.5% throughout the 15 weeks of the study. From Day 28 to the last carpet sampling, a few dead larvae were observed only in the Petri dishes containing the carpet discs in contact with the treated cats.

The number of emerged fleas observed from the 16 carpet samplings from the two rooms with treated cats was significantly different ($p < 0.01$) over the course of the study. Among the carpet samples from each room, significantly ($p < 0.01$) more fleas emerged from A2 than from A1 and A3 and from A5 than A4 and A6. The technicians reported that the cats spent less time in A2 and A5 compared to the other locations (personal communication).

DISCUSSION AND CONCLUSIONS

Topical treatments with (S)-methoprene or pyriproxyfen inhibit the development of flea eggs by different mechanisms,[16] and these are usually considered to be effects occurring on the treated pet. Published studies describing efficacies against the development of newly emerged fleas are based on experimental designs in which cats were infested with adult fleas and eggs were collected in pans below the cats.[17,18] The design in the study reported here differed in that we examined the inhibitory effect on flea emergence as a result of contact of the treated

Table 2. Arithmetic mean number of fleas obtained from carpet discs after incubation (inhibition of adult flea emergence).

	Day	Week													
	-1	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mean flea count in discs from control carpets (n=6)	43.3	43.2	43.5	44.7	41.2	41.3	39.8	45.3	39.2	43.3	36.5	37.7	43.2	38.7	38.2	41.2	38.7
Mean flea count in discs from carpets in cat rooms (n=6)	43.7	5.7	3.3	5.5	2.7	0.8	1.2	0.7	1.8	1.2	1.2	2.3	2.7	2.5	1.3	4.7	4.5
Efficacy (%)	-	86.9	92.3	87.7	93.5	98.0	97.1	98.5	95.3	97.3	96.8	93.8	93.8	93.5	96.5	88.7	88.4
p-value	-	<0.005															

cats with carpets in their environment. There was no assessment of flea infestations on cats, nor was there an opportunity for flea eggs to come in contact with the cat's skin.

In this experimental design, cat resting areas, where the cats had free access to the supports covered by carpets, mimicked a home environment. The results represent what could happen under natural conditions in households. The data show that two carpets exposed to the treated cats, A2 and A5, supported significantly greater flea emergence than the other carpets. While the sites where the cats spent time were not recorded, we surmise that the cats used these two carpets less for resting (personal observation from the technicians). One contributing factor could be that the space between the carpets and the top of the enclosure was less for A2 and A5 compared to A1 and A4. This mimics real conditions, in which cats choose their resting sites, and is consistent with observations that cats tend to seek more secluded, low traffic areas as resting sites, using elevated areas as vantage points, or enclosed areas (i.e. cat box) to hide.[10,11] In this study, the challenge (# of eggs applied to the carpet sample) was the same, whether the cats lay on the carpets or not. In the home environment, flea eggs would be predominantly deposited in preferred resting areas, where cats lie down and groom themselves.

This study demonstrated that topical flea control products containing IGRs can act directly on inhibiting environmental infestations via the presence of insect growth regulator molecules in the pet's environment.

CONFLICT OF INTEREST

This clinical study was funded by Boehringer Ingelheim Animal Health,[29] Avenue Tony Garnier, 69007 Lyon, France of which Wilfried Lebon and Frédéric Beugnet are employees. Mike Murray and Doug Carithers are employees of Boehringer Ingelheim Animal Health in the United States. Ecole Nationale Vétérinaire de Toulouse, of which the other co-authors are employee, is an independent French Veterinary school contracted to conduct the study. All authors voluntarily publish this article and have no personal interest in these studies other than publishing the scientific findings that they have been involved in via planning, initiating, monitoring and conducting the investigations and reporting the results.

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DISCLAIMER

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