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Efficacy of a topically applied formulation of metaflumizone on cats against the adult cat flea, flea egg production and hatch, and adult flea emergence

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Abstract

A spot-on metaflumizone formulation was evaluated to determine its adulticidal efficacy, effect upon egg production, and ovicidal activity when applied to flea infested cats. Eight male and eight female adult domestic shorthair cats were randomly assigned to either serve as non-treated controls or were treated topically with a minimum of 40 mg/kg metaflumizone in single spot-on Day 0. On Days –2, 7, 14, 21, 28, 35, 42, 49, and 56, each cat was infested with approximately 100 unfed cat fleas, *Ctenocephalides felis felis*. On Days 1, 2, and 3, and at 48 and 72 h after each post-treatment reinfestation, flea eggs were collected and counted. At approximately 72 h after treatment or infestation, each cat was combed to remove and count live fleas. Egg viability was determined by examining hatched eggs after 5 days and adult emergence was determined 28 days after egg collection. Metaflumizone provided $\geq 99.6\%$ efficacy against adult fleas from Days 3 to 45 following a single application. Following treatment, egg production fell by 51.6% within 24 h and 99.2% within 48 h. Following subsequent weekly infestations egg production from treated cats was negligible out to Day 38, with $\geq 99.5\%$ reduction relative to non-treated cats. Where there were eggs to evaluate, metaflumizone treatment did not have any apparent effect on the hatching of eggs or on the development and emergence of adult fleas from the eggs produced by fleas from treated animals.

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1. Introduction

The cat flea, *Ctenocephalides felis felis*, is likely the most important ectoparasite of companion animals in most areas of the world (Dryden, 1993; Rust and Dryden, 1997).

Currently, most flea control efforts are based on convenient on-animal spot-on treatments or oral

medications (Dryden and Broce, 2002; Rust, 2005). The primary focus of flea control today is to force fleas into “extinction” in a localized environment (home or yard) by preventing reproduction (Dryden and Broce, 2002; Chin et al., 2005). Elimination of an existing flea infestation can therefore be accomplished either by killing newly acquired fleas with a residual on-animal adulticide before they can initiate reproduction or directly affecting the viability of the eggs with the use of insect growth regulators or other ovicidal compounds (Dryden and Broce, 2002).

After cat fleas acquire a host, they mate and after 24 h females start laying eggs. Shortly thereafter, each female

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flea on the animal is producing up to 40–50 eggs/day (Rust and Dryden, 1997). Fleas deposit their eggs into the haircoat of the pet and the eggs fall off into the premises where they develop in a few weeks to the adult flea (Dryden, 1989). Therefore, in order for a topical insecticide to prevent egg production, the residual activity must be sufficient to kill newly acquired fleas with 24 h, or at least produce sufficient toxicity to stop blood feeding and therefore reproduction.

As mentioned above, the reproductive process can also be prevented by administration of ovicidal compounds such as topical or systemic insect growth regulators. Compounds such as lufenuron, methoprene, and pyriproxyfen have demonstrated pronounced ovicidal and larvicidal activity (Olsen, 1985; Zakson et al., 1992; Palma et al., 1993; Blagburn et al., 1994; Hink et al., 1994; Young et al., 2004). In addition selamectin has been shown to provide prolonged ovicidal activity against flea eggs (Dryden et al., 2002).

Metaflumizone is a semicarbazone insecticide with no known cross-resistance to other chemical compounds (Salgado and Hayashi, this volume). Metaflumizone has been found to have potent activity against fleas after a single topical application to cats (Holzmer et al., this volume). This study was undertaken to evaluate the efficacy of metaflumizone applied to cats in a spot-on formulation (ProMeris[®] for cats, Fort Dodge Animal Health, Overland Park, KS, USA) against adult fleas, flea egg production and hatch, and the development of fleas from eggs to adults.

2. Materials and methods

2.1. Animals

Eight males and eight females adult (>6-month-old; 2.6–4.8 kg), purpose bred domestic shorthair cats were used in this study. Cats used in this investigation had no exposure to an ectoparasiticide prior to treatment and were in good health throughout the study.

On Day –6, each of 19 cats were infested with approximately 100 *C. felis* (cat flea) from the wildcat strain established and maintained as a closed colony at Kansas State University. On Day –4, flea comb counts were conducted to assess the ability of cats to maintain infestations. Cats were combed with a fine-toothed flea comb having 12–13 teeth/cm (Safari Flea Comb, Whitco, Centereach, NY, USA). Flea removal was achieved by combing each cat thoroughly for 10 min. If five or more fleas were recovered during this period, the cat was combed for an additional 5 min. If any fleas were recovered during the second combing period, the

cats were combed for an additional 5 min. The 16 cats used in the study were selected from these 19 cats based on highest pretreatment flea counts and had maintained at least 30 fleas, the minimum number of parasites required for inclusion. These cats were ranked in descending order by flea count and gender and randomly allocated into two groups.

Cats were housed individually in indoor cages. Each cage was approximately 0.6 m × 0.6 m × 0.6 m and constructed of stainless steel with solid sides and back, with a steel barred door. Each individual cage was identified by animal number and color-code only and was not identified by treatment. Cats were physically separated by the solid stainless steel cage walls. Cats were fed a commercial dry cat-food ration. Water was available *ad libitum*. No baths, shampoos, or pesticides were administered to the cats during the preconditioning phase or during the course of the study. All animal care procedures conformed to guidelines established by the Institutional Animal Care and Use Committee at Kansas State University (approval no. 2369).

2.2. Experimental design and methods

Cats (4M:4F) in Group A remained non-treated and served as controls. Cats (4M:4F) in Group B were treated with a metaflumizone formulation containing 200 mg active ingredient/ml. Animals were dosed with a minimum of 40 mg metaflumizone/kg according to pretreatment body weight. Cats weighing ≤4 kg received 0.8 ml of formulation and cats weighing >4 kg received 1.6 ml of formulation. The dose was applied to the skin at a single spot on the dorsal neck at the base of the skull.

On Days –2, 7, 14, 21, 28, 35, 42, 49, and 56, each cat was infested with approximately 100 unfed cat fleas. On Days 1, 2, and 3, and at 2 and 3 days after each post-treatment reinfestation, flea eggs were collected from the pan under each cat cage. At approximately 72 h after treatment or infestation, each cat was combed to remove and count live fleas. The fleas were not replaced on the animals following the 72 h count. Prior to the egg collections, cats were massaged/brushed vigorously by hand for ~20 s in their cages to dislodge any flea eggs from the cat's hair coat, allowing the eggs to fall into the drop pan below the cage. Eggs were collected, placed in a glass Petri dish and counted under a dissecting microscope.

Viability of eggs was determined by attaching up to 50 flea eggs from each collection (two replicates of about 25 eggs) to the lids of glass Petri dishes using water based, non-toxic glue (Glue Stic, Avery Inc.,

Brea, CA, USA). The lid was inverted and placed over a corresponding lower dish containing growth media and held in a growth chamber at approximately 75–80% R.H. and 27–28 °C. Eggs were examined using a dissecting microscope 5 days after attachment to lids to determine hatch. Hatched larvae were allowed to continue development in the growth media. At 10–12 days after egg collection, pupae (and any larvae that had not completed cocoon formation) were sifted from the media and placed into plastic vials with lids. Adult emergence was determined by counting adult fleas at about 28 days after egg collection. Personnel conducting comb counts, egg collections and viability assessments were blinded to treatment group allocation.

2.3. Data analysis

Statistical analyses were performed separately for flea and egg counts, percent egg hatch and percent adult emergence, for each examination day. Flea and egg counts were transformed by the $\log_{10}(\text{count} + 1)$ transformation prior to analysis in order to stabilize the variance and normalize the data. Using the PROC MIXED procedure (SAS 8.2, Cary NC, USA), post-treatment transformed count data were analyzed by an analysis of variance (ANOVA) with a model that considered treatment as a fixed effect and block as a random effect. The treatment effect was tested against the residual error at the 5% level of significance. Least square means (LSMeans) for each group and each day were computed and compared. The ANOVA *F*-test was used to determine the treatment effect. Half of the *P*-value from the *F*-test was used to provide the one-sided *P*-value to determine if the reduction in counts of the treated group relative to the non-treated control group was significant at the 5% level.

Percent efficacies, relative to the non-treated control group and based on geometric means, were calculated as follows:

$$\text{efficacy (\%)} = \frac{\text{GMean control} - \text{GMean treated}}{\text{GMean control}} \times 100$$

Percent egg hatch and percent adult emergence were calculated as follows:

$$\frac{\text{number of hatched eggs or number of emerged adults}}{\text{number of eggs incubated}}$$

Percent egg hatch and percent adult emergence values were transformed using the arcsine square root

transformation ($\sin^{-1} \sqrt{\text{value}/100}$) prior to analysis in order to normalize the data and stabilize the variance.

Transformed percentages were analyzed by a two-way ANOVA that considered treatment as a fixed effect and block as a random effect. Treatment effect was tested against the residual error at the 5% level of significance. LSMs for each group and each day were computed and compared. The ANOVA *F*-test was used to determine the treatment effect.

Back-transformed means (BMeans) and percent efficacy, based on back-transformed means and relative to the non-treated control, were computed for percent egg hatch and percent adult emergence values. Percent efficacy was calculated as

$$\text{efficacy (\%)} = \frac{\text{BMean of control} - \text{BMean of treated}}{\text{BMean of control}} \times 100$$

where back-transformed means were calculated as

$$\text{BMean} = [\sin(\text{LSMean})]^2$$

3. Results

All cats included in the study demonstrated adequate pretreatment flea retention with Day -4 flea counts ranging from 42 to 88. Non-treated cats also maintained adequate flea infestations throughout the study with geometric mean flea counts ranging from 44.4 to 80.5 (Table 1).

Treated cats received an average dose of 53.0 mg metaflumizone/kg (39.0–76.2 mg/kg). This dose of metaflumizone resulted in significantly lower geometric mean flea counts on treated cats than counts on non-treated controls throughout the entire 8 weeks of the study ($P < 0.05$, Table 1). Treatment with metaflumizone provided $\geq 99.6\%$ efficacy for 6 weeks post-treatment and then 91.7 and 86.5% at 7 and 8 weeks post-treatment, respectively.

Geometric mean flea egg counts for the metaflumizone-treated cats were also significantly lower than those for non-treated controls at all post-treatment evaluations ($P < 0.05$, Table 2). Egg production on metaflumizone-treated cats was reduced by 55.3% within 24 h after treatment and $>99\%$ over the next 24 h. For subsequent infestations, egg production was negligible from treated cats out to at least 5 weeks after treatment (Day 38), with $\geq 99.5\%$ reduction relative to non-treated cats at all evaluations (Table 2). Geometric mean number of eggs produced from fleas from Days 8 to 38 post-treatment ranged from 0.0 to 1.5 and 264.7 to 1398.9 on treated and

Table 1
Mean adult flea counts and percent efficacy relative to non-treated controls for cats treated with metaflumizone spot-on

Treatment	Count day									
	–4	3	10	17	24	31	38	45	52	59
Non-treated	65.9 a	57.0 a	80.5 a	61.3 a	72.9 a	52.6 a	45.3 a	45.7 a	44.4 a	45.1 a
Metaflumizone	65.6 b	0.2 b (99.7)	0.2 b (99.8)	0.2 b (99.8)	0.0 b (100)	0.0 b (100)	0.2 b (99.6)	0.2 b (99.6)	3.7 b (91.7)	6.1 b (86.5)

Percent efficacy, with respect to the non-treated control, is in parentheses. Geometric means in each row with the same letters are not significantly different; $\alpha = 0.05$.

control cats, respectively. Egg production from treated cats increased slightly over the next 3 weeks, but was still reduced by 87.0 and 93.1% (based on geometric means) on Days 58 and 59 post-treatment.

The metaflumizone treatment did not have any effect on the hatching of eggs produced by fleas from treated animals. Where there were eggs to evaluate, there were no significant differences between the percent hatches of flea eggs from treated or non-treated cats ($P > 0.05$, Table 2). Throughout the study the percent hatch (based on geometric means) was consistent for both groups and ranged from about 75 to 100%.

Similarly, the metaflumizone treatment did not have effects on the development and emergence of adult fleas from the eggs produced by fleas from treated animals.

Where there were eggs to evaluate, there were no significant differences between the percent hatch of flea eggs from treated or non-treated cats ($P > 0.05$, Table 2) from Days 1 to 30, and from 52 on. While adult emergence was significantly lower for eggs from treated animals collected from Days 37 to 51, this may not truly reflect a treatment effect as these comparisons were based on only a small number of eggs from treated animals (Table 1). Significant reductions were in the order of 25–50%. Some relatively low rates of percent adult emergence were seen for both treatment groups early in the study (e.g. Days 1, 2, 3, and 10) This was due to too much glue being applied to the inside of the Petri dish lid which meant the glue was not dry when larvae hatched from eggs and were subsequently

Table 2
Geometric mean flea egg counts, back-transformed mean percent egg hatch and adult emergence and percent reductions relative to non-treated controls for cats treated with metaflumizone spot-on

Count day	Egg count		Egg hatch (%)		Adult emergence (%)	
	Non-treated control	Metaflumizone	Non-treated control	Metaflumizone	Non-treated control	Metaflumizone
0	819.1 a	708.6 a	92.65 a	93.58 a	71.98 a	57.98 a
1	666.8 a	298.3 b (55.3)	94.93 a	96.78 a (0.0)	44.30 a	45.01 a (0.0)
2	871.7 a	7.3 b (99.2)	91.79 a	95.80 a (0.0)	32.04 a	35.70 a (0.0)
3	1118.9 a	0.6 b (99.9)	93.03 a	100 a (0.0)	56.56 a	50.38 a (10.9)
9	1279.6 a	0.0 b (100)	96.17	–	68.68	–
10	1038.8 a	0.0 b (100)	71.73	–	35.68	–
16	471.8 a	0.1 b (>99.9)	82.71	–	63.42	–
17	1183.6 a	0.0 b (100)	89.54	–	80.72	–
23	717.7 a	0.6 b (99.9)	91.56 a	85.36 a (6.8)	80.98 a	50.00 a (38.3)
24	1398.9 a	0.0 b (100)	94.79	–	84.76	–
30	264.7 a	0.6 b (99.8)	93.17 a	75.36 a (19.1)	84.66 a	76.50 a (9.6)
31	653.6 a	0.0 b (100)	89.32	–	79.77	–
37	274.5 a	1.5 b (99.5)	97.93 a	97.56 a (0.4)	89.14 a	53.21 b (40.3)
38	361.6 a	0.1 b (>99.9)	96.63	–	93.29	–
44	201.7 a	7.0 b (96.5)	91.85 a	78.64 a (14.38)	71.55 a	35.00 b (51.1)
45	265.8 a	0.8 b (99.7)	94.82 a	95.85 a (0.0)	91.31 a	53.06 b (41.9)
51	354.5 a	48.5 b (86.3)	94.42 a	90.71 a (3.9)	90.61 a	69.34 b (23.5)
52	536.8 a	10.5 b (98.0)	96.34 a	95.53 a (0.9)	90.42 a	81.73 a (9.6)
58	414.8 a	53.9 b (87.0)	90.09 a	88.54 a (1.7)	79.77 a	67.87 a (14.9)
59	476.4 a	476.4 a	33.1 b (93.1)	33.1 b (93.1)	87.85 a	83.54 a (4.9)

Percent reduction, with respect to the non-treated control, is in parentheses. Geometric means for each parameter in each row with the same letters are not significantly different; $\alpha = 0.05$. –, No eggs/adults to evaluate.

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trapped in the glue. This tended to affect both treatment groups similarly and thus, had no effect on the comparative evaluations.

4. Discussion

Veterinarians should expect that correctly applied metaflumizone will control the existing flea burden on a cat and that there should be a rapid reduction in egg production from the existing flea infestation. Treatment of cats with a metaflumizone spot on almost completely halted egg production (99.2% reduction) within 48 h of application and provided 99.7% control of the existing flea burden within 72 h. Therefore, within 2 days of treatment, no egg dissemination should occur from treated cats.

Currently available topical spot-on insecticide formulations such as fipronil-(S) methoprene, imidacloprid, and selamectin are marketed to provide at least 30 days of effective flea control. A single application of metaflumizone provided greater than 99% control of adult cat fleas for at least 42 days after treatment and should provide highly effective flea control when used as a monthly treatment.

Following treatment, egg production was reduced by over 99% for at least 5 weeks. At 6 weeks post-treatment, the control of egg production at 48 h after reinfestation was 96.5%, and 99.7% between 48 and 72 h after reinfestation. Consumption of blood is necessary before cat fleas can initiate reproduction (Rust and Dryden, 1997) and egg production does not begin until 24 h after females take their first blood meal (Akin, 1984; Dryden, 1989). Therefore, if a residual insecticide can kill or produce toxicity in newly acquired fleas within 24 h, egg production should be markedly reduced or halted. While this study did not directly evaluate the residual speed of kill of metaflumizone or reduction of blood consumption by adult fleas, it did demonstrate that metaflumizone had a profound effect upon egg production. This indirectly indicates that metaflumizone is producing rapid toxicity and thus markedly reducing blood feeding by fleas.

When eggs were available for evaluation from treated animals, the metaflumizone treatment appeared to have little effect on the hatchability of eggs or the survival and development of fleas from eggs recovered.

5. Conclusion

This study demonstrated that a single dose of metaflumizone provided greater than 99% control of

flea egg production for at least 5 weeks and control of adult *C. felis* on cats for 6 weeks.

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