

Evaluation of the Ovicidal Activity of Lufenuron and Spinosad on Fleas' Eggs from Treated Dogs

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ABSTRACT

This study was conducted to compare the production and viability of flea eggs on dogs treated with either the combination of milbemycin oxime-lufenuron or spinosad. Fifteen dogs were randomly distributed into three treatment groups and housed individually. Dogs in group 1 served as untreated controls, dogs in group 2 were treated with milbemycin oxime-lufenuron, and dogs in group 3 were treated with spinosad. Products were administered orally according to label directions on days 0, 30, and 60. All dogs were infested with 250 cat fleas, *Ctenocephalides felis*, KS1 strain, on days 14, 28, 42, 49, 58, 70, 77, and 88. Forty-eight hours after each infestation, dogs were placed into egg collection cages for 3 hours. Flea eggs were collected, counted, and placed

into rearing media to determine adult flea emergence. The percent adult flea emergence from eggs collected from untreated control dogs ranged from 19.3% to 69.4% throughout the study. Eggs collected from dogs treated with spinosad had percent adult emergence of 49.2%, 20.3%, and 37.9% on collection days 30, 60, and 90, respectively. Even though eggs were collected on days 51, 72, and 79, those eggs were not viable. No adult fleas developed from eggs collected from dogs treated with lufenuron-milbemycin oxime over the entire 90-day study.

INTRODUCTION

The elimination of fleas on dogs and cats and in the surrounding environment can be achieved by killing adult fleas on the host and eliminating environmental life stages. Several residual insecticides such as dinotefuran, fipronil, imidacloprid, selamectin, and spinosad do an excellent job of killing existing adult fleas on the pet.¹⁻⁸ However, after

treatment, the pet still lives in an infested home environment with flea eggs, larvae, pupae, and emerging fleas. These remaining life stages provide an ongoing source of adult fleas that will continually reinfest pets treated only with adulticidal compounds.⁹ Historically, repeated application of insecticides and insect growth regulators formulated as environmental sprays were used in an attempt to eliminate the premises flea life stages.^{9,10} However, client compliance with environmental treatment protocols was disappointing and often resulted in recurrent infestations and client dissatisfaction.

New insecticides and insect growth regulators in convenient dosage forms (spot-ons, tablets, oral suspensions, and injectables), applied directly to the pet, provide prolonged residual activity and have dramatically improved pet owner compliance, helped to eliminate recurrent infestations, and improved client satisfaction.¹¹⁻¹²

Although residual flea adulticides such as dinotefuran, fipronil, imidacloprid, selamectin, and spinosad provide prolonged adulticidal activity, efficacy may decrease throughout the month following administration.^{2,7,13-17} As the residual efficacy decreases, adult female fleas may live longer than 24 hours and start producing eggs.^{13,14,16,18} The production of viable eggs may extend the infestation within the home.

Providing ovicidal (lufenuron, methoprene, pyriproxyfen, or selamectin) activity as part of a flea treatment protocol is extremely beneficial because any eggs produced by adults surviving adulticide treatment will be rendered nonviable.^{6,13,19-21}

An orally administered residual adulticide flea product containing spinosad has been approved for use in the U.S. market. In 2010 and 2011, studies indicated that under certain conditions or with some flea strains, the residual efficacy of spinosad decreases markedly towards the end of the month.^{7,15} In a presentation on spinosad residual efficacy given at the 55th Annual Meeting of the American Association of Veterinary Parasitologists, results from three studies

conducted in Alabama, North Carolina, and Ireland, using dogs 14 to 16 weeks of age, was given.¹⁵ The combined efficacy of the three trials on day 29 was remarkably only 2%.¹⁵ It is unknown if this poor residual efficacy was due to variability in flea strain or the age of the dogs.

Then, in 2011, two studies were published using the KS1 flea strain.⁷ This publication detailed two studies where the efficacy of spinosad against the KS1 flea strain in dogs was 22.1% and 32.5%, respectively, on day 29 post-treatment.⁷ In each of the above reported studies, adulticidal efficacy began to wane at approximately day 15 post-treatment. Additionally, in a 2011 study conducted in France, the residual efficacy of spinosad and fipronil against fleas infesting dogs at 24 and 48 hours, the efficacy of spinosad was 85.0% and 89.0%, respectively on day 28 post-infestation was evaluated.¹⁶

Because the adulticidal efficacy of spinosad has been shown to wane in several of the aforementioned studies, we conducted this study to determine if fleas survived long enough on spinosad-treated dogs to feed, mate, and produce viable eggs. Therefore, this study was conducted to compare the production and viability of flea eggs on dogs treated with either the combination of milbemycin oxime/lufenuron or spinosad and infested with the KS1 flea strain. We used lufenuron as a positive reference control for egg viability.

MATERIALS AND METHODS

Fleas

The KS-1 strain of *Ctenocephalides felis* was used in the study. The KS1 cat flea strain of *Ctenocephalides felis* has been maintained as a closed colony at Kansas State University since 1990. The colony was originally established by removing fleas from dogs and cats at a local animal shelter in Riley county Kansas. *In-vitro* and *in-vivo* evaluations have indicated that the KS1 strain has some level of resistance or reduced susceptibility to carbaryl, chlorpyrifos, fenthion, fipronil, imidacloprid, permethrin, pyrethrins, and spinosad.^{2,7,13,18, 22-26}

Due to the hardness of this flea strain, it has been used extensively as an indicator of flea product performance. In fact, almost every major flea product has been tested against this flea strain either before or shortly after introduction to the US market.

Animals and Housing

This study used 15 purpose bred mongrel dogs between 6 and 12 months of age housed in individual kennels. The line of dogs is one approved by the FDA for pivotal studies; the age range is standard and usual for conducting investigations. Dogs must be housed in individual kennels for individual observation and to obtain accurate data. During the 14-day preconditioning phase, no medications or pesticides were administered or used, and none of the dogs were bathed or shampooed. All animal care and procedures conformed to the established guidelines of the Kansas State University Institutional Animal Care and Use Committee and those of Novartis Animal Health.

Animal Selection and Randomization

On day 1, the dogs were weighed and randomly distributed into three groups, five dogs in each group.

Treatments and Infestation

Dogs allocated to group 1 served as untreated controls. Dogs in group 2 were treated with Sentinel® Flavor Tabs® (Novartis Animal Health) milbemycin oxime - lufenuron (minimum dosage 0.5mg/kg milbemycin oxime, 10mg/kg lufenuron) per label directions immediately after a meal. Dogs in group 3 were treated with the appropriate size Comfortis® (Elanco) spinosad tablet (minimum dosage 30 mg/kg) per label directions, with a meal.

Dogs were observed to ensure they had consumed a meal before treatment and observed for 1 hour after tablet administration to monitor for emesis. Dogs were treated on days 0, 30, and 60.

Flea Infestations and Evaluation of Egg production and Ovicidal Activity

All dogs were infested with 250 fleas, KS1 strain, 1 to 5 days post emergence, on days

14, 28, 42, 49, 58, 70, 77, and 88. This corresponds to 14 and 28 days following the first treatment; 12, 19, and 28 days following the second treatment; and 10, 17 and 28, days following the third dosing.

Forty-eight hours after each flea infestation, the dogs were removed from their individual kennels and placed into stainless steel metabolic cages with expanded metal floors over stainless steel solid surface collection pans for flea egg collections. Dogs were housed in the metabolic cages for 3 hours. After 3 hours, the dogs were brushed to dislodge any remaining eggs. The dogs were then removed from the metabolic cages, given one dose of Capstar® (nitenpyram) (minimum dose 1.0 mg/kg) orally, and returned to their original kennels.

All flea eggs collected in the stainless steel pans were counted. Up to 100 eggs from each dog were placed in glass Petri dishes containing growth media (sand, ground dog chow, brewer's yeast, and dried blood), and held in a growth rearing chamber (I-30B, Percival Manufacturing Co, Boone, IA; 27-28° C, 70-80% relative humidity, 24 hours dark). 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 emerged adult fleas at about 28 days after egg collection. Personnel conducting egg counts, egg collections, and viability assessments were blinded to treatment group allocation.

Statistics

An analysis of variance (ANOVA, SAS PROC MIXED) was used to compare the percent of adult fleas that developed from eggs recovered from each dog in the milbemycin oxime - lufenuron and spinosad treatment groups. Data on the percent of adult fleas was transformed using an (arcsine (square root (percent flea hatch))) transformation to normalize the variance. The ANOVA was performed at all days post-treatment.

The following hypothesis was tested:

Table 1: Evaluation of the Ovicidal Activity of Lufenuron and Spinosad on Flea Eggs from Treated Dogs.

		Collection days			Collection days				Collection days		
	Treatment Day 0a	Day 16b	Day 30	Treatment Day 30	Day 44	Day 51	Day 60	Treatment Day 60	Day 72	Day 79	Day 90
Controls											
Total eggs incubated ^c		326	500		400	430	398		278	230	226
Total adults produced ^d		63	347		156	201	116		122	68	48
% Development ^e		19.3 ^b	69.4 ^b		39 ^b	46.7 ^b	29 ^b		43.9 ^b	29.6 ^b	21.2 ^b
Lufenuron – milbemycin oxime											
Total eggs incubated		500	500		500	500	500		500	317	ND*
Total adults produced		0	0		0	0	0		0	0	0
% Development		0 ^a	0 ^a		0 ^a	0 ^a	0 ^a		0 ^a	0 ^a	0 ^a
Spinosad											
Total eggs incubated		0	380		0	13	256		2	36	256
Total adults produced		0	187		0	0	52		0	0	97
% Development		0 ^{na}	49.2 ^b		0 ^{na}	0 ^a	20.3 ^b		0 ^b	0 ^b	37.9 ^b

a Dogs in group #2 were orally administered Sentinel® Flavor Tabs®(Novartis Animal Health) milbemycin oxime - lufenuron (minimum dosage 0.5mg/kg milbemycin oxime, 10mg/kg lufenuron) per label directions immediately after a meal. Dogs in group #3 were orally administered the appropriate size of Comfortis® (Elanco) spinosad (minimum dosage 30 mg/kg) per label directions, with a meal.

b Dogs were infested with 250 *C. felis* 48 hours prior to each egg collection day.

c. Five dogs in each treatment group and a maximum of 100 eggs/dog used for viability assessments.

d Number of adult fleas emerging 28 days following egg collection.

e Percent of adult fleas emerging from eggs collected.

*ND – no data: no eggs were collected due to procedural error.

Numbers in columns with unlike superscripts are significantly different at $p < 0.05$

a) Ho: Mean (Trans(Group X parasites +1)) = Mean (Trans(Group Y parasites +1)), vs Ha: Mean (Trans(Group X parasites +1)) < Mean (Trans(Group Y parasites+1))

Where:

Trans = (arcsine (square root (percent flea hatch))), and, Parasites = percent of adult fleas recovered from a random subset of the total eggs collected for group comparisons where $X \neq Y$, where group comparisons were: milbemycin oxime - lufenuron vs untreated control, spinosad vs untreated control, and milbemycin oxime - lufenuron

vs spinosad .

Rejection of the null hypothesis (statistical significance) at a specific time point indicated that the product under test was superior (lower number of adult fleas) to the comparator product at that time point.

The data were grouped by month and over all months, and categorized as either positive for adults developed from collected eggs and assigned a score of '1,' or negative for adults developed from collected eggs and assigned a score of '0.' The percent of dogs exhibiting inhibition was then calculated

as the number of dogs exhibiting complete control (ie, no adults developed) divided by the total number of dogs in the group.

RESULTS

Eggs collected from untreated control dogs had percent adult emergence ranging from a low of 19.3% to a high of 69.4% (Table 1). Viability of eggs in some of the collections was likely reduced due to urine and feces contamination of egg collection pans. Urine and feces contamination occurred sporadically in all treatment groups.

Eggs collected from dogs treated with spinosad had percent adult emergence of 49.2%, 20.3%, and 37.9% on collection days 30, 60, and 90, respectively. Even though eggs were collected on days 51, 72 and 79, those eggs were not viable (Table 1). At no collection period over the 90 day study did any adult fleas develop from eggs collected from dogs treated with lufenuron-milbemycin oxime (Table 1).

DISCUSSION

While a previously published study had shown a remarkably high level of residual activity of spinosad,⁸ several other investigations demonstrated a significant reduction in adulticide efficacy after Day 15 against a variety of flea strains.^{7,15,16} This current study revealed that egg production from spinosad treated dogs infested with the KS1 flea strain began within 12 to 19 days following treatment, and that by day 30 post-treatment for each dosing period, many of those eggs were viable.

When conducting efficacy trials with laboratory maintained flea strains, it is often difficult to assess how relevant the data is to real world “in-clinic” situations. How the data generated in individual studies relates to clinical practice is often difficult to determine. Efficacy results are specific to the conditions of the individual study and the susceptibility of the laboratory flea strain used in those studies. The KS1 flea strain has been extensively evaluated over the past 2 decades, and while the KS1 flea strain is one of the least susceptible labo-

ratory maintained flea strains for various insecticides,^{2,7,13,18,22-26} numerous field collected cat flea strains have been identified that were even less susceptible.²⁶⁻²⁸ Several field collected cat flea strains were found to be less susceptible to organophosphates and pyrethroids than the KS1 strain.²⁶ In 2001, a strain designated as R6 from a veterinary practice in Lakeland Florida was significantly less susceptible to fipronil than the KS1 strain.²⁷ Additionally, a number of field collected flea strains were found to be less susceptible to imidacloprid than the KS1 strain.²⁸

Lufenuron provided 100% ovicidal activity against the KS1 flea strain throughout this study; no viable eggs were collected from dogs treated with lufenuron-milbemycin oxime.

Flea infestations can be dramatically reduced or eliminated using topical and/or systemic flea adulticides if flea death occurs prior to initiating reproduction or if reproduction is directly inhibited. One previous study provided evidence of remarkable residual adulticidal efficacy (100% at 40mg/kg) of spinosad at day 30 post-treatment.⁸ However, another study demonstrated reduced adulticidal efficacy (89% at day 30) with limited production of flea eggs from dogs 30 days following treatment with spinosad.¹⁶

Data from the current study shows that viable eggs were produced on spinosad treated dogs infested with the KS1 strain of cat fleas between monthly doses. Therefore, variability in susceptibility to spinosad may also exist in other flea populations.

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Conflict of Interest Statements:

This study was funded in part by Novartis Animal Health US, Inc. Dr Dryden performs consulting work for Novartis Animal Health and other pharmaceutical companies. Drs Dryden and Payne have been sponsored to present lectures by Novartis Animal Health and other pharmaceutical companies. Vicki Smith and Debra Ritchie are research technicians working in laboratories super-

vised by Drs Dryden and Payne. Dr Allen is an employee of Novartis Animal Health US, Inc, and participated in the study design, analysis, interpretation of the data, and writing and submission of the manuscript for consideration. Statistical analysis was performed by Louis Luempert, a biostatistician employed by Novartis Animal Health US, Inc.

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