Adulticidal Efficacy of a Formulation of Fipronil/(S)-methoprene/cyphenothrin Against KS1 (Kansas State 1) *Ctenocephalides felis felis* (Bouché) Fleas

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ABSTRACT

Background

A study was conducted to evaluate the efficacy of FRONTLINE® TRITAK® For Dogs (fipronil/(S)-methoprene/cyphenothrin, Merial, Inc., Duluth, GA) against a known strain of fleas with a history of reduced susceptibility to fipronil and pyrethroids, KS1 (Kansas State 1), of *Ctenocephalides felis felis* fleas.

Methods

One day after topical treatment with placebo or active, dogs (n = 32) were infested with 100 unfed adult KS1 fleas, with repeat infestations on Days 7, 14, 21 and 28. The number of live fleas was counted at 12 hours post-infestation for Groups 1 (n=8) and 3 (n=8) and at 24 hours post-infestation for Groups 2 (n=8) and 4 (n=8).

Results

Observed efficacies were 97.1-99.8% at 12-hour assessments on Day 1 through Day 28 and 99.8-100% at 24-hour assessments, occurring on Day 2 through Day 29.

Conclusions

A single dose of FRONTLINE® TRITAK® For Dogs (fipronil/(S)-methoprene/cyphenothrin) (0.67 ml or 1.34 ml) provided excellent efficacy against the KS1 *Ctenocephalides felis felis* flea strain for up to 4 weeks after treatment.

BACKGROUND

Elimination and prevention of flea infestations is accomplished by both killing the existing flea population on a pet and having

Table 1. *Geometric mean[1] flea counts 12 hours after infestation (and percent efficacy) for dogs treated with FRONTLINE TRITAK, or remaining untreated*

Day of flea removal	Control	FRONTLINE TRITAK	Efficacy	p-value
Day 1	60.6	1.8[B]	97.1%	<0.0001[U]
Day 7	81.0	0.1[B]	99.8%	<0.0001[E]
Day 14	78.5	0.4[B]	99.5%	<0.0001[E]
Day 21	45.4	0.1[B]	99.8%	<0.0001[E]
Day 28	56.4	1.4[B]	97.5%	<0.0001[U]

1 Based on transformation to natural logarithm of (count + 1)
B Significantly different from control (p<0.01)
E Results from t-test for means with poolable variances
U Results from t-test for means with unequal variances

residual protection throughout the labeled duration of activity by rapidly killing any newly acquired fleas that the pet may pick up. The cat flea, *Ctenocephalides felis*, is the most common and prevalent flea species infesting dogs.[1] These fleas can act as vectors to transmit pathogens to animals and cause allergic dermatitis, which is why the ability to eliminate them quickly and continuously is important.

The KS1 strain of *Ctenocephalides felis* is a laboratory strain that has been maintained at Kansas State University as a closed colony since the 1990s. Previous research has shown that the KS1 strain has reduced susceptibility to compounds such as fipronil and pyrethroids.[2] Other studies have documented decreased levels of persistent activity of fipronil against KS1 fleas over the course of the month.[3,4,5]

The purpose of this study was to evaluate in a well-controlled study the efficacy of a fipronil/(S)-methoprene/cyphenothrin combination against the KS1 *Ctenocephalides felis* strain of fleas at both 24 and 48 hours after flea infestations for 1 month.

METHODS

Experimental Design

A study was conducted to demonstrate the efficacy of fipronil/(S)-methoprene/cyphenothrin against the KS1 strain of fleas. These studies were performed at Kansas State University and were designed in accordance with standard methods for evaluating the efficacy of parasiticides for the control of flea infestations.[6] Animals were handled in compliance with both Merial Institutional Animal Care and Use Committee (IACUC) (#13-116M) and Kansas State IACUC (#3388) approvals, and were in compliance with the Animal Welfare Act. The trial facility meets USDA-APHIS animal welfare requirements.

Animals

The study involved 32 purpose bred beagle canines (8 per group), each identified by a unique numerical tattoo. Sixteen males and 16 females, approximately 8-9 months old, and weighing 15.0-20.0 lbs. (6.8-9.1 kg) were included. All dogs were in good health, and none had been treated with an ectoparasiticide product, either topically or orally, within 3 months prior to the study and treatment. Pre-treatment flea infestations, with removal counts, were performed on Day -2 to ensure that dogs were capable of maintaining adequate flea infestations, and then allocated to their assigned treatment group. Dogs were not anesthetized or sedated prior to any of the study infestations. Dogs were housed individually. Health observations were conducted daily throughout the study, which included every hour for 4 hours following the treatment with the fipronil/(S)-methoprene/cyphenothrin on Day 0.

Study Design

A total of 38 dogs (19 males and 19

Table 2. *Geometric mean[1] flea counts 24 hours after infestation (and percent efficacy) for dogs treated with FRONTLINE TRITAK, or remaining untreated*

Day of flea removal	Control	FRONTLINE TRITAK	Efficacy	p-value
Day 2	58.2	0.1[B]	99.8%	<0.0001[E]
Day 9	67.5	0.0[B]	100%	<0.0001[U]
Day 15	55.4	0.0[B]	100%	<0.0001[U]
Day 22	39.8	0.0[B]	100%	<0.0001[U]
Day 29	51.2	0.1[B]	99.8%	<0.0001[E]

1 Based on transformation to natural logarithm of (count + 1)
B Significantly different from control (p<0.01)
E Results from t-test for means with poolable variances
U Results from t-test for means with unequal variances

females) were infested with approximately 100 *Ctenocephalides felis* fleas prior to Day -2, and comb-counted 24 hours later. The six dogs with the lowest pre-treatment flea counts were not allocated. The remaining 32 dogs (16 males and 16 females) with the highest flea counts were ranked within sex by decreasing pre-treatment flea counts. Studies followed a controlled, randomized block design. Eight replicates of four animals each were formed. The four dogs with the highest pre-treatment flea counts formed replicate 1; the next four highest formed replicate 2, and so on, until all dogs were allocated. Within replicates, each dog was randomly allocated to one of the four treatment groups. All dogs remained in their assigned groups for the duration of the study.

Dogs were weighed prior to Day 0 and the appropriate treatment size was selected based on the animal's weight. On Day 0, treatment with commercially available product was applied according to label instructions, topically by parting the hair between the shoulder blades and applying the formulation directly to the skin. Dogs in groups 1 and 2 were designated as the control dogs, and they were treated with the weight-appropriate dose of mineral oil of either 1.0 mL or 2.0 mL. Dogs in groups 3 and 4 were treated with the weight- appropriate dose of FRONTLINE® TRITAK® For Dogs (fipronil/(S)-methoprene/cyphenothrin) of either 0.67 mL or 1.34 mL.

Dogs were treated once on Day 0 per allocation. Dogs were each infested on Days 1, 7, 14, 21, and 28 post-treatment with 100 *Ctenocephalides felis* fleas from the KS1 flea strain. At 12 hours post-infestation, dogs in Group 1 and 3 were systematically combed with a standard flea comb, removing and counting all live fleas. At 24 hours post-infestation, dogs in Group 2 and 4 were also thoroughly combed to remove and enumerate live fleas.

All dogs were combed, using a fine-toothed flea comb, having 12 - 13 teeth/cm, for at least 10 minutes. If five or more live fleas were recovered during this period, the dog was combed for an additional 5 minutes. If any live fleas were recovered during the second combing period, the dog was combed for an additional 5 minutes to ensure no live fleas remained. Different flea combs were used for collection with the treated and the control group dogs.

Data Analysis

All analyses and calculations were performed using SAS Version 9.3. Statistical significance was declared at a two-sided p-value of 0.05.

Adult flea counts were transformed to the natural logarithm of (count + 1) to calculate geometric means. Percent efficacy for each treatment group on each day was calculated as: 100 * (GMC –GMT) /GMC, where GMC = geometric mean of the control group and GMT = geometric mean of

the treated group.

The transformed data were analyzed using t-tests for means with poolable variances or for means with unequal variances, as appropriate; variances were compared using the maximum-F test and Satterthwaite's Approximation was used to determine the degrees of freedom for the unequal-variance tests. When one group had zero variance, variances were declared unequal by definition. The t-test is equivalent to one-way ANOVA when variances are poolable, and is more appropriate when variances are found to be unequal. Each treated group was compared to the corresponding control group.

RESULTS

All animals remained in apparent good health throughout the study. The geometric mean counts of the live fleas in the control and treated groups 1 and 3 at 12 hours post-infestation ranged between 45.4 to 81.0, and 0.1 to 1.8, respectively (Table 1). The geometric mean counts of the live fleas in the control and treated groups 2 and 4 at 24 hours post-infestation ranged between 39.8 to 58.2 and 0.0 to 0.1, respectively (Table 2).

The 12-hour post-infestation efficacies observed in dogs that were infested with the KS1 strain of *Ctenocephalides felis* fleas on Days 1, 7, 14, 21, and 28 were 97.1%, 99.8%, 99.5%, 99.8%, and 97.5% (Table 1). The 24-hour post-infestation efficacies observed in dogs that were infested with the KS1 strain of *Ctenocephalides felis* fleas on Days 1, 7, 14, 21, and 28 and counted on Days 2, 8, 15, 22, and 29 were 99.8%, 100%, 100%, 100%, and 99.8% (Table 2). There was a highly significant difference ($p<0.0001$) between the treated and control dogs at both 12 and 24 hour time points through Day 29 against the KS1 strain of *Ctenocephalides felis* fleas.

DISCUSSION

In this study, a single dose of fipronil/(S)-methoprene/cyphenothrin was highly effective in controlling the KS1 strain of *Ctenocephalides felis* felis. Considering the standard EPA and FDA regulatory approval for flea efficacy is a geometric mean of 90%, fipronil/(S)-methoprene/cyphenothrin outperformed this standard demonstrating a geometric mean control level at 12 hours post-infestation of >97% efficacy, and at 24 hours post-infestation, efficacy remained >99% throughout the duration of the study.

Previous research has shown that the KS1 flea strain has reduced susceptibility to fipronil, imidacloprid, pyrethrins, and spinosad with efficacy dropping below 90% often well before 28 days post-treatment.[3,4,5,7,8,9] . While FRONTLINE® TRITAK® contains both fipronil and cyphenothrin, a pyrethroid insecticide, diminished activity was not seen against KS1 fleas. In this study the combination demonstrated very high efficacy throughout the entirety of the study consistently reducing the number of KS1 fleas present at both 12 and 24 hours post-infestation on Days 1, 7, 14, 21, and 28 by 97.1-100%.

CONCLUSIONS

The study demonstrated the efficacy of a single dose of fipronil/(S)-methoprene/cyphenothrin against KS1 (Kansas State 1) *Ctenocephalides felis* felis. Following the single dose, new flea infestations were rapidly cleared and the residual control against fleas was provided and continued for at least a month.

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Competing Interests

The work reported herein was funded by Merial Inc., Duluth, GA, USA. DC is employed by Merial, Inc., SJG is a PhD independent contract biostatistician. MWD has been sponsored to present lectures and has research projects funded at Kansas State University by Merial Limited, manufacturers of FRONTLINE® TRITAK®. RS is a contractor for Merial, Inc.

Authors' Contributions

DC & MWD were authors of study design and MWD served as primary study investi-

gator. All authors reviewed and approved the final manuscript.

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