Residual Adulticidal Activity of a Dinotefuran-pyriproxyfen Topical Spot-on Formulation Applied to Dogs Against Weekly Infestations with the KS1 Flea Strain

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ABSTRACT

The residual efficacy of a dinotefuran (22% w/w)-pyriproxyfen (3% w/w) topical spot-on formulation against the KS1 flea strain infesting dogs was determined.

Dogs were allocated to two treatment groups. Eight dogs in treatment group 1 were treated with the dinotefuran-pyriproxyfen formulation according to label directions and the eight dogs in treatment group 2 served as untreated controls. Therapeutic efficacy was determined on dogs infested with 100 fleas, on day -2 , treated on day 0 and assessed for fleas removed by combing 24 hours post-treatment. Residual efficacy was determined by re-infesting dogs with 100 fleas on days 7, 14, 21, 28, 35, 42, 49, and 56 days post-treatment, and then removing fleas from dogs 24 hours post re-infestation by use of a flea comb. The dinotefuran-pyriproxyfen topical spot-on formulation eliminated all fleas from treated dogs within 24 hours of administration of treatment. Efficacy was 100% within 24 hours of infestation on day 7, was 99.4% after the day 14 infestation, and declined minimally to 96.1% following the day 56 infestation.

INTRODUCTION

The cat flea, *Ctenocephalides felis*, is the most important ectoparasite of cats and dogs worldwide.[1] Veterinarians and pet owners are now armed with an array of new flea control technologies. But as new product technologies are continually developed, the goals of flea control have not changed.[2] The resident flea population on the pet must be

killed, the infestation in the premises must be eliminated, and recurrence of flea infestation prevented.

Flea control on dogs and cats and their in-home premises is often based on the ability of topical or systemic treatments to not only to kill newly acquired fleas, but also to prevent flea reproduction.[2-7] Flea infestations on pets and in the in-home premises are being controlled because these various formulations either kill most newly acquired fleas prior to initiation of egg laying and/or rendering the vast majority of eggs non-viable.[5-7]

Once *C felis* acquires a host, the males and females will mate rapidly, and females begin laying eggs within the hair-coat of their host within 24–48 hours.[8-9] After the eggs are laid, they drop off the host into the premises, where they ultimately develop from eggs to larvae, to pupae, and then adult fleas.[1]

Female cat fleas can produce 40–50 eggs/day, and massive levels of infestation can occur rapidly if reproduction is not stopped.[10] Therefore, if a residual insecticide is going to be able to stop flea reproduction it must be able to kill or render newly acquired fleas moribund within 24 hours. Dinotefuran is a quick-kill furanicotinyl insecticide, and previous studies have documented that different topical formulations exhibit rapid residual speed of flea kill.[11,12]

In one study when dogs were infested with KS1 fleas 28 days after administration of a topical spot-on dinotefuran (22% w/w)-pyriproxyfen (3% w/w) formulation, 99.5% of the fleas were dead within 24 hours. [12]

The purpose of the current study was to determine the 24-hour residual efficacy of a topical spot-on dinotefuran (22% w/w)-pyriproxyfen (3% w/w) formulation for an extended period (56 days) after a single application. Fleas used in the current investigation were from the KS1 cat flea, *C felis* strain. The KS1 flea strain has been maintained as a closed colony at Kansas State University since 1990. Previous in-vitro and in-vivo evaluations demonstrate that the KS1 strain has some level of resistance or reduced susceptibility to carbaryl, chlorpyriphos, fenthion, fipronil, imidacloprid, permethrin, pyrethrins, and spinosad.[13-20]

MATERIALS AND METHODS

Fleas

Cat fleas, *Ctenocephalides felis*, the KS-1 strain, which have been maintained at Kansas State University as a closed colony since July 1990.

Animals and Housing

Twenty Dogs (10 male, 10 female; 8.8 ± 1.7 kg of body weight; 8–- 9 months of age) were housed in individual cages. No drugs, baths, shampoos, or pesticides were administered to the dogs during the preconditioning phase or the course of the study other than what was described in the protocol. All animal care procedures conformed to guidelines established by the Institutional Animal Care and Use Committee at Kansas State University (IACUC protocol #3424).

Animal Selection and Allocation

All dogs were infested with 100 fleas on day -5 and combed to remove all fleas on day -4 . The eight females and eight males with the highest flea counts were retained for the study. Within each gender the eight dogs were ranked in descending order by flea count. For each of the eight dogs in each gender group, a random number generated by EXCEL was assigned to each dog in rank order.

Dogs were grouped into replicates of two based on descending flea counts. The two dogs within each of the replicates were allocated to treatment groups (1 and 2). Each replicate contained one dog in each of the two treatment groups. The highest random number in each block was assigned to treatment number 1 and the second highest number to treatment number 2. This was repeated for each block of animals in the study. Thus, at the end of the process, there were eight dogs in each treatment group.

Treatment

Dogs were weighed prior to treatment. Dogs in treatment group 1 (8) were admin-

Table 1. *Geometric mean flea counts and percent efficacy against the KS1 cat flea strain infesting dogs treated with a dinotefuran (22% w/w) - pyriproxyfen (3% w/w) topical spot-on formulation.*

	Day 0		Day 7[5]		Day 14		Day 21		Day 28	
Treatment[1]	Mean # of fleas[2,3]	% control[4]	Mean # of fleas	% control	Mean # of fleas	% control	Mean # of fleas	% control	Mean # of fleas	% control
24 hours post-treatment or infestation										
Control	15.1[5]		53.5		58.2		47.8		61.1	
DP	0.0[a]	100	0.0[b]	100	0.3[b]	99.4	0.7b	98.6	0.9[b]	98.5
	Day 35		Day 42		Day 49		Day 56			
Control	49.4		43.4		41.4		47.9			
DP	0.9[b]	98.2	1.0 [b]	97.7	1.1 [b]	97.2	1.9 [b]	96.1		

[1] 16 dogs were used in this study. The 8 dogs in the control group received no treatment. The 8 dogs in the DP (dinotefuran 22% w/w - pyriproxyfen 3.00% w/w) group were administered the topical spot-on according to label directions on Day 0.

[2] Each dog (8 – 11 months of age) was infested with approximately 100 adult Ctenocephalides felis from the KS1 strain on days -2, 7, 14, 21, 28, 35, 42, 49 & 56.

[3] Geometric mean # of live fleas recovered from dogs per treatment group.

[4] % reduction = ((geometric mean count control -geometric mean count treatment)/ geometric mean count treatment)) x 100

[a] geometric mean of treatment group was significantly different from control (P =0.002).

[b] geometric mean of treatment group was significantly different from control (P <0.001).

[5] Two of the dogs in the control group were extremely fastidious groomers and had single digit flea numbers after the initial infestation therefore, they were removed from the study and the control group contained 6 dogs from day 7 through the end of the study.

istered a dinotefuran 22.0% w/w (24.86% w/v) - pyriproxyfen 3% w/w (3.39% w/v) (Vectra® for Dogs and Puppies – CEVA) topical spot-on according to label directions. Dogs assigned to group 2 (8) served as untreated controls.

Infestations and Efficacy Evaluations

Dogs were infested with 100 fleas 1 to 5 days post emergence, on Day -2 , and fleas were removed by combing 24 hours post-treatment. Residual efficacy was determined by re-infesting dogs with 100 fleas on days 7, 14, 21, 28, 35, 42, 49, and 56 days post-treatment, and then removing fleas from dogs 24 hours post re-infestation by use of a flea comb.

Fleas were removed by combing each dog with a fine-toothed flea comb having 12-13 teeth per centimeter. Only live fleas were enumerated. Flea removal was conducted by combing each dog thoroughly for 10 minutes by two members of the flea team. If five or more live fleas were recovered during this period, the dog was combed for an additional 5 minutes. If any fleas were recovered during this second combing period, the dog was combed for an additional 5 minutes.

Analysis

All analyses and calculations were performed using SAS version 9.3. Flea counts were transformed to the natural logarithm of (count+1) to calculate geometric means. Percent efficacy for each treatment group on each study day was calculated with the Abbott formula as follows:

$$\text{Efficacy } (\%) = 100 \times ((MC - MT))/MC$$

Where MC = mean of the control group (geometric or arithmetic) and MT = mean of the treated group (geometric or arithmetic).

Treatments were compared using a GLIMMIX procedure (The GLIMMIX procedure fits statistical models to data with

correlations or non-constant variability, and where the response is not necessarily normally distributed). In the results by day, treatments were also compared using a t-test procedure. Assumptions of equal variances were tested using Folded F test, in case of rejection of homogeneity the Satterthwaite's correction was applied.

RESULTS

Even though a pretreatment assessment was conducted to eliminate dogs that were poor retainers of fleas, two control dogs exhibited extreme grooming behavior following the day -2 infestation and upon combing on day 1. Each had less than 10 fleas. This resulted in a geometric flea burden on control dogs of only 15.1. (Table 1) These two dogs were removed from the study, dropping the control group from eight to six dogs for the remainder of the study. Geometric mean flea burdens on controls thereafter ranged from 43.4 to 66.1 (Table 1).

The dinotefuran-pyriproxyfen topical spot-on formulation eliminated all fleas from treated dogs within 24 hours of administration of treatment (Table 1). Efficacy was 100% within 24 hours of the day 7 infestation, was 99.4% after the day 14 infestation, and declined minimally to 96.1% following the day 56 infestation. (Table 1).

DISCUSSION

It had been previously demonstrated that several flea products did not perform well against the KS1 flea strain, either due to resistance or innate reduced susceptibility.[13-20] Certain organophosphate and pyrethroid-based products had remarkably poor residual efficacy against this flea strain. Residual speed of kill of organophosphate and pyrethroid based products was so slow that substantial egg production occurred between weekly flea infestations.[19] Evaluation of a 65% w/w permethrin spot-on, 13.8% w/w fenthion spot-on, and an 8% w/w chlorpyriphos collar revealed that these products only provided 49.1%, 57.7%, and 8.5% control of eggs following flea infestations 3 weeks post-treatment, respectively.[19] Because egg production begins around 24 hours or later post-infestation, these data clearly indicate very poor residual efficacy of those insecticides against the KS1 flea strain infesting dogs.

Imidacloprid, fipronil, and spinosad based products have also demonstrated poor performance against this strain. When any of these three compounds were administered to dogs or cats, all of these products produced <90% adulticide efficacy against the KS1 flea strain prior to the next labeled monthly application.[13,18-20] This study demonstrated that the dinotefuran-pyriproxyfen topical spot-on formulation produced prolonged residual activity against the KS1 flea strain with >96.1% control of fleas within 24 hours of treatment and within 24 hours of infestation through 8 weeks.

In a previous study published in 2011 conducted in our laboratory using the KS1 flea strain, it was determined that the 24-hour residual efficacy on day 28 post-treatment of this same dinotefuran-pyriproxyfen formulation was 99.5%.[12] That is similar to efficacy observed during this current study in the same time frame of 98.5%, indicating fairly consistent results with this formulation and strain several years apart.

This dinotefuran - pyriproxyfen formulation is labeled for monthly application to control fleas on dogs. This study clearly demonstrated that adulticidal efficacy well above 90% was maintained substantially beyond the labeled reapplication period. Pet owners do not always remember to reapply an ectoparaciticide monthly. Reapplication may occur several days or occasionally much later than is recommended. A formulation that provides substantial efficacy beyond the scheduled reapplication time would clearly be beneficial to non-compliant pet owners.

Because efficacy was determined by removing fleas 24 hours after each post-treatment infestation, no direct measurement on egg production occurred. However, the high level of 24-hour residual efficacy at every time point after treatment would clearly

indicate this formulation has a profound effect upon flea reproduction.

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