Efficacy of Topically Applied Dinotefuran Formulations and Orally Administered Spinosad Tablets Against the KS1 Flea Strain Infesting Dogs.

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ABSTRACT

Two studies were conducted to evaluate and compare the efficacy of two different topical dinotefuran formulations and orally administered spinosad against the KS1 flea strain infesting dogs. In study 1. treatment groups were untreated controls, dinotefuran - pyriproxyfen - permethrin topical spot-on (DPP) and spinosad chewable tablet. In study 2, treatment groups were untreated controls, dinotefuran – pyriproxyfen (DP) topical spot-on and spinosad chewable tablet. All dogs were infested with 100 fleas on Day -2, 7, 14, 21 and 28. In study #1, spinosad and the DPP formulation provided 100 and 97.2% efficacy at 6 hours post-treatment. On day 28, the 24-hour efficacy of the spinosad formulation dropped to 22.1%. The DPP formulation still provided 92.3% efficacy 6 hrs post infestation on day 28. In study # 2 the DP formulation provided 100 - 99.5% efficacy through day 28 at 6 and 24 hour comb counts. The spinosad formulation was 100% 6 hours post-treatment, but efficacy dropped to 32.5% 24 hrs post-reinfestation on day 28. The dinotefuran topical formulations were highly effective against the KS1 flea strain infesting dogs, whereas the spinosad oral tablets, while highly effective against established infestations, did not provide a high level of residual efficacy on days 21 and 28 when dogs were combed at 24 hrs post-infestation.

INTRODUCTION

While great advances have been made in flea product technology, the three primary goals of flea control have not changed.[1] When flea infested pets are presented to a veterinarian, the resident flea population on the pet must be eliminated rapidly, thereby providing rapid relief, and elimination of the infestation in the premises that results in continual reinfestation of pets and prevent future flea infestations. With regard to to eliminating adult fleas already feeding on pets, and killing newly acquired fleas, speed of kill is important. Rapid flea kill helps to manage Flea Allergy Dermatitis, reduces

reproduction, decreases the possibility of transmission of vector borne diseases, and can increase client satisfaction. The purpose of the two studies described in this article was to evaluate and compare the initial and residual speed of kill of a spinosad chewable tablet and two different dinotefuran based formulations against the KS1 flea strain. While published data is currently lacking on the speed of flea kill on dogs of dinotefuran based formulations, previous evaluations of orally administered spinosad have demonstrated that it has rapid initial speed of flea kill and potent residual activity.[2-4] Dinotefuran is a quick-kill nitroguanidine insecticide first discovered in 1993.[5]

The compound was structured and synthesized with acetylcholine as the lead, distinguishing it from other neonicotinoids that are based on nicotine. Specifically, Dinotefuran mimics the action of a neurotransmitter acetylcholine. Acetylcholine normally activates a nerve impulse at the synapse, but its effects are terminated very quickly.[5] Dinotefuran binds to one of the same receptor sites as acetylcholine, and activates the nerve impulse. The binding is permanent, causing continuous nerve stimulation, which in turn results in tremors, uncoordinated movement, and death of the insect. Most importantly, Dinotefuran is specific, and does not bind to mammalian acetylcholine receptor sites.[5]

Fleas used in the current investigations were the KS1 cat flea, *Ctenocephalides felis*, a strain that has been maintained as a closed colony at Kansas State University since 1990. In-vitro and in-vivo evaluations have indicated that the KS1 strain has some level of resistance or reduced susceptibility to carbaryl, chlorpyriphos, fenthion, fipronil, imidacloprid, permethrin, and pyrethrins.[6-12]

MATERIALS AND METHODS

The two studies conducted in these investigations had almost identical study designs.

Animals and Housing:

Each study used 34 purpose bred mongrel dogs (17m:17f) between 8 and 11 months of age. Dogs were housed in indoor concrete runs with cinder block walls seperating each run. Dogs used in these studies had no drugs, baths, shampoos, or pesticides administered 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 approval #2857 and #2882)

Animal Selection and Randomization:

On day–7, the 34 dogs in each study were infested with 100 cat fleas, *Ctenocephalides felis*, (KS1 strain) 1 to 5 days post emergence. On day 5, flea comb counts were performed to assess the ability of dogs to maintain infestations. Dogs were combed with a fine-toothed flea comb having 12–13 teeth/cm. Flea removal was achieved by combing each dog thoroughly for 10 min. If five or more fleas were recovered during this period, the dog was combed for an additional 5 min. If any fleas were recovered during the second combing period, the dogs were combed for an additional 5 min.

The 15 male dogs and 15 female dogs retaining the highest flea levels were retained for the study. Within each gender the 15 dogs were ranked in descending order by flea count. Dogs were randomly grouped into replicates of three based on descending flea counts and allocated into one of three treatment groups (10 dogs; 5m/5f). Each group of 10 dogs was then randomly divided into two subgroups of 5 dogs each.

Treatments:

In study #1, treatment groups were untreated controls, dinotefuran (4.95% w/w) - pyriproxyfen (0.44% w/w) , permethrin (36.08% w/w) (DPP), (Vectra 3D®; Ceva Animal Health, Rutherford, NJ. Formerly Summit VetPharm) topical spot-on, and spinosad 30 – 60 mg/kg (Comfortis®; Elanco Animal Health, Greenfield IN) chewable tablet.

In study #2 treatment groups were untreated controls, dinotefuran 22% w/w - pyriproxyfen 3.00% w/w (DP), (Vectra for Dogs and Puppies®; Ceva Animal Health,

Rutherford, NJ. Formerly Summit Vet-Pharm) topical spot-on applied and spinosad 30 – 60 mg/kg (Comfortis; Elanco) chewable tablet. In both studies, the DPP and DP were applied according to label directions. Spinosad tablets were administered only after dogs had been observed to have eaten and dogs were observed carefully for 1 hour after administration to ensure they did not vomit up the tablet.

Efficacy Evaluations:

To evaluate efficacy of the formulations in eliminating an existing flea infestation,all dogs were infested with 100 fleas on Day –2. Treatments were applied on Day 0 and efficacy was determined by removing fleas from five dogs in each treatment group at 6 hours and five dogs at 24 hours post-treatment. Residual efficacy was determined by reinfesting dogs with 100 fleas on days 7, 14, 21 & 28 post-treatment and then removing fleas from five dogs in each treatment group at 6 hours and five dogs at 24 hours post-reinfestation. Fleas were removed using previously described flea combing procedure.

DATA ANALYSIS

All analyses and calculations were performed using SAS Version 9.2. Statistical significance was declared at a two-sided p-value of 0.05. 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.

Because at least one treatment group had zero variance for some days, and variances were frequently significantly ($p<0.05$) different using the maximum-F test for other days.

Table 1: *Geometric mean flea counts and percent efficacy against the KS1 cat flea strain infesting dogs treated with either a dinotefuran (4.95% w/w) - pyriproxyfen (0.44% w/w) - permethrin (36.08% w/w) topical spot-on or orally administered spinosad (30 – 60 mg/kg) chewable tablet.*

	Day 0		Day 7		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
6 hours post-treatment or infestation										
Controls	91.35[a]		68.1[a]		63.9[a]		68.0[a]		69.0[a]	
DPP	2.6[b]	97.2	0.1[b]	99.8	0.00[b]	100	1.4[b]	98.0	5.3[b]	92.3
Spinosad	0.0[c]	100	0.1[b]	99.8	3.9[b]	93.9	30.6[a]	55.0	44.6[a]	35.4
24 hours post-treatment or infestation										
Controls	112.25[a]		59.1[a]		59.1[a]		72.5[a]		58.7[a]	
DPP	0b	100	0[b]	100	0.3[b]	99.5	0.9[b]	98.8	3.0[b]	94.8
Spinosad	0b	100	0.9[b]	98.4	9.0[c]	84.8	21.5[a]	70.4	46.7[a]	22.1

[1] 30 dogs were used in this study. The 5 dogs in each the control group received no treatment. The 5 dogs in each DPP (dinotefuran 4.95% w/w - pyriproxyfen 0.44% w/w - permethrin 36.08% w/w) or spinosad (30 – 60mg/kg) group were administered the topical spot-on or oral chewable tablet, 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.

[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

[5] Note that one dog in each of the control group had in excess of 100 fleas removed at combing due to counting errors at infestation.

[a,b,c] geometric means within a column with unlike letter superscripts are significantly different (P <0.05).

Treatments were compared using a t-test for means with poolable variances or for means with unequal variances, as appropriate. Variances were compared using an F-test and Satterthwaite's Approximation was used to determine the degrees of freedom for the unequal-variance tests. When one variance was 0, the variances were unequal by definition, and where both variance were 0, no comparison was possible.

RESULTS

In study #1, the spinosad chewable tablet and the DPP topical spot-on formulation provided 100 and 97.2% efficacy within 6 hours post-treatment (Table 1). Both formulations provided > 93.9% efficacy within 6 hours post-infestation through day 14. However on day 21 the efficacy of the spinosad chewable tablet 6 hours post infestation had decreased to 55.0% (Table 1). The DPP topical spot-on formulation still provided 92.3% and 94.8% control at 6 and 24 hours post infestation on day 28, whereas, the spinosad chewable tablet provided only 22.1% efficacy 24 hours post infestation on day 28 (Table 1).

In study # 2, the DP topical spot-on formulation and the spinosad chewable tablet provided 100% efficacy within 6 hours post-treatment (Table 2). The DP topical spot-on provided 99.5-100 % control through day 28 at the 6 and 24 hour comb counts. While the spinosad chewable tablets provided 100% and 96.6% control 24 hours post-infestation on days 7 and 14 respectively, efficacy dropped rapidly thereafter (Table 2). Efficacy of the spinosad chewable tablets 24 hours post-infestation was only 32.5% on day 28 (Table 2). There were no adverse events associated with treatments in either study.

DISCUSSION

These studies demonstrated that both dinotefuran formulations and the spinosad chewable tablets had rapid initial speed of kill against the KS1 flea strain. It was also demonstrated that both dinotefuran formulations provided high levels of residual activity

Table 2. *Geometric mean flea counts and percent efficacy against the KS1 cat flea strain infesting dogs treated with either a dinotefuran (22% w/w) - pyriproxyfen (3.0% w/w) topical spot-on or orally administered spinosad (30 – 60 mg/kg) chewable tablet.*

	Day 0		Day 7		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
6 hours post-treatment or infestation										
Controls	69.5[a]		79.0[a]		59.9[a]		63.6[a]		66.5[a]	
DP	0.0[b]	100	0.0[b]	100	0.0[b]	100	0.0[b]	100	0.0[b]	100
Spinosad	0.0[b]	100	6.5[b]	91.7	13.9[c]	76.8	67.1[a]	0.0	70.7[a]	0.0
24 hours post-treatment or infestation										
Controls	61.6[a]		74.2[a]		55.9[a]		64.6[a]		60.5[a]	
DP	0.0[b]	100	0.0[b]	100	0.0[b]	100	0.1[b]	99.8	0.3[b]	99.5
Spinosad	0.0[b]	100	0.0[b]	100	1.9[b]	96.6	22.8[c]	64.7	40.8[a]	32.5

[1]30 dogs were used in this study. The 5 dogs in each control group received no treatment. The 5 dogs in each DP (dinotefuran 22% w/w - pyriproxyfen 3.00% w/w) or spinosad (30 – 60mg/kg) group were administered the topical spot-on or oral chewable tablet 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.

[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,b,c] geometric means within a column with unlike letter superscripts are significantly different ($P < 0.05$).

against this flea strain with > 92.3% control of fleas within 6 hours of infestation through day 28. Because the two studies were conducted separately, direct statistical comparisons of the two dinotefuran formulations is not possible. However, it does appear that the DP formulation with the higher concentration of dinotefuran (22%w/w) provided a more rapid residual speed of flea kill, providing >99.5% control throughout the study. These are the first published studies to evaluate the speed of flea kill attributes of these dinotefuran formulations on dogs.

It has previously been demonstrated that several flea products do not perform well against the KS1 flea strain either due to resistance or innate reduced susceptibility.[6-12] In one study, the residual speed of kill of organophosphate and pyrethroid based products was poor against this flea strain.[12] In that study a 65% permethrin spot-on, 13.8% fenthion spot-on and an 8% chlorpyriphos collar were applied to dogs to determine if these formulations could kill fleas rapidly enough to inhibit egg production. These formulations reduced egg production by only 49.1%, 57.7% and 8.5% when dogs were infested with the KS1 flea strain three weeks after application.[12]

Fipronil and imidacloprid are two other compounds shown to have less than optimal residual activity against the KS1 strain. This is interesting, since these insecticides were introduced into the U.S. as flea products 6 years after this strain was colonized. Numerous studies using other cat flea strains have reported that the 28-30 day residual efficacy of fipronil and imidacloprid based flea products to range from 95% to 100%.[6, 13-21]

However, when these fipronil and imidacloprid formulations were evaluated against the KS1 strain the 28-30 day residual efficacy was markedly reduced.[6,9-11] In studies conducted on cats, the residual efficacy of fipronil was dose-dependent and ranged from 71.4% at 7.5mg/kg to 89.8% at [15] mg/kg.[6,11] In another trial using cats, the residual efficacy of a spot-on formulation of imidacloprid against the KS1 strain 30 days post-treatment was only 72.6%.[9] While some insecticides do not have high residual activity against the KS1 strain, others have demonstrated good residual efficacy, including metaflumizone and selamectin.[9-11] Based on the current studies, dinotefuran is also highly effective against this flea strain, with rapid speed of kill, killing >92% of fleas in 6 hrs after 28 days, and should provide effective initial and residual flea control on flea infested dogs.

When the spinosad chewable tablets were administered to dogs in these studies, the initial activity against established flea infestations was pronounced with 100% flea kill within 6 hours. But the residual activity of spinosad against the KS1 flea strain infesting dogs dropped off markedly after 2 weeks. In these studies there was no significant activity against the KS1 flea strain when efficacy was evaluated 24 hours after infestation on day 28. It should be noted that the evaluation of residual efficacy was performed at 24 hours after each reinfestation in these studies, and not at the standard 48 hours. It is unknown why spinosad has reduced activity against the KS1 flea strain.

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