Study of the Sustained Speed of Kill of the Combination Fipronil/Amitraz/ (S)-methoprene and the Combination Imidacloprid/Permethrin Against Newly Acquired *Dermacentor variabilis* (American Dog Tick)

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ABSTRACT

The acaricidal efficacy of the commercial topical combination of fipronil/amitraz/(S)-methoprene (CERTIFECT™) and imidacloprid/permethrin (K9 ADVANTIX®) was evaluated against *Dermacentor variabilis*. The study was conducted with three treatment groups comprised of eight mixed breed dogs per group. Group 1 was the untreated control group; Group 2 was the CERTIFECT treated group, and Group 3 was the K9 ADVANTIX treated group. Each treatment was administered topically once on Day 0, according to the recommended label dose and instructions for use. All dogs were infested weekly beginning on Day 7 with 50 adult unfed *D. variabilis* over a period of 6 weeks. Ticks were counted 24 hours after each infestation. The percent reduction in the number of ticks by CERTIFECT ranged from 100% to 92.8 % when compared to untreated controls for all 6 weekly infestations. The percent reduction for K9 ADVANTIX ranged from 98.2% to 58.8%. CERTIFECT treated dogs had significantly ($P<0.05$) fewer ticks than K9 ADVANTIX

™ Merial has applied for the trademark of CERTIFECT in the United States and it is a registered trademark in other countries.

FRONTLINE Plus® is a registered trademark of Merial.

®K9 ADVANTIX is a registered trademark of Bayer

® ALLERGROOM is a registered trademark of Virbac Corporation

treated dogs at 24h counts at weeks 1, 3, 4, 5, and 6 assessments.

INTRODUCTION

Dermacentor tick species are present worldwide and can be vectors of disease for humans and animals.[1,2] In North America, *Dermacentor variabilis* is a common tick species affecting dogs. It is one of the vectors of *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever and tick attachment can cause a paralytic syndrome in dogs. Effective control of tick infestation in dogs is based mainly on the regular application of an acaricide.[3] Spot-on formulations of insecticide and/or acaricide drugs provide a convenient method for external parasite control in dogs and cats. Effective environmental control measures are complex and have variable success rates.

In this study, two veterinary-dispensed topical insecticide/acaricide combination products with labelled activity against ticks, including *Dermacentor variabilis*, were chosen for comparison: fipronil/amitraz/(S)-methoprene (CERTIFECT™ and imidacloprid/permethrin (K9 ADVANTIX®).

The combination of fipronil and (S)-methoprene formulated for topical application to dogs and cats (FRONTLINE® Plus) provides a broad spectrum of activity against insects (including fleas, flea eggs, and flea larvae, and lice) and acarids (including ticks and other mites).

The addition of amitraz to fipronil and (S)-methoprene potentiates the acaricidal effects of fipronil to significantly increase the speed of kill of ticks,[4,5] cause ticks to detach[6,7] and extend the high level of efficacy throughout the monthly application period.[8] Imidacloprid is an insecticide with no labelled activity against acarids. Thus, permethrin was added to imidacloprid to broaden the range of efficacy to include ticks. The tick efficacy guidelines in both the European Union and USA typically require a minimal activity of 90% of efficacy at 48 hour counts.[9] Faster acting acaricidal activity is desirable from both an aesthetic and risk of disease transmission perspective. The purpose of the study reported here was to assess the observed sustained activity of both products 24 hours after tick infestations at weekly intervals for up to 6 weeks after treatment.

MATERIALS AND METHODS

Study Design

This study was a negative (untreated) and positive (reference drug treated) controlled efficacy study. The study experimental unit was the individual healthy dog, each identified by a unique number, treated, and assessed for the study variables on an individual basis. Dogs had not been exposed to ectoparasiticides during the previous 3 months. All dogs were shampooed with Allergroom® Shampoo, a non-insecticidal shampoo, for approximately 5 minutes on Day -10.

Twenty-eight mixed breed dogs were allocated based on pre-treatment tick infestations conducted on Day-7 and counted at Day-5. Two 14-dog weight groups were formed on Day -5. Dogs in Weight Group 1 weighed 10.7 to 14.6 kg, and dogs in Weight Group 2 weighed 19.2 to 24.9 kg. Two dogs from Weight Group 2 and one dog from Weight Group 1 with the lowest pre-qualification tick count were removed from the pool of dogs to be utilized on the study. One dog from Weight Group 1 was removed for health reasons.

Three groups of eight dogs were randomly allocated based on decreasing body weight. The three dogs with the highest body weights formed Replicate 1, the next formed Replicate 2, and so on, until all dogs were allocated. Within replicates, each dog was randomly allocated to one of the three treatment groups.

The *D. variabilis* were obtained from the Oklahoma State University Tick Rearing Facility. These ticks were from a strain which is not known to be resistant to any ectoparasiticide.

Personnel involved with evaluation of efficacy were blinded as to treatment.

Each drug/formulation was applied topically to each dog per label directions, but

Table 1: *Categorization of Ticks for counting:*

Category	General Findings	Attachment status	Interpretation
1	Live	Free	Acaricidal effect NOT demonstrated
2	Live	Attached; unengorged	Acaricidal effect NOT demonstrated
3	Live	Attached; engorged1	Acaricidal effect NOT demonstrated
4	Dead	Free	Acaricidal effect demonstrated
5	Dead	Attached; unengorged	Acaricidal effect demonstrated
6	Dead	Attached; engorged[1]	Acaricidal effect NOT demonstrated

Adapted from Marchiondo et al., 2007
1 Engorged tick: a tick with a conspicuous enlargement of the alloscutum that has blood in its digestive tract, as shown by squeezing/crushing of the tick on white paper.

application occurred only once on Day 0. All dogs whose weights were not equivalent to a whole pound had their weights rounded up to the next whole pound.

Dogs in Treatment Group 1 were untreated. Dogs in Treatment Group 2 were treated with the appropriate pipette size of CERTIFECT to deliver a minimum dose of 6.7 mg/kg of fipronil, 8 mg/kg of amitraz and 6.03 mg/kg of (S)-methoprene on dogs. For treatment administration, the total volume was applied on two separate spots placed on the midline of the neck and at the base of the neck, between the shoulder blades.

Bodyweight Range	Pipette Size	Pipette Volumes (mL)
23 – 44 lbs (10.5 – 20 kg)	M	2.14
45 – 88 lbs (20.5 – 40 kg)	L	4.28

Dogs in Treatment Group 3 were treated with the appropriate pipette of K9 ADVANTIX delivering a minimum dose of 10 mg/kg of imidacloprid and 50 mg/kg of permethrin per dog. For treatment administration, the total volume was applied according to label direction by parting the hair and applying directly on the skin on 3 sites on dogs weighing less than 44 lbs (20 kg) and four sites for dogs weighing 45 lbs (20.5 kg) or more along the midline from the shoulder blades to the base of the tail.

Bodyweight Range	Pipette Color	Pipette Volumes (mL)
21 – 55 lbs (9.55 – 25 kg)	Red	2.5

Specification of Study Variables

Each dog was infested with unfed *D.variabilis* (50 +5) on Days 7, 14, 21, 28, 35, and 42. The tick infestations were made weekly and ticks were counted at 24 (+2) hours post-infestation. The ticks were categorized according to criteria described in Table 1. An infestation rate > 25% in at least six controls was considered to be an adequate infestation.

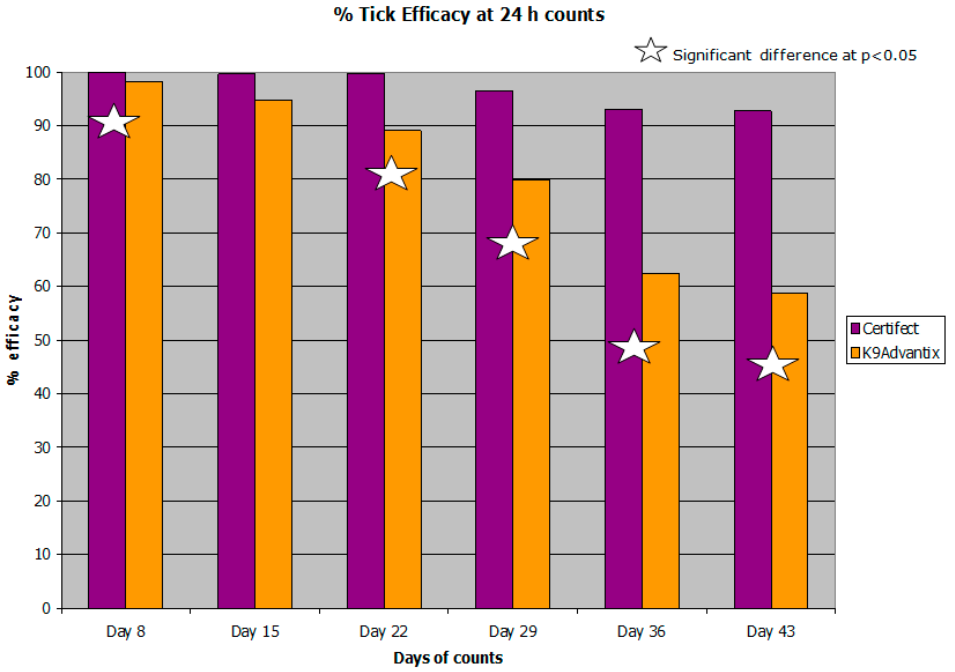
Data Analysis

The ticks were categorized as proposed by Marchiondo et al., 2007 (Table 1). Total counts of adult ticks in categories 1, 2, 3 and 6 were transformed to the natural logarithm of (count +1) for calculation of geometric means by treatment group at each time point. The ticks in the three 'Live' categories, as well as in the 'Dead, attached, engorged' category, were interpreted as

Table 2: *Results of comparative efficacy on Dermacentor variabilis*

Treatment Group		Tick Count on Day					
		8	15	22	29	36	43
Untreated Control	Geometric Mean	26.6	20.1	28.9	26.6	26.6	33.4
	Std Dev	8.2	6.6	8	8.7	10	8.3
CERTIFECT	Geometric Mean	0	0.1	0.1	0.9	1.9	2.4
	Std Dev	0	0.4	0.4	1.3	7.4	7.7
	Efficacy (%)	100	99.6	99.7	96.5	93	92.8
K9 ADVANTIX	Geometric Mean	0.5	1.1	3.1	5.4	10	13.8
	Std Dev	0.7	2.3	1.4	5.2	6.5	5.8
	Efficacy (%)	98.2	94.8	89.1	79.8	62.5	58.8

Figure 1: *Comparative Sustained Efficacy at 24h Counts Against Dermacentor variabilis*



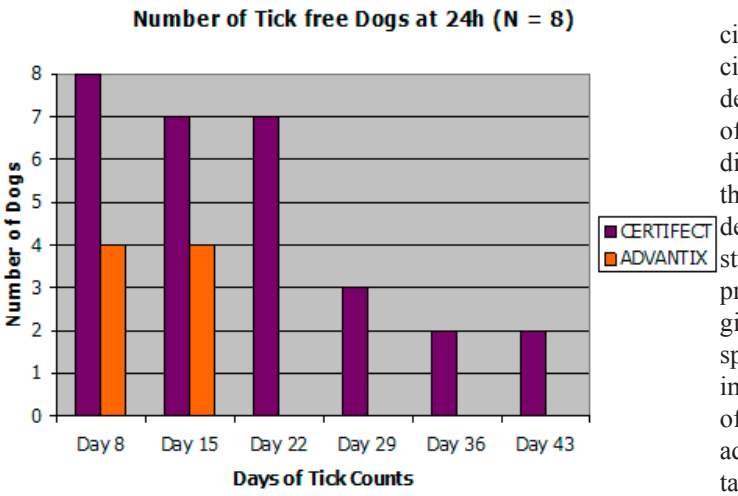
treatment failures. Their counts were combined and the total was used in the subsequent analysis.

Percent reduction from the negative control group (Treatment Group 1) mean was calculated for Treatment Groups 2 and 3 at every post-treatment time point using the formula [(C - T) / C] x 100, where C is the geometric mean for the negative control group and T is the geometric mean for Treatment Group 2 or 3. Treatment Group 2 was compared to each of the other treatment groups (Treatment Groups 1 and 3) using Analysis of Variance on log count. All testing was two-sided at the significance level α=0.05.

Animal Management

Animals were managed similarly and with due regard for their well-being. Animals were handled in compliance with the

Figure 2: *Number of Tick Free Dogs at 24hour*



relevant Institutional Animal Care and Use Committee approvals and other local applicable regulations and requirements.

RESULTS AND DISCUSSION

The tick counts at 24 hours and percentage of efficacy against *D.variabilis* are shown in Table 2 and Figure 1. The efficacy of CERTIFECT against *D. variabilis* at the 24 hour counts post-infestation ranged from 100% to 92.8% during the 6 week duration of the study. The percent reduction in the number of *D.variabilis* ticks when compared to the controls was significant ($P < 0.001$) at all 6 weekly time points. The efficacy of K9 ADVANTIX ranged from 98.2% to 94.8% for weeks 1 through 2 at 24 hours post-infestation. During the following 4 weeks (weeks 3 to 6) the efficacy of K9 ADVANTIX decreased from 89.1% to 58.5%. CERTIFECT, when compared to K9 ADVANTIX, had a significantly ($P<0.05$) greater percent efficacy against *D.variabilis* at Day 8 and at Days 22, 29, 36, and 43. After Day 15, there were no Tick Free dogs in the K9 ADVANTIX group, whereas some CERTIFECT-treated dogs remained free of ticks throughout the study (Figure 2).

Often, in tick efficacy studies, the tick counts rates are reported at 48 hours after infestation. It is documented in published references that the 48 hour efficacy of K9 ADVANTIX is usually greater than 90% for 3 to 4 weeks or more, based on the tick species.[10,11,12,13,14] Some specific study designs have determined the percentage of ticks that do not immediately or quickly infest the dogs,[13] which was not determined in the present study. The 24-hour data presented in this study gives an indication of the speed of kill, which is important for the benefit of the pet owners who are adverse to observing attached engorged ticks on their dogs. Moreover, the significant efficacy observed within 24 hours of tick infestation and the high percentage of tick free dogs treated with CERTIFECT should greatly reduce the risk of transmission of pathogens by ticks. With the proven synergistic combination[7] of fipronil and amitraz, the new topical combination formulation, CERTIFECT, provided a more rapid speed of kill of *Dermacentor variabilis* than K9 ADVANTIX in this study for a duration of at least 6 weeks.

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