A Study to Evaluate the Acaricidal Efficacy of a Single Topical Treatment with a Topical Combination of Fipronil/Amitraz/(S)-Methoprene Against Dermacentor Variabilis on Dogs

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ABSTRACT

The objective of the study was to confirm the acaricidal efficacy of a single topical treatment of the combination of fipronil/amitraz/(S)-methoprene (CERTIFECT®) against induced infestations of *Dermacentor variabilis*. Sixteen healthy mixed breed mongrels (8 males and 8 females), approximately 9.6 to 13.7 months of age on Day 0, and weighing 16.2 to 33.4 lbs, were selected from a group of 20 dogs based on Day -1 pre-treatment tick counts to be utilized in this randomized, blinded efficacy study. Eight dogs were randomly assigned to one of the two treatment groups: Group 1- placebo (vehicle), Group 2 - fipronil/amitraz/(S)-methoprene (delivering at least 6.7mg fipronil/kg body weight (bw), 8.0 mg amitraz/kg bw, and 6.0 mg (S)-methoprene/kg bw). The vehicle or treatment was applied directly onto the skin at two separate spots on the neck of each dog, once on Day 0, per label dose and directions for use. All dogs were infested with 50 (± 5) unfed adult *D. variabilis* on Day 1, then weekly on Days 7, 14, 21, 28, and 35. Ticks were thumb counted at 24 (±3) hours following each tick infestation and subsequently were counted and removed from each dog at 48 (±3) hours after tick infestation.

Thumb counts were performed on all dogs at 24 hours following each infestation. Efficacies on Days 2, 8, 15, 22, 29, and 36 were 98.6, 100, 99.7, 96.6, 86.6, and 90.1%, respectively. All dogs treated with the fipronil/amitraz/(S)-methoprene product had significantly ($p<0.05$) fewer ticks than placebo (vehicle-treated) control animals.

Removal counts were performed at 48 hours after each infestation. Efficacies for Days 3, 9, 16, 23, 30, and 37 were 100, 100, 100, 100, 99.4, and 97.2%, respectively. Again, all dogs treated with the fipronil/amitraz/(S)-methoprene product had significantly ($p<0.05$) fewer ticks than placebo (vehicle-treated) control animals.

No treatment related adverse events

were observed during the study, including during four observations performed within 5 hours post-treatment.

The results of this study confirm rapid and effective control of *D. variabilis* ticks on CERTIFECT-treated dogs.

INTRODUCTION

Globally, Ixodid ticks (hard-bodied ticks) transmit most of the important tick-borne pathogens found in people and animals, including dogs. While several genera are represented in this family, *Dermacentor spp*. are represented worldwide, and most are capable vectors of disease. In North America, *Dermacentor variabilis* is the primary representative of the genus by distribution, the major vector of Rocky Mountain Spotted Fever (RMSF), responsible for the spread of Tularemia, and has been implicated in the transmission of Cytauxzoonosis.[1] In addition, *Dermacentor variabilis* is one of the ticks responsible for causing tick paralysis.

Transmission of tick-borne disease typically requires ticks to attach and feed, followed by a reactivation period prior to transmission.[2] Therefore, a window of opportunity exists during which tick removal or acaricide product may reasonably interrupt transmission.[2] While the amount of time before a particular disease is transmitted is not clearly defined for many pathogens, with some tick-borne diseases, such as *Borrelia burgdorferi*, the process necessary for the organism to become infective and the subsequent transmission time is well established, with the highest transmission potential occurring by 48-72 hours following attachment.[3,4,5,6] Time following attachment and transmission of RMSF by *Dermacentor spp*. is less well defined, with transmission potentially occurring quickly or possibly as long as 48 hours after attachment.[7,8,9]

Regardless of how rapidly a particular pathogen is transmitted, with a reduction in tick exposure times, the potential for tick-borne transmission to occur is also diminished. This study was conducted to evaluate the acaricidal efficacy of a fipronil/amitraz/(S)-methoprene topical spot-on formulation against *Dermacentor variabilis* at both 24 and 48 hours following weekly infestations of dogs for 35 days following a single treatment.

MATERIALS AND METHODS

Study Design

This study was a blinded, placebo-controlled efficacy study using a randomized block design where blocks were based on pre-treatment tick counts within sex. None of the dogs considered for use in this study had been treated with ectoparasiticides (either topical or systemic) within 3 months of the start of the study. Each dog was shampooed on Day -7 with a non-insecticidal shampoo ALLERGROOM® for approximately 5 minutes. All dogs had a physical examination on Day -5 to ensure their healthy status. All animals received the same feed, which was given once or twice daily depending on recommended ration. Water was available *ad libitum*. All dogs were managed similarly and with due regard for their well-being in compliance with Kansas State University (IACUC #2903), local, and Merial Institutional Animal Care and Use Committee approvals and in accordance with any applicable laws and regulations. Each dog was individually housed throughout the study. All dogs used in this study were retained in the Kansas State University Veterinary School colony upon completion of the study.

On Day -3, 20 healthy purpose-bred mixed-breed mongrel dogs (10 males and 10 females) were infested with 50 (±5) *D. variabilis* once, pre-treatment, for selection purposes. The two dogs of each sex with the lowest pre-treatment tick count were not allocated and excluded from the remainder of the study. Thus, the remaining 16 dogs were ranked by decreasing pre-treatment tick counts within sex and eight replicates of two animals each were formed. The two male dogs with the highest pre-treatment tick counts formed Replicate 1; the next two highest formed Replicate 2, and so on, until all eight males were allocated. This process was repeated for females. Within replicates, each dog was randomly allocated to one of

the two treatment groups using the randomization function of Excel®. Dogs ultimately used in this study ranged in weight from 16.2-33.4 lbs and averaged 24.2 lbs. Ages of these dogs ranged from 9.6 months to 13.7 months, and averaged 11.3 months.

Treatments were dosed according to weight on Day 0, with dogs not weighing exactly on a whole pound having their weight rounded up to the next whole pound. Either a placebo (1 or 2 mL of vehicle) or the appropriate pipette of CERTIFECT (fipronil/amitraz/(S)-methoprene) was applied topically on two separate spots on the dorsal midline of the neck according to the label directions of the CERTIFECT product. For each dog, the first half of the application was deposited on the surface of the skin at the base of the neck in front of the shoulder blades. The second half of the application was applied just behind the base of the skull on the midline of the neck. For dogs in the treated group, the formulation delivered at least 6.7 mg fipronil/kg body weight (bw), 8.0 mg amitraz/kg bw, and 6.0 mg (S)-methoprene/kg bw. Personnel involved with subsequent evaluation of efficacy were unaware as to the treatment assignments of the animals. Treatment group designations were not revealed to personnel involved with the evaluations of efficacy, and dogs were coded for use in separation of groups during counting.

Ticks used in this study were sourced from an established colony at the Oklahoma State University Entomology department colony. The genetics of this colony are updated on a regular basis, using tick strains not known to be resistant to any ectoparasiticide. The ticks were received and re-counted to aliquots of 50 unfed adult ticks per infestation.

Placebo (vehicle)

Bodyweight Range (lbs)	Total Dose Volume (mL)
up to 22	1.0 mL
23 – 44	2.0 mL

CERTIFECT*

Bodyweight Range (lbs)	Pipette Volumes (mL)
up to 22	1.07
23 – 44	2.14

**Fipronil 6.4%, Amitraz 7.6%, and (S)-methoprene 5.8% of total volume*

Specification of Study Variables

All dogs were infested with *Dermacentor variabilis* on Days 1, 7, 14, 21, 28, and 35. Adult unfed *D. variabilis* were used for infestations. Animals were placed in lateral recumbency in a stainless steel tub, and ticks were applied along the left or right lateral side of the animal. No ticks were placed on the dorsal midline of the animals near treatment sites. Following tick application, each dog remained in the tub for ten minutes and if any ticks dropped or crawled off into the tub, they were picked up by hand and placed back on the dog. Following the period of tick exposure, each dog was returned to its original housing.

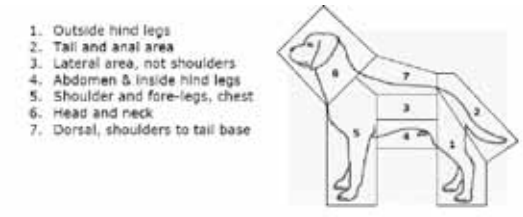
Thumb-counts were performed 24 hours later (±3 hours), and the number and status of any ticks present were recorded in the raw data. Thumb counts were carried out by parting and feeling through the dog's hair with finger tips. Dogs were examined by region, and ticks were counted and categorized appropriately. As ticks were counted, each tick was marked on the most posterior end with a small dot using a paint marker. Disposable gloves and aprons were worn and changed between each of the treatment groups.

The marking of ticks was performed to help ensure that individual ticks were counted once. The regions noted in Figure 1 were examined, individually, and counts recorded for each region.

The ticks were categorized according to Table 1. Categorization of the ticks allow calculation of the percent efficacy (killing effect), as well as the calculation of the attachment rate, in comparison with the untreated control group.

Tick removal counts were performed at 48 (±3) hours following each post-treatment infestation. Counting of ticks was carried out by parting and feeling through the dog's

Figure 1: *Regional tick-count mapping description*



hair with finger tips. When suspected ticks were found, the hair was further parted, visual confirmation of tick's presentation was made, and ticks were removed with forceps. After an area was cleared by this method, a flea comb was applied to the area for secondary confirmation of tick removal. The living status of the ticks was confirmed at removal, and ticks were disposed of in containers of soap solution. Each treatment group had separate comb/tweezers pairs assigned to it. Disposable gloves and aprons were worn and changed between each of the treatment groups.

Data Analysis

To measure the killing effect (% efficacy), the total counts of adult ticks in categories 1 through 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 treatment failures in this study. Their counts were combined, and the total for each dog was used in the subsequent analysis. Percent reduction from the negative control group (Treatment Group 1) mean was calculated for Treatment Group 2 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. Treatment Group 2 was compared to treatment group 1 using Analysis of Variance on log count. All testing was two-sided at the significance level α=0.05.

RESULTS

Dogs treated with fipronil/(S)-methoprene/amitraz had significantly ($p<0.05$) fewer ticks (live free, live attached unengorged, and dead attached engorged) than placebo (vehicle treated) control animals at every 24-hour post-infestation tick count. Efficacies for Days 2, 8, 15, 22, 29, and 36 were 98.6, 100, 99.7, 96.6, 86.6, and 90.1%, respectively (Table 2; Figure 2).

Dogs treated with fipronil/amitraz/(S)-methoprene had significantly ($p<0.05$) fewer ticks (live free, live attached unengorged, and dead attached engorged) than placebo (vehicle treated) control animals at every 48

Table 1: *Categorization of ticks for counting*[a]

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

[a]*Adapted from Marchiondo et al., 200710*

[b]*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.*

Table 2. *Summary of Tick Counts Post-treatment by Day at 24 and 48 Hours after Infestation*

Post- Treatment/ Infestation (hour)	Study Day	Placebo **(Treatment Group 1)** Geometric Mean[a] (Arithmetic Mean)	CERTIFECT **(Treatment Group 2)** Geometric Mean (Arithmetic Mean)	Efficacy[b] (%)	P-value[c]
24	2	31.8 (32.6)	0.4 (0.6)	98.6	<.0001
	8	25.4 (30.1)	0.0 (0.0)	100	<.0001
	15	33.3 (35.5)	0.1 (0.1)	99.7	<.0001
	22	33.0 (34.4)	1.1 (1.6)	96.6	<.0001
	29	25.9 (28.3)	3.5 (4.8)	86.6	0.0006
	36	34.0 (35.6)	3.4 (4.6)	90.1	0.0003
48	3	31.8 (33.6)	0.0 (0.0)	100	<.0001
	9	30.4 (36.1)	0.0 (0.0)	100	<.0001
	16	35.0 (37.8)	0.0 (0.0)	100	<.0001
	23	36.8 (38.3)	0.0 (0.0)	100	<.0001
	30	30.4 (32.6)	0.2 (0.3)	99.4	<.0001
	37	33.8 (36.3)	0.9 (1.3)	97.2	<.0001

[a] Based on transformation to the natural logarithm(count+1). There were 8 animals per treatment.
[b] Efficacy 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.
[c] Probability value from analysis of variance on log count.

hour post-infestation tick count. Efficacies for Days 3, 9, 16, 23, 30, and 37 were 100, 100, 100, 100, 99.4, and 97.2%, respectively (Table 2; Figure 2).

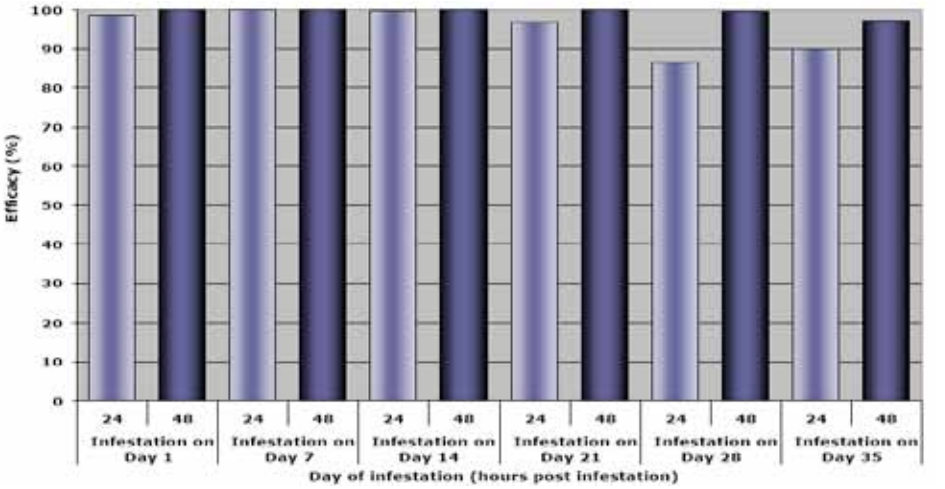
No treatment related adverse events occurred during the study.

DISCUSSION

A number of studies have been conducted to evaluate the efficacy of acaricides against ticks infesting dogs, but the standard timeframe for measuring acaricidal efficacy against ticks is typically 48 hours after experimental infestation. In this study, excellent efficacy was observed by 24 hours, as well as at the 48-hour assessment. Regarding transmission of many tick-borne diseases, it is true that following host acquisition, some time is needed for tick attachment, feeding, and pathogen activation before transmission.[11] Although the time necessary for this process varies, the more rapidly ticks can be killed or disengaged from the host, the less likely a pathogen will be transferred. It has been stated that if ticks are prevented from feeding for longer than 24 hours, the transmission rates of *Borrelia burgdorferi, Anaplasma phagocytophilum, R. rickettsii* and *Babesia microti* drop significantly.[12] Therefore, application of a residual acaracide that provides rapid kill and detachment of ticks within 24 hours should drastically limit the potential for transmission of these and other less well-studied tick borne diseases.

Because the fipronil/amitraz/(S)-methoprene combination affords a more rapid killing effect on ticks, 24 hour acaricidal efficacy was assessed in the current study, in addition to 48 hour assessments. Four previous studies have been published by other laboratories evaluating the 24 hour residual efficacy of CERTIFECT (fipronil/amitraz/(S)-methoprene) against different strains of *D. variabilis* infesting dogs.[12,13,14] Although performed in different laboratories, the residual efficacy in those studies is similar to the data generated in this study. In the current study, the efficacy achieved

Figure 2. *Tick Efficacy Post-treatment by Day at 24 and 48 Hours after Infestation*



24 and 48 hours after the day 28 infestation was 86.6% and 99.4%, respectively. In the study reported by Baker et al. (2011) the efficacy achieved 24 and 48 hours after the day 28 infestation was 83.2% and 98.5%, respectively. In the two studies reported by Prullage et al. (2011), the efficacy achieved 24 hours after the day 28 infestations was 87.4% and 95.9%. In the study reported by Kunkle et al. (2012), the ticks were counted at 24 hours after the day 28 infestation and efficacy was slightly higher, at 96.5%. The consistently high efficacy observed in five separate studies demonstrates the repeatability of this formulation's performance against multiple strains of this tick species.

Observed efficacy of acaricides often varies between trials, laboratories, or tick strains. This can be demonstrated by comparing three separate 48-hour efficacy studies of other acaricidal products performed against *Dermacentor variabilis*. Two efficacy trials conducted in the same laboratory at Kansas State University against *D. variabilis* demonstrate variability due to tick strains. In one trial, fipronil/(S)-methoprene and imidacloprid-permethrin were respectively 72.3% and 17.5% efficacious against a *D. variabilis* strain from Oklahoma at 48 hours on day 30.[15] In the second KSU trial, using a strain from California, fipronil/(S)-methoprene, and imidacloprid-permethrin were 83.2% and 92.0% efficacious, respectively on day 30.[16]

A third study illustrates variable results in different laboratories using the same tick strain. In this case, using the same California strain of *D. variabilis* tick as the second KSU study, another laboratory found the fipronil/(S)-methoprene was 93.8% efficacious on Day 28. [17] The variability of the results of these three acaricidal studies is a marked contrast to the consistent 24-hour performance CERTIFECT provided against *D. variabilis* ticks, which was observed in five separate trials, performed by four separate laboratories, and against multiple strains of D. variabilis.

Dermacentor spp. are well-known for their ability to cause tick paralysis and transmit tick-borne diseases.[1] Thus, rapid acaricidal efficacy causing ticks to be killed prior to attachment or rapidly detach throughout the treatment interval can be highly desirable traits of a topically applied acaricide. The fipronil/(S)-methoprene/amitraz was highly efficacious in affecting *Dermacentor variabilis* attachment (live or dead ticks) at both 24 and 48 hours after infestation throughout the month-long post- application period. As exposure times are reduced, the potential for tick-borne transmission to occur is also diminished. Because most ticks on the fipronil/amitraz/(S)-methoprene

treated dogs were detached or killed within 24 hours, disease transmission would be less likely to occur on these dogs.

CONCLUSIONS

For the comparison of CERTIFECT (fipronil/amitraz/(S)-methoprene) vs. placebo (vehicle), all post-infestation counts showed a significant treatment effect at α=0.05. Efficacy levels exceeded US EPA Guideline thresholds at all time-points and were >90% at all 24 hour counts through Day 22, 86.6% (p=0.0006) on Day 29, and 90.1% on Day 36. The results at 24 hours post-infestation demonstrate excellent and rapid tick control efficacy against *Dermacentor variabilis* throughout the study.

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Acknowledgements, Funding and Conflict of Interest Statements:

This study was funded by Merial Limited, Duluth, GA. Dr. Dryden performs consulting work for Merial Limited and other pharmaceutical companies. Drs. Carithers and Kunkle are employees of Merial Limited and participated in the study design, analysis, interpretation of the data, and writing and submission of the manuscript for consideration.

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