Efficacy of FRONTLINE® Plus (Fipronil/(S)-Methoprene) and FRONTLINE Gold (Fipronil/(S)-Methoprene/Pyriproxyfen) Against Ixodes scapularis at Twenty Four Hours Following Weekly Tick Infestations on Dogs

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ABSTRACT

Two separate studies were performed to evaluate the 24-hour efficacy of FRONTLINE® Plus for Dogs (fipronil/(S)-methoprene, Boehringer Ingelheim, Duluth, GA, USA) and FRONTLINE Gold for Dogs (fipronil/(S)-methoprene/pyriproxyfen, Boehringer Ingelheim, Duluth, GA, USA) against *Ixodes scapularis* ticks on Days 2, 9, 16, 23, and 30. From a pool of 28 dogs, 24 dogs with the highest tick counts were allocated to one of three treatment groups: Group A comprised eight control dogs treated with 0.5 mL of mineral oil each, Group B comprised eight dogs treated with FRONTLINE Plus (0.67 mL for dogs 5 to 22 lbs, or 1.34 mL for dogs 23 to 44 lbs), and Group C comprised eight FRONTLINE Gold-treated dogs (0.67 mL for dogs 5 to 22 lbs, or 1.34 mL for dogs 23 to 44 lbs). The first study compared Groups A and B, and the second study compared Groups A and C. Both studies utilized the same control dogs. Both studies were performed at the same time, on the same days, at the same facility. Combining the two studies, 24 dogs were treated with mineral oil (Group A), FRONTLINE Plus (Group B), or FRONTLINE Gold (Group C) on Day 0. All dogs were infested with 50 live, unfed *Ixodes scapularis* ticks on Days 1, 8, 15, 22, and 29 post-treatment, and all ticks were removed and counted 24 hours later on Days 2, 9, 16, 23, and 30. Dogs treated with FRONTLINE Plus had significantly ($p<0.01$) fewer live ticks than

the controls at 24 hours post-infestation on Days 2, 9, 16, 23, and 30. Geometric mean efficacy was 100% at Days 9 and 23; >99% at Days 2 and 16; and 93.2% at Day 30. Dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live ticks than the controls at 24 hours post-infestation on Days 2, 9, 16, 23, and 30. Geometric mean efficacy was 100% at Days 9, 16, and 23; >99% at Day 2; and 98.4% at Day 30. Both products demonstrated excellent efficacy and persistent activity against *Ixodes scapularis* tick infestations at 24 hours for a full 30 days.

INTRODUCTION

Publications typically note that ticks and mosquitoes are the most important vectors of disease to animals and humans.[1,2] While mosquito-borne infections are readily and rapidly transmissible, most of the currently identified tick-borne infections are not immediately transmissible, which allows an opportunity to interfere with tick-feeding and prevent or reduce transmission of the disease causative agent. *Ixodes scapularis* ticks have the ability to be competent vectors of a number of diseases, including Lyme disease, babesiosis, and anaplasmosis. This is due to their protracted life cycle and feeding patterns. *Ixodes scapularis* ticks have a 2-year life cycle.

One adult female tick typically produces about 2,000 eggs in the spring. The larvae that emerge are not infected with Borrelia burgdorferi, the causative agent of Lyme disease. The larvae (or nymphs) become infected when they feed on infected white-footed mice, *Peromyscus leucopus*. After feeding, the larvae lie dormant in the leaf-litter until the following spring, when they molt into nymphs. If the nymphs were infected as larvae, they are capable of infecting susceptible mice or other mammalian hosts, including humans and dogs, when they feed. If they were not infected, the nymphs can become infected by feeding on infected mice, and will be capable of transmitting disease as adults. The nymphs then remain dormant until late fall, when they mature into adult ticks. The adult ticks attach to deer or other mammalian hosts and mate, after which the females engorge, and then drop off the host. The female ticks overwinter under the leaf-litter until spring, using the acquired blood meal to develop their eggs, expel the eggs, then die.[3]

Borrelia burgdorferi transmission in dogs typically occurs between 36 and 72 hours after tick attachment,[4] and the transmission potential rises throughout that interval. Therefore, in order for acaricides to reduce transmission of Lyme disease, they need to be highly effective in killing ticks by 24 hours, throughout the treatment interval. FRONTLINE Plus[5] demonstrated the capability to prevent Lyme disease transmission in a well-controlled study so we compared the activity of FRONTLINE Gold efficacy to FRONTLINE Plus against *Ixodes scapularis* ticks.

MATERIALS AND METHODS

Animal Welfare

Two studies were conducted by the same experienced, independent contract research facility. Dogs in both studies were managed similarly and handled with due regard for their welfare. All animals were handled in compliance with local and Boehringer Ingelheim Institutional Animal Care and Use Committee (IACUC) approvals. The trial facility used for both studies meets USDA-APHIS animal welfare requirements. The Investigator ensured that all personnel were appropriately trained, and that procedures were in compliance with each protocol.

All dogs were allowed to acclimate to the test facility for 7 days prior to the initiation of each study. All dogs were housed individually in accordance with the Animal Welfare Act. All dogs received 1-2 cups of commercial dry canine ration (Loyall, Adult Maintenance Formula, Nutrena), once daily, meeting their daily nutritional requirements, and fresh water from the city water supply was provided ad libitum.

Animal Management and Study Inclusion

A total of 24 dogs were utilized in these

Table 1. *Summary of 24 hour geometric mean[1] tick (Ixodes scapularis) weekly counts and results of the t-tests comparing dogs treated with mineral oil (control), FRONTLINE Plus, or FRONTLINE Gold*

Day	Control geometric mean	FRONTLINE Plus geometric mean (efficacy)	FRONTLINE Gold geometric mean (efficacy)	Control vs. FRONTLINE Plus p-value	Control vs. FRONTLINE Gold p-value	FRONTLINE Gold vs. FRONTLINE Plus p-value
2	29.3	0.1[A] (99.7%)	0.1[A] (99.7%)	<0.0001[E]	<0.0001[E]	>0.10[E]
9	25.3	0.0[A] (100%)	0.0[A] (100%)	<0.0001[U]	<0.0001[U]	--
16	28.2	0.1[A] (99.5%)	0.0[A] (100%)	<0.0001[E]	<0.0001[U]	>0.10[E]
23	33.1	0.0[A] (100%)	0.0[A] (100%)	<0.0001[U]	<0.0001[U]	--
30	26.6	1.8[A] (93.2%)	0.4[A] (98.4%)	0.0003[U]	<0.0001[E]	>0.10[U]

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

two studies combined (16 dogs in each study), and both studies utilized the same control dogs (Group A). For the first study, the dogs ranged from 16.5 to 32.4 pounds (as weighed on Day -4), and were approximately 3 to 5 years of age, with eight males and eight females included. For the second study, the dogs ranged from 16.7 to 35.2 pounds (as weighed on Day -4), and were approximately 2.5 to 5 years of age, with nine males and seven females included. For both studies, all dogs were in good health, and none had been treated with a topical ectoparasiticide within 3 months prior to study initiation, nor had they been treated with an isoxazoline ectoparasiticide within 1 year prior to study initiation. No dogs which were debilitated, suffering from disease or injury, fractious, or otherwise unsuitable for study inclusion, were included in the study.

Study Design

The studies were designed in accordance with the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention, and control of flea and tick infestation on dogs and cats.[6] Both studies were controlled efficacy studies using a randomized block design based on pre-treatment tick infestation counts on dogs, and all evaluations of efficacy were performed by personnel in blinded conditions. Each dog was an experimental unit.

Both studies were performed at the same time, on the same days, at the same facility, and both studies utilized the same eight control dogs.

For the challenge studies, *Ixodes scapularis* ticks used were from an Oklahoma State University tick colony, maintained on sheep and rabbits, and originating in 1991 from a natural population in Stillwater, Oklahoma. For allocation purposes, Rhipicephalus sanguineus ticks used were from a BerTek, Inc. colony, maintained on dogs and rabbits, and originating in July 2009 from a natural population in Greenbrier, Arkansas.

Allocation

For both studies, 28 dogs were infested with 50 live, unfed Rhipicephalus sanguineus ticks on Day -5 prior to treatment to ensure the dogs considered for inclusion were not adept at removing ticks. On Day -4, 24 hours post-infestation, all ticks were removed and counted. Twenty four dogs with the highest tick counts were selected for allocation. Eight replicates of three dogs each were formed; the three dogs with the highest pre-treatment tick counts formed the first replicate. The next three dogs with the highest pre-treatment tick counts formed the second replicate, and so on, until all dogs were allocated. Within replicates, each dog was randomly allocated to one of three treatment groups. The first group comprised

mineral oil-treated control dogs, the second group comprised FRONTLINE Plus-treated dogs, and the third group comprised FRONTLINE Gold-treated dogs. The dogs remained in their assigned groups throughout the duration of each study.

Treatment

Dogs in both studies were weighed on Day -4. Per the allocation, on Day 0, the appropriate dose of FRONTLINE Plus or FRONTLINE Gold was applied to dogs in Group B and Group C, respectively (0.67 mL for dogs 5 to 22 lbs, or 1.34 mL for dogs 23 to 44 lbs). Also on Day 0, Group A dogs were treated with 0.5 mL of mineral oil, regardless of weight. FRONTLINE Plus was applied according to label instructions: topically by parting the hair between the shoulder blades, applying the entire formulation directly to the skin in one single spot, at the base of the neck. FRONTLINE Gold and mineral oil were applied to their respective dogs according to label instructions: topically by parting the hair between the shoulder blades and applying the formulation directly to the skin to form a stripe down the dog's back to the base of the tail. Dogs in both studies were checked hourly for 4 hours post-treatment to ensure there were no adverse reactions to the applied treatment.

Tick Counts

For both studies, all dogs were infested with 50 live, unfed *Ixodes scapularis* ticks each, which were placed along the dorsal midline of the body from the shoulders to the hips, on Days 1, 8, 15, 22, and 29. At 24 hours post-infestation on Days 2, 9, 16, 23, and 30, all ticks were removed from all dogs and counted. Ticks were removed with forceps, and afterward, a standard, fine-toothed flea comb was used to further confirm that all ticks had been removed.

Statistical analysis

All analyses and calculations were performed using SAS Version 9.4. Statistical significance was declared at a two-sided p-value of 0.05.

Adult tick 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. When both groups had zero variance, no comparison could be made. Each active treatment was compared to the control group, and the two active treatments were compared.

The data and results of the t-tests are summarized in Table 1.

RESULTS

Adverse reactions

No adverse events were reported in any dog, at any time, during either of the two studies.

Antiparasitic efficacy

In the first study, dogs treated with FRONTLINE Plus had significantly ($p<0.01$) fewer live ticks than the controls at 24 hours post-infestation on Days 2, 9, 16, 23, and 30. Twenty four-hour efficacy was 100% at Days 9 and 23; >99% at Days 2 and 16; and 93.2% at Day 30.

In the second study, dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live ticks than the controls at 24 hours post-infestation on Days 2, 9, 16, 23, and 30. Twenty four-hour efficacy was 100% at Days 9, 16, and 23; >99% at Day 2, and 98.4% at Day 30.

DISCUSSION

One could theorize that the addition of pyriproxyfen - on top of fipronil and (S)-methoprene - in the FRONTLINE Gold formulation might affect the performance of fipronil

against ticks. This study demonstrated that the activity of both products against *Ixodes scapularis* at 24 hours following weekly tick infestations was excellent throughout the month-long study. During this time there was no significant difference ($p>0.10$) in efficacy between the products, with both products demonstrating greater than 93% efficacy at 24 hours against *Ixodes scapularis* infestations throughout the treatment interval, which is an important consideration in the transmission of Borrelia burgdorferi.[4,5]

CONFLICT OF INTEREST

These clinical studies were funded by Boehringer Ingelheim Animal Health, of which Doug Carithers is an employee, and Jordan Crawford is a contractor. BerTek, Inc., of which William Russell Everett is an employee, is an independent contract research facility contracted to conduct these studies. Sheila Gross is an independent statistician.

All authors voluntarily publish this article and have no personal interest in these studies, other than publishing the scientific findings in which they have been involved via planning, initiating, monitoring, and conducting the investigations, as well as analyzing the results.

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