Comparative Efficacy of fipronil/(S)-methoprene-pyriproxyfen (FRONTLINE® Gold) and Sarolaner (Simparica®) Against Induced Infestations of Ixodes scapularis on Dogs

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ABSTRACT

A study was performed to compare the 12-hour efficacy of FRONTLINE® Gold (fipronil/(S)-methoprene/pyriproxyfen) with that of SIMPARICA® (sarolaner) against *Ixodes scapularis* tick infestations on dogs. Based on pre-treatment tick count, 24 dogs were allocated to one of three groups, eight dogs in each group. On Day 0, each dog in Group B was treated with a dose of commercially available FRONTLINE Gold for Dogs appropriate for its weight, and each dog in Group C was treated with a dose of commercially available SIMPARICA appropriate for its weight. Group A dogs remained untreated throughout the duration of the study. On each of Days 1, 7, 14, 21, and 28, all dogs were infested with 50 live, unfed *Ixodes scapularis* ticks, and at 12 hours post-infestation on Days 2, 8, 15, 22, and 29, ticks were removed from all dogs and counted. Using arithmetic means for all calculations, dogs treated with FRONTLINE Gold had significantly ($p<0.01$) fewer live ticks than the controls at 12 hours post-infestation on all evaluation days from Day 2 to Day 29. FRONTLINE Gold 12-hour arithmetic mean efficacy was 99.1%, 100%, 100%, 100%, and 94.7% on the same days. Dogs treated with SIMPARICA had significantly ($p<0.01$) fewer live ticks than the controls at 12 hours post-infestation on the same days. SIMPARICA 12-hour arithmetic mean efficacy was 97.0%, 96.1%, 92.6%, 87.3%, and 84.5% on the same days, respectively. Dogs treated with FRONTLINE Gold had significantly ($p<0.05$) fewer live ticks than dogs treated with SIMPARICA on Day 15.

INTRODUCTION

Ticks act as vectors for a number of organisms capable of causing disease in humans and animals, including dogs. One such disease is Lyme disease, caused by Borrelia burgdorferi, which in North America is

transmitted by *Ixodes scapularis* and *Ixodes pacificus* ticks. Infection with *B. burgdorferi* in mammals can range from asymptomatic to life-threatening, with the primary manifestation of clinical signs in dogs manifesting as lameness.[1] *I. scapularis* ticks can be found in many regions throughout the eastern United States and *I. pacificus* in the west, with the distribution of both, expanding.[2] Due to the increasing risk of tick exposure and potential health risks to dogs, it is recommended that pet owners not only vaccinate dogs against Lyme disease, but also take measures to reduce tick populations in the dogs' environment, frequently examine and carefully remove any ticks found on the dog, and to use an approved product year-round to protect dogs against exposure to ticks.[3]

Within the past several decades, many topical and more recently oral acaricide formulations have been available to control tick infestations on dogs. The goals of a successful tick control program are to eliminate ticks quickly and continuously for the comfort of the dog and satisfaction of the pet owner, and to prevent ticks from transmitting diseases, such as Lyme disease, to the dog. While the minimum transmission time has not been established, maximum risk of transmission is estimated between 24 to 36 hours after tick attachment.[4] Additionally, the risk of Lyme disease transmission increases with increased duration of tick attachment. Therefore, a product that eliminates ticks quickly is desirable.

The purpose of the present study was to assess and compare the 12-hour efficacy of the topically applied FRONTLINE Gold for Dogs and orally administered SIMPARICA against *Ixodes scapularis* tick infestations on dogs.

MATERIALS AND METHODS

Animal Welfare

This study was conducted at BerTek, Inc., an experienced, independent contract research facility. All animals were managed similarly and with due regard for their welfare. All animals were handled in compliance with the Boehringer Ingelheim Institutional Animal Care and Use Committee (IACUC) approvals. The trial facility used for the study meets USDA-APHIS animal welfare requirements. The Investigator ensured that all personnel were appropriately trained, and that procedures were in compliance with the protocol. Concomitant veterinary care and therapy, as well as any adverse events, were recorded.

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

Animal Management and Study Inclusion

Twenty-four dogs (12 males and 12 females) were selected for study inclusion based on pre-treatment tick counts. The dogs were aged between approximately 2 to 5 years, and weighed between 8.4 and 14.7 kilograms (as weighed on Day -4). No dogs younger than 8 weeks or weighing less than 5.0 kilograms were considered for use in this study. No animals which may have been debilitated, suffering from disease or injury, fractious, presenting abnormalities at the application sites, or otherwise unsuitable for inclusion were considered for use. All dogs were in good health, and none had been treated with a monthly ectoparasiticide within 3 months prior to study initiation, nor had they been treated with a topical 3-month ectoparasiticide within 1 year of the study initiation. Individual dog information, as assessed before study inclusion, is listed in Table 1.

Allocation

For allocation, and to ensure that study dogs would retain ticks, a total of 28 dogs were infested with 50 Rhipicephalus sanguineus ticks on Day -5. On Day -4, 24 hours post-infestation, the dogs were combed and ticks removed and counted. The 24 dogs with

Table 1. *Individual dog information and results of allocation (Day -4)*

Treatment group	Dog ID	Age (years)	Sex	Weight (kg) Day -4	Dose given
Group A (untreated controls)	MC4692	2	M	13.5	N/A
	F0205	4	F	8.9	
	MC5208	2	F	12.6	
	MC1744	2	F	14.7	
	MC4581	3	M	10.0	
	NE1217	5	M	14.5	
	MC2256	4	F	10.3	
	F0187	4	F	8.4	
Group B (FRONTLINE Gold-treated)	MC2983	3	M	9.0	0.67 mL
	MC5436	2	M	10.7	1.34 mL
	MC4645	2	M	11.0	1.34 mL
	MC4441	2	M	12.7	1.34 mL
	F0101	5	F	9.9	0.67 mL
	MC4195	4	M	10.3	1.34 mL
	MC4722	2	M	13.4	1.34 mL
	MC4172	3	M	12.2	1.34 mL
Group C (SIMPARICA-treated)	F0121	5	F	12.5	40.0 mg
	MC4537	3	F	13.2	40.0 mg
	MC5599	2	F	9.9	20.0 mg
	MC4684	3	M	9.0	20.0 mg
	MC2844	2	F	9.9	20.0 mg
	MC4114	3	F	10.9	40.0 mg
	MC0365	3	M	11.3	40.0 mg
	F0194	4	F	10.7	40.0 mg

the highest tick counts were selected and ranked based on pre-treatment tick infestation counts. 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. Group A comprised untreated control dogs, Group B comprised FRONTLINE Gold-treated dogs, and Group C comprised SIMPARICA-treated dogs. The dogs remained in their assigned groups throughout the duration of the study. The results of the allocation process are shown in Table 1.

Study Design

This study was a well-controlled efficacy study using a randomized block design based on dog pre-treatment tick infestation counts, and all evaluations of efficacy were performed by personnel in blinded conditions. Each dog was an experimental unit.

The *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. The Rhipicephalus sanguineus ticks used for qualification were from the BerTek, Inc. colony, maintained on dogs and rabbits, and originating in July 2009 from a natural population in Greenbrier, Arkansas.

Table 2. *Summary of geometric mean1 and arithmetic mean tick (Ixodes scapularis) counts (with efficacies) for dogs treated with FRONTLINE Gold or SIMPARICA, or remaining as untreated controls*

Day	Control AM	FRONTLINE Gold AM (Efficacy)	SIMPARICA AM (Efficacy)
2	29.4	0.3[B] (99.1%)	0.9[B] (97.0%)
8	25.9	0.0[B] (100%)	1.0[B] (96.1%)
15	28.9	0.0[B,C] (100%)	2.1[B] (92.6%)
22	30.5	0.0[B] (100%)	3.9[B] (87.3%)
29	25.9	1.4[B] (94.7%)	4.0[B] (84.5%)

[1] *Based on transformation to the natural logarithm of (count + 1)*
[B] *Significantly different from control (p<0.01)*
[C] *Significant difference between FRONTLINE Gold and SIMPARICA (p<0.05)*
AM=Arithmetic mean

Treatment

All dogs were weighed on Day -4, and the appropriate product and dose were selected for each dog based on its weight. On Day 0, Group B dogs were treated with either 0.67 mL (for dogs 2.3 to 10.0 kg) or 1.34 mL (for dogs 10.4 to 20.0 lbs) of FRONTLINE Gold for Dogs, which was applied according to label instructions: topically by parting the hair between the shoulder blades, applying the formulation directly to the skin at the base of the neck, and dragging it down the spine in a single line. Also on Day 0, Group C dogs were treated with either 20.0 mg (for dogs 5.0 to 10.0 kg) or 40.0 mg (for dogs 10.0 to 20.0 kg) of SIMPARICA, which was administered according to label instructions: by administering one whole chewable orally. Group A dogs remained untreated throughout the duration of the study.

Tick Counts

On each of Days 1, 7, 14, 21, and 28, all dogs were infested with 50 *Ixodes scapularis* ticks (sex ratio 1:1), which were placed on the lateral aspect of the body to avoid potential direct contact with the product application site. At 12 hours following each infestation, on Days 2, 8, 15, 22, and 29, all ticks were removed from all dogs, counted, and discarded.

STATISTICAL ANALYSIS

The statistician was responsible for the calculation of efficacy. The statistical unit was the individual dog, and the primary assessment variable in this study was the decrease in the number of live ticks. The average percent reduction in tick counts for each group was calculated using arithmetic means:

Efficacy (%) against ticks = 100 x (AMC–AMT)/AMC, where AMC = arithmetic mean number of live ticks in the control group, and AMT = arithmetic mean number of live ticks on dogs in the treated group.

The 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. Each treated group was compared to the control group, and the two treated groups were compared.

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

The data and results of the t-tests are summarized in Tables 2 and 3.

RESULTS

Adverse Reactions

All dogs remained in apparent good health throughout the study, no adverse events were noted, and no dogs were removed from the study.

Tick Efficacy

Using arithmetic means for all calculations, dogs treated with FRONTLINE Gold had

Table 3. *Results of the t-tests of log-transformed data comparing each treated group to the control group, and comparing FRONTLINE Gold to SIMPARICA*

Day	Control vs. FRONTLINE Gold AM p-value	Control vs. SIMPARICA AM p-value	FRONTLINE Gold vs. SIMPARICA AM p-value
2	<0.0001[U]	<0.0001[U]	>0.10[E]
8	<0.0001[U]	<0.0001[U]	0.0856[U]
15	<0.0001[U]	<0.0001[U]	0.0211[U]
22	<0.0001[U]	<0.0001[E]	>0.10[U]
29	<0.0001[U]	<0.0001[E]	>0.10[E]

[E] *Results from t-test for means with poolable variances*
[U] *Results from t-test for means with unequal variances*
AM=Arithmetic mean

significantly ($p<0.01$) fewer live ticks than the controls at 12 hours post-infestation on all evaluation days from Day 2 to Day 29. Dogs treated with SIMPARICA had significantly ($p<0.01$) fewer live ticks than the controls at 12 hours post-infestation on the same days.

FRONTLINE Gold 12-hour arithmetic mean efficacies were 99.1%, 100%, 100%, 100%, and 94.7% on the same days, respectively. SIMPARICA 12-hour arithmetic mean efficacies were 97.0%, 96.1%, 92.6%, 87.3%, and 84.5% on the same days, respectively. Dogs treated with FRONTLINE Gold had significantly ($p<0.05$) fewer live ticks than dogs treated with SIMPARICA on Day 15 as seen in Figure 1.

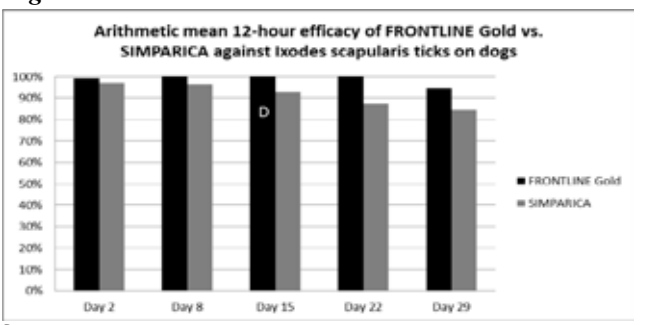
DISCUSSION AND CONCLUSIONS

In a previous study, FRONTLINE Gold for Dogs demonstrated excellent efficacy against *Ixodes scapularis* ticks. In that study, at 24 hours post-infestation on Days 2, 9, 16, 23, 30, and 37, FRONTLINE Gold killed 99.7%, 100%, 100%, 100%, 98.4%, and 96.5% of ticks, respectively[5].

The present study demonstrated that treatment with a single dose of FRONTLINE Gold resulted in a rapid reduction in live tick numbers just 12 hours post-infestation, throughout a 29-day period.

The ability of FRONTLINE Gold to kill *Ixodes scapularis* ticks by 12 hours post-infestation, throughout the month, provides several benefits for the dog and its owner. Ticks will have less opportunity to bite and feed, providing rapid relief for the dog and greater satisfaction for the pet owner. Topically applied and locally active acaricides and orally administered and systemically active acaricides can both be effective. Pet owners have different preferences for which type of acaricide to use on their dog. Additionally, topically applied and locally active acaricides can start affecting ticks soon after they come into contact with the pet's hair coat, and do not require the ticks to feed to be active. In the study reported here, FRONTLINE Gold rapidly killed *Ixodes scapularis* ticks, which can transmit Lyme

Figure 1:



[D] *Significant difference between FRONTLINE Gold and SIMPARICA ($p<0.05$)*

disease, throughout the monthly application period.

CONFLICT OF INTEREST

These clinical studies were funded by Boehringer Ingelheim, 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 the present study. 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.

DISCLAIMER

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