ABSTRACT

The speed of kill of a spot-on formulation of fipronil (Frontline® Top Spot®, Merial Limited, Duluth, GA) against adult cat fleas (Ctenocephalides felis) and brown dog ticks (Rhipicephalus sanguineus) was evaluated in dogs in a commercial laboratory setting. Forty dogs were allocated to 20 replicates of two based on sex and pretreatment flea counts. Within each replicate, dogs were randomly allocated to an untreated control group or to treatment with fipronil, administered topically as a spot-on per label instructions. The technical staff performing the flea and tick counts were unaware of treatment group assignments. Each dog was infested with approximately 100 unfed adult fleas on Day –8 or –6 and on Day –1. Each dog also was infested with approximately 50 unfed adult ticks on Day –1. Treatments were administered on Day 0 according to body weight. Flea and tick counts were performed on four randomly selected dogs from each treatment group at approximately 6, 12, 18, 24, and 48 hours after treatment. Flea and tick count reductions for dogs treated with fipronil were significant (P < .05), as compared with untreated controls, at 18, 24, and 48 hours after treatment. Controlled efficacy of fipronil against C. felis and R. sanguineus ranged from 94% to 100% at these evaluation times. This study demonstrated that the speed of kill of fipronil, applied topically as a spot-on, was 100% against C. felis fleas on dogs within 12 to 18 hours after treatment and 100% against R. sanguineus ticks between 24 and 48 hours after treatment.

INTRODUCTION

Fipronil, a phenylpyrazole compound with a high degree of insecticidal/acaricidal activity, acts as an antagonist at the insect GABA receptor.1,2 Two commercial formulations of fipronil have been developed for use as long-acting, water resistant topical products to kill fleas and ticks on dogs and cats: Frontline® Spray (Merial Limited, Duluth, GA) is applied on the animal’s haircoat, while Frontline® Top Spot® is applied...
directly on a very small area of the skin. The latter formulation translocates over the animal’s body by passive diffusion in the sebum, via hairs and skin, thus providing adequate levels of fipronil to kill fleas and ticks on any part of the body. Fipronil kills cat fleas (*Ctenocephalides felis*), dog fleas (*Ctenocephalides canis*), brown dog ticks (*Rhipicephalus sanguineus*), American dog ticks (*Dermacentor variabilis*), lone star ticks (*Amblyomma americanum*), and deer ticks (*Ixodes scapularis*). Monthly applications of fipronil are recommended for tick control. Fipronil is not a repellent, so it is not uncommon (especially when challenge is heavy) to observe fleas or ticks transiently on the skin of a treated animal. Ticks may attach, begin the feeding process, and may even slightly engorge. However, fleas and ticks coming in contact with the skin of a treated dog or cat will be exposed to lethal levels of fipronil.

Speed of kill is an important feature for a flea and tick control product, since it is desirable to have the product work early enough to break the life cycle—killing the arthropods before they have a chance to lay eggs and reinfest households and pets. Flea allergy dermatitis is a common condition among dogs and cats, and a fast-acting product is essential in the management of this condition, which can cause pronounced discomfort to the animal. Additionally, ticks are vectors for various diseases, and a fast-acting tick control product could help reduce disease transmission. Lastly, complete and rapid speed of kill enhances client satisfaction with a flea and tick control product. The objective of this trial was to evaluate the speed of kill of the spot-on formulation of fipronil against adult cat fleas (*C. felis*) and the brown dog tick (*R. sanguineus*) in dogs.

**MATERIALS AND METHODS**

**Test Animals**

Forty random-source dogs, including mongrels, beagles, and other breeds, of both sexes and various ages were selected from the kennel population at the test site. The dogs were washed with a noninsecticidal shampoo once during the 14-day pretrial acclimation period. The dogs had not been recently treated with flea or tick adulticides either topically or systemically. The dogs were housed individually in stainless-steel cages or runs, which were cleaned daily. The housing units prevented physical contact between animals during the study. A complete and balanced diet was fed once daily, and fresh drinking water was available ad libitum.

**Infestations**

*Ctenocephalides felis* adults (both sexes) were supplied from an in-house colony at the test facility. The adult parasites were reared in accordance with standard operating procedures of the facility and had been in colony at the facility for at least 15 years. Outside sources of adult cat fleas are periodically added to the colony in an effort to influence the genetic make-up of the colony. *Rhipicephalus sanguineus* adults of both sexes were supplied from the in-house colony, which had been in colony at the facility for at least 12 years.

Each dog was infested with approximately 100 unfed adult fleas on either Day −8 or −6, and on Day −1 as follows: The cage or run door was opened, the animal’s identity was checked, and the fleas were swirled in their collection tubes and dumped quickly *en masse* between the shoulder blades of the dog. On Day −1, all 40 dogs were also infested with unfed ticks, as follows. Atropine (10 mg/ml SQ) was given as a preanesthetic. Approximately 10 to 20 minutes later, each dog was sedated by simultaneous intravenous injection of xylazine and acepromazine. Each dog was then placed in an infestation chamber, and 50 unfed adult ticks were deposited between the shoulder
blades. The ticks were allowed at least 20 minutes to crawl into the haircoat and select an attachment site. Any ticks falling off the dogs within 20 minutes were replaced between the shoulder blades and given an additional opportunity to attach. After the allotted time, the dog was returned to its housing unit. The infestation chambers were cleaned with alcohol between each animal.

Allocation and Treatments
Dogs were allocated to replicates based on their sex and flea populations on Day –7 or Day –5. Counts were determined by collection of all fleas from the dog, using a flea comb. The two most heavily infested female dogs formed the first replicate, the next two most heavily infested female dogs formed the second replicate, and so forth until 10 replicates were formed. Within each replicate thus formed, dogs were randomly allocated to two treatment groups by placing two slips of paper, equal in size, and lettered A or B, in a beaker. The first slip was drawn from the container and the letter on it assigned to the first female dog (the lower identification number) in the first replicate. The code slips were returned to the container and the process was continued for each replicate pair until all 20 female dogs were allocated to treatment A or B. This same process was used to allocate the 20 male dogs to treatment groups. After all dogs were allocated, treatments were randomly assigned to each replicate pair. Dogs in one group served as untreated controls, and those in the second group were treated with Frontline® (fipronil) Top Spot®, by topical application according to label instructions.

On Day 0, each dog in the fipronil group was treated with the appropriate number of Frontline® Top Spot® applicators by holding the applicator upright, snapping the tip open, and placing the tip of the applicator on the skin of the dog between the shoulder blades. The tube applicator was squeezed, applying the entire contents in a single spot to the skin. Body weights recorded earlier were used to calculate the appropriate dose.

Five replicates of eight dogs each (four per treatment group, based on cage location) were formed immediately following treatment. One replicate was randomly selected for flea and tick counting at each of five evaluation times (6, 12, 18, 24, or 48 hours) after treatment. At each evaluation, dogs were combed and fleas and ticks were removed. All dogs within a treatment group were combed at approximately the same time. In addition, gloves and gowns were changed between treatment groups to decrease the potential for cross-contamination. All fleas and ticks removed from each dog were placed in a container labeled with the dog’s identification number, for subsequent counting.

Evaluations
Counts of live fleas and ticks collected at each evaluation time were transformed to the natural logarithm of (count + 1) for analysis and calculation of geometric means. Mean numbers of live fleas and ticks recovered from dogs after treatment were analyzed using t-tests for means with unequal or equal variances, as appropriate, for each examination.

Controlled efficacy of Frontline® Top Spot® against cat fleas and brown dog ticks was calculated at each evaluation, using the following formula:

\[
\% \text{ Controlled efficacy} = \left( \frac{\text{Control group mean} - \text{Treated group mean}}{\text{Control group mean}} \right) \times 100
\]

RESULTS
Untreated control dogs each had approximately 47 to 73 live fleas recovered at post-treatment evaluations (Table 1, Figure 1). Dogs treated with fipronil had a mean of ap-
approximately 18 fleas each at 6 hours after treatment (74% efficacy) and 4 fleas per dog at 12 hours. At 18, 24, and 48 hours after treatment, no live fleas were recovered from any dog treated with fipronil (< .05).

A mean of approximately 25 or 26 live ticks each was collected from untreated control dogs at 6 and 12 hours after treatment, compared with a mean of 9 (65% efficacy at 6 hours) and 5 (79% efficacy at 12 hours) ticks per dog in the group treated with fipronil (Table 1, Figure 1). At 18, 24, and 48 hours, controlled efficacy of fipronil against ticks ranged from 94% to 100% (< .05).

**DISCUSSION**

Few parasites can inflict more discomfort in dogs and cats than fleas. These tiny pests are much more than an annoyance, and the presence of even a few fleas can initiate severe flea allergy dermatitis in some pets. Additionally, *C. felis* and *C. canis* are intermediate hosts for the common tapeworm, *Dipylidium caninum*. Therefore, the faster these insects are killed, the more comfortable the animal will be and the less chance there is for an allergic reaction. While neither prevalence nor severity of flea allergy dermatitis was measured in this study, it was demonstrated that there was already a high degree of activity of the spot-on formulation of fipronil against cat fleas on dogs as early as 6 hours after treatment, and 100% kill was achieved between 12 and 18 hours after application. At 24 and 48 hours after treatment, flea counts remained at zero for dogs treated with fipronil (< .05).

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<th>Hours after Treatment</th>
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<th>Live Tick Counts</th>
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<tr>
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aFrontline® Top Spot®, Merial Limited, Duluth, GA.

*Significantly less than mean for control group at same time (P < .05).

**Figure 1.** Controlled efficacy of fipronil against induced infestations of fleas (*C. felis*) and ticks (*R. sanguineus*) on dogs evaluated at various times after treatment.
Ticks are recognized as being very important vectors of debilitating and deadly diseases, such as Lyme disease, ehrlichiosis, Rocky Mountain spotted fever, and babesiosis, which occur in humans as well as in cats and dogs throughout the United States. The highest risk of disease transmission from tick to host has been reported to occur more than 10 hours after attachment for *Rickettsia* and 48 to 72 hours for *Borrelia* and *Babesia*. Although it is beyond the scope of this study and there is no claim for Frontline® brand products for prevention of tick-borne diseases, the prompt removal of ticks is considered to be among the most effective prophylactic tools available. A significant (*P* < .05) reduction in the tick population was observed in this study as early as 6 hours after treatment. The effect of the spot-on formulation of fipronil against *R. sanguineus* was 95% by the 18-hour posttreatment observation and reached 100% between 24 and 48 hours after treatment.

The results of this study indicate that the spot-on formulation of fipronil provides fast, effective action against induced infestations of cat fleas and brown dog ticks. The benefits of this rapid onset of action of fipronil against fleas and the control of flea allergy dermatitis in both dogs and cats have been previously reported. Further research to evaluate the effect of speed of kill on tick-borne diseases may be considered in the future; however, these studies are far more complex due to the involvement of several tick species and stages and the consideration of many environmental factors.

**CONCLUSION**

Findings in this study demonstrated that the speed of kill of fipronil, applied topically as a spot-on, was 100% against *C. felis* fleas on dogs within 12 to 18 hours after treatment and was 100% against *R. sanguineus* ticks between 24 and 48 hours after treatment.

**REFERENCES**