Repellency and Efficacy of 65% Permethrin and 9.7% Fipronil Against *Ixodes ricinus*

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*ABSTRACT*

Two topically applied spot-on products—65% permethrin (Defend® Exspot® Treatment for Dogs, Schering-Plough Animal Health, Union, NJ) and 9.7% fipronil (Frontline® Spot On Dog, Merial Limited, Iselin, NJ)—used for canine flea and tick control were evaluated for repellency against *Ixodes ricinus*, the tick species that is the primary vector of Lyme disease in Europe. Eighteen dogs were randomly assigned to the following treatment groups (n = 6): (1) 65% permethrin, (2) 9.7% fipronil, or (3) untreated control. Dogs were exposed to ticks in individually assigned cages with a carpet that covered *≈*70% of the cage bottom. Dogs in treatment groups 1 and 2 were treated in accordance with label directions for each respective product on study day 0. Fifty unfed, male and female adult ticks were placed in the cages 15 to 30 minutes before the dogs. The dogs were placed in the cages for a 2-hour exposure period at 2, 7, 14, 21, 28, 35, and 41 days after treatment. After a 2-hour exposure period, dogs were removed from the cages and live (attached and unattached) and dead ticks were counted on the dogs, on the carpets, and in the cages. Cages were thoroughly cleaned and new carpet was used for each tick exposure period. Treatment of dogs with 65% permethrin reduced tick numbers on dogs by 99.1% at 2 days, 99.0% at 1 week, 95.9% at 2 weeks, 88.5% at 3 weeks, 87.1% at 4 weeks, and 48.0% at 6 weeks after application. In contrast, treatment of dogs with 9.7% fipronil reduced tick numbers on dogs by 61.4% at 2 days, 51.6% at 1 week, 37.0% at 2 weeks,
33.7% at 3 weeks, 10.8% at 4 weeks, and 0% at 6 weeks after application. The efficacy of 65% permethrin was significant ($P \leq .05$) when compared to the control at all challenges, whereas the efficacy of 9.7% fipronil was not significant as compared to controls at 21, 28, 35, and 41 days after treatment. The 65% permethrin killed significantly more *Ixodes ricinus* ticks ($P \leq .05$) than 9.7% fipronil from 2 to 41 days after treatment. The 65% permethrin repelled 1.9-, 2.0-, 3.0-, 43.3-, 3.9-, 8.9-, and 17.3-fold more *Ixodes ricinus* than did 9.7% fipronil at 2, 7, 14, 21, 28, 35, and 41 days after treatment, respectively, and all differences in repellency were significant ($P \leq .05$).

**INTRODUCTION**

Permethrin, a photostable synthetic pyrethroid, was first described in 1973 and since that time has been developed for the control of a wide variety of arthropod species. It has been used to provide effective control of ticks on both food-producing and companion animals. In addition to its acaricidal properties, permethrin when applied to cloth has been shown to repel both insects and ticks.

In Europe, *Ixodes ricinus* Linnaeus is the primary vector of *Borrelia burgdorferi sensu lato*, the etiologic agent of Lyme disease prevalent among human populations throughout Europe. The geographic distribution of *I. ricinus* includes Scandinavia, the British Isles, Central Europe, Germany, France, the Iberian peninsula, Italy, the Balkans, Eastern Europe, North Africa, and Iran. Dogs seropositive for *B. burgdorferi sensu lato* have been identified in Norway, Denmark, the Netherlands, and Spain. Ticks infected with *B. burgdorferi sensu lato* have been recently collected in Finland, Sweden, Germany, the Netherlands, Switzerland, Croatia, and Tunisia. In addition, *B. burgdorferi sensu lato* has been identified in ticks from museum specimens collected throughout Britain as early as 1897. Therefore, the association between ticks and *B. burgdorferi* is widespread throughout Europe and North Africa. Schreck recommended that repellency studies should be conducted with the pest species of concern and the appropriate species from which protection was sought rather than with surrogate model species. In keeping with that concept, this study was designed to determine the duration of repellency of two topically applied spot-on products—65% permethrin and 9.7% fipronil—for the protection of dogs against *I. ricinus* infestation.

**MATERIALS AND METHODS**

**Experimental Design**

The objective of this study was to determine the duration of 65% permethrin and 9.7% fipronil repellency against the primary vector of Lyme disease in Europe, *I. ricinus*. Eighteen dogs were randomly assigned to the following treatment groups ($n = 6$): (1) 65% permethrin; (2) 9.7% fipronil; or (3) untreated control. Dogs were exposed to ticks in individually assigned cages with a carpet that covered $\approx 70\%$ of the cage bottom. Dogs in groups 1 and 2 were treated in accordance with label directions for the respective product on study day 0. Fifty unfed, male and female adult ticks were placed in each cage 15 minutes before the dogs. Each dog was placed in the cage for a 2-hour exposure period on days 2, 7, 14, 21, 28, 35, and 41 after treatment. After a 2-hour exposure period, dogs were removed from the cages and live (attached and unattached) and dead ticks were counted on the dogs, the carpet, and the cage. The study was conducted in accordance with *Good Clinical Practice for the Conduct of Clinical Trials for Veterinary Medicinal Products* published by the Committee for Veterinary Medicinal Products (CVMP; July 1994).
Dogs

Twenty-four Beagle dogs from the laboratory animal colony began the acclimation period. Eighteen of the dogs were determined to be healthy and free of skin disease and were selected by veterinary examination for inclusion in the study. The 18 dogs were of mixed sex, 8.5 to 34.5 months of age, and weighed 8.9 to 13.3 kg. A number tattooed in the ear individually identified each dog. All dogs were determined by veterinary examination to be in good health and free of ectoparasites at the beginning of the study. Before initiation of the study, the dogs had been acclimated for 7 days and washed with a noninsecticidal soap (Gullick's Pet Care, ultra-mild shampoo). The dogs had not been treated with any parasiticide for at least 28 days before treatment. Dogs were fed commercial dry dog food either once or twice daily and water was available ad libitum.

Ticks

Laboratory-reared adult *I. ricinus* with a male:female ratio of approximately 50:50 were obtained from Dr. L. Jones, National Environmental Research Council, Institute of Virology and Environmental Microbiology, Oxford, UK (study days 2, 7, 14, 21, 28) and from Professor B. Bertschart, University of Neuchatel, Neuchatel, Switzerland (study days 35 and 41).

Test Formulations

Defend® Exspot® Treatment for Dogs, also called Pulvex®, is a 65% permethrin spot-on in a novel proprietary formulation produced by Schering-Plough Animal Health Corporation, Union, NJ. Frontline® Spot On Dog is a 9.7% fipronil spot-on formulation produced by Merial Limited, Iselin, NJ.

Allocation to Treatment

Each dog was randomly assigned to both a holding cage and a test cage in order to prevent cross contamination. Three male and three female dogs were assigned to each treatment group.

Application of Treatments

One treatment application was made to each treated dog on study day 0 in accordance with the respective label directions. In treatment group 1, all dogs weighed <15 kg and in accordance with label directions received 1 mL of 65% permethrin applied to the skin between the shoulder blades with a calibrated syringe. In treatment group 2, 9.7% fipronil from the commercial package was applied to the skin between the shoulder blades at the dose rate of 0.67 mL for dogs that weighed <10 kg and 1.34 mL for dogs that weighed 10 to 20 kg.

Test Cage

Each test cage with solid sides and bottom was made of heavy-gauge plastic with smooth impermeable surfaces and was $1.1 \times 0.7 \times 0.5$ m (length, width, and height). The lid included a window covered with a fine-mesh screen to allow gas exchange and was attached securely to prevent ticks from escaping. Approximately 70% of the floor area of the test cage was covered by a carpet. The carpet material was light colored nylon with a nap of 12 to 15 mm. Cages were thoroughly washed and carpets were discarded after each use. Test cages were assigned to individual dogs throughout the study to remove the possibility of cross contamination.

Challenge Procedures

Carpets were lightly sprayed with water; then 50 adult *I. ricinus* with an approximately equal male:female ratio were placed on the carpet in each test cage. After a 15-minute acclimation period, dogs were placed in their respective test cages, lids were securely attached,
and dogs remained in the test cages for 2 hours. Although in close proximity to the dogs, ticks had a choice between getting on the dogs to feed or remaining in the test cage/carpet. After the 2-hour exposure period, dogs were removed from the cages and transported to a counting table by two handlers wearing disposable, impermeable gloves and aprons. Each dog was thoroughly examined by two investigators and the number of live and dead ticks on each dog was determined by parting the hair and combing with a fine-tooth comb. Each test cage was examined and the number of live and dead ticks on the carpet and in the test cage was determined. Approximately 1 hour was required to count ticks on each dog and its respective test cage. Live and dead ticks found on the dogs or in the cages were destroyed after ticks were enumerated, and cages were thoroughly washed to remove any potential insecticide residues.

Statistical Analysis

Statistical analyses on tick counts were performed separately for live ticks on each dog (efficacy) as well as for the number of live or dead ticks in each cage and the number of dead ticks on each dog (repellency). The analysis considered that not all ticks were recovered and therefore a proportion was calculated based on the total number of ticks accounted for. The proportions were calculated as follows:

**Efficacy:** \[ \frac{\text{No. of Live Ticks on Dog}}{\text{Total No. of Ticks Accounted For}} \times 100 \]

**Repellency:** \[ \frac{[\text{Total No. Ticks in Cage (Dead + Live)} + \text{No. Dead Ticks on Dog}]}{\text{Total No. of Ticks Accounted For}} \]

Tick counts were transformed by the log_{10} (count + 1) transformation before analysis in or-
der to stabilize the variance; however, no transformation was performed on the calculated proportions. The transformed counts and proportions were analyzed by a one-way Analysis of Variance (ANOVA) with the treatment term in the model. The treatment effect was tested against the residual error at the 5% level (statistical significance). The differences between the least squares means (LS means) and the $P$ values associated with these differences were computed and compared using a two-sided t-test (comparing treated groups) or one-sided t-test (comparing control to treated groups) procedure at the fifth percentile of significance. The SAS Proc Mixed procedure was used for these analyses.

The percentage of efficacy relative to the control group and based on either arithmetic means (counts and proportions) or geometric means (counts only) was calculated for live ticks that were recovered on the dog. The percentage of repellency based on arithmetic or geometric means was calculated for number of ticks dead or alive in the cage and number of dead ticks on the dog. Efficacy and repellency were calculated as follows:

\[
\text{Geometric Mean No. Ticks/Dog (Control)} - \text{Geometric Mean No. Ticks/Dog (Treated)} \times 100
\]

\[
\text{Efficacy Proportion} = \frac{\text{No. of Live Ticks/Dog}}{\text{Total No. Ticks (live + dead)/Dog} \times \text{Total No. Ticks (live + dead)}} \times 100
\]

where the geometric mean was calculated as:

\[
\text{Geometric mean} = 10^{\text{LSMEAN} - 1}
\]

**RESULTS**

A high percentage of ticks exposed to dogs treated with 65% permethrin died within the 2-hour exposure period; therefore results are presented for both efficacy and repellency.

**Efficacy**

The geometric mean number of live ticks per dog and the percentage of efficacy (i.e., percentage killed) based on geometric means
and relative to the untreated control are shown in Table 1 and Figure 1. Treatment of dogs with 65% permethrin reduced tick numbers on dogs by 99.1% at 2 days, 99.0% at 1 week, 95.9% at 2 weeks, 88.5% at 3 weeks, 87.1% at 4 weeks, 74.1% at 5 weeks, and 48.0% at 6 weeks after application. In contrast, treatment of dogs with 9.7% fipronil reduced tick numbers on dogs by 61.4% at 2 days, 51.6% at 1 week, and 37.0% at 2 weeks after application; no substantial efficacy was observed from 3 to 6 weeks after treatment. The efficacy of 65% permethrin was significant ($P \leq .05$) when compared with the controls at all challenges, whereas the efficacy of 9.7% fipronil was not significant when compared with the controls at 21, 28, 35, and 41 days after treatment. The 65% permethrin formulation killed significantly more $I. ricinus$ ($P \leq .05$) than did the 9.7% fipronil formulation from 2 to 41 days after treatment.

### Repellency

The geometric mean number of ticks that were repelled (i.e., total number of live and dead ticks in the cages/carpets and the number of dead ticks found on the dogs) and the percentage of repellency based on geometric means relative to the untreated control are shown in Table 2. The relative repellency based on the numbers of ticks collected is shown in Figure 2 for 65% permethrin and Figure 3 for 9.7% fipronil. Calculated repellency relative to the untreated control of 65% permethrin was $-77.0$, $-157.1$, $-238.7$, $-69.8$, $-80.0$, $-58.3$, and $-112.0$ at 2, 7, 14, 21, 28, 35, and 41 days after treatment.
after treatment, respectively. These values represent 1.9-, 2.0-, 3.0-, 4.3-, 3.9-, 8.9-, and 17.3-fold greater repellency of 65% permethrin than was observed for 9.7% fipronil at 2, 7, 14, 21, 28, 35, and 41 days after treatment, respectively. The repellency of 65% permethrin was significantly different ($P \leq 0.05$) from the control at all challenges, whereas the repellency of 9.7% fipronil was not significantly different from the control at 21, 28, 35, and 41 days after treatment. The 65% permethrin repelled significantly more *I. ricinus* ($P \leq 0.05$) than did the 9.7% fipronil from 2 to 41 days after treatment.

## DISCUSSION

Cloth sprayed or impregnated with permethrin has been shown to effectively repel several members of the genus *Ixodes*, which includes *I. pacificus* from the western United States, *I. scapularis* from the eastern US, and *I. ricinus* from Europe. Permethrin concentrations on cloth in these reports ranged from 0.004 mg/cm$^2$ to 0.25 mg/cm$^2$. With exposure periods of ticks to permethrin ranging from 10 seconds to 1 hour, >90% of larvae, nymph, and adults of the *Ixodes* species were shown to be either incapacitated or killed on subsequent examination. In addition, the residual quantity of permethrin adherent to cloth after several hot water/detergent washings was sufficient to provide effective protection. The repellency studies conducted with permethrin applied to cloth have demonstrated that factors affecting repellency include the concentration of permethrin on the substrate and the duration of contact between ticks and permethrin. Furthermore, Heller-Haupt and Varma have shown that susceptibility to pyrethroids, including permethrin, among ticks increases as the age of the tick increases. Extrapolation of permethrin concentrations on cloth to those expected on dogs after topical applications have not been made because of inherent difficulty in calculating the combined surface area of skin and hair.

This study was designed with a relatively brief, 2-hour exposure period of ticks, *I. ricinus*, to the insecticides on dogs. The objective was to show that with a brief exposure period ticks were repelled and not killed because previous experiments for product registration with longer exposure periods of 3 to 5 days resulted in the death of most ticks within a few days af-

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**Figure 2.** Mean numbers of adult ticks, *Ixodes ricinus*, killed and repelled by a 2-hour exposure to dogs treated with 65% permethrin. Dogs (*n* = 6) were exposed to 50 unfed adult ticks for a 2-hour period in an enclosed cage 2, 7, 14, 21, 28, 35, and 41 days after treatment. Line C represents the mean number of ticks recovered per cage (live and dead) that were not on dogs. Line D represents the mean total number of ticks per dog that were killed. Line L represents the mean number of live ticks found on dogs. The shaded area between Line C and Line L represents the mean total number of ticks per dog that were repelled. The shaded area between Line D and Line L represents the mean total number of repelled ticks that were killed.
ter exposure to treated dogs. The finding that *Ixodes* species exposed to dogs treated with 65% permethrin killed nearly all ticks during the week after treatment was consistent with previous observations of *Ixodes* species exposed to permethrin-treated cloth. More than 95% and 87% of ticks exposed to permethrin in this study were killed 14 and 28 days after treatment, respectively, and thereafter the percentage of ticks killed declined to 48% 42 days after treatment. It was noteworthy that as the number of ticks killed by a 2-hour exposure to dogs treated with 65% permethrin decreased over time, the percentage of ticks found alive in the cage (repelled) but not on the dogs increased. In contrast, the efficacy of 9.7% fipronil as measured by the number of live ticks observed on the dog did not exceed 61% at any time after treatment, and 9.7% fipronil exhibited little repellent effect at any time after treatment.

The test system was closed and designed so that all ticks placed into the cages could be recovered. In the untreated control group, 80.2% of the 50 ticks were recovered. This suggests that the dogs may have ingested some ticks during grooming or that some were not observed because of their small size. Approximately 1.8% of ticks were found dead on the dogs in the control group, which indicates that ticks used in the study were healthy and experienced little mortality in the 2-hour period of exposure to the insecticides. It was noteworthy that slightly more than 50% of ticks began attaching to the dogs within a 2-hour period. Substantially lower attachment rates have been observed in other experiments with *Ixodes* species.

**CONCLUSION**

These data indicate that *Ixodes ricinus* are highly susceptible to permethrin and these data suggest that dogs treated with 65% permethrin once will be protected from infestation with *I. ricinus* for at least 4 to 6 weeks. The level of protection would be expected to increase with a longer exposure period of ticks to treated dogs as ticks attempt to attach and begin the 4- to 8-day feeding period. The remarkably high level of protection from infestation with *I. ricinus* afforded to dogs treated with 65% permethrin (Defend® Exspot® Treat-
REFERENCES


