Evaluation of an Imidacloprid (8.8% w/w)–Permethrin (44.0% w/w) Topical Spot-On and a Fipronil (9.8% w/w)–(*S*)-Methoprene (8.8% w/w) Topical Spot-On to Repel, Prevent Attachment, and Kill Adult *Rhipicephalus sanguineus* and *Dermacentor variabilis* Ticks on Dogs*

M. W. Dryden, DVM, PhD[a]
P. A. Payne, DVM, PhD[a]
V. Smith, RVT[a]
J. Hostetler, DVM[b]

[a]*Department of Diagnostic Medicine/Pathobiology*
College of Veterinary Medicine
Kansas State University
Manhattan, KS 66506

[b]*Bayer Animal Health*
P.O. Box 390
Shawnee Mission, KS 66201

CLINICAL RELEVANCE

This study evaluated the effectiveness of two topical spot-on formulations, imidacloprid (8.8% w/w)–permethrin (44.0% w/w) and fipronil (9.8%w/w)–(*S*)-methoprene (8.8% w/w), to repel, prevent the attachment of, and kill adult *Rhipicephalus sanguineus* and *Dermacentor variabilis* on dogs. Twelve purpose-bred beagles were distributed into three groups of four dogs each; one group served as untreated controls, and each of the other two groups received one of the test products. Dogs were exposed to 25 adult ticks of each species for 10 minutes on posttreatment days 3, 7, 14, 21, and 28. Unattached or repelled ticks were collected and evaluated for viability, and on-dog tick counts were conducted at 3, 24, and 48 hours after tick exposure. The imidacloprid–permethrin formulation provided significant repellency of *R. sanguineus* and *D. variabilis* for up to 3 and 4 weeks after treatment, respectively, and provided good overall control for *R. sanguineus* and *D. variabilis* during the study period. The fipronil–(*S*)-methoprene formulation provided good overall tick control during the study period. Additionally, the imidacloprid–permethrin formulation provided significant morality of repelled *R. sanguineus* and *D. variabilis* at every posttreatment time point.

*This study was funded in part by a grant from Bayer Animal Health, Shawnee Mission, Kansas.

INTRODUCTION

Two tick species that commonly parasitize dogs in the United States are *Rhipicephalus sanguineus* (brown dog tick) and *Dermacentor variabilis* (American dog tick).[1] These ticks are vectors of several important bacterial and protozoal pathogens that cause disease in dogs. *R. sanguineus* is the primary vector of *Ehrlichia canis* (canine monocytic ehrlichiosis) and *Babesia canis* and has been implicated as a vector of the agent of Rocky Mountain spotted fever in North America. *D. variabilis* is the primary vector of *Rickettsia rickettsii* (Rocky Mountain spotted fever), transmits *Francisella tularensis,* and has been shown to transmit *Ehrlichia canis.*[1]

R. sanguineus is widely distributed across much of the southern United States; in temperate regions, however, it is most common within kennels and homes because it appears to be cold intolerant.[1] Like the other significant tick species that parasitize dogs, *R. sanguineus* is a three-host tick but is unique in that all feeding life stages prefer to feed on dogs. *D. variabilis* occurs widely across the central and eastern United States from Florida to southern New England and from the Atlantic coast to eastern sections of the plains states.[2] Populations also occur along the Pacific coast.

Control of *R. sanguineus* and *D. variabilis* on dogs is important not only to prevent irritating tick infestations but also to reduce dogs' chances of acquiring tick-transmitted disease-causing organisms. Several studies have been published evaluating the efficacy of fipronil and imidacloprid–permethrin formulations against *R. sanguineus* and *D. variabilis* on dogs.[3–10] In addition, a few studies have been conducted to evaluate the ability of these formulations not only to repel but also to kill repelled *R. sanguineus* and *D. variabilis.*[9,10] These repellency studies were conducted by placing treated dogs in ventilated dog crates for 2 hours and counting both live and dead ticks immediately after the 2-hour exposure period. No differentiation was made between live or dead repelled ticks.

The study reported here was conducted to evaluate the effectiveness of an imidacloprid (8.8% w/w)–permethrin (44.0% w/w) combination spot-on and a fipronil (9.8% w/w)–(*S*)-methoprene (8.8% w/w) spot-on to both repel and kill adult *R. sanguineus* and *D. variabilis.* Viability of repelled ticks was assessed for up to 48 hours.

MATERIALS AND METHODS

Housing

Twelve purpose-bred beagles (six males and six females), 6 to 24 months of age, were distributed by pairs based on cohabitability, gender, and weight into indoor–outdoor pens or runs. The indoor pen area was 122 × 244 cm (4 × 8 ft), and pens were separated by stainless-steel solid sides. When dogs were infested with ticks, they were confined to the indoor pens and the entrance was lined with a strip of petroleum jelly and double-sided sticky tape to prevent tick movement out of and between pens. The outdoor run area was 12 × 304 cm (4 × 10 ft), and pens were divided by chain-linked fencing covered by fiberglass siding 132 cm (52 inches) high. The outdoor area was completely covered by a roof.

Each dog was identified by an individual alphanumeric ear tattoo. Housing was in full compliance with all USDA Animal and Plant Health Inspection Service requirements. Dogs were fed a commercially available high-quality dog food that met National Research Council nutritional requirements. Water was available ad libitum. After being exposed to ticks, dogs were monitored twice daily for general health. In addition, daily observations were made to ensure there were no adverse clinical signs after administration of the test material. Dogs were weighed on arrival and before treatment.

Treatments

Treatment groups were based on gender and weight. Dogs were treated on study day 0:

- **Group 1—**Four dogs (two males and two females; mean weight, 10.43 kg) were treated with an imidacloprid (8.8% w/w)–permethrin (44.0% w/w) topical spot-on (K9 Advantix, Bayer Animal Health) according to label dosing recommendations; the dose was applied evenly to four spots on top of the back from the shoulder to the base of the tail.
- **Group 2—**Four dogs (two males and two females; mean weight, 10.84 kg) were treated with a fipronil (9.8% w/w)–(*S*)-methoprene (8.8% w/w) spot-on (Frontline Plus, Merial) according to label dosing recommendations; the entire dose was applied on one spot between the shoulder blades.
- **Group 3—**Four dogs (two males and two females; mean weight, 11.02 kg) served as untreated controls.

Personnel conducting treatments, tick exposures, and tick counts were provided with personal protective gear, including full-body disposable coveralls, shoe covers, and latex gloves.

Tick Exposures

Dogs were infested with a combination of 25 adult *R. sanguineus* and 25 adult *D. variabilis* on posttreatment days 3, 7, 14, 21, and 28. Ticks were purchased from EL Labs (Soquel, CA) and shipped to Kansas State University by overnight courier. Before being placed on the dogs, ticks were maintained in the laboratory at Kansas State University for 1 to 3 days at room temperature and 92% to 94% relative humidity.

Dogs were placed in lateral recumbency in a stainless-steel tub. Dogs were manually restrained by two technicians for the tick infestation procedure. After the lid of the tick shipping container was removed, a laboratory technician exhaled slowly over the top of the container to stimulate tick activity. Ticks were then immediately deposited along the lateral thorax and abdomen of each dog while it was in the steel tub. Not all of the ticks moved immediately into the haircoat in all dogs. Some of the ticks dropped or crawled off the dogs. As this occurred, the ticks were collected and placed back on the dogs. Dogs were gently held in the tub and exposed to ticks in this manner for 10 minutes. After the tick exposure, dogs were returned to the indoor runs. It should be noted that when ticks were placed on dogs, an immediate assessment was conducted to ensure that all ticks were alive.

Evaluation of Rapid Repellent-Like Activity ("Repellency") and Viability of Unattached Ticks

Unattached ticks found in the tub after the 10-minute exposure were collected, sorted by species, and placed in glass vials. Perforated caps were placed over the vials to prevent ticks from escaping but to allow for air exchange. Recovered ticks were placed according to treatment groups in separate humidity chambers at 92% to 94% relative humidity and room temperature. The effect of the short-term (10-minute) acaricide exposure on unattached ticks was evaluated by assessing the viability of the ticks immediately as they were collected (10 minutes after initial exposure) and again at 3, 24, and 48 hours after exposure. Ticks were assessed as follows:

- **Live—**Tick could move forward in a natural motion using all its legs for locomotion and could right itself when placed on its dorsal surface (back).
- **Moribund—**Tick could not move forward in a natural motion using all its legs (moribund ticks often moved in a slow, uncoordinated, and staggered fashion with the legs

TABLE 1. Repellent Effect of an Imidacloprid (8.8% w/w)–Permethrin (44.0% w/w) Combination Topical Spot-On and a Fipronil (9.8% w/w)–(*S*)-Methoprene (8.8% w/w) Topical Spot-On against Adult *Rhipicephalus sanguineus* and *Dermacentor variabilis* Exposed to Treated Dogs for up to 10 Minutes

	DAYS AFTER TREATMENT					
	Day 3			*Day 7*		
TREATMENT GROUP[a]	*Mean*[b]	*% Repelled*[c]	*% Difference vs Control*	*Mean*[b]	*% Repelled*[c]	*% Difference vs Control*
R. sanguineus						
Controls	0.7	4.0[d]	—	2.2	9.0[d]	—
Imidacloprid–permethrin	20.7	83.0[e]	79.0	13.1	53.0[e]	44.0
Fipronil–(*S*)-methoprene	0.0	0.0[d]	–4.0	2.2	9.0[d]	0.0
D. variabilis						
Controls	2.0	9.0[d]	—	1.6	9.0[d]	—
Imidacloprid–permethrin	23.2	93.0[e]	84.0	21.1	86.0[e]	77.0
Fipronil–(*S*)-methoprene	1.8	11.0[d]	2.0	4.2	18.0[d]	9.0

[a]Each of four dogs in the control group received no treatment. Each of four dogs in the imidacloprid–permethrin or fipronil–(*S*)-methoprene treatment group received a topical dose of the formulated product according to label directions on day 0. Each dog was infested with 25 adult *R. sanguineus* and 25 adult *D. variabilis* on days 3, 7,14, 21, and 28.

curled) and was unable to right itself when placed on its dorsal surface (back).

- **Dead—**Tick displayed no movement, including no leg motion, when examined.

It was often necessary to remove ticks from the glass vials and place them on the palm of a gloved hand or the bottom of a plastic tray to make these observations. For data analysis, moribund and dead ticks were recorded together as dead.

Evaluation of Tick Kill

At 3, 24, and 48 hours after being exposed to ticks, each dog was placed on a stainless-steel table and visually inspected for attached ticks. Examiners wore personal protective gear and were blinded as to treatment group allocation. The visual examination procedure was conducted by running a flea comb or fingers against the lay of the hair so that the hair could be parted to visually inspect for ticks. Routine examination began on the head and proceeded to the back, sides, abdomen, chest, front legs and feet (with careful inspection between the toes), and finally the hind legs and feet. Each dog was examined for 30 minutes. Any tick observed alive and attached was counted and identified by species. Ticks were not removed after the 3- and 24-hour tick counts, and dogs were returned to the indoor runs; after the 48-hour evaluations, ticks were removed and dogs were returned to the pens and allowed access to both the indoor and outdoor runs.

Statistical Methods

A repeated measures analysis of variance (selecting the best covariance structure [smallest Akaike information criterion]) was conducted. If "treatment × day" interaction was significant,

DAYS AFTER TREATMENT								
Day 14			Day 21			Day 28		
Mean[b]	% Repelled[c]	% Difference vs Control	Mean[b]	% Repelled[c]	% Difference vs Control	Mean[b]	% Repelled[c]	% Difference vs Control
4.6	20.0[d]	—	2.7	14.0[d]	—	10.2	42.0[d]	—
9.5	39.0[e]	19.0	10.1	42.0[e]	28.0	11.1	47.0[d]	5.0
4.3	18.0[d]	–2.0	3.2	16.0[d]	2.0	7.9	37.0[d]	–5.0
1.8	8.0[d]	—	3.1	17.0[d]	—	4.0	19.0[d]	—
14.1	63.0[e]	55.0	21.6	87.0[e]	70.0	15.9	68.0[e]	49.0
1.2	9.0[d]	1.0	2.1	9.0[d]	–8.0	1.3	7.0[d]	–12.0

[b]Geometric mean number of ticks recovered in tub from four dogs/treatment group after 10 minutes' exposure to treated or control dogs.
[c]Average of each dog's calculated percent repelled, based on the number of ticks initially infested.
[d,e]For the percent repelled data, different column letters indicate a statistically significant difference ($P < .05$).

"treatment" effects were tested for each time period. If no interaction was noted, a "main treatment" effect was used to show group differences. Least squares means were calculated for both "treatment" and "treatment × day," and differences were compared. All P values < .05 were deemed statistically significant. Geometric means were calculated using antilog (average natural log + 1)-1 algorithm. Repellent effects (Table 1) were analyzed using proportions (transformed with arcsine square root). Note: For viability assessments (Tables 2 and 3), a repeated measures analysis of covariance (using day 3 total tick counts from tub as a covariate and selecting best covariance structure [smallest Akaike information criterion]) was used. Although these two tables express the efficacy in terms of percent dead or moribund, the actual analysis was performed using the number of live ticks found on a dog at the respective time interval.

RESULTS

Evaluation of Repellent-Like Effect (Repellency)

R. sanguineus

The rapid irritation or repellent-like effect (repellency) of the imidacloprid–permethrin formulation against *R. sanguineus* was evident on posttreatment days 3 through 21 (Table 1). On posttreatment days 3, 7, 14, 21, and 28, 79%, 44%, 19%, 28%, and 5% more *R. sanguineus*, respectively, were recovered in the tub after ticks were exposed to imidacloprid–permethrin–treated dogs than control dogs (Table 1). The fipronil–(*S*)-methoprene formulation did not display a similar repellent-like effect against *R. sanguineus*.

D. variabilis

The repellent of effect of imidacloprid–permethrin against *D. variabilis* was evident on

TABLE 2. Viability Assessment of Unattached or Repelled Adult *Rhipicephalus sanguineus* after 10 Minutes' Exposure to Dogs Treated with Either an Imidacloprid (8.8% w/w)–Permethrin (44.0% w/w) Combination Topical Spot-On or a Fipronil (9.8% w/w)–(*S*)-Methoprene (8.8% w/w) Topical Spot-On

Treatment Group[a]	*Mean No. of Ticks*[b]	*% Dead or Moribund*[c]			
		10 Min[d]	*3 Hr*	*24 Hr*	*48 Hr*
Posttreatment day 3					
Controls	0.7	0.0[e] (*n* = 2)	0.0[e] (*n* = 2)	16.7[e] (*n* = 2)	16.7[e] (*n* = 2)
Imidacloprid–permethrin	20.7	100.0[f]	100.0[f]	100.0[f]	100.0[f]
Fipronil–(*S*)-methoprene	0.0	NA	NA	NA	NA
Posttreatment day 7					
Controls	2.2	0.0[e]	0.0[e]	0.0[e]	12.5[e]
Imidacloprid–permethrin	13.1	93.2[f]	100.0[f]	100.0[f]	100.0[f]
Fipronil–(*S*)-methoprene	2.2	0.0[e]	0.0[e]	29.2[e]	100.0[f]
Posttreatment day 14					
Controls	4.6	0.0[e]	0.0[e]	5.0[e]	0.0[e]
Imidacloprid–permethrin	9.5	24.2[e]	80.0[f]	96.9[f]	96.9[f]
Fipronil–(*S*)-methoprene	4.3	0.0[e]	0.0[e]	0.0[e]	16.7[e]
Posttreatment day 21					
Controls	2.7	0.0[e]	0.0[e]	0.0[e]	0.0[e]
Imidacloprid–permethrin	10.1	44.2[e]	100.0[f]	100.0[f]	100.0[f]
Fipronil–(*S*)-methoprene	3.2	0.0[e]	0.0[e]	0.0[e]	25.0[e]
Posttreatment day 28					
Controls	10.2	0.0[e]	0.0[e]	0.0[e]	0.0[e]
Imidacloprid–permethrin	11.1	39.1[f]	85.7[f]	79.5[f]	77.4[f]
Fipronil–(*S*)-methoprene	7.9	0.0[e]	5.5[e]	9.9[e]	1.6[e]

[a]Each dog in the control group (*n* = 4) received no treatment; each dog in the imidacloprid–permethrin (*n* = 4) or fipronil–(*S*)-methoprene (*n* = 4) treatment group received a topical dose of the formulated product according to label directions on day 0. Each dog was infested with 25 adult *Rhipicephalus sanguineus* on days 3, 7, 14, 21, and 28.

[b]Geometric mean number of ticks recovered in tub from four dogs/treatment group after 10 minutes' exposure to treated or control dogs.

[c]A tick was classified as dead or moribund if it displayed no movement, including leg motion, when examined; if it moved in a slow, uncoordinated, and staggered fashion with its legs curled; or if it was unable to right itself when placed on its dorsal surface (back).

[d]Percent of ticks dead immediately following exposure to treated dogs.

[e,f]For the percent dead or moribund data, different column letters indicate a statistically significant difference ($P < .05$).

NA = not able to calculate

posttreatment days 3 through 28 (Table 1). On posttreatment days 3, 7, 14, 21, and 28, 84%, 77%, 55%, 70%, and 49% more *D. variabilis*, respectively, were recovered in the tub after ticks were exposed to imidacloprid–permethrin–treated dogs than control dogs (Table 1). The fipronil–(*S*) methoprene formulation did not display a similar immediate repellent-like effect against *D. variabilis* at any time point after treatment (Table 1).

TABLE 3. Viability Assessment of Unattached or Repelled Adult *Dermacentor variabilis* after 10 Minutes' Exposure to Dogs Treated with Either an Imidacloprid (8.8% w/w)–Permethrin (44.0% w/w) Combination Topical Spot-On or a Fipronil (9.8% w/w)–(*S*)-Methoprene (8.8% w/w) Topical Spot-On

Treatment Group[a]	*Mean No. of Ticks*[b]	*% Dead or Moribund*[c]			
		10 Min[d]	*3 Hr*	*24 Hr*	*48 Hr*
Posttreatment day 3					
Controls	2.0	0.0[e]	0.0[e]	6.3[e]	6.3[e]
Imidacloprid–permethrin	23.2	99.0[f]	100.0[f]	100.0[f]	100.0[f]
Fipronil–(*S*)-methoprene	1.8	0.0[e] (*n* = 3)	4.8[e] (*n* = 3)	33.3[e] (*n* = 3)	33.3[e] (*n* = 3)
Posttreatment day 7					
Controls	1.6	0.0[e] (*n* = 3)	0.0[e] (*n* = 3)	0.0[e] (*n* = 3)	0.0[e] (*n* = 3)
Imidacloprid–permethrin	21.1	81.8[f]	93.2[f]	100.0[f]	100.0[f]
Fipronil–(*S*)-methoprene	4.2	0.0[e]	0.0[e]	12.5[e]	41.7[e]
Posttreatment day 14					
Controls	1.8	0.0[e]	0.0[e]	0.0[e]	0.0[e]
Imidacloprid–permethrin	14.1	36.8[e]	78.9[f]	90.6[f]	84.3[f]
Fipronil–(*S*)-methoprene	1.2	0.0[e] (*n* = 2)	0.0[e] (*n* = 2)	25.0[e] (*n* = 2)	25.0[e] (*n* = 2)
Posttreatment day 21					
Controls	3.1	0.0[e] (*n* = 3)	0.0[e] (*n* = 3)	6.7[e] (*n* = 3)	0.0[e] (*n* = 3)
Imidacloprid–permethrin	21.6	35.2[e]	96.6[f]	100.0[f]	100.0[f]
Fipronil–(*S*)-methoprene	2.1	0.0[e]	8.3[e]	8.3[e]	8.3[e]
Posttreatment day 28					
Controls	4.0	0.0[e]	0.0[e]	0.0[e]	0.0[e]
Imidacloprid–permethrin	15.9	50.2[e]	62.2[f]	57.8[f]	37.4[e]
Fipronil–(*S*)-methoprene	1.3	0.0[e] (*n* = 3)	0.0[e,f] (*n* = 3)	0.0[e,f] (*n* = 3)	0.0[e] (*n* = 3)

[a]Each dog in the control group (*n* = 4) received no treatment; each dog in the imidacloprid–permethrin (*n* = 4) or fipronil–(*S*)-methoprene (*n* = 4) treatment group received a topical dose of the formulated product according to label directions on day 0. Each dog was infested with 25 adult *D. variabilis* on days 3, 7, 14, 21, and 28.

[b]Geometric mean number of ticks recovered in tub from four dogs/treatment group after 10 minutes' exposure to treated or control dogs.

[c]A tick was classified as dead or moribund if it displayed no movement, including leg motion, when examined; if it moved in a slow, uncoordinated, and staggered fashion with its legs curled; or if it was unable to right itself when placed on its dorsal surface (back).

[d]Percent of ticks dead immediately following exposure to treated dogs.

[e,f]For the percent dead or moribund data, different column letters indicate a statistically significant difference ($P < .05$).

NA = not able to calculate

Viability of Unattached (Repelled) Ticks

R. sanguineus

There was a significant effect on the viability of unattached or repelled *R. sanguineus* that dropped off imidacloprid–permethrin–treated dogs on every exposure day after treatment (Table 2). At 3 days after treatment, 100% of unattached or repelled *R. sanguineus* were dead within 10 minutes. Thereafter, on days 7 and 21, 100% were dead within 3 and 48 hours,

respectively. On posttreatment day 28, 77.4% of unattached or repelled *R. sanguineus* were dead within 48 hours (Table 2). The unattached *R. sanguineus* from the fipronil–(*S*)-methoprene–treated dogs had significant mortality compared with controls only at 7 days after treatment.

D. variabilis

There was also a significant effect on the viability of unattached or repelled *D. variabilis* that dropped off imidacloprid–permethrin–treated dogs on posttreatment days 3 through 21 (Table 3). At 3, 7, 14, and 21 days after treatment, 100%, 100%, 84.3%, and 100% of the repelled *D. variabilis* were dead, respectively, within 48 hours (Table 3). There was no significant mortality of unattached *D. variabilis* exposed to the fipronil–(*S*)-methoprene–treated dogs.

Evaluation of Tick Kill

R. sanguineus

Day 3 geometric mean on-dog *R. sanguineus* counts 3 hours after exposure were significantly reduced compared with controls for dogs treated with imidacloprid–permethrin and fipronil–(*S*)-methoprene (Table 4). On day 3, geometric mean on-dog *R. sanguineus* counts 3 hours after exposure to control dogs or dogs treated with imidacloprid–permethrin or fipronil–(*S*)-methoprene were 14.0, 0.0, and 4.6, respectively (Table 4). Three-hour postexposure on-dog *R. sanguineus* counts conducted on days 3 through 28 were significantly reduced on dogs treated with imidacloprid–permethrin compared with controls and on dogs treated with fipronil–(*S*)-methoprene compared with controls.

Both imidacloprid–permethrin and fipronil–(*S*)-methoprene–treated dogs had significant reductions in geometric mean *R. sanguineus* counts at 24 hours after exposure on posttreatment days 3 through 14, and imidacloprid–permethrin–treated dogs also had significant reductions in geometric mean tick counts at days 21 and 28. Both imidacloprid–permethrin– and fipronil–(*S*)-methoprene–treated dogs had significant reductions in geometric mean *R. sanguineus* counts 48 hours after exposure at every posttreatment time point. The 48-hour efficacy of the imidacloprid–permethrin formulation on days 3, 7, 14, 21, and 28 after treatment was 100%, 100%, 100%, 88.8%, and 100%, respectively (Table 4). The 48-hour efficacy of the fipronil–(*S*)-methoprene formulation on days 3, 7, 14, 21, and 28 after treatment was 100%, 100%, 100%, 91.9%, and 86.3%, respectively (Table 4).

D. variabilis

Three-hour postexposure on-dog *D. variabilis* counts conducted on days 3 through 28 were significantly reduced on dogs treated with imidacloprid–permethrin compared with the controls. Three-hour postexposure *D. variabilis* counts on dogs treated with imidacloprid–permethrin were reduced by 100% and 70.7% on posttreatment days 3 and 28, respectively (Table 5).

Both imidacloprid–permethrin– and fipronil–(*S*)-methoprene–treated dogs had significant reductions in geometric mean *D. variabilis* counts at 24 hours after exposure on posttreatment days 3 and 7. Dogs treated with imidacloprid–permethrin also had significant reductions in geometric mean *D. variabilis* counts at 24 hours after exposure on posttreatment days 14, 21, and 28 (Table 5). Both imidacloprid–permethrin– and fipronil–(*S*)-methoprene–treated dogs had significant reductions in geometric mean *D. variabilis* counts at 48 hours after exposure at every posttreatment time point. The 48-hour efficacy of the imidacloprid–permethrin formulation against *D. variabilis* on posttreatment days 3, 7, 14, 21, and 28 was 100%, 100%, 92.9%, 100%, and

TABLE 4. Geometric Mean Tick Counts[a] and Percent Control[b] of Adult *Rhipicephalus sanguineus* Infesting Dogs Treated with Either an Imidacloprid (8.8% w/w)–Permethrin (44.0% w/w) Combination Topical Spot-On or a Fipronil (9.8% w/w)–(*S*)-Methoprene (8.8% w/w) Topical Spot-On

	DAYS AFTER TREATMENT									
	Day 3		***Day 7***		***Day 14***		***Day 21***		***Day 28***	
TREATMENT GROUP[c]	*Mean No. of Ticks*	*% Control*	*Mean No. of Ticks*	*% Control*	*Mean No. of Ticks*	*% Control*	*Mean No. of Ticks*	*% Control*	*Mean No. of Ticks*	*% Control*
3 hr after infestation										
Controls	14.0[d]	—	12.2[d]	—	9.1[d]	—	10.7[d]	—	7.5[d]	—
Imidacloprid–permethrin	0.0[f]	100.0	1.4[e]	88.1	2.5[e]	72.9	1.3[e]	87.9	2.3[e]	69.9
Fipronil–(*S*)-methoprene	4.6[e]	66.9	5.7[d]	53.6	9.0[d]	0.8	9.3[d]	12.9	6.9[d]	8.2
24 hr after infestation										
Controls	8.2[d]	—	7.4[d]	—	6.7[d]	—	8.4[d]	—	4.2[d]	—
Imidacloprid–permethrin	0.0[e]	100.0	0.6[f]	92.4	0.9[e]	87.2	0.8[e]	90.7	0.9[e]	79.4
Fipronil–(*S*)-methoprene	0.0[e]	100.0	0.0[e]	100.0	1.2[e]	82.0	3.5[d,e]	57.8	2.6[d,e]	38.8
48 hr after infestation										
Controls	5.7[d]	—	7.7[d]	—	6.1[d]	—	3.9[d]	—	2.3[d] (n = 3)	—
Imidacloprid–permethrin	0.0[e]	100.0	0.0[e]	100.0	0.7[e]	88.8	0.0[e]	100.0	0.0[e]	100.0
Fipronil–(*S*)-methoprene	0.0[e]	100.0	0.0[e]	100.0	0.0[f]	100.0	0.3[e]	91.9	0.3[e]	86.3

[a]Geometric mean number of ticks attached on four dogs/treatment group.
[b]Percent control = [(Geometric Mean Count Control – Geometric Mean Count Treatment)/Geometric Mean Count Treatment] × 100.
[c]Each dog in the control group (n = 4) received no treatment; each dog in the imidacloprid–permethrin (n = 4) or fipronil–(*S*)-methoprene (n = 4) treatment group received a topical dose of the formulated product according to label directions on day 0. Each dog was infested with 25 adult *R. sanguineus* on days 3, 7, 14, 21, and 28.
[d,e,f]For the actual tick count on each dog (log transformed), different column letters indicate a statistically significant difference (P < .05).

92.0%, respectively (Table 5). The 48-hour efficacy of the fipronil–(*S*)-methoprene formulation against *D. variabilis* on posttreatment days 3, 7, 14, 21, and 28 was 96.1%, 100%, 87.2%, 78.9%, and 83.23%, respectively (Table 5).

DISCUSSION

The imidacloprid–permethrin treatment produced significant repellent-like activity (repellency) against *R. sanguineus* and *D. variabilis* compared with controls for 21 and 28

TABLE 5. Geometric Mean Ticks Counts[a] and Percent Control[b] of Adult *Dermacentor variabilis* Infesting Dogs Treated with Either an Imidacloprid (8.8% w/w)–Permethrin (44.0% w/w) Combination Topical Spot-On or a Fipronil (9.8% w/w)–(*S*)-Methoprene (8.8% w/w) Topical Spot-On

	DAYS AFTER TREATMENT									
	Day 3		***Day 7***		***Day 14***		***Day 21***		***Day 28***	
TREATMENT GROUP[c]	*Mean No. of Ticks*	*% Control*	*Mean No. of Ticks*	*% Control*	*Mean No. of Ticks*	*% Control*	*Mean No. of Ticks*	*% Control*	*Mean No. of Ticks*	*% Control*
3 hr after infestation										
Controls	10.3[d]	—	8.4[d]	—	10.5[d]	—	10.1[d]	—	11.5[d]	—
Imidacloprid–permethrin	0.0[e]	100.0	0.3[e]	96.2	2.3[e]	77.9	0.8[e]	92.3	3.4[e]	70.7
Fipronil–(*S*)-methoprene	7.6[d]	26.1	8.1[d]	4.0	8.6[d]	17.4	11.8[d]	–16.2	8.2[d,e]	29.1
24 hr after infestation										
Controls	7.0[d]	—	8.0[d]	—	7.2[d]	—	9.1[d]	—	10.1[d]	—
Imidacloprid–permethrin	0.0[e]	100.0	0.2[e]	97.6	1.6[e]	77.9	0.2[e]	97.9	1.1[e]	88.7
Fipronil–(*S*)-methoprene	0.8[e]	88.8	0.8[e]	90.2	2.7[d,e]	62.8	7.5[d]	17.3	5.1[d,e]	49.9
48 hr after infestation										
Controls	4.9[d]	—	5.8[d]	—	4.4[d]	—	6.1[d]	—	8.5[d] (n = 3)	—
Imidacloprid–permethrin	0.0[e]	100.0	0.0[e]	100.0	0.3[e]	92.9	0.0[f]	100.0	0.7[e]	92.0
Fipronil–(*S*)-methoprene	0.2[e]	96.1	0.0[e]	100.0	0.6[e]	87.2	1.3[e]	78.9	1.4[e]	83.2

[a]Geometric mean number of ticks attached on four dogs/treatment group.
[b]Percent control = [(Geometric Mean Count Control – Geometric Mean Count Treatment)/Geometric Mean Count Treatment] × 100.
[c]Each dog in the control group (n = 4) received no treatment; each dog in the imidacloprid–permethrin (n = 4) or fipronil–(*S*)-methoprene (n = 4) treatment group received a topical dose of the formulated product according to label directions on day 0. Each dog was infested with 25 adult *Dermacentor variabilis* on days 3, 7, 14, 21, and 28.
[d,e,f]For the actual tick count on each dog (log transformed), different column letters indicate a statistically significant difference (P < .05).

days, respectively, after treatment (Table 1). The fipronil–(*S*)-methoprene formulation did not produce any repellency against either tick species. The repellency of the imidacloprid–permethrin formulation demonstrated in this study has been observed previously and is likely related to the rapid irritating or repellent effect of permethrin.[9,10] Of interest is that in previous repellency studies, tick exposures were conducted by placing imidacloprid–perme-

thrin–treated dogs and ticks into ventilated crates, whereas in our study treated dogs were exposed to ticks in open tubs. In the previous studies in which *R. sanguineus* and *D. variabilis* were exposed to dogs in crates, the imidacloprid–permethrin formulation had significant repellency against those ticks for 28 and 35 days after treatment, respectively, about 1 week longer than in the current study.

All the repelled or unattached *R. sanguineus* recovered from the imidacloprid–permethrin–treated dogs on posttreatment days 3, 7, and 21 were moribund or dead within 10 minutes and 3 hours after exposure, respectively. On day 28, 77.4% the ticks were dead by 48 hours. The effect of imidacloprid–permethrin on repelled *D. variabilis* was similar on days 3 through 21 but decreased significantly on day 28. This is a further indication of the rapid neurotoxic effect of permethrin on susceptible ticks. A 10-minute exposure to fipronil–(*S*)-methoprene–treated dogs had minimal effect on unattached ticks, except at day 7 with *R. sanguineus*. Our inability to evaluate the effect on ticks from short-term exposure to fipronil–(*S*)-methoprene–treated dogs was partly because so few ticks were repelled by this formulation.

The imidacloprid–permethrin and fipronil–(*S*)-methoprene formulations provided good efficacy against *R. sanguineus*, with 100% and 86.3% control achieved, respectively, by the 48-hour tick counts 28 days after treatment (Table 4). Similarly, the imidacloprid–permethrin and fipronil–(*S*)-methoprene formulations provided good efficacy against *D. variabilis*, with 92.0% and 83.2% control achieved, respectively, by the 48-hour tick counts 28 days after treatment (Table 5). Although both formulations provided good control of ticks, the imidacloprid–permethrin–treated dogs had fewer ticks of either species at the 3-hour post-exposure counts compared with fipronil–(*S*)-methoprene–treated dogs on days 3 through 21. These lower tick counts may be may be the result of fewer ticks initially attaching to the dogs because so many ticks were repelled by the imidacloprid–permethrin formulation.

In this study design, we were unable to account for every tick placed on the dogs. Most tick loss likely occurred either from ticks' being killed by the acaricides and dropping off in the runs or from ingestion by dogs during grooming.

CONCLUSION

This study demonstrates that the imidacloprid–permethrin topical formulation can produce a repellent-like effect (repellency) against *R. sanguineus* and *D. variabilis* exposed to treated dogs. In addition, the imidacloprid–permethrin topical formulation also produced significant morality of repelled ticks. Both the imidacloprid–permethrin and fipronil–(*S*)-methoprene formulations provided good overall control of *R. sanguineus* and *D. variabilis* on dogs during the study period.

ACKNOWLEDGMENTS

The authors thank Terry Settje, BA, Biostatistician, Pharmaceutical R&D, Bayer Animal Health, Shawnee Mission, Kansas, for statistical evaluation of the data. Thanks also to the numerous students who provided invaluable assistance in conducting tick counts.

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