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 *Ixodes scapularis* and *Amblyomma americanum* Ticks on Dogs*

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CLINICAL RELEVANCE

This study evaluated the effectiveness of topical two 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 *Ixodes scapularis* and *Amblyomma americanum* 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 against *I. scapularis* for up to 3 weeks after treatment, and both formulations provided good overall control of *I. scapularis* and *A. americanum* during the study period.

INTRODUCTION

Two of the most common ticks parasitizing dogs in the eastern half of the United States are *Amblyomma americanum* (lone star tick) and *Ixodes scapularis* (black-legged tick).[1] Both tick species are vectors of several important bacterial pathogens causing vectorborne disease in dogs. *I. scapularis* is the vector of *Borrelia burgdorferi*

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and *Anaplasma phagocytophilum*, whereas *A. americanum* is a vector of *Ehrlichia chaffensis*, *Ehrlichia ewingii,* and *Francisella tularensis.*[1]

I. scapularis is widely distributed in the eastern and central United States and has been reported in at least 35 states.[2] Distribution of this tick ranges from Florida north to Maine, west to Minnesota, and then south to central Texas. The range of *A. americanum* has been expanding; while it was once considered primarily a southern tick, focal populations now exist in many northern states, including Connecticut, Maine, Massachusetts, Michigan, New Jersey, and New York.[1,3] The distribution of both of these tick species is linked to the distribution and abundance of their primary reproductive host, the white-tailed deer.[1,3,4]

Control of* I. scapularis *and* A. americanum *on dogs is important in reducing a dog's chance of acquiring tick-transmitted disease-causing organisms.

Control of *I. scapularis* and *A. americanum* on dogs is important not only to prevent irritating tick infestation but also to reduce a dog's chance of acquiring tick-transmitted disease-causing organisms. Several studies have been conducted to evaluate the ability of amitraz, fipronil, or an imidacloprid–permethrin combination to prevent the transmission of *B. burgdorferi* and/or *A. phagocytophilum* from *I. scapularis* to dogs.[5–10] However, few articles have been published evaluating the efficacy of acaricides to repel, prevent attachment of, or kill *I. scapularis.*[8] In addition, there are very few published studies evaluating the efficacy of any acaricide against *A. americanum* infesting dogs.[11]

To prevent the transmission of tick-vectored disease-causing organisms, ticks must be killed rapidly once they acquire a host, either before or shortly after attachment, or hosts must be rendered unattractive to ticks, such as by applying a compound that has "repellent" characteristics. The definition of *repellency* is not always clear[12] and has been defined as a substance that causes orientation away from its source.[13] The mode of action of repellents is also unclear and is likely not the same for all compounds or parasites. It has been proposed that the classic insect repellent DEET "repels" arthropods by blocking their ability to perceive certain host chemicals that otherwise would serve as an attractant.[14] However, DEET may not be a repellent but rather an "inhibitor of attraction."[14] Another compound that has reported repellent-like activity is amitraz. Here, the "repellent-like" effect or demonstrated prevention of attachment and feeding is likely directly related to the rapid neurotoxicity of amitraz.[15,16] Permethrin can produce rapid neurotoxicity and has repellent-like activity in insects and arachnids[17,18]; it is used as a "repellent" in numerous dog, livestock, and human formulations to prevent bites from blood-feeding arthropods.[18] The rapid neurotoxicity results in quick death (knockdown) or causes irritability (repellent-like effect) to the arthropod, resulting in prevention of attachment and feeding.[11,18–20]

This study 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 repel, prevent attachment of, and kill adult *I. scapularis* and *A. americanum.*

MATERIALS AND METHODS

Housing

Twelve purpose-bred beagles (6–24 months of age; six males and six females) were distributed by pairs based on cohabitability, gender, and weight into indoor–outdoor pens and runs. 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. The indoor pen area was 122 × 244 cm (4 × 8 ft), and pens were separated by stainless-steel solid sides. While they were infested with ticks, the dogs were confined to the indoor pens and the doors in front of the pens were lined with a strip of petroleum jelly and double-sided sticky tape to prevent tick movement out of and between pens. Between tick infestation challenge periods, dogs also had access to outdoor runs. The outdoor run area was 120 × 304 cm (4 × 10 ft), and the runs were divided by chain-linked fencing covered by fiberglass siding 132 cm (52 inches) high. The outdoor area was completely covered by a roof.

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 observations. In addition, daily observations were made to ensure that 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, 10.06 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, 10.18 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, 10.52 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 *A. americanum* and 25 adult *I. scapularis* on posttreatment days 3, 7, 14, 21, and 28. Ticks were purchased from Oklahoma State University and shipped by overnight courier to Kansas State University, where they were maintained in the laboratory at room temperature and 92% to 94% relative humidity for 1 to 3 days before being placed on the dogs. Because of a counting error, 30 adult *A. americanum* were placed on the dogs along with the standard 25 *I. scapularis* on posttreatment day 28.

For tick infestations, dogs were placed on their side in a stainless-steel tub. The lid of the tick shipping container, containing the designated number of ticks for each dog, was removed and a person exhaled slowly over the top of the container to stimulate tick activity. Ticks were then immediately deposited alongside each dog. On treated and untreated control dogs, the tick infestations were typical in that not all ticks moved immediately into the haircoat. Dogs

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 *Amblyomma americanum* and *Ixodes scapularis* Exposed to Treated Dogs for up to 10 Minutes

	DAYS AFTER TREATMENT					
	Day 3			*Day 7*		
TREATMENT GROUP[a]	*Mean No. of*[b]	*% Repelled*[c]	*% Difference vs Control*	*Mean No. of*[b]	*% Repelled*[c]	*% Difference vs Control*
A. americanum						
Controls	12.1	49.0[d]	—	7.3	30.0[d]	—
Imidacloprid–permethrin	21.3	86.0[e]	37.0	16.8	68.0[f]	38.0
Fipronil–(*S*)-methoprene	16.8	68.0[f]	19.0	6.3	26.0[d]	–4.0
I. scapularis						
Controls	0.0	0.0[d]	—	0.0	0.0[d]	—
Imidacloprid–permethrin	17.8	72.0[f]	72.0	12.8	52.0[f]	52.0
Fipronil–(*S*)-methoprene	0.6	3.0[d]	3.0	0.0	0.0[d]	0.0

[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 *I. scapularis* on days 3, 7, 14, 21, and 28; with 25 adult *A. americanum* on days 3, 7, 14, and 21; and with 30 adult *A. americanum* on day 28.

were held in the tub and exposed to ticks for 10 minutes. Some of the ticks dropped or crawled off the dogs. As this occurred, the ticks were picked up and placed back on the dogs during the initial 10-minute infestation period. Following the tick exposure period, dogs were returned to the indoor runs. At least two people handled each dog during tick infestations.

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

Unattached ticks found in the tub after the 10-minute exposure were collected, sorted by species, and placed into glass vials. Perforated caps were placed over these 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 room temperature and 92% to 94% relative humidity. The effect of the short-term (10-minute) acaricide exposure on the unattached ticks was evaluated by assessing the viability of the ticks immediately as they were collected (i.e., 10 minutes after initial exposure) and then 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 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.

DAYS AFTER TREATMENT								
Day 14			Day 21			Day 28		
Mean No. of[b]	*% Repelled*[c]	*% Difference vs Control*	*Mean No. of*[b]	*% Repelled*[c]	*% Difference vs Control*	*Mean No. of*[b]	*% Repelled*[c]	*% Difference vs Control*
11.6	47.0[d]	—	12.2	49.0[d]	—	25.5	85.0[d]	—
17.7	73.0[d]	26.0	17.7	71.0[f]	22.0	23.4	78.3[d]	–6.7
16.7	71.0[d]	24.0	13.6	56.0[d]	7.0	23.4	78.3[d]	–6.7
0.2	1.0[d]	—	0.2	1.0[d]	—	0.4	2.0[d]	—
8.4	38.0[e]	37.0	3.1	13.0[f]	12.0	1.1	5.0[d]	3.0
1.2	6.0[d]	5.0	1.2	7.0[d,f]	6.0	0.7	4.0[d]	2.0

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

It was often necessary to remove ticks from glass vials and place them on the palm of a gloved hand or into 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 visibly inspected for attached ticks. Examiners wore personal protective gear and were blinded as to treatment group allocation. The examination procedure involved running a flea comb or fingers against the lay of the hair so the hair could be parted to visually inspect for ticks. The examination commenced on the dog's head, proceeded to the back, each side, abdomen, chest, front legs and feet (with careful inspection between the toes), and then the hind legs and feet. Each dog was examined for 30 minutes. Any tick observed alive and attached was counted and identified by species, and the location where it was found was recorded. 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, "treatment" effects were tested for each time period (for the individual tick species). If no interaction was noted, a "main treatment" effect was used to show group differences.

TABLE 2. Viability Assessment of Unattached or Repelled Adult *Amblyomma americanum* 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 Groups[a]	*Mean No. of Ticks*[b]	*% Dead or Moribund*[c]			
		10 Min[d]	*3 Hr*	*24 Hr*	*48 Hr*
Posttreatment day 3					
Controls	12.1	8.6[e]	8.6[e]	8.6[e]	8.6[e]
Imidacloprid–permethrin	21.3	100.0[f]	100.0[f]	100.0[g]	100.0[g]
Fipronil–(*S*)-methoprene	16.8	0.0[e]	17.6[e]	53.0[f]	72.9[f]
Posttreatment day 7					
Controls	7.3	0.0[e]	5.0[e]	10.0[e]	13.1[e]
Imidacloprid–permethrin	16.8	42.5[f]	100.0[f]	100.0[f]	100.0[f]
Fipronil–(*S*)-methoprene	6.3	0.0[e]	0.0[e]	7.1[e]	14.3[e]
Posttreatment day 14					
Controls	11.6	0.0[e]	0.0[e]	0.0[e]	1.8[e]
Imidacloprid–permethrin	17.7	29.8[e]	83.3[f]	94.6[f]	96.3[f]
Fipronil–(*S*)-methoprene	16.7	0.0[e]	3.3[e]	4.3[e]	4.3[e]
Posttreatment day 21					
Controls	12.2	2.1[e]	5.9[e]	8.0[f]	4.2[e,f]
Imidacloprid–permethrin	17.7	0.0[e]	67.6[f]	8.3[f]	8.3[f]
Fipronil–(*S*)-methoprene	13.6	0.0[e]	0.0[e]	0.0[e]	0.0[e]
Posttreatment day 28					
Controls	25.5	0.0[e]	0.0[e]	0.0[e]	0.0[e]
Imidacloprid–permethrin	23.4	0.0[e]	0.0[e]	6.6[e]	4.1[e]
Fipronil–(*S*)-methoprene	23.4	1.2[e]	1.2[e]	4.3[e]	5.4[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 *A. americanum* on days 3, 7, 14, and 21 and with 30 adult *A. americanum* on day 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,g]For the percent dead or moribund data, different column letters indicate a statistically significant difference ($P < .05$).

Least squares means were calculated for both "treatment" and "treatment × day," and the 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 was

TABLE 3. Viability Assessment of Unattached or Repelled Adult *Ixodes scapularis* 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	0.0	NA	NA	NA	NA
Imidacloprid–permethrin	17.8	96.4	100.0	100.0	100.0
Fipronil–(*S*)-methoprene	0.6	50.0 (*n* = 2)	50.0 (*n* = 2)	100.0 (*n* = 2)	100.0 (*n* = 2)
Posttreatment day 7					
Controls	0.0	NA	NA	NA	NA
Imidacloprid–permethrin	12.8	92.4	100.0	100.0	100.0
Fipronil–(*S*)-methoprene	0.0	NA	NA	NA	NA
Posttreatment day 14					
Controls	0.2	0.0 (*n* = 1)	0.0 (*n* = 1)	100.0 (*n* = 1)	0.0 (*n* = 1)
Imidacloprid–permethrin	8.4	95.8	100.0	100.0	100.0
Fipronil–(*S*)-methoprene	1.2	66.7 (*n* = 3)	100.0 (*n* = 3)	100.0 (*n* = 3)	100.0 (*n* = 3)
Posttreatment day 21					
Controls	0.2	0.0 (*n* = 1)	0.0 (*n* = 1)	0.0 (*n* = 1)	100.0 (*n* = 1)
Imidacloprid–permethrin	3.1	100.0	100.0	100.0	100.0
Fipronil–(*S*)-methoprene	1.2	100.0 (*n* = 3)	66.7 (*n* = 3)	100.0 (*n* = 3)	100.0 (*n* = 3)
Posttreatment day 28					
Controls	0.4	100.0 (*n* = 2)	100.0 (*n* = 2)	100.0 (*n* = 2)	100.0 (*n* = 2)
Imidacloprid–permethrin	1.1	50.0 (*n* = 3)	100.0 (*n* = 3)	100.0 (*n* = 3)	100.0 (*n* = 3)
Fipronil–(*S*)-methoprene	0.7	100.0 (*n* = 2)	100.0 (*n* = 2)	100.0 (*n* = 2)	100.0 (*n* = 2)

[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 *I. scapularis* 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). Statistical analyses of the % dead or moribund data exhibited no "treatment × day" interaction, with a significant overall treatment effect. However, pairwise comparisons could be performed (nonestimated least square means). Statistical results are inconclusive.

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

NA = not able to calculate.

used (using day 3 total tick counts from tub as a covariate and selecting best covariance structure [smallest Akaike information criterion]). 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)

A. americanum

The rapid irritation or repellent-like effect of the imidacloprid–permethrin topical spot-on formulation against *A. americanum* was evident on days 3, 7, and 21 after treatment (Table 1). More *A. americanum* were displaced in the fipronil–(*S*)-methoprene group than in the control group on day 3. Seven days after treatment, repelled geometric means of 16.8, 6.3, and 7.3 *A. americanum* were recovered in the tub 10 minutes after ticks were exposed to dogs in Group 1 (imidacloprid–permethrin), Group 2 [fipronil–(*S*)-methoprene], and Group 3 (control), respectively (Table 1).

I. scapularis

The repellency effect of the imidacloprid–permethrin topical spot-on formulation against *I. scapularis* was evident on posttreatment days 3, 7, 14, and 21 (Table 1). The fipronil–(*S*)-methoprene formulation did not appear to have any significant repellency effect against *I. scapularis* at any posttreatment time point (Table 1).

Viability of Unattached (Repelled) Ticks

A. americanum

The imidacloprid–permethrin formulation significantly affected the viability of unattached (repelled) *A. americanum* on posttreatment days 3, 7, and 14. As shown in Table 2, 100% of the repelled *A. americanum* were dead within 10 minutes on posttreatment day 3 and within 3 hours on posttreatment day 7. *A. americanum* repelled from the fipronil–(*S*)-methoprene–treated dogs had significantly greater mortality versus controls 3 days after treatment. At day 14 after treatment, 96.3% and 4.3% of *A. americanum* repelled from Group 1 (imidacloprid–permethrin) and Group 2 [fipronil–(*S*)-methoprene] dogs, respectively, were dead 48 hours after exposure (Table 2). Of particular interest was the viability assessment of *A. americanum* repelled from imidacloprid–permethrin–treated dogs 3 weeks after initial treatment: Although 67.6% of the repelled ticks were classified as either dead or moribund 3 hours after exposure (Table 2), most of those ticks had revived and were classified as live at the 24-hour postexposure assessment. Neither product produced significant mortality of repelled *A. americanum* 28 days after treatment.

I. scapularis

Unattached *I. scapularis* were remarkably susceptible to both products (Table 3). None of the repelled *I. scapularis* recovered from tubs after exposure to the imidacloprid–permethrin–treated dogs were alive 3 hours after exposure from days 3 to 28 (Table 3). Even though very few *I. scapularis* were repelled from the fipronil–(*S*)-methoprene–treated dogs, those that were recovered in the tub always died within 24 hours (Tables 1 and 3).

Evaluation of Tick Kill

A. americanum

Day 3 geometric mean on-dog *A. americanum* counts 3 hours after exposure were significantly reduced on treated dogs (Groups 1 and 2) versus control animals (Group 3) (Table 4). Seven days after treatment, only the geometric mean *A. americanum* counts on dogs treated with imidacloprid–permethrin were significantly reduced compared with counts on control dogs. On day 7, the geometric mean on-dog *A. americanum* counts 3 hours after exposure were 10.7, 2.0, and 10.7 for control dogs, dogs treated with imidacloprid–permethrin, and dogs treated with fipronil–(*S*)-methoprene, respectively (Table 4). On days 14, 21, and 28 after treatment, the numbers of *A. americanum* at the

TABLE 4. Geometric Mean Tick Counts[a] and Percent Control[b] of Adult *Amblyomma americanum* 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	7.6[d]	—	10.7[d]	—	9.0[d]	—	9.0[d]	—	2.9[d]	—
Imidacloprid–permethrin	0.9[e]	88.6	2.0[e]	81.1	1.9[e]	78.9	3.7[e]	58.9	1.1[e]	63.1
Fipronil–(*S*)-methoprene	2.4[e]	67.8	10.7[d]	0.5	2.3[d,e]	74.3	4.8[d]	46.9	1.9[d,e]	33.4
24 hr after infestation										
Controls	6.3[d]	—	10.1[d]	—	8.9[d]	—	10.1[d]	—	2.4[d]	—
Imidacloprid–permethrin	0.0[e]	100.0	0.2[f]	98.1	0.6[e]	93.6	0.8[e]	92.3	0.9[e]	63.6
Fipronil–(*S*)-methoprene	0.0[e]	100.0	1.3[d]	86.7	1.6[e]	81.6	4.9[d]	51.7	0.9[e]	63.6
48 hr after infestation										
Controls	NA	—	10.5[d]	—	7.4[d]	—	9.8[d]	—	1.9[d]	—
Imidacloprid–permethrin	NA	NA	0.0[e]	100.0	0.3[e]	95.7	0.3[e]	96.8	0.0[f]	100.0
Fipronil–(*S*)-methoprene	NA	NA	0.0[e]	100.0	0.0[e]	100.0	0.8[e]	92.1	0.7[e]	64.4

[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 *A. americanum* on days 3, 7, 14, and 21 and with 30 adult *A. americanum* on day 28.
[d,e,f]For the actual tick count on each dog (log transformed), different column letters indicate a statistically significant difference ($P < .05$).
NA = not able to calculate.

3-hour postexposure counts were similar for dogs treated with fipronil–(*S*)-methoprene or imidacloprid–permethrin.

The imidacloprid–permethrin–treated dogs had significant reductions in geometric mean *A. americanum* counts 24 hours after exposure at every time point from day 3 to day 28. The dogs treated with fipronil–(*S*)-methoprene had significant reductions in geometric mean *A. americanum* counts at 24 hours after exposure

TABLE 5. Geometric Mean Ticks Counts[a] and Percent Control[b] of Adult *Ixodes scapularis* 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	10.2[d]	—	15.4[d]	—	13.5[d]	—	11.8[d]	—	19.8[d]	—
Imidacloprid–permethrin	0.7[e]	92.9	4.2[f]	72.5	3.4[d]	74.6	13.8[d]	–16.4	10.5[d]	46.7
Fipronil–(*S*)-methoprene	1.1[e]	89.1	7.0[e]	54.8	11.1[d]	17.2	11.4[d]	3.9	13.5[d]	31.6
24 hr after infestation										
Controls	4.8[d]	—	7.7[d]	—	9.2[d]	—	9.1[d]	—	13.4[d]	—
Imidacloprid–permethrin	0.0[e]	100.0	0.0[e]	100.0	0.0[e]	100.0	0.0[f]	100.0	0.7[f]	94.9
Fipronil–(*S*)-methoprene	0.0[e]	100.0	0.2[e]	97.5	0.6[e]	93.9	2.0[e]	78.2	1.9[e]	85.7
48 hr after infestation										
Controls	NA	—	5.0[d]	—	7.7[d]	—	7.9[d]	—	12.0[d]	—
Imidacloprid–permethrin	NA	NA	0.0[e]	100.0	0.0[e]	100.0	0.0[e]	100.0	0.4[e]	96.5
Fipronil–(*S*)-methoprene	NA	NA	0.0[e]	100.0	0.0[e]	100.0	0.0[e]	100.0	0.2[e]	98.4

[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 *I. scapularis* 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).
NA = not able to calculate.

on days 3, 7, 14, and 28. On days 7 and 21, the counts on the imidacloprid–permethrin–treated dogs were significantly lower than on the fipronil–(*S*)-methoprene–treated dogs.

The 48-hour efficacy of the imidacloprid–permethrin formulation on posttreatment days 7, 14, 21, and 28 was 100%, 95.7%, 96.8%, and 100%, respectively (Table 4). The 48-hour efficacy of the fipronil–(*S*)-methoprene formulation on posttreatment days 7, 14, 21, and 28 was 100%, 100%, 92.1%, and 64.4%, respectively (Table 4). However, because the geo-

metric mean *A. americanum* infestation rate on control dogs had declined to only 1.9 ticks/dog on day 28, caution needs to be taken before making any comparative efficacy claims at this point. The 48-hour geometric mean *A. americanum* counts on day 28 was 0.0 and 0.7 for dogs treated with imidacloprid–permethrin and fipronil–(*S*)-methoprene, respectively.

I. scapularis

Day 3 geometric mean on-animal *I. scapularis* counts 3 hours after exposure to control dogs and dogs treated with imidacloprid–permethrin or fipronil–(*S*)-methoprene were 10.2, 0.7, and 1.1, respectively (Table 5). The 3-hour postexposure *I. scapularis* counts were statistically similar for both formulations on days 3, 14, 21, and 28. On day 7, the geometric mean on-animal *I. scapularis* counts 3 hours after exposure on the imidacloprid–permethrin–treated dogs were lower than those on the fipronil–(*S*)-methoprene–treated dogs.

The 24-hour postexposure efficacy of both products against *I. scapularis* was similar on days 3, 7, and 14. On posttreatment days 21 and 28, however, the geometric mean counts of *I. scapularis* on dogs treated with imidacloprid–permethrin were significantly reduced compared with the counts on dogs treated with fipronil–(*S*)-methoprene (Table 5). The 24-hour postexposure efficacy of the fipronil–(*S*)-methoprene formulation on days 21 and 28 was 78.2% and 85.7%, respectively, whereas the 24-hour postexposure efficacy of the imidacloprid–permethrin formulation on days 21 and 28 was 100.0% and 94.9%, respectively (Table 5).

The 48-hour postexposure efficacy of both products against *I. scapularis* was similar from day 7 to day 28 after treatment. The 48-hour postexposure efficacy of the fipronil–(*S*)-methoprene formulation against *I. scapularis* was 100% on days 7, 14, and 21 and decreased to 98.4% on day 28. Similarly, the 48-hour postexposure efficacy of the imidacloprid–permethrin formulation against *I. scapularis* was 100% on days 7, 14, and 21 and decreased to 96.5% on day 28 (Table 5).

DISCUSSION

The imidacloprid–permethrin treatment produced a significant repellent-like activity against *I. scapularis* compared with controls for up to 21 days after treatment. The percent of *I. scapularis* not attaching (repelled) was 72% on day 3 and declined to 13% on day 21 (Table 1). The fipronil–(*S*)-methoprene formulation did not produce any repellent-like effect against *I. scapularis*. The repellent effect of the imidacloprid–permethrin formulation against *A. americanum* was not as evident in this study. Statistical differences in numbers of *A. americanum* not attaching on Group 1 dogs versus control animals (Group 3) were observed on days 3, 7, and 21, but the differences were not as pronounced as with *I. scapularis*. The reasons for these differences in repellent-like effect between the two tick species are unknown. Unless similar patterns of repellency are observed for these species from other sources (e.g., colonized strains, field-collected isolates), it is difficult to determine whether this is a true species difference. The repellent-like effect 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.[18–20]

Some of the reduced differences in repellency observed between *I. scapularis* and *A. americanum* on imidacloprid–permethrin–treated dogs can likely be attributed to the low overall attachment rate of the ticks in this trial (Tables 4 and 5). Whereas the geometric mean counts of *I. scapularis* attached to control dogs at the 3-hour time point ranged from 10.2 to 19.8, *A. americanum* attachment rates on control

dogs ranged from a high of 10.7 to a low of 2.9. Tick repellency data on day 3 illustrates these differences (Table 1). After ticks were exposed to control dogs for 10 minutes and dogs were removed from the tubs, no *I. scapularis* but almost half (49%) of the *A. americanum* originally placed in the tubs were found. It is unknown if these attachment differences are related to the tick species in general or are particular to *A. americanum* from the Oklahoma State University colony. The reason for the even more marked reduction in *A. americanum* attachment on day 28 is unknown. It may be related to the recent molting from nymphs; numerous molting cuticles were evident in the shipping vials.

As previously mentioned, the lethal effect of short-term (10-minute) exposure of unattached *A. americanum* to dogs treated with the imidacloprid–permethrin formulation had diminished greatly by 21 days after treatment. Of particular interest is the apparent "revival"

***The imidacloprid–permethrin formulation significantly affected the viability of unattached* A. americanum.**

All the repelled or unattached *A. americanum* from the imidacloprid–permethrin–treated dogs on posttreatment days 3 and 7 died within 10 minutes and 3 hours after exposure, respectively. On day 14, 83.3% of the ticks were dead within 3 hours and 96.3% by 48 hours. Apparently, the concentration of permethrin in the dog's haircoat during the first 2 weeks after treatment was sufficient to kill almost all the unattached *A. americanum* after only 10 minutes' exposure to treated dogs. The fipronil–(*S*)-methoprene formulation had a significantly lethal effect on unattached *A. americanum* only at 3 days after treatment.

The decreasing concentration and corresponding lethal activity of the imidacloprid–permethrin formulation from application through day 28 were displayed by the mortality data of unattached *A. americanum* (Table 2). On posttreatment days 3, 7, 14, and 21, *A. americanum* mortality was 100%, 42.5%, 29.8%, and 0.0%, respectively, within 10 minutes after exposure. Similarly, the high attachment rates of *I. scapularis* on control dogs allowed for a reasonable assessment of decreasing repellent-like activity of the imidacloprid–permethrin formulation throughout the month after treatment (Table 1). The percentage of *I. scapularis* repelled or not attached on posttreatment days 3, 7, 14, 21, and 28 were 72%, 52%, 38%, 13%, and 5%, respectively.

of *A. americanum:* Although 67.6% of unattached ticks were recorded as dead or moribund 3 hours after exposure, only 8.3% of the ticks were found to be dead or moribund by 24 hours. This is likely evidence that the concentration of the acaricide (permethrin) had diminished sufficiently by 3 weeks after treatment to permit "knockdown," but the ticks were able to detoxify the reduced levels of residual insecticide rather than being killed. Permethrin works as a contact insecticide and acaricide by quickly paralyzing the nervous system of arthropods, producing a quick knockdown effect on treated arthropods. The rapid neurotoxicity or knockdown can occur within several minutes. However, knockdown is different from mortality. It is not uncommon for arthropods to recover within several hours after being knocked down.[21,22]

Because almost all of the *I. scapularis* initially attached to control dogs, it was difficult to

make any statistical evaluations of the effects of the two formulations on the viability of repelled or unattached *I. scapularis* (Table 3). All unattached or repelled *I. scapularis* exposed to dogs treated with either formulation died within 24 hours.

The imidacloprid–permethrin and fipronil–(*S*)-methoprene formulations provided good acaricidal efficacy against *A. americanum*, with 96.8% and 92.1% control achieved, respectively, by the 48-hour tick counts 21 days after treatment (Table 4). On day 28, however, 100% of the ticks were killed within 48 hours on the imidacloprid–permethrin–treated dogs whereas only 64.4% were killed on the fipronil–(*S*)-methoprene–treated dogs. Although these differences were statistically significant, the low attachment rates on controls render the accuracy of the differences suspect.

The imidacloprid–permethrin and fipronil–(*S*)-methoprene formulations provided good efficacy against *I. scapularis*, with 96.5% and 98.4% control achieved, respectively, by the 48-hour tick counts 28 days after treatment (Table 5). While both formulations provided control of *I. scapularis* on dogs, the imidacloprid–permethrin formulation provided a faster kill on days 21 and 28 after treatment. The imidacloprid–permethrin formulation provided 100% and 94.9% efficacy against *I. scapularis* 24 hours after exposure on posttreatment days 21 and 28, respectively (Table 5), whereas the 24-hour postexposure efficacy of the fipronil–(*S*)-methoprene formulation against *I. scapularis* was 78.2% and 85.7% on posttreatment days 21 and 28, respectively.

CONCLUSION

This study further demonstrates that the imidacloprid–permethrin formulation can produce a repellent-like effect against *I. scapularis* exposed to treated dogs. It was also observed that there were differences in the numbers of ticks repelled and the viability of repelled ticks between the *A. americanum* and *I. scapularis* used in this study. While the repellent-like effect was pronounced during the first 3 weeks after treatment, this activity diminished over time. Both the imidacloprid–permethrin and fipronil–(*S*)-methoprene formulations provided good overall control of *I. scapularis* and *A. americanum* on dogs during the study period.

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