Efficacy of Imidacloprid (8.8% w/w) Plus Permethrin (44% w/w) Spot-On Topical Solution against *Amblyomma americanum* Infesting Dogs Using a Natural Tick Exposure Model*

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CLINICAL RELEVANCE

This study evaluated the efficacy of an imidacloprid (8.8% w/w) + permethrin (44% w/w) spot-on topical solution (K9 Advantix, Bayer Animal Health) against *Amblyomma americanum* using a natural field exposure model. Sixteen beagles were divided into two groups of eight dogs each. One group of dogs was treated with K9 Advantix, and the other group served as untreated controls. On day –1 and at 3, 7, 14, 21, and 28 days after treatment, the dogs were walked for 80 minutes in an *A. americanum*–infested habitat at the Konza Prairie Biological Station in northeastern Kansas. Postexposure tick counts (efficacy evaluations) were conducted on each dog at 3 and 48 hours after exposure. At 3 days after treatment, the efficacy of K9 Advantix within 3 hours of natural tick exposure was 88.0% and declined slowly during the study. The 48-hour postexposure efficacy remained above 93.5% throughout the study.

INTRODUCTION

The tick species most commonly infesting dogs in North America are *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *Dermacentor andersoni* (Rocky Mountain wood tick), *Ixodes pacificus* (western black-legged tick), *Ixodes scapularis* (black-legged tick), and *Rhipicephalus sanguineus* (brown dog tick).[1] In eastern Kansas, *A. americanum* and *D. variabilis* are the most common tick species encountered.[2] *I. scapularis, R. sanguineus,* and *A. maculatum* are less commonly encountered.[2] In the past 4 years, during the spring and early summer, *A. americanum* has become the tick species most commonly submit-

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ted to Kansas State University (KSU) College of Veterinary Medicine for identification.[3]

A. americanum was once considered to dwell primarily in the southeastern and Gulf Coast regions; however, its range is rapidly expanding, and populations now occur in many northern states, including Connecticut, Maine, Massachusetts, Michigan, New Jersey, and New York.[4] Several factors have contributed to the increased range and frequency of the identification of *A. americanum*, such as its increasing habitat and wide host range that encompasses coyotes, deer, dogs, small mammals, birds (including turkeys), and humans.[4] White-tailed deer, the preeminent host for all life stages of *A. americanum*, and the wild turkey, which supports immature stages, are considered the hosts primarily responsible for this tick's expansion.[4] *A. americanum* is rapidly becoming one of the most common ticks infesting dogs across the south, midwest, southern plains, east, and southeastern United States. The lone star tick is a major nuisance parasite and is also a vector of *Ehrlichia chaffeensis* (human monocytic ehrlichiosis), *Ehrlichia ewingii*, and *Borrelia lonestari,* which causes a Lyme disease–like illness called *southern tick–associated rash illness.*[1,5] It has also been implicated in the transmission of *Francisella tularensis* (tularemia).[1]

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Efficacy evaluations of topically applied acaricides against this tick species in its natural habitat will provide beneficial "real world" information to veterinarians and pet owners. Previously, evaluations of topical acaricides used to kill ticks on dogs were either laboratory studies using laboratory-colonized ticks or in-clinic evaluations of client-owned animals with natural exposures. These types of studies, while valuable, do not always provide "real world" information on the performance of topically applied products. This report describes the evaluation of an imidacloprid (8.8% w/w) plus permethrin (44% w/w) spot-on topical solution (K9 Advantix, Bayer Animal Heath) to prevent infestation and kill *A. americanum* infesting dogs in a natural tick exposure model.

MATERIALS AND METHODS

Dogs

Sixteen purpose-bred beagles (male and female; 6–24 months of age) were housed in indoor–outdoor pens and runs (2 dogs/pen or run). The indoor pen area was 122 × 244 cm (4 × 8 ft), and pens were separated by stainless-steel solid sides. After being exposed to the tick-infested habitat, 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 ticks from leaving or moving between pens. The outdoor run area was 120 × 304 cm (4 × 10 ft), and pens were divided by chain-link fencing covered by fiberglass siding 132 cm (52 inches) high. The outdoor area was completely covered by a roof.

Housing was in full compliance with all USDA Animal and Plant Health Inspection Service requirements. Each dog was identified by an individual alphanumeric ear tattoo. 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 before treatment; two dogs were allotted to each pen based on cohabitability, gender, and weight. After tick exposure ceased, dogs were treated with doxycycline (10 mg/kg once daily for 10 days) as a preventive for possible exposure to tick-transmitted diseases.

Over a 14-day period, dogs were trained to walk on a leash using the Gentle Leader Headcollar system (Premier Pet Products, Midlothian, VA). This was done to facilitate handling and walking of the dogs in the naturally tick-infested habitat.

Pretreatment Tick Counts and Group Allocations

On day –1, dogs were fitted with Gentle Leader Headcollars and placed in individual plastic dog crates, and the crates were loaded on a trailer. Dog handlers sprayed their clothing and exposed skin with Deep Woods Off (23.8% DEET; S. C. Johnson). Dogs were then transported (approximately 30-minute ride) in the trailer to the Konza Prairie Biological Station (www.konza.ksu.edu) in the Flint Hills region of northeastern Kansas (39°05′ N, 96°35′ W), an area where numerous white-tailed deer and wild turkeys are frequently observed. Several areas of the Biological Station had been evaluated by "dragging" for tick collection with a white flannel cloth before the study. A site in Section AL was chosen based on accessibility and the presence of numerous naturally occurring *A. americanum* adults

Figure 1. *Questing female* Amblyomma americanum *at the Konza Prairie Biologic Station.*

(Figure 1) and nymphs and a smaller number of *D. variabilis* adults.

After arriving at the site, personnel dressed in full-body disposable coveralls, shoe covers, and latex gloves, and the gloves and boots were sealed at the ankles and wrists with duct tape. Dogs were then let out of the crates and placed on 6-foot leads. Each handler walked two dogs. For pretreatment tick exposures, dogs were kept in pairs based on pen assignment.

Eight tick exposure grid zones with an area of 465 m^2 (30.5 × 15.25 m [5,000 sq ft; 100 × 50 ft]) were staked out on the Konza tick exposure site. Grids were adjoining with four grids running west to east and four grids immediately adjacent to the south running east to west (Figure 2). Four grids (zones 5, 6, 7, and 8) were located in tall grass prairie habitat (big bluestem, Indian grass, little bluestem, and switch grass); the other four grids (zones 1, 2,

1	**2**	**3**	**4**
8	**7**	**6**	**5**

Figure 2. *Tick exposure grid layout; each grid is 30.5 × 15.25 m (100 × 50 ft).*

Figure 3. *Konza dog walk site.*

3, and 4) were located within a gallery forest containing predominately bur and chinquapin oaks, as well as green ash, hackberry, elm, and black walnut trees (Figure 3).

A handler and two dogs were randomly allocated to a designated grid zone. Dogs were then walked in the grid area for 10 minutes. Handlers were instructed to walk the dogs throughout a grid zone so that the entire zone was covered. After 10 minutes, dogs and handler moved to the next grid zone in a clockwise fashion until the dogs had been walked in each of the eight grids for a total exposure time of 80 minutes. Dogs were then walked back to the trailer, where they were given access to water before being returned to the crates. Once dogs were crated, handlers removed their disposable coveralls. Dog crates were loaded on the trailer for transport back to the KSU animal housing facility.

On-Animal Tick Infestation Evaluations

Three hours after being exposed to the tick-infested grid, dogs were examined for presence of attached ticks by placing each dog on a stainless-steel table for visual examination. Each dog was held by one person while a second observed and counted ticks. Examiners wore gowns and latex gloves while examining and holding the dogs. The examination procedure was conducted by running a flea comb or fingers against the lay of the hair so the hair could be parted to visibly inspect for ticks. Examination commenced on the head, proceeded to the back, each side, abdomen, chest, front legs and feet (to include inspection between the toes), and then the hind legs and feet. Each dog was examined for 30 minutes. Any tick observed attached to the dogs was counted, identified by species, and mapped on individual records. After recording the pretreatment tick counts, all ticks were manually removed and the dogs were returned to their respective runs.

Treatments and Posttreatment Tick Exposures

Because dogs had been previously placed into pairs based on weight, gender, and cohabitability, dogs were allocated to treatment groups based on the pretreatment pen pair tick counts. Dogs were treated on day 0.

- **Treatment group 1—**Eight dogs (four males and four females; mean weight, 10.39 kg) served as untreated controls.
- **Treatment group 2—**Eight dogs (four males and four females; mean weight, 11.22 kg) were treated with K9 Advantix in applicator pipettes according to label dosing recommendations based on body weight: The hair along the dorsal midline was parted, and the solution was evenly distributed on the visible skin to four locations from the top of the shoulder to the base of the tail.

No abnormal clinical signs were noted in any treated dog at any time during the study.

On posttreatment day 3, dogs were fitted with Gentle Leader Headcollars, placed in individual plastic dog crates, taken to the Konza Prairie Biological Station, and exposed to ticks as previously described. Dogs were kept in

TABLE 1. Pre- and Posttreatment Geometric Mean Live Adult Flea Counts[a]

	Study Day[c]					
Group[b]	*–1*	*3*	*7*	*14*	*21*	*28*
2–3 hr after exposure						
Controls	34.7	27.6	34.6	17.7	38.9	12.1
Imidacloprid–permethrin	34.0	3.3[d]	7.8[d]	5.6[d]	13.9[d]	3.8[d]
Efficacy (%)		88.0	77.5	68.1	64.3	68.5
48 hr after exposure						
Controls		32.2	37.6	18.1	38.1	12.8
Imidacloprid–permethrin		2.1[d]	0.4[d]	1.0[d]	2.3[d]	0.4[d]
Efficacy (%)		93.5	98.9	94.6	94.1	96.6

[a]On study days 3–28, ticks counted at 2–3 hr postexposure were not removed; ticks were removed after counting at 48 hr.
[b]Each of eight dogs in the control group received no treatment. Each of eight dogs in the treated group received the label dose of K9 Advantix (imidacloprid [8.8% w/w] + permethrin [44% w/w]; Bayer Animal Health) on day 0.
[c]On each day indicated, dogs were walked for 80 minutes in an area predetermined to be naturally infested with adults and nymphs of *Amblyomma americanum;* the terrain was approximately 60% gallery forest and 40% open native prairie.
[d]Means values were significantly different from controls ($P < .05$)

pairs based on treatment and pen assignment. Dogs in the different treatment groups were separated at all times. A handler and dog pair were allocated to a grid zone by alternating treatment and control groups within the grid pattern. Placement of a treatment group into even- or odd-numbered grids was determined by coin toss, and placement of dog–handler units into a specific grid was made by random drawing of pen number. Dogs were then walked for 10 minutes in each grid zone for total exposure time of 80 minutes and loaded on the trailer for transport back to KSU as previously described.

Evaluation of Repellency and Prevention of Attachment

Three hours after being returned to KSU, the dogs were examined for ticks as previously described, except ticks were not removed at this time. Following the 3-hour postexposure examination, dogs were returned to their respective indoor runs.

Evaluation of Tick Kill 48 Hours after Exposure

Dogs were again examined for live attached ticks 48 hours after tick exposure. Dogs were examined and live ticks counted as previously described, except that all ticks observed at the 48-hour count were manually removed. After this final tick count, dogs were returned to their runs and allowed access to both indoor and outdoor runs.

Evaluation of Residual Acaricide Activity

The described process of tick exposure and tick counts was repeated 1, 2, 3, and 4 weeks after treatment to evaluate residual efficacy.

Statistical Methods

Repeated measures analysis of covariance (using pretreatment counts as a covariate) and best covariance structure (smallest Akaike information criterion) was used to evaluate the tick count data. All P values < .05 were deemed statistically significant. Geometric means were

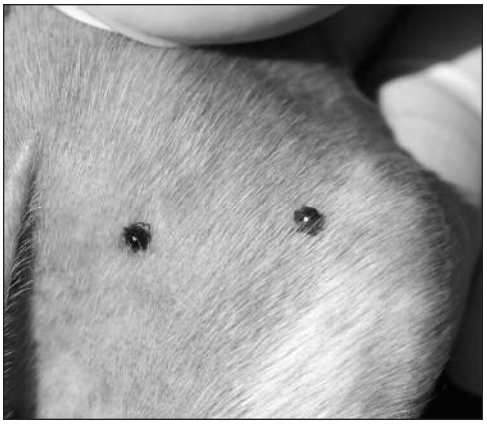

Figure 4. *Two adult female* Amblyomma americanum *ticks attached to the ear of an untreated control dog after it was walked through a naturally tick-infested habitat.*

calculated using antilog (average natural log + 1)-1 algorithm. Efficacy was determined using the formula in the box on page 105.

RESULTS

Pretreatment geometric mean *A. americanum* counts were 34.7 and 34.0 ticks/dog for groups 1 and 2, respectively (Table 1). Geometric mean *A. americanum* ticks/dog counts on control dogs fluctuated from a high of 38.9 on day 21 to a low of 12.1 on day 28. During this investigation, virtually all (>98%) of the ticks found attached to the dogs were adult and nymph stages of *A. americanum* (Figure 4). Pretreatment geometric mean *D. variabilis* counts were 2.2 and 2.5 ticks/dog for groups 1 and 2, respectively. Over the next 4 weeks, geometric mean *D. variabilis* counts on control dogs ranged from a low of 0.8 to a high of 3.3 ticks/dog. Because of the low numbers of *D. variabilis* encountered in this trial, only the *A. americanum* counts were used to evaluate product efficacy. While both adults and nymphs of *A. americanum* were recovered from dogs, the few *D. variabilis* that were recovered were adults.

Efficacy of the imidacloprid–permethrin formulation against *A. americanum* was 88.0% within 3 hours of natural tick exposure (repellency) (Table 1) on posttreatment day 3. Three-hour postexposure efficacy on posttreatment days 7 and 14 was 77.5% and 68.1%, respectively. Three-hour postexposure efficacy of the formulation declined slowly during the remainder of the study, with 64.3% and 68.5% control on days 21 and 28, respectively.

The 48-hour postexposure (acaricidal) efficacy of the imidacloprid–permethrin spot-on topical solution against *A. americanum* was 93.5%, 98.9%, 94.6%, 94.1%, and 96.6% on days 3, 7, 14, 21, and 28 respectively (Table 1).

DISCUSSION

The area chosen for this natural exposure model was documented[3] to be heavily infested with *A. americanum.* Geometric mean *A. americanum* ticks/dog counts ranged from a low of 12.1 to a high of 38.9. During the study, more than 1,300 ticks were removed from the eight untreated control dogs after the six tick exposures. These tick attachment rates were considered an excellent challenge for evaluation of product performance. Laboratory studies using colonized ticks have displayed tick counts on control dogs that were similar to or occasionally lower than those observed in this study.[7–13]

The imidacloprid–permethrin spot-on topical solution produced significantly lower tick counts 3 hours after exposure at all time points and all observations compared with the untreated control group of dogs. These reductions in the number of ticks on dogs (clinical repellency) within just 3 hours of exposure are possibly due to the rapid neurotoxic effect of permethrin.[14] The rapid neurotoxicity of permethrin can result in quick death (knockdown), tick agitation, prevention of attachment (repellency), and inhibition of feeding of ticks.[9–12] While the imidacloprid–permethrin

$$\text{Efficacy} = \frac{(\text{Geometric Mean No. of Live Adult Ticks on Control Dogs} - \text{Geometric Mean No. of Live Ticks on Treated Dogs})}{\text{Geometric Mean No. of Live Adult Ticks on Control Dogs}} \times 100$$

formulation did produce significant reductions in tick counts within 3 hours of exposure, it could not be determined from this study whether ticks were prevented from attaching or if they attached and then were rapidly killed.

The efficacy of the imidacloprid–permethrin formulation against *A. americanum* at 48 hours after exposure remained above 93.5% throughout the study. The natural variation in the number of ticks attaching to controls provided meaningful insight into veterinarians' and clients' perceptions of tick product performance. Three weeks into the study, the geometric mean tick counts on these naturally exposed dogs were 38.1 ticks/dog on the controls and 2.3 ticks/dog on treated dogs, translating to an efficacy rate of 94.1%. However, just 1 week later, the number of ticks in the natural environment declined, resulting in a geometric mean of 12.8 ticks/dog in the control dogs. At this time, a corresponding level of efficacy (96.6%) was observed based on less than 1 tick/dog (0.4). The variation in tick counts on control dogs and the corresponding efficacy of the product demonstrate the importance of the magnitude of tick exposure on the perception of product performance. Dogs traveling through environments with heavy tick infestations will often present with a few ticks even if the product's efficacy remains above 90% to 95%.

CONCLUSION

This study represents the first published account of a natural field exposure model evaluating a topically applied tick product within North America. The data from this study provide veterinarians with "real world" information related to product performance in an actual tick challenge. This model enabled the evaluation of a topically applied tick product against one of the most important tick species east of the Rocky Mountains, *A. americanum*. The data indicate that the imidacloprid–permethrin spot-on topical solution produced a rapid reduction in *A. americanum* tick numbers within 3 hours, and efficacy remained at or above 93.5% for the entire study duration.

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