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Efficacy of a novel oral formulation of sarolaner (Simparica™) against five common tick species infesting dogs in the United States

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ABSTRACT

The efficacy of a single oral treatment with sarolaner (Simparica™, Zoetis), a novel isoxazoline compound, was evaluated against five tick species known to infest dogs in the United States. A total of 10 laboratory studies, two against each species, were conducted using adult purpose-bred mongrels or Beagle dogs. In each study, 16 dogs were randomly allocated to one of two treatment groups based on pre-treatment host-suitability tick counts. Dogs were infested with approximately 50 unfed adult *Amblyomma americanum, Amblyomma maculatum, Dermacentor variabilis, Ixodes scapularis* or *Rhipicephalus sanguineus* ticks on Days -2, 5, 12, 19, 26 and 33. On Day 0, dogs were treated with a placebo or a sarolaner tablet providing a minimum dose of 2 mg/kg. Tick counts were conducted 48 h after treatment and after each subsequent weekly re-infestation.

There were no treatment-related adverse reactions during any of the studies. Dogs in the placebo-treated group maintained tick infestations throughout the studies. Geometric mean live tick counts were significantly lower ($P \leq 0.0001$) in the sarolaner-treated group compared to the tick counts in the placebo group at all timepoints. Treatment with sarolaner resulted in ≥99.6% efficacy against existing infestations of all five tick species within 48 h. The efficacy against weekly post-treatment re-infestations of all tick species was ≥96.9% for at least 35 days after treatment.

Thus, a single dose of sarolaner administered orally at the minimum dosage of 2 mg/kg, resulted in excellent efficacy within 48 h against existing tick infestations, and against weekly re-infestations for 35 days after treatment. These studies confirmed that administration of the minimum dose of sarolaner will provide rapid treatment of existing infestations and give at least one month of control against re-infestation by the common tick species affecting dogs in the US.

1. Introduction

Ticks are recognized as one of the most prevalent ectoparasites in domestic animals. In addition to the local irritation resulting from the bite, ticks may also directly injure animals by producing generalized hypersensitivity reactions, and by causing tick paralysis resulting from a toxin produced by the salivary glands (Muller and Kirk, 2013). Ticks are also the vectors of many zoonotic diseases in the United States including Lyme disease (caused by *Borrelia burgdorferi*), which is transmitted by *Ixodes spp*. and Rocky Mountain spotted fever (caused by *Rickettsia rickettsia*) which is transmitted by *Dermacentor* spp. Other tick-borne pathogens cause dog-specific infections, such as *Babesia canis* which is primarily transmitted by *Dermacentor reticulatus*, and *Babesia vogeli* and *Ehrlichia canis*, which are primarily transmitted by *Rhipicephalus sanguineus* (Chomel, 2011; Dantas-Torres et al., 2012).

Tick paralysis toxins and tick-borne pathogens can cause subclinical to life threatening diseases in dogs and humans (Blagburn and Dryden, 2009). Recently, possibly due to changes in awareness, climate and or lifestyle, the incidence of tick-borne diseases has been rising markedly (Blagburn and Dryden, 2009; Jaenson et al., 2012; Lindgren et al., 2000; Chomel, 2011).

Tick prevention and control programs for companion animals were originally based on the topical application of acaricides. The

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most widely used products are generally applied as spot-on applications on a monthly schedule or as collars and may include compounds with efficacy against both fleas and ticks. A number of chemical classes are used for tick control and include: phenyl pyrazoles e.g., fipronil; pyrethroids e.g., permethrin and deltamethrin; and octopamines e.g., amitraz (Rust, 2005). Programs to prevent the transmission of tick-borne pathogens to dogs have largely relied on routine use of these types of products coupled with other means of vector avoidance.

Recently, a new class of compounds, the isoxazolines, have shown excellent efficacy following oral administration against fleas, along with enhanced efficacy against ticks (Rohdich et al., 2014; Shoop et al., 2014). High efficacy via oral administration is likely to increase compliance from pet owners by eliminating the difficulties of administering topical products, and removing the need to temporarily isolate the treated-pet from children and other pets required with some topical products where contact must be avoided until the product is dry. Sarolaner (Simparica™, Zoetis) is a novel isoxazoline with potent systemic activity against ticks and fleas after oral administration that was developed specifically for use in dogs (McTier et al., 2016a) and has demonstrated month long efficacy against ticks after a single oral dose (McTier et al., 2016b).

Here we report laboratory studies conducted to confirm the efficacy of sarolaner, at a single oral dose of 2 mg/kg, against existing infestations and subsequent weekly re-infestations of five tick species commonly infesting dogs in the US for up to five weeks after treatment.

2. Materials and methods

Two studies were conducted to confirm the efficacy of sarolaner against each of the following tick species commonly infesting dogs in the United States: *Amblyomma americanum* (Lone Star tick); *Amblyomma maculatum* (Gulf Coast tick); *Dermacentor variabilis* (American dog tick); *R. sanguineus* (brown dog tick); and *Ixodes scapularis* (black legged tick). The studies were conducted at research laboratories in Arkansas (AR), Texas (TX) and California (CA). All studies were conducted in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats (Marchiondo et al. 2013) and complied with Good Clinical Practices, (EMEA, 2000). Study protocols were reviewed and approved by the local and/or Zoetis Institutional Animal Care and Use Committee.

2.1. Animals

All dogs used in these studies had not been treated with an ectoparasiticide for at least 60 days and demonstrated good tick retention prior to treatment, and were in good health at enrollment. Sixteen purpose-bred dogs, including both sexes, were enrolled in each study. Dogs were Beagles or mixed-breed, ranging in age from 7 months to 13 years, and weighing between 5.6 kg and 35.0 kg. Female dogs were confirmed not to be pregnant or lactating. Each dog was individually identified by a unique and permanent code (microchip or tattoo). Dogs were housed in individual indoor pens such that no physical contact was possible between them, and the possibility of tick transfer among animals was minimal. Dogs were fed an appropriate maintenance ration of a commercial canine diet for the duration of the study. Water was available ad libitum.

2.2. Experimental design and methods

Day 0 for each study was the day dogs were administered the study treatment. Dogs were acclimated to the study conditions for at least 7 days prior to treatment. The dogs were observed for general health at least once daily throughout the studies. A physical exam was performed on each dog by a veterinarian to determine health and suitability prior to inclusion in the trial. For tick infestations, a pre-counted aliquot of approximately 50 (1:1 sex ratio) adult unfed ticks were placed onto the hair coat and allowed to disperse on the dog. At the California and Arkansas sites, dogs were placed in travel crates for 2–4 h after infestation to restrict the dogs' movement and facilitate tick (*A. americanum*, *A. maculatum*, *D. variabilis*, *R. sanguineus*) attachment. For *A. americanum* infestations at the California facility, the dogs were also lightly sedated prior to infestation to further enhance attachment for this species.

Tick counts were performed by personnel trained in the standard procedures in use at the test facility. Personnel changed protective clothing between dogs to avoid any cross-contamination, and personnel conducting parasite or other observations were unaware of treatment assignments. Initially, the entire dog's entire body was examined, pushing the hair against its natural nap, exposing, counting and removing the ticks. After the manual inspection, an extra-fine tooth comb was used to comb the animal to remove any missed ticks. Each dog was examined for at least 10 min. If ticks were encountered in the last minute, combing was continued in one minute increments until no ticks were encountered. The ticks were examined to assess viability and the numbers of live and dead ticks was quantified.

At least 16 animals arrived into the housing facilities on or before Day-11. General health observations were performed at least once a day from the start of the acclimation period. All dogs were given a physical examination to evaluate general health and suitability for inclusion into the study. The dogs were examined to ensure they were free of ticks and were then infested to determine the host suitability between Day-11 and Day-7. The live attached ticks present on each dog were counted and removed at 48 (±2) hours after infestation. When more than 16 dogs were available, those with the highest counts were selected for inclusion. Dogs were ranked by decreasing tick count into blocks of two and randomly allocated within block to treatment with placebo or sarolaner tablets. Blocks of dogs were randomly assigned to adjacent pens within the test facility. Dogs were moved into their allocated pens on or before Day-2.

Dogs were weighed and infested with ticks on Day-2. On Day 0, the dogs were dosed orally with one to three tablets (placebo or sarolaner strengths of 5, 10, 20, or 40 mg) such that the sarolaner dose was as close as possible to 2 mg/kg without under-dosing.

Each dog was offered its regular food ration ~20 min before dosing. Dogs were hand-pilled to ensure accurate dose delivery. Each dog was observed for several minutes after dosing for evidence that the dose was swallowed, and for potential adverse events associated with treatment and then for up to 2 h for any signs of emesis. Dogs were observed for general health and any reaction to treatment approximately 1, 3 and 6 h after treatment. On Day 2, each dog was examined to remove and count ticks. Subsequently, all animals were re-infested with ticks on Days 5, 12, 19, 26 and 33. All dogs were examined, combed and parasite counted on Days 7, 14, 21, 28 and 35. Ticks exhibiting movement after gentle touching or exposure to CO_2 were considered alive.

2.3. Parasites

Ticks of each species were obtained from four different laboratory maintained colonies (Tables 1–5). These ticks were originally isolated from the field and had wild caught ticks introduced into each tick colony every 1–2 years or generations. One *I. scapularis* study (TX) utilized wild caught adult ticks from South Carolina.

Table 1
Geometric (arithmetic) mean live *Amblyomma americanum* (lone star tick) counts and ranges for placebo control and treated dogs and percent efficacy relative to controls for dogs treated once orally with sarolaner chewable tablets at 2 mg/kg in two laboratory studies.

Laboratory/tick strain	Day	Placebo		Sarolaner		% Efficacy[b]
		Mean	Range	Mean	Range	
California/Stillwater, OK	2	23.3 (23.8)	16–30	0.0[a] (0.0)	0–0	100
	7	19.2 (20.8)	9–32	0.2[a](0.3)	0–1	99.0 (98.8)
	14	15.6 (20.8)	1–32	0.0[a] (0.0)	0–0	100
	21	22.3 (24.0)	9–38	0.1[a] (0.1)	0–1	99.6 (99.5)
	28	26.1 (28.0)	12–42	0.0[a](0.0)	0–0	100
	35	24.7 (25.1)	13–33	0.8[a] (1.4)	0–5	96.9 (94.5)
Arkansas/College station, TX	2	23.7 (26.3)	6–39	0.1[a] (0.1)	0–1	99.6 (99.5)
	7	14.3 (15.0)	7–19	0.0[a](0.0)	0–0	100
	14	18.8 (19.1)	13–23	0.0[a] (0.0)	0–0	100
	21	13.2 (14.3)	4–20	0.0[a] (0.0)	0–0	100
	28	17.3 (18.0)	9–25	0.0[a] (0.0)	0–0	100
	35	15.1 (15.4)	11–21	0.0[a] (0.0)	0–0	100

[a] Geometric mean live tick count significantly lower than placebo ($P \leq 0.0001$).
[b] Efficacy calculated using arithmetic mean live tick counts is shown in parenthesis.

Table 2
Geometric (arithmetic) mean live *Amblyomma maculatum* (gulf coast tick) counts and ranges for placebo control and treated dogs and percent efficacy relative to controls for dogs treated once orally with sarolaner chewable tablets at 2 mg/kg in two laboratory studies.

Laboratory/tick strain	Day	Placebo		Sarolaner		% Efficacy[b]
		Mean	Range	Mean	Range	
Arkansas/College station, TX	2	30.1 (32.3)	12–48	0.0[a] (0.0)	0–0	100
	7	31.1 (34.6)	8–50	0.0[a] (0.0)	0–0	100
	14	26.6 (28.4)	15–47	0.1[a] (0.1)	0–1	99.7 (99.6)
	21	25.8 (27.5)	17–50	0.0[a] (0.0)	0–0	100
	28	28.0 (29.5)	18–50	0.0[a] (0.0)	0–0	100
	35	20.6 (22.4)	8–35	0.0[a] (0.0)	0–0	100
Texas/Stillwater, OK	2	21.5 (25.8)	6–46	0.0[a] (0.0)	0–0	100
	7	25.0 (28.3)	8–48	0.0[a] (0.0)	0–0	100
	14	32.5 (34.5)	15–60	0.1[a] (0.3)	0–2	99.5 (99.3)
	21	28.6 (29.6)	15–40	0.0[a](0.0)	0–0	100
	28	30.6 (32.4)	17–51	0.1[a] (0.3)	0–2	99.5 (99.2)
	35	26.1 (29.0)	10–48	0.2[a] (0.3)	0–1	99.3 (99.1)

[a] Geometric mean live tick count significantly lower than placebo ($P<0.0001$).
[b] Efficacy calculated using arithmetic mean live tick counts is shown in parenthesis.

Table 3
Geometric (arithmetic) mean live *Dermacentor variabilis* (American dog tick) counts and ranges for placebo control and treated dogs and percent efficacy relative to controls for dogs treated once orally with sarolaner chewable tablets at 2 mg/kg in two laboratory studies.

Laboratory/tick strain	Day	Placebo		Sarolaner		% Efficacy[b]
		Mean	Range	Mean	Range	
Arkansas/Greenbrier, AR	2	21.3 (22.4)	15–35	0.0[a] (0.0)	0–0	100
	7	18.6 (20.1)	10–38	0.1[a](0.1)	0–1	99.5 (99.4)
	14	20.8 (21.0)	15–26	0.1[a](0.3)	0–2	99.3 (98.8)
	21	15.6 (17.5)	5–33	0.0[a](0.0)	0–0	100
	28	16.2 (17.4)	10–31	0.0[a] (0.0)	0–0	100
	35	17.9 (18.9)	8–25	0.0[a] (0.0)	0–0	100
California/Henderson, NC	2	33.4 (35.5)	13–50	0.1[a] (0.1)	0–1	99.7 (99.6)
	7	35.2 (36.0)	24–51	0.2[a] (0.3)	0–1	99.5 (99.3)
	14	28.0 (29.9)	17–50	0.4[a] (0.5)	0–1	98.5 (98.3)
	21	32.4 (34.6)	15–54	0.1[a] (0.1)	0–1	99.7 (99.6)
	28	22.4 (23.5)	12–37	0.2[a] (0.3)	0–1	99.2 (98.9)
	35	22.6 (24.9)	11–53	0.1[a] (0.3)	0–2	99.3 (99.0)

[a]Geometric mean live tick count significantly lower than placebo ($P<0.0001$).
[b]Efficacy calculated using arithmetic mean live tick counts is shown in parenthesis.

2.4. Data analysis

The individual dog was the experimental unit and the primary endpoint was live tick counts. Tick counts were transformed by the $\log_e(\text{count} + 1)$ transformation prior to analysis in order to stabilize the variance and normalize the data. Using the PROC MIXED procedure (SAS 9.2, Cary NC), transformed counts were analyzed using a mixed linear model by timepoint. The model included the fixed effect of treatment. The random effects included block and error. Testing was two-sided at the significance level $\alpha = 0.05$. Percent efficacy was calculated using Abbott's formula:

$$\%\text{Efficacy} = \frac{(\text{Mean placebo} - \text{mean treated})}{\text{Mean placebo}} \times 100$$

Table 4
Geometric (arithmetic) mean live *Ixodes scapularis* (black legged tick) counts and ranges for placebo control and treated dogs and percent efficacy relative to controls for dogs treated once orally with sarolaner chewable tablets at 2 mg/kg in two laboratory studies.

Laboratory/tick strain	Day	Placebo		Sarolaner		% Efficacy
		Mean	Range	Mean	Range	
Arkansas/Stillwater, OK	2	24.7 (25.3)	17–35	0.0[a] (0.0)	0–0	100
	7	28.4 (28.9)	20–35	0.0[a] (0.0)	0–0	100
	14	24.8 (25.1)	18–30	0.0[a] (0.0)	0–0	100
	21	17.4 (20.5)	3–30	0.0* (0.0)	0–0	100
	28	20.9 (22.4)	11–38	0.0[a] (0.0)	0–0	100
	35	25.8 (27.3)	10–36	0.0[a](0.0)	0–0	100
Texas/SC wild caught adults	2	14.7 (15.1)	8–21	0.0 (0.0)	0–0	100
	7	8.6 (10.8)	2–19	0.0[a](0.0)	0–0	100
	14	15.3 (16.3)	7–28	0.0[a] (0.0)	0–0	100
	21	10.7 (12.1)	3–21	0.0[a](0.0)	0–0	100
	28	10.8 (11.5)	5–18	0.0[a] (0.0)	0–0	100
	35	15.5 (16.1)	10–25	0.0[a] (0.0)	0–0	100

[a] Geometric mean live tick count significantly lower than placebo ($P<0.0001$).

Table 5
Geometric (arithmetic) mean live *Rhipicephalus sanguineus* (brown dog tick) counts and ranges for placebo control and treated dogs and percent efficacy relative to controls for dogs treated once orally with sarolaner chewable tablets at 2 mg/kg in two laboratory studies.

Laboratory/tick strain	Day	Placebo		Sarolaner		% Efficacy[b]
		Mean	Range	Mean	Range	
California/Henderson, NC	2	32.7 (34.5)	14–52	0.0[a](0.0)	0–0	100
	7	30.4 (31.1)	22–47	0.1[a] (0.1)	0–1	99.7 (99.6)
	14	26.9 (27.5)	18–35	0.0[a] (0.0)	0–0	100
	21	27.9 (29.8)	14–51	0.0[a] (0.0)	0–0	100
	28	24.2 (26.1)	11–36	0.1[a] (0.1)	0–1	99.6 (99.5)
	35	26.1 (27.0)	15–33	0.8[a] (0.9)	0–2	97.1 (96.8)
Arkansas/Greenbrier, AR	2	24.3 (25.5)	15–36	0.0[a] (0.0)	0–0	100
	7	17.9 (18.4)	13–26	0.0[a] (0.0)	0–0	100
	14	18.0 (19.8)	7–34	0.0[a] (0.0)	0–0	100
	21	19.0 (20.6)	8–31	0.0[a] (0.0)	0–0	100
	28	19.1 (20.6)	10–33	0.0[a](0.0)	0–0	100
	35	20.7 (22.0)	13–36	0.0[a] (0.0)	0–0	100

[a] Geometric mean live tick count significantly lower than placebo ($P<0.0001$).
[b] Efficacy calculated using arithmetic mean live tick counts is shown in parenthesis.

3. Results

3.1. Efficacy

Dogs in the placebo-treated groups generally maintained tick infestations throughout the studies (Tables 1–5). Mean tick recovery from the placebo dogs ranged from about 25–70% of the applied infestation. The use of sedation of the dogs for tick infestation (at the CA site) appeared to improve tick recovery rates for *A. americanum* (Table 1). However, there was variation in tick retention among study sites and tick strains that was most obvious when wild caught versus colony-reared *I. scapularis* were used (Table 4).

For *A. americanum*, efficacy against existing infestations was 100 and 99.6% at 48 h after treatment in the two studies. For subsequent weekly re-infestations, efficacy was ≥96.9% in the CA study and 100% in the AR study through 35 days post treatment (Table 1). Efficacy against existing infestations of *A. maculatum* was 100% at 48 h after treatment in both studies, and efficacy against subsequent weekly re-infestations was ≥99.3% in the TX study and ≥99.7 in the AR study through 35 days post treatment (Table 2). Efficacy against existing *D. variabilis* infestations was 100 and 99.7% at 48 h after treatment. Following weekly re-infestations, efficacy against this tick was ≥98.5% in the CA study and ≥99.3 in the AR study through 35 days post treatment (Table 3). For *I. scapularis*, efficacy was 100% against existing infestations at 48 h after treatment and at 48 h after subsequent weekly re-infestations for 35 days in both studies (Table 4). In the AR study with *R. sanguineus*, efficacy was 100% against the existing infestation at 48 h after treatment and at 48 h after subsequent weekly re-infestations through 35 days post treatment (Table 5). In the CA *R. sanguineus* study, efficacy was 100% at 48 h after treatment and efficacy against subsequent weekly re-infestations was ≥97.1% for 35 days post treatment. For all tick species, the mean tick counts for sarolaner-treated dogs were significantly lower than those for placebo-treated dogs at all post treatment counts ($P \leq 0.0001$)

3.2. Health observations

No adverse events related to treatment with sarolaner were reported in any study.

4. Discussion

Until recently there were no orally administered marketed products that could quickly kill ticks and also provide a high level of residual efficacy for at least a month or longer. With the introduction of the isoxazolines, pet owners and veterinarians now have a highly effective alternative to topical spot-on formulations for the control of fleas and ticks on dogs.

The dose of sarolaner was selected to provide immediate treatment of existing infestations and one month's consistent control of new infestations of the least sensitive tick commonly found on dogs (*A. maculatum*, McTier et al., 2016b). Thus, it was expected that a single oral dose of sarolaner at the minimum dose of 2 mg/kg would provide robust efficacy against the main ticks infesting dogs in the US for at least a month.

The 10 studies reported here confirmed the consistent, high efficacy of sarolaner against the most common ticks found on dogs in

the US for 35 days. A single oral dose of sarolaner at 2 mg/kg provided 100% efficacy against existing infestations of *I. scapularis*, *R. sanguineus* and *A. maculatum*, and ≥99.6% efficacy against *A. americanum* and *D. variabilis* within 48 h of dosing. Following weekly re-infestations of all five species, the single treatment resulted in significant and ≥96.9% reductions in live ticks for 35 days.

An initial attachment and feeding of at least 24–48 h is required before transmission of most tick-borne pathogens can occur (Little, 2007; Salinas et al., 2010). If the infected ticks are killed within that time period, the transmission may be prevented (Wengenmayer et al., 2014). In the studies presented here, on all but three of the fifty post treatment tick evaluations for all species, the efficacy was ≥99%. This consistent high level of efficacy against the common tick species suggests that monthly treatment with sarolaner should reduce the risk of transmission of tick-borne diseases, which is important given that the incidence and spread of tick-borne diseases has been rising markedly (Blagburn and Dryden, 2009; Lindgren et al., 2000; Chomel, 2011; Jaenson et al., 2012).

5. Conclusions

The robust and consistent efficacy of a single oral treatment of sarolaner at the minimum dose of 2.0 mg/kg against the five major US tick species was confirmed against existing infestations and weekly re-infestations for 5 weeks. Efficacy of ≥99.6% was achieved versus existing infestations within 48 h after treatment. Efficacy was maintained at greater than 96.9% within 48 h after re-infestation for the 35 day duration of all studies.

Conflict of interest

The studies reported here were funded by Zoetis, Florham Park, NJ. RHS, SPM, NAH, MRM, SH, SC and JJR are current employees of Zoetis. WRE, LC and DRY are independent investigators contracted for these studies. All authors assisted with the design and conduct of the studies, interpretation of the data and manuscript review. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

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