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Determination of the effective dose of a novel oral formulation of sarolaner (Simparica™) for the treatment and month-long control of fleas and ticks on dogs

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ABSTRACT

Three laboratory studies were conducted to determine the appropriate dose of sarolaner, a novel isoxazoline, for the treatment and month-long control of infestations of fleas and ticks on dogs. In the first study, dogs were treated orally with sarolaner suspension formulations at 1.25, 2.5 or 5.0 mg/kg, and infested with *Dermacentor reticulatus, Rhipicephalus sanguineus* ticks and with *Ctenocephalides felis felis* (cat flea) prior to treatment and then weekly for up to 8 weeks. Fleas and ticks were counted 48 h after treatment and after each subsequent infestation at 24 h for fleas and 48 h for ticks. The lowest dose of sarolaner (1.25 mg/kg) provided 100% efficacy against fleas from treatment through Day 35 and 98.4% at Day 56. This dose of sarolaner resulted in 99.7–100% control of both species of ticks through Day 28. In Study 2, dogs were dosed orally with placebo or sarolaner suspension formulations at 0.625, 1.25 or 2.5 mg/kg and infested with *Ixodes scapularis* prior to treatment and weekly for 6 weeks, *Amblyomma americanum* (pretreatment and Day 26), *Dermacentor variabilis* (Day 33) and *A. maculatum* (Day 41). *Ixodes scapularis* was the most susceptible; the lowest dose (0.625 mg/kg) providing >95% efficacy through Day 43. Efficacy against *D. variabilis* on Day 35 was >95% at 1.25 and 2.5 mg/kg, whereas the 0.625 mg/kg dose gave only 61.4% efficacy. *Amblyomma* spp. were the least susceptible ticks; efficacy of the 1.25 mg/kg dose at Day 28 for *A. americanum* was markedly lower (88.5%) than achieved for *D. reticulatus* (100%) at Day 28 and also lower than for *D. variabilis* at Day 35 (96.2%). In Study 3, dogs were dosed orally with placebo or sarolaner in the proposed commercial tablet (Simparica™) at 1.0, 2.0 or 4.0 mg/kg, and infested with *A. maculatum*, one of the ticks determined to be dose limiting, prior to treatment and then weekly for 5 weeks. All doses gave 100% control of the existing infestation. The two highest dosages resulted in >93% control of subsequent challenges for 5 weeks. There was no significant improvement in efficacy provided by the 4.0 mg/kg dose over the 2.0 mg/kg dose ($P > 0.05$) at any time point. The 2.0 mg/kg dose was superior to the 1.0 mg/kg on Day 14 ($P = 0.0086$) and as efficacy for 1.0 mg/kg declined below 90% at Day 28, a single 1 mg/kg dose would not provide a full month of tick control. Thus, 2.0 mg/kg was selected as the sarolaner dose rate to provide flea and tick control for at least one month following a single oral treatment.

1. Introduction

The most common permanent or semi-permanent ectoparasites occurring on dogs are fleas, ticks and mites. *Ctenocephalides felis felis,* the cat flea, is considered the most important ectoparasite of dogs and cats (Rust and Dryden, 1997) and is endemic worldwide. Adult fleas are blood feeders, are recognized as a major cause of allergic skin disease in dogs, and when present in sufficient numbers are capable of causing anemia (Krämer and Mencke, 2001). They are intermediate hosts for the dog tapeworm, *Dipylidium caninum,* and can transmit a number of zoonotic pathogens, including *Bartonella henselae*, the causative agent for cat scratch fever, as well as other zoonotic Bartonellas, such as *B. clarrigeae* and *B. koehlerae* (Chomel and Kasten, 2010).

Tick infestations can range from an occasional nuisance to a continuous infestation and can cause serious, even life-threatening disease (Dryden and Payne, 2004). During feeding, large amounts of blood are taken up by ticks; excess water is removed and

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returned to the host in the form of saliva, which contains a number of pharmacologically active substances, including anticoagulants and immunomodulators, with the components varying between species. Tick-borne pathogens are normally passed to their next host in saliva and some species excrete toxins within their saliva (Needham and Teel, 1991). In severe infestations, tick numbers may be high enough to cause anemia due to physical blood loss through their feeding. Ticks are responsible for the transmission of a number of pathogens, some dog-specific, and others zoonotic. *Babesia canis* and *Ehrlichia canis* are both primarily dog-specific infections, the former primarily transmitted by *Dermacentor* spp. (*D. variabilis*, American Dog tick and *D. reticulatus*, Ornate Cow tick) and the latter by the Brown Dog tick, *Rhipicephalus sanguineus* (Chomel, 2011; Dantas-Torres et al., 2012). Zoonotic infections include Lyme disease, caused by *Borrelia burgdorferi,* and human granulocytic anaplasmosis (HGA), caused by *Anaplasma phagocytophilum,* which are transmitted by *Ixodes* spp., and Rocky Mountain Spotted Fever, caused by *Rickettsia rickettsii,* which is transmitted primarily by ticks in the genera *Amblyomma, Dermacentor, Ixodes* (Dryden and Payne, 2004; Chomel, 2011) and *Rhipicephalus* (Demma et al., 2005). *Amblyomma americanum,* the Lone Star tick, and *A. maculatum*, the Gulf Coast tick, have been determined to harbor a number of other *Erlichia, Borrelia* and *Rickettsia* spp. (Mixon et al., 2006; Jiang et al., 2012; Moncayo et al., 2010; Nicholson et al., 2009; Paddock et al., 2010).

Control of fleas and ticks is primarily based on the use of parasiticides, and until recently convenient on-animal treatments applied as spot-on or collar applications have been the standard accepted method (Dryden and Payne, 2004; Rust, 2005). The most widely used products include compounds with efficacy against fleas and ticks, as well as specific insecticides or acaricides. Most have direct insecticidal/acaricidal activity and control the parasites on the animal. In addition, there are products such as insect growth regulators that may be orally dosed or applied to the pet and control fleas by disrupting the off-host life stages (eggs and larvae), and others that may be used for environmental applications. Despite the variety of available products and application methods, both fleas and ticks remain an ongoing problem for many pet owners. Formulations of spinosad (for fleas only) and more recently, isoxazoline insecticide/acaricides have been introduced that provide control of these ectoparasites for a month or more after a single oral dose (Robertson-Plouch et al., 2008; Rohdich et al., 2014; Shoop et al., 2014). There are obvious advantages to orally administered products, which remove environmental/user exposure concerns that may accompany topically applied products. In addition, oral products are not affected by bathing/water exposure as are some topically administered products, thus ensuring a more uniform performance during the treatment period.

Sarolaner is a novel isoxazoline with potent activity against fleas and ticks that was developed specifically for companion animals (McTier et al., 2016). Here we report studies conducted to determine the appropriate dose rate of an oral formulation of sarolaner (Simparica™, Zoetis) given as a single dose to dogs to provide treatment of existing infestations and at least one month control of fleas and ticks.

2. Materials and methods

Three studies were conducted to: 1) compare the relative susceptibilities of fleas and ticks; 2) determine which of the common ticks found on dogs in the US was the least susceptible and; 3) identify the minimum dose required to treat and control the least sensitive parasite (s) for at least one month following a single treatment.

Study 1 was conducted in South Africa and Studies 2 and 3 were conducted in the USA. The 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). Study 3 additionally complied with Good Clinical Practices, (VICH guideline GL9) (EMEA, 2000). Study protocols were reviewed and approved by the ClinVet and/or Zoetis Institutional Animal Care and Use Committee (s). Masking of all studies was assured through the separation of functions. All personnel conducting observations or animal care, or performing infestations and counts were masked to treatment allocation.

2.1. Animals

Individually identified, adult, purpose-bred Beagles ≥8 months of age and ≤ 20 kg were used in each study. The dogs had not been treated with an ectoparasiticide for at least 60 days and were in good health at the time of treatment. Dogs were housed individually in indoor runs that conformed to accepted animal welfare guidelines. Dogs were fed an appropriate maintenance ration of a commercial dry canine feed for the duration of the study. Water was available *ad libitum*. Study 1 included 48 dogs (24 male and 24 female), Study 2 used 32 male dogs and Study 3 had 32 dogs (15 male, 17 female).

2.2. Experimental design and methods

General methods: Day 0 represents the day that dogs received the study treatment. Dogs were acclimated to the study conditions for at least 14 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 study. For infestations in Studies 1 and 2, approximately 100 cat fleas (*C. felis felis*, ~1:1 sex ratio) and/or approximately 50 ticks (~1:1 sex ratio) of each species to be assessed were applied directly to a site proximal or adjacent to the shoulder blades and allowed to crawl into the hair coat. In study 3, dogs were sedated prior to tick infestation and confined in shipping crates for approximately 6 h to reduce animal movement and enhance tick attachment. Forty eight hours after treatment and each tick infestation and/or 24 h after each flea infestation, every dog was thoroughly examined and combed to remove and count fleas and ticks. Flea and tick counts were performed by personnel trained in the standard procedures in use at the test facility. Protective gloves and clothing were changed between dogs, and personnel conducting parasite counts or other observations were unaware of treatment assignments.

Study 1: On Day−7, each dog was infested with brown dog ticks (*R. sanguineus*). On Day−5, the ticks on each dog were removed and counted; dogs were ranked by descending tick count into eight blocks of six, and randomly allocated within blocks to six treatment groups. On Day−2, the dogs were weighed and infested with *R. sanguineus* and *Dermacentor reticulatus* ticks. On Day−1, the dogs were infested with fleas.

On Day 0, the eight animals in each group were treated via oral gavage at 0.5 mL/kg body weight with one of the following: placebo; sarolaner suspension (10 mg/mL) to provide a dose of 5 mg/kg; sarolaner suspension (5 mg/mL) to provide a dose of 2.5 mg/kg; sarolaner suspension (2.5 mg/mL) to provide a dose of 1.25 mg/kg; another isoxazoline analog suspension (5 mg/mL) to provide a dose of 2.5 mg/kg (results not provided) or sarolaner solution (5 mg/mL) to provide a dose of 2.5 mg/kg. The results for the latter experimental treatment are reported in McTier et al. (2016). Dogs were observed for general health and any reaction to treatment approx-

imately 1, 3 and 6 h after treatment on Day 0, then once daily for the remainder of the study.

On Day 2, each dog was examined and combed to count and remove fleas and ticks. Subsequently, all animals were infested with *R. sanguineus* and *D. reticulatus* on Days 5, 12, 19, 25 and 33, and with fleas on Days 6, 13, 20, 26 and 34. The dogs in the placebo group and the group treated with sarolaner at 1.25 mg/kg were additionally infested with these ticks on Days 41 and 55 and with fleas on Days 42 and 56. All dogs were examined, combed and parasite counted on Days 7, 14, 21, 27 and 35; placebo dogs and dogs treated at 1.25 mg/kg were also counted on Days 43 and 56.

For the counts, all dogs were first examined visually, and any ticks detected were removed using forceps. Ticks were examined to determine their viability. Any tick able to move in a coordinated manner was considered live. The dogs were then thoroughly flea combed to count and remove fleas and any remaining ticks. Fleas able to stand up right and/or move in a coordinated manner were considered live. Commercial fine-toothed flea combs were used. Dogs were systemically combed using repeated strokes initially while standing starting from the head, then proceeding caudally along the dorsum. The dog was then turned on each side and then on its back for combing of the sides and ventral surfaces. Dogs were repeatedly combed until no fleas were recovered for about 5 min. Each animal was examined for a minimum of 10 min.

Study 2: On Day–9, each dog was infested with *I. scapularis*. On Day–7, the ticks on each dog were removed and counted, the dogs ranked by descending tick count into eight blocks of four, and randomly allocated within blocks to four treatment groups. On Day–2, the dogs were weighed and infested with *I. scapularis* and *A. americanum* ticks.

On Day 0, the eight animals in each group were treated via oral gavage at 0.5 mL/kg body weight with one of the following: placebo; sarolaner suspension (5 mg/mL) to provide a dose of 2.5 mg/kg; sarolaner suspension (2.5 mg/mL) to provide a dose of 1.25 mg/kg; or sarolaner suspension (1.25 mg/mL) to provide a dose of 0.625 mg/kg. Dogs were observed for general health and any reaction to treatment approximately 1, 3 and 6 h after treatment on Day 0, then once daily for the remainder of the study.

On Day 2, each dog was examined and combed to remove and count ticks (as described above). Subsequently, all animals were infested with *I. scapularis* on Days 5, 12, 19, 26, 33 and 41, and additionally with *A. americanum* on Day 26, *D. variabilis* on Day 33 and *A. maculatum* on Day 41. All dogs were examined and combed and live parasites were counted on Days 7, 14, 21, 28, 35 and 43.

Study 3: On Day–9, each dog was infested with *A. maculatum*. On Day–7, the ticks on each dog were removed and counted, the dogs ranked by descending tick count into eight blocks of four, and randomly allocated within blocks to four treatment groups. On Day–2, the dogs were weighed and infested with *A. maculatum*.

On Day 0, the 8 animals in each group were treated with one of the following tablet formulations: placebo; sarolaner at 1.0 mg/kg; sarolaner at 2.0 mg/kg; or sarolaner at 4.0 mg/kg. Tablets were individually shaved and/or sanded to deliver the appropriate dosage for the dog's body weight. Tablets were placed at the back of the tongue and the dog was encouraged to swallow. Food was offered prior to and after dosing. Dogs were observed for general health and any reaction to treatment approximately 1, 3 and 6 h after treatment on Day 0, then at least once daily for the remainder of the study.

On Day 2, each dog was examined and combed to remove and count ticks (as described above). Subsequently, all animals were infested with *A. maculatum* on Days 5, 12, 19, 26, and 33. On Days 6, 13, 20, 27 and 34, the ticks on each dog were counted *in situ* and not removed (thumb counted) and the numbers of live (attached and free) were determined. All dogs were examined, combed and ticks were counted and removed on Days 7, 14, 21, 28 and 35.

2.3. Parasites

Study 1: Cat fleas were from a laboratory colony, which was initiated with fleas originally obtained from Hannover University, Germany. *R. sanguineus* ticks were from a laboratory colony, which was initiated with ticks originally obtained from a colony in France. The strain had been maintained for 12 generations in the laboratory. *D. reticulatus* were from a laboratory colony, which was initiated with ticks originally obtained from a colony in Ireland. The strain had been maintained for six generations in the laboratory and additional wild caught ticks collected in the Netherlands had been introduced approximately two years prior to the study conduct.

Study 2: All tick colonies were initiated with wild caught ticks from Oklahoma and additional ticks collected locally are introduced into the colonies at least once every two years.

Study 3: The *A. maculatum* colony was initiated with local wild-caught ticks from Oklahoma in 1991 and additional engorged females from the field had been introduced every two years; the latest introduction was approximately one year prior to the study.

2.4. Data analysis

The individual dog was the experimental unit and the primary endpoint was live flea and/or tick count. Flea and 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 for repeated measures. The model included the fixed effect of treatment, day of study and the interaction between treatment and day of study. The random effects included room, block within room, the interaction between block and treatment within room (animal term) and error. In Studies 1 and 2, *a priori* contrasts were used to compare treatment means to the control at the one-sided significance level $\alpha = 0.05$. In Study 3, *a priori* contrasts were used to assess pair wise comparisons between treatments at each time point. Testing was two-sided at the significance level $\alpha = 0.05$. Percent efficacy, relative to the control group and based on geometric means, was calculated as follows:

$$\%\text{Efficacy} = \frac{(\text{Mean Control - Mean Treated})}{\text{Mean Control}} \times 100$$

3. Results

3.1. Efficacy

Study 1: Placebo-treated animals maintained flea and tick infestations throughout the study (Tables 1–3). Live flea and tick counts for all sarolaner dose groups were significantly lower than the placebo group ($P \leq 0.0032$) on all post-treatment count days.

Sarolaner administered in a suspension formulation at 1.25, 2.5, or 5.0 mg/kg provided 100% reduction in live flea counts 48 h after treatment of the existing infestation, and 100% reduction in live flea counts when evaluated 24 h after each weekly re-infestation for 35 days (Table 1). The 1.25 mg/kg treatment group had efficacies of 99.9 and 98.4% on Days 43 and 56, respectively.

Against *R. sanguineus*, all sarolaner treatments resulted in 100% efficacy within 48 h after treatment and 99.5–100% efficacy against subsequent infestations through Day 28 (Table 2). The higher doses 2.5 and 5.0 mg/kg gave 100% efficacy at Day 35, while the 1.25 mg/kg dose resulted in 96.7% efficacy on Day 35 and this declined to 88.1% on Day 56. Similar efficacy to that shown against *R. sanguineus* was seen with all dosages and formulations of sarolaner

Table 1
Geometric mean flea counts for placebo and sarolaner-treated dogs and percent efficacy relative to placebo for dogs treated orally with sarolaner suspension formulations (Study 1).

Count Day	Placebo	Sarolaner (mg/kg)		
		1.25	2.5	5.0
2	59.8	0.0* (100)	0.0* (100)	0.0* (100)
7	71.2	0.0* (100)	0.0* (100)	0.0* (100)
14	77.8	0.0* (100)	0.0* (100)	0.0* (100)
21	80.1	0.0* (100)	0.0* (100)	0.0* (100)
28	75.3	0.0* (100)	0.0* (100)	0.0* (100)
35	69.4	0.0* (100)	0.0* (100)	0.0* (100)
43	84.5	0.1* (99.9)	–	–
56	76.6	1.3* (98.4)	–	–

$P \leq 0.05$. Percent efficacy is given in parentheses.
* Geometric mean counts are significantly lower than placebo.

Table 2
Geometric mean *Rhipicephalus sanguineus* counts for placebo and sarolaner-treated dogs and percent efficacy relative to placebo for dogs treated orally with sarolaner suspension formulations (Study 1).

Count Day	Placebo	Sarolaner (mg/kg)		
		1.25	2.5	5.0
2	19.3	0.0* (100)	0.0* (100)	0.0* (100)
7	26.0	0.0* (100)	0.0* (100)	0.0* (100)
14	12.7	0.0* (100)	0.0* (100)	0.0* (100)
21	22.3	0.0* (100)	0.1* (99.6)	0.0* (100)
28	17.1	0.0* (100)	0.1* (99.5)	0.0* (100)
35	25.4	0.8* (96.7)	0.0* (100)	0.0* (100)
43	24.6	0.3* (98.6)	–	–
56	23.3	2.8* (88.1)	–	–

$P \leq 0.05$. Percent efficacy is given in parentheses.
* Geometric mean counts are significantly lower than placebo.

Table 3
Geometric mean *Dermacentor reticulatus* counts for placebo and sarolaner-treated dogs and percent efficacy relative to placebo for dogs treated orally with sarolaner suspension formulations (Study 1).

Count Day	Placebo	Sarolaner (mg/kg)		
		1.25	2.5	5.0
2	24.3	0.0* (100)	0.0* (100)	0.0* (100)
7	33.1	0.0* (100)	0.1* (99.7)	0.0* (100)
14	31.9	0.0* (100)	0.0* (100)	0.0* (100)
21	31.0	0.1* (99.7)	0.1* (99.6)	0.0* (100)
28	31.2	0.0* (100)	0.2* (99.4)	0.2* (99.4)
35	25.7	0.5* (98.2)	0.2* (99.3)	0.0* (100)
43	31.5	0.4* (98.6)	–	–
56	29.4	15.4* (47.7)	–	–

$P \leq 0.05$. Percent efficacy is given in parentheses.
* Geometric mean counts are significantly lower than placebo.

against *D. reticulatus* with efficacy maintained at 98.2–100% for 43 days (Table 3).

Study 2: Placebo-treated animals maintained tick infestations following each challenge, though recoveries of *A. americanum* (approx. 3/dog) were markedly lower than the other three tick species (approx. 14–28 per dog) (Tables 4 and 5). Live tick counts for all sarolaner dose groups were significantly lower than the placebo group ($P \leq 0.0279$) on all post-treatment count days for all tick species except for the 0.625 mg/kg group against *A. maculatum* on Day 43 ($P = 0.2827$).

Against an existing infestation of *I. scapularis*, sarolaner at all doses provided 100% reduction in live tick counts 48 h after treatment (Table 4). The two highest doses 1.25 and 2.5 mg/kg resulted in ≥98.8% efficacy against subsequent re-infestations up to Day 43. Persistent efficacy for the 0.625 mg/kg dose was slightly lower at 98.2, 96.6 and 95.0% on Days 28, 35 and 43, respectively.

Table 4
Geometric mean *Ixodes scapularis* counts for placebo and sarolaner-treated dogs and percent efficacy relative to placebo for dogs treated orally with sarolaner suspension formulations (Study 2).

Count Day	Placebo	Sarolaner (mg/kg)		
		0.625	1.25	2.5
2	15.8	0.0* (100)	0.0* (100)	0.0* (100)
7	15.4	0.0* (100)	0.1* (99.4)	0.2* (98.8)
14	17.0	0.3* (98.3)	0.0* (100)	0.1* (99.5)
21	16.0	0.1* (99.4)	0.0* (100)	0.0* (100)
28	16.3	0.3* (98.2)	0.0* (100)	0.0* (100)
35	14.2	0.5* (96.6)	0.0* (100)	0.0* (100)
43	16.2	0.8* (95.0)	0.1* (99.1)	0.0* (100)

$P \leq 0.05$. Percent efficacy is given in parentheses.
* Geometric mean counts are significantly lower than placebo.

Table 5
Geometric mean *Amblyomma americanum* (*A.a*), *Dermacentor variabilis* (*D.v*), and *Amblyomma maculatum* (*A.m*) counts for placebo and sarolaner-treated dogs and percent efficacy relative to placebo for dogs treated orally with sarolaner suspension formulations (Study 2).

Count Day	Tick	Placebo	Sarolaner (mg/kg)		
			0.625	1.25	2.5
2	*A.a*	3.5	0.2* (94.5)	0.4* (89.5)	0.1* (97.4)
28	*A.a*	3.2	1.1* (66.8)	0.4* (88.5)	0.2* (94.0)
35	*D.v*	28.3	10.9* (61.4)	1.1* (96.2)	0.1* (99.7)
43	*A.m*	22.8	15.4 (32.6)	7.2* (68.3)	0.9* (96.1)

$P \leq 0.05$. Percent efficacy is given in parentheses.
* Geometric mean counts are significantly lower than placebo.

Table 6
Geometric mean *Amblyomma maculatum* thumb counts at 24 h after infestation for placebo and sarolaner-treated dogs and percent efficacy relative to placebo for dogs treated orally with sarolaner tablet formulation (Study 3).

Count Day	Placebo	Sarolaner (mg/kg)		
		1.0	2.0	4.0
6	22.6^{a}	2.4^{b} (89.5)	0.4^{c} (98.4)	1.7^{bc} (92.4)
13	29.6^{a}	10.0^{b} (66.2)	2.3^{c} (92.3)	1.5^{c} (94.8)
20	24.5^{a}	7.5^{b} (69.2)	4.9^{b} (79.9)	2.1^{c} (91.6)
27	20.3^{a}	15.4^{ab} (24.4)	12.1^{b} (40.7)	6.8^{c} (66.3)
34	20.9^{a}	14.3^{ab} (31.9)	11.6^{b} (44.7)	4.8^{c} (76.9)

$P > 0.05$. Percent efficacy is given in parentheses.
Geometric mean counts with the same superscript within rows are not significantly different.

For *A. americanum*, Day 2 efficacy versus the existing infestation ranged from 89.5 to 97.4% (Table 5). At Day 28, efficacies for the 0.625, 1.25 and 2.5 mg/kg doses were 66.8, 88.5 and 94.0%, respectively. For *D. variabilis* on Day 35, the respective efficacies were 61.4, 96.2 and 99.7%, and for *A. maculatum* (Day 43) these were 32.6, 68.3 and 96.1%, respectively. Generally, efficacy against these three species was markedly lower than that for *I. scapularis* assessed at the same dose and time point (Tables 4 and 5).

Study 3: Placebo-treated animals maintained *A. maculatum* infestations following each challenge, with mean recoveries of 20.3–29.6 ticks per dog at 24 h thumb counts (Table 6) and 15.2–27.6 ticks per dog at the 48 h comb counts (Table 7). Live tick counts for all sarolaner dose groups were significantly lower than the placebo group ($P \leq 0.0290$) on all post-treatment count days except for the 1.0 mg/kg group thumb counts on Days 27 ($P = 0.1355$) and 34 ($P = 0.1174$).

Tick counts determined by thumb counts showed that high efficacy was achievable within 24 h of infestation but this was variable. The 2 and 4 mg/kg dosages resulted in >90% reduction for 2 and 3 weeks post-treatment, respectively (Table 6).

Table 7
Geometric mean *Amblyomma maculatum* counts at 48 h after treatment and infestation for placebo and sarolaner-treated dogs and percent efficacy relative to placebo for dogs treated orally with sarolaner tablet formulation (Study 3).

Count Day	Placebo	Sarolaner (mg/kg)		
		1.0	2.0	4.0
2	15.2[a]	0.0[b] (100)	0.0[b] (100)	0.0[b] (100)
7	24.6[a]	0.3[b] (98.8)	0.2[b] (99.2)	0.0[b] (100)
14	27.6[a]	0.6[b] (97.7)	0.0[c] (100)	0.1[c] (99.6)
21	23.3[a]	0.0[b] (100)	0.4[b] (98.4)	0.4[b] (98.2)
28	20.9[a]	2.2[b] (89.5)	1.2[bc] (94.2)	0.2[c] (99.1)
35	20.7[a]	3.4[b] (83.4)	1.4[bc] (93.2)	0.4[c] (98.6)

$P > 0.05$. Percent efficacy is given in parentheses.
Geometric mean counts with the same superscript within rows are not significantly different.

All three sarolaner dosages resulted in 100% efficacy against the existing infestation at 48 h. For post-treatment infestations, live tick counts for the 1.0 mg/kg dose were not significantly different to those for the 2.0 mg/kg dose ($P > 0.05$) at all time-points except Day 14, and counts were not different for the 2.0 and 4.0 mg/kg dosages on any day. The 1.0 mg/kg dose only provided >90% efficacy through Day 21 and control declined to <90% thereafter. Both the 2.0 and 4.0 mg/kg doses provided >93% control through Day 35 (Table 7).

3.2. Health observations

There were no adverse health events related to treatment with sarolaner noted in any study.

4. Discussion

In the first study, sarolaner at all doses, was highly effective for the treatment and control of flea and tick infestations on dogs for at least one month. Efficacy against fleas was 100% from treatment through Day 35 and >95% through Day 56. Ticks were less susceptible, though treatment at the lowest dose resulted in ~100% control of both species for at least 4 weeks. In this study, *D. reticulatus* was the least sensitive species, followed by *R. sanguineus* and then by fleas, and the lowest dose of 1.25 mg sarolaner/kg provided treatment and control (>96%) of all three parasite species for at least one month after treatment.

The second study titrated sarolaner doses from 0.625 to 2.5 mg/kg against *I. scapularis* and included point evaluations against three other tick species. *Ixodes scapularis* was highly susceptible, with the lowest dose of sarolaner providing 100% reduction for the existing infestation on Day 2 and >95% efficacy through Day 43. On Day 35 efficacy against *D. variabilis* was >96% for the 1.25 and 2.5 mg/kg dosages, but the 0.625 mg/kg dose only resulted in 61.4% reduction, which was similar to that achieved for *D. reticulatus* in the previous study. Point tests with *Amblyomma* spp. on Days 2, 28, and 43 indicated that this genus was the least susceptible of the ticks tested, as no dose tested resulted in 100% control of an existing infestation of *A. americanum,* and efficacy at Day 28 for this species was markedly lower than that achieved for *D. reticulatus* at the same dose rates in the previous study. Also, the Day 28 efficacy at all dose rates was generally lower for *A. americanum* than for *D. variabilis* at Day 35. Efficacy versus *A. maculatum* at Day 43 was similarly low with the two lower doses (0.625 and 1.25 mg/kg) resulting in <70% control.

Collectively, these two studies indicated that ticks were less susceptible than fleas to sarolaner and that *Amblyomma* spp. were the least sensitive ticks. In Study 2, a dose of 1.25 mg/kg resulted in <90% control of the existing infestation of *A. americanum* and against post-treatment challenge on Day 28, while a dose of 2.5 mg/kg yielded >94% control of an existing infestation of *Amblyomma* spp. and post-treatment challenges for at least 43 days. This suggested that a minimum dose of between 1.25–2.5 mg/kg would be needed to ensure effective treatment and control of fleas and ticks for a month following a single treatment. Based on the markedly better tick recovery from placebo dogs for *A. maculatum* (22.8 ticks/dog) versus *A. americanum* (~3.5 ticks/dog) and the similar efficacy, *A. maculatum* was selected as the species for dose determination in Study 3.

All dose levels of sarolaner (1.0, 2.0 and 4.0 mg/kg) provided 100% efficacy against an existing infestation of *A. maculatum* within 48 h post-treatment in Study 3. The lowest dose (1.0 mg/kg) provided >90% reduction of subsequent infestations at 48 h through Day 21 but efficacy declined to <90% thereafter, while both the 2.0 and 4.0 mg/kg doses resulted in >90% efficacy through Day 35. There was no significant improvement in efficacy provided by the 4.0 mg/kg dose over the 2.0 mg/kg dose ($P > 0.05$) at any time point. The 2.0 mg/kg dose was not significantly superior to the 1.0 mg/kg on any day except Day 14 ($P = 0.0085$), however, efficacy for the 1 mg/kg dose declined below 90% at Day 28, indicating that a single dose was unlikely to provide optimal tick control for a full month. Thus, the 2.0 mg/kg dose rate was selected as the minimum dose rate to provide flea and tick control for at least one month following a single oral treatment.

5. Conclusions

These studies demonstrated that a dose rate of 2.0 mg sarolaner/kg provided effective treatment and control of fleas and ticks on dogs for one month after a single oral treatment.

Conflict of interest

The studies reported here were funded by Zoetis, Florham Park, NJ. TLM, RHS, AP, LH, SPM, and MRM are current employees of Zoetis. JJF was an independent investigator contracted for the study conducted in RSA. 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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