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Efficacy of sarolaner in the prevention of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* transmission from infected *Ixodes scapularis* to dogs

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ABSTRACT

The efficacy of sarolaner (Simparica™, Zoetis) to prevent transmission primarily of *Borrelia burgdorferi* and secondarily of *Anaplasma phagocytophilum* from infected wild-caught *Ixodes scapularis* to dogs was evaluated in a placebo-controlled laboratory study. Twenty-four purpose-bred laboratory Beagles seronegative for *B. burgdorferi* and *A. phagocytophilum* antibodies were allocated randomly to one of three treatment groups: placebo administered orally on Days 0 and 7, or sarolaner at 2 mg/kg administered orally on Day 0 (28 days prior to tick infestation) or on Day 7 (21 days prior to tick infestation). On Day 28, each dog was infested with approximately 25 female and 25 male wild caught adult *I. scapularis* that were determined to have prevalence of 57% for *B. burgdorferi* and 6.7% for *A. phagocytophilum* by PCR. *In situ* tick counts were conducted on Days 29 and 30. On Day 33, all ticks were counted and removed. Acaricidal efficacy was calculated based on the reduction of geometric mean live tick counts in the sarolaner-treated groups compared to the placebo-treated group for each tick count. Blood samples collected from each dog on Days 27, 49, 63, 77, 91 and 104 were tested for the presence of *B. burgdorferi* and *A. phagocytophilum* antibodies using the SNAP® 4Dx® Plus Test, and quantitatively assayed for *B. burgdorferi* antibodies using an ELISA test. Skin biopsies collected on Day 104 were tested for the presence of *B. burgdorferi* by bacterial culture and PCR.

Geometric mean live tick counts for placebo-treated dogs were 14.8, 12.8, and 19.1 on Days 29, 30, and 33, respectively. The percent reductions in mean live tick counts at 1, 2, and 5 days after infestation were 86.3%, 100%, and 100% for the group treated with sarolaner 21 days prior to infestation, and 90.9%, 97.1%, and 100% for the group treated with sarolaner 28 days prior to infestation. Geometric mean live tick counts for both sarolaner-treated groups were significantly lower than those for the placebo group on all count days ($P < 0.0001$).

There were no adverse reactions to treatment with sarolaner. Transmission of *B. burgdorferi* to all eight placebo-treated dogs was confirmed by positive antibody (6 of 8 dogs), PCR (7 of 8 dogs), and/or culture (7 of 8 dogs). Similarly, transmission of *A. phagocytophilum* was confirmed by the presence of antibodies in four placebo-treated dogs. In contrast, treatment with a single dose of sarolaner prevented transmission of *B. burgdorferi* from infected ticks to dogs infested 21 or 28 days after treatment as demonstrated by negative antibody, PCR, and culture results. Prevention of transmission of *A. phagocytophilum* was demonstrated by negative antibody results in all sarolaner-treated dogs.

1. Introduction

Ticks are vectors for a number of serious disease causing organisms affecting dogs, man and other animals. Canine Lyme disease, caused by *Borrelia burgdorferi*, and canine anaplasmosis, caused by *Anaplasma phagocytophilum*, are bacterial diseases transmitted by ticks that can affect humans and animals wherever the vector and a pathogen reservoir is present. These pathogens are transmitted by various *Ixodes* tick species in different parts of the world. In the United States the primary vector is *Ixodes scapularis*, with the exception of the pacific coast where it is *I. pacificus*. *Ixodes ricinus* is the primary vector for both organisms in Europe (Chomel, 2011).

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In Asia, *Ixodes persulcatus* is a vector for both organisms, and *Dermacentor silvarum* is also a vector for *A. phagocytophilum* (Chomel, 2011).

Infection with either *B. burgdorferi* or *A. phagocytophilum* can cause a variety of clinical signs in dogs, ranging from asymptomatic sub-clinical infection to acute life-threatening or chronic conditions, and co-infection can increase the incidence and severity of these signs (Beall et al., 2006; Littman et al., 2006). *I. scapularis* can be found throughout the northeastern, north central, southeastern and south central United States (Dennis et al., 1998; Brownstein et al., 2003), and appears to be expanding into new areas (Ogden et al., 2009). In endemic Lyme areas, prevalences of *B. burgdorferi* and *A. phagocytophilum* in *I. scapularis* adults of 50.3% and 61%, respectively, have been demonstrated (Schulze et al., 2005). Because of the increasing recognition of the importance of infections with tick-borne pathogens on the health of dogs, it is currently recommended that pet owners take measures to reduce tick populations in the dogs' environment, frequently examine and immediately remove any ticks found on the dog, and to use an approved product year-round to protect dogs against unexpected exposure to ticks (Companion Animal Parasite Council, 2015).

Sarolaner (Simparica™, Zoetis) is a new isoxazoline that has been developed for the treatment and month long control of ticks and fleas on dogs following a single oral dose (McTier et al., 2016). Laboratory studies confirmed that sarolaner was effective for the entire month against the major species of ticks infesting dogs in Europe and the United States (Geurden et al., 2016; Six et al., 2016a). The current study was conducted to evaluate the efficacy of a single oral treatment of sarolaner at a dosage of 2 mg/kg to protect dogs primarily from *B. burgdorferi* and secondarily from *A. phagocytophilum* infections transmitted by *I. scapularis*.

2. Materials and methods

This laboratory study was conducted in the United States 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). The study protocol was reviewed and approved by the Zoetis Institutional Animal Care and Use Committee.

2.1. Animals

Purpose bred, Beagles obtained from a commercial supplier were used. The 12 male and 12 female dogs ranged in age from 11 to 13 months, and weighed between 6.0 and 13.3 kg at the time of treatment. Each dog was uniquely identified by an ear tattoo. Dogs had not been previously infested with ticks, vaccinated against *B. burgdorferi*, nor used in any experimental evaluation.

Dogs were housed individually in indoor stainless steel raised runs approximately 4 feet by 6 feet with tender-foot flooring. Enclosures conformed to accepted animal welfare guidelines and ensured no direct contact between dogs. Dogs were fed an appropriate maintenance ration of a commercial dry canine feed for the duration of the study. Water was available *ad libitum*. From Day 0 (the day of first study treatment) to the end of the study, study participants handling dogs used separate protective clothing (e.g., laboratory coat, coveralls or apron, disposable gloves, and booties) for dogs in each treatment group.

2.2. Masking

All study personnel who were involved in post-treatment health observations, tick counts, selection of biopsy sites, biopsy collection, serology analysis, and tissue analysis were unaware of treatment allocation.

2.3. Health observations

A physical exam was performed on each dog by a veterinarian to determine health and suitability prior to inclusion in the study. Dogs were assessed at least twice daily for the duration of the study for signs of abnormal health. Dogs were examined at 1, 3, 6, and 24 h after each treatment for possible adverse reactions. Beginning six days after infestation with ticks, daily observations included evaluation for clinical signs specific to tick-borne disease, including but not limited to lameness, ataxia and lethargy.

2.4. Allocation to treatment

Dogs were ranked within sex by decreasing body weight into blocks of three and randomly allocated to one of three treatment groups: placebo administered on Days 0 and 7, sarolaner administered on Day 0 (28 days prior to tick infestation), or sarolaner administered on Day 7 (21 days prior to tick infestation). To maintain masking, placebo was administered to dogs in the sarolaner groups on days when sarolaner was not administered. Blocks of dogs were randomly assigned to adjacent pens within the test facility. Dogs were moved into their allocated pens on Day -2.

2.5. Treatment administration

The commercial Simparica™ chewable tablet formulation was used. The placebo formulation was similar to the active formulation, but did not contain sarolaner. For dogs in the sarolaner-treated groups, 40 mg sarolaner tablets were shaved and/or sanded based on each dog's individual bodyweight to deliver a sarolaner dosage of 2.0 mg/kg. Dogs in the placebo-treated group received a single placebo tablet that matched the active 40 mg tablet in size and appearance. Dogs were hand pilled to ensure accurate and complete dose delivery. Each dog was observed for several minutes to ensure the dose was swallowed and then periodically for up to two hours for signs of emesis.

2.6. Ticks

Adult *I. scapularis* ticks were collected from Lyme endemic regions in Rhode Island between October and November, 2012. Ticks were maintained at 5–15 °C under saturated humidity conditions for 4–8 weeks until used to infest the dogs. A random sample of 30 adult *I. scapularis* female and male ticks of this field collected population was tested by PCR for the presence of *B. burgdorferi* and *A. phagocytophilum* DNA using published methods (McCall et al., 2011).

2.7. Tick infestation and counting

On Day 28, a pre-counted aliquot of approximately 25 female and 25 male adult *I. scapularis* were placed onto the hair coat of each dog and allowed to disperse. Following infestation, dogs were placed in travel crates in a darkened room for approximately six hours to enhance tick attachment.

Tick counts were performed by personnel trained in the standard procedures in use at the test facility. On Days 29 and 30, the numbers of live ticks (attached and free) were determined *in situ* by careful and systematic examination of the entire dog's body and by pushing the hair against its natural nap to expose ticks. On Day 33, ticks were counted and removed. After the manual inspection, an

extra-fine tooth comb (commercial flea comb) was used to comb the dog to remove any otherwise missed ticks.

2.8. Sample collection and testing

2.8.1. Blood

Blood samples were collected from each dog on Days -6, 27, 49, 63, 77, 91 and 104 (i.e., before treatment, before tick infestation, and at 21, 35, 49, 63, and 76 days after tick infestation).

At each time point, serum was separated from each sample and an aliquot assayed using the SNAP® 4Dx® Plus test (IDEXX Laboratories, Inc., Westbrook, Maine, USA) using the procedures described in the package insert. The SNAP® 4Dx® Plus Test kit is an ELISA for simultaneous detection of canine antibodies to *Ehrlichia canis, Ehrlichia ewingii, B. burgdorferi, A. phagocytophilum*, and *A. platys,* and for *Dirofilaria immitis* antigen. Sensitivity of this test for *B. burgdorferi* has been reported to be 94.4% when compared to a combination of immunofluorescence assay and Western blot (O'Connor et al., 2004), and the specificity to be 99.5% (Duncan et al., 2004). The *A. phagocytophilum* sensitivity and specificity have been reported to be 99.1% and 100%, respectively, relative to the immunofluorescence assay (Chandrashekar et al., 2007).

A second aliquot of serum was tested for *B. burgdorferi* quantitative antibody levels to OspA by ELISA. Briefly, 96-well microtiter plates were coated with recombinant purified OspA antigen in 0.01 M Borate buffer and incubated overnight at 4 °C. After discarding the coating solution, plates were blocked with 200 μL 1% Casein in PBS containing 0.05% Tween 20® and incubated at 37 °C for 60 (±5) min. Blocked plates were washed three times with PBS-Tween 20®. Test serum samples were diluted in PBS-Tween 20® to the appropriate start dilution. Positive and negative control samples (OspA) were diluted accordingly. After transferring the test and control samples to the test plate, plates were incubated at 37 °C for 60 (±5) min. Plates were washed for three times with PBS-Tween 20®. Conjugate [goat anti-dog IgG(H + L) antibody, peroxidase labeled] was diluted to the appropriate dilution in PBS-Tween 20® and added to each well. Plates were incubated at 37 °C for 60 (±5) min and then washed for three times with PBS-Tween 20®. ABTS substrate was prepared and added to each well and plates were incubated for 10–15 min at room temperature. Optical densities (OD) were determined and test sample titers were calculated from the average plus three standard deviations of the negative control OD value. Test sample dilutions above the negative control OD represented the end point titer. Testing was repeated if the positive or negative controls were not in the expected range.

2.8.2. Skin biopsies

On Day 33, after all ticks were removed, the hair from the four areas with the highest number of attached ticks or with the most notable inflammation from an attached tick on each dog was shaved to identify the sites for future biopsy. If an area of the face, ears or head was one of the identified sites, an area of the dog's neck close to the site was shaved to reduce animal discomfort. If less than four areas of tick attachment or inflammation were present, up to four of the most common areas of tick attachment or inflammation from dogs with infestations were shaved so that a total of four sites were shaved on each dog. To maintain site location, the same sites were shaved again on Days 49, 63, 77, 91 and 104.

On Day 104, two 4.0 mm punch skin biopsies were collected from each of the four shaved locations using standard aseptic surgical procedures. One biopsy from each site was used for bacterial culture and PCR testing, and the second sample was stored at ≤20 °C as a backup.

Skin biopsies were cultured to isolate *B. burgdorferi* and tested for the presence of *B. burgdorferi* DNA by PCR using published methods (Nocton et al., 1994; Chang et al., 2001).

2.9. Data analysis

The individual dog was the experimental unit. Statistical analyses were performed using SAS 9.2 (SAS, Cary NC).

2.9.1. Tick counts

Live tick counts were subjected to the $\log_e$(count +1) transformation prior to analysis in order to stabilize the variance and normalize the data. Using the PROC MIXED procedure, transformed live tick counts (attached and free) were analyzed using a mixed linear model for repeated measures. The model included the fixed effects of sex, treatment, day of study and the interaction among those effects. The random effects included block within sex, the interaction between block and treatment within sex (animal term) and error. *A Priori* contrasts were used to assess pair-wise comparisons between treatments at each time point.

No interactions involving both sex and time point were significant, but there were significant treatment effects therefore treatment differences were assessed at each time point. Testing was two-sided at the significance level α=0.05. Percent efficacy, relative to the placebo group was calculated as follows:

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

2.9.2. B. burgdorferi *antibody, culture and PCR, and* A. phagocytophilum *antibody*

Frequency distributions of positive/negative antibodies, cultures and PCR for *B. burgdorferi* and antibodies for *A. phagocytophilum* were calculated for each treatment at each time point. Frequency distributions of 'ever positive' (a dog was defined as being 'ever positive' if it ever tested positive for *B. burgdorferi* by the presence of antibodies, or positive culture, or positive PCR test) for *B. burgdorferi* and positive for *A. phagocytophilum* antibody were calculated for each treatment and a Fisher's exact test was used to test for overall treatment effects and treatment differences.

2.9.3. *Quantitative* B. burgdorferi *antibody titers by ELISA*

Antibody titers were transformed $\log_e$(titer) prior to analysis. The transformed titers were analyzed with a general linear mixed model for repeated measures. The model included the fixed effects of sex, treatment, time point and all interactions among those effects. The random effects included block within sex, the interaction of block and treatment within sex (animal term), and error. Mean centered Day -6 $\log_e$(titer) data was used as a covariate for the repeated measures model for log transformed antibody titers to adjust for differences in log transformed titers prior to treatment.

No interactions involving both sex and time point were significant, but there were significant treatment effects therefore treatment differences were assessed at each time point. All tests were done at the two-tailed 5% level of significance. Least squares means at each time point and 95% confidence intervals were back-transformed to obtain the geometric means and their corresponding confidence intervals.

3. Results

3.1. *Live* I. scapularis *counts and infection prevelance*

PCR analysis of a random sample of 30 ticks from the study population confirmed prevalence rates of 57% for *B. burgdorferi* and 6.7%

Table 1
Efficacy of a single oral treatment of sarolaner (2 mg/kg): Live *Ixodes scapularis* counts, ranges, and number of dogs with ticks.

Treatment group	Time of count after infestation	Number of dogs with live ticks	Live *I. scapularis* count		
			Range	Geometric mean	Percent reduction relative to placebo
Placebo	1 day	8 of 8	12–21	14.8	–
	2 days	8 of 8	8–17	12.8	–
	5 days	8 of 8	15–25	19.1	–
Sarolaner	1 day	6 of 8	0–6	1.3*	90.9%
28 days prior to infestation	2 days	3 of 8	0–2	0.4*	97.1%
	5 days	0 of 8	0–0	0.0*	100%
Sarolaner	1 day	7 of 8	0–5	2.0*	86.3%
21 days prior to infestation	2 days	0 of 8	0–0	0.0*	100%
	5 days	0 of 8	0–0	0.0*	100%

* Geometric mean count significantly lower than placebo ($P < 0.0001$).

for *A. phagocytophilum*. Live ticks were found on all eight placebo-treated dogs on all count days, with a geometric mean (range) of 14.8 (12–21), 12.8 (8–17), and 19.1 (15–25) live ticks at 1, 2, and 5 days after infestation, respectively (Table 1). One day after infestation, live ticks were found on 13 of 16 sarolaner-treated dogs (live tick count range 0–6), two days after infestation, live ticks were found on only three of eight dogs treated 28 days prior to infestation (live tick count range 0–2) and no ticks were found on the dogs treated 21 days prior to infestation. Five days after infestation, no live ticks were found on any sarolaner-treated dog. Relative to placebo, live tick counts at 1, 2, and 5 days after infestation were reduced by 90.9%, 97.1%, and 100% for dogs treated 28 days prior to infestation, and by 86.3%, 100%, and 100% for dogs treated 21 days prior to infestation. Geometric mean live tick counts were significantly lower than placebo on all Days ($P < 0.0001$).

3.2. B. burgdorferi *and* A. phagocytophilum *serum antibody by SNAP® 4Dx® plus*

All dogs were negative for antibodies to *B. burgdorferi* prior to study treatment. *B. burgdorferi* antibody was detected in six of the eight placebo dogs on at least one post-infestation day. Two dogs were positive on Day 63, five on Days 77 and 91, and six on Day 104. None of the 16 sarolaner-treated dogs were positive for *B. burgdorferi* antibody at any time-point (Table 2).

All dogs were negative for antibodies to *A. phagocytophilum* prior to study treatment. *A. phagocytophilum* antibody was detected in four of the eight placebo dogs on at least one post-infection day. One dog was positive on Day 63, and four on Days 77, 91, and 104. None of the 16 sarolaner-treated dogs were positive for *A. phagocytophilum* antibody at any time point (Table 3). The number of sarolaner-treated dogs positive for *A. phagocytophilum* antibody was not significantly different from placebo-treated dogs ($P = 0.0769$).

3.3. B. burgdorferi *PCR and culture from skin biopsies*

In seven of the eight placebo-treated dogs, the presence of *B. burgdorferi* in two or more of the four skin biopsy samples was confirmed by PCR and culture. *B. burgdorferi* was not detected by PCR or culture in any of the four skin biopsies collected from one of the placebo-treated dogs (Table 4). *B. burgdorferi* was not detected by PCR or culture in any of the skin biopsies collected from any of the 16 sarolaner-treated dogs.

3.4. *Dogs 'ever positive' for* B. burgdorferi

For placebo-treated dogs, *B. burgdorferi* antibody was present on at least one post-infestation time-point, or *B. burgdorferi* was detected by PCR or isolated by culture in at least one skin biopsy. Thus, all eight placebo-treated dogs were 'ever positive' for *B. burgdorferi*. For sarolaner-treated dogs, *B. burgdorferi* antibody was not detected at any time-point, and *B. burgdorferi* was not detected by PCR nor isolated by culture in any of the four skin biopsies. Therefore none of the sarolaner-treated dogs were 'ever positive' for *B. burgdorferi*. The number of sarolaner-treated dogs 'ever positive' for *B. burgdorferi* was significantly lower than that for the placebo-treated dogs ($P = 0.0002$).

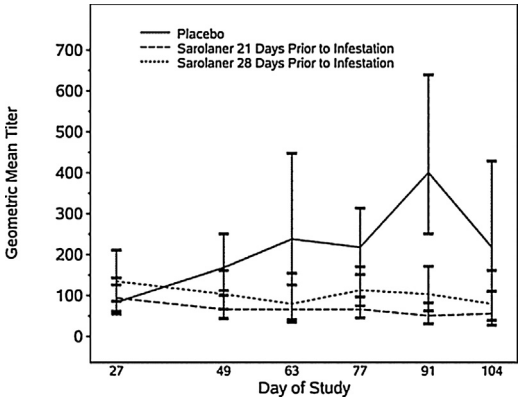

Fig. 1. *Borrelia burgdorferi* ELISA titers in dogs treated orally with placebo or sarolaner (2 mg/kg) on Day 0 or 7 and infested with infected *Ixodes scapularis* on Day 28. Error bars represent back-transformed 95% confidence intervals.

3.5. *Quantitative* B. burgdorferi *antibody titers by ELISA*

Following tick infestation, antibody titers to *B. burgdorferi* in placebo-treated dogs increased markedly, while those for both sarolaner-treated groups remained generally unchanged (Fig. 1). Antibody titers for the group treated with sarolaner 21 days prior to infestation were significantly lower than those for the placebo group on all post-infestation days ($P \leq 0.0078$). Antibody titers for the group treated with sarolaner 28 days prior to infestation were not significantly different than placebo on Day 49 ($P = 0.1227$), but were significantly lower on all subsequent post-infestation days ($P \leq 0.0475$). Antibody titers for the sarolaner group treated 21 days prior to infestation were not significantly different than those for the group treated 28 days prior to infestation on any post-infestation day ($P \geq 0.0552$).

3.6. *Health observations*

There were no adverse events related to treatment with sarolaner. One placebo-treated dog was noted to have forelimb lameness on Days 64 and 92. Both occurrences responded to symptomatic treatment with oral carprofen.

Table 2
SNAP® 4Dx® Plus test results for *Borrelia burgdorferi* antibody in dogs treated orally with placebo or sarolaner (2 mg/kg) on Day 0 or 7 and infested with infected *Ixodes scapularis* on Day 28.

		Borrelia burgdorferi antibody negative (−)/positive (+)						
Treatment group	Animal	Day -6	Day 27	Day 49	Day 63	Day 77	Day 91	Day 104
Placebo	1879066	–	–	–	–	+	+	+
	1892208	–	–	–	+	+	+	+
	1901011	–	–	–	–	+	+	+
	1908040	–	–	–	+	+	+	+
	1908538	–	–	–	–	–	–	+
	1909241	–	–	–	–	+	+	+
	1909577	–	–	–	–	–	–	–
	1911962	–	–	–	–	–	–	–
Sarolaner21 or 28 days prior to infestation		All sarolaner-treated dogs were negative for *B. burgdorferi* antibody on all Days						

Table 3
SNAP® 4Dx® Plus test results for *Anaplasma phagocytophilum* antibody in dogs treated orally with placebo or sarolaner (2 mg/kg) on Day 0 or 7 and infested with infected *Ixodes scapularis* on Day 28.

		Anaplasma phagocytophilum antibody negative (−)/positive (+)						
Treatment group	Animal	Day-6	Day 27	Day 49	Day 63	Day 77	Day 91	Day 104
Placebo	1879066	–	–	–	–	+	+	+
	1892208	–	–	–	–	–	–	–
	1901011	–	–	–	–	–	–	–
	1908040	–	–	–	+	+	+	+
	1908538	–	–	–	–	–	–	–
	1909241	–	–	–	–	+	+	+
	1909577	–	–	–	–	–	–	–
	1911962	–	–	–	–	+	+	+
Sarolaner21 or 28 days prior to infestation		All sarolaner-treated dogs were negative for *A. phagocytophilum* antibody on all Days						

Table 4
Borrelia burgdorferi PCR and culture results from skin biopsies collected on Day 104 (76 days after infestation with infected *Ixodes scapularis* on Day 28) from dogs treated orally with placebo or sarolaner (2 mg/kg).

		Borrelia burgdorferi PCR and culture results negative (−)/positive (+)							
		Biopsy site 1		Biopsy site 2		Biopsy site 3		Biopsy site 4	
Treatment group	Animal	PCR	Culture	PCR	Culture	PCR	Culture	PCR	Culture
Placebo	1879066	+	+	+	+	+	+	–	–
	1892208	+	+	+	+	–	–	–	–
	1901011	+	+	+	+	+	+	–	–
	1908040	+	+	+	+	+	+	–	–
	1908538	+	+	+	+	–	–	+	–
	1909241	–	–	–	–	–	–	–	–
	1909577	+	+	+	+	+	+	+	+
	1911962	+	+	+	+	+	+	–	–
Sarolaner21 or 28 days prior to infestation		Skin biopsies collected from sarolaner-treated dogs were negative for *B. burgdorferi* by PCR and bacterial culture							

4. Discussion

B. burgdorferi was successfully transmitted to all eight placebo-control dogs, but not to any of the sarolaner-treated dogs. Despite the markedly lower prevalence of *A. phagocytophilum* (~10% of that for *B. burgdorferi*), four of the eight placebo-control dogs became infected. Although not significantly different to the placebo-control group, *A. phagocytophilum* was not transmitted to any of the sarolaner-treated dogs. Therefore in this study, sarolaner protected dogs against the transmission of *B. burgdorferi* and *A. phagocytophilum* when exposed to infected *I. scapularis* ticks up to 28 days after treatment.

The time required for a tick to feed before pathogen transmission occurs is dependent on many factors, and varies between tick species and disease agents. Laboratory studies have shown that *I. scapularis* only occasionally transmits *B. burgdorferi* before 24–48 h of feeding, and that transmission occurs to a greater extent with longer feeding periods (Kidd and Breitschwerdt, 2003). This period of time between initiation of feeding and pathogen transmission is likely a result of the time it takes for the organism to migrate within the tick from the mid gut to the salivary glands where it can be transmitted via saliva during feeding (de Silva and Fikrig, 1995; Piesman, 1995; Schwan and Piesman, 2000). *A. phagocytophilum* has been found in the salivary glands of unfed *I. scapularis,* and therefore it is postulated that it is more likely to be quickly transmitted (de Silva and Fikrig, 1995; Kidd and Breitschwerdt, 2003). However, transmission times demonstrated in laboratory studies with mice have been variable, ranging from less than 24 to more than 40 h (Kidd and Breitschwerdt, 2003).

In a study conducted to evaluate the speed of kill of a single oral dose of sarolaner at a dosage of 2 mg/kg against *I. scapularis*, live tick counts were significantly reduced relative to placebo at eight hours after treatment, and efficacy reached 98.8% at 12 h, and was 100% at 24 h post-treatment (Six et al., 2016b). A rapid speed of kill was maintained throughout the month with ≥95.7% efficacy at 24 h after re-infestation for 28 days. This rapid speed of kill, combined with the prevention of pathogen transmission observed in the current study suggests that the infected *I. scapularis* ticks were killed before *B. burgdorferi* and *A. phagocytophilum* transmission could occur.

The ability of a topical application of fipronil, amitraz, and (S)-methoprene to prevent the transmission of *B. burgdorferi* and *A. phagocytophilum* from infected *I. scapularis* to dogs has been previously reported (McCall et al., 2011). One of the perceived benefits of topically applied acaricidal products is that ticks will be killed before attachment and commencement of feeding, therefore reducing the risk of pathogen transmission. The results of the current study indicate that an orally administered acaricide with a consistent and rapid speed of kill can also reduce the risk of tick-borne pathogen transmission.

In order to reduce the risk of tick-borne disease, it is recommended that pet owners take steps to reduce pet exposure to ticks, including the year-round use of approved ectoparasiticides to protect pets from unexpected tick exposure. The recommendation for year-round use is especially important with regards to *I. scapularis,* because the adults are most active and likely to feed on dogs in the cooler months, when the need for ectoparasite control might not typically be considered. Laboratory studies have confirmed the efficacy of sarolaner for the entire month against the major species of ticks infesting dogs in Europe and the United States (Geurden et al., 2016; Six et al., 2016a). As the speed of kill and consequent interruption of feeding is critical to reduce the risk of tick-borne pathogen transmission, laboratory studies were also conducted to evaluate the speed of kill of sarolaner against *I. ricinus* and *A. maculatum* (Six et al., 2016b). Following treatment, live tick counts were significantly reduced relative to placebo at eight hours post treatment, reached 90.1% against *I. ricinus* and 99.2% against *A. maculatum* within 12 h, and provided 100% efficacy at 24 h after treatment against both species. This rapid speed of kill was maintained throughout the month with ≥98.7% and ≥89.6% efficacy against *I. ricinus* and *A. maculatum*, respectively, at 24 h after re-infestation at least through Day 28.

An initial attachment and feeding of at least 24–48 h is required before transmission of most tick-borne pathogens can occur (Salinas et al., 2010; Little et al., 2014). If the infected ticks are killed within that time period, the transmission may be prevented (Wengenmayer et al., 2014). With its rapid speed of kill and sustained high efficacy within 24 h for a full month after a single dose, sarolaner is likely to effectively reduce the chances of ticks surviving and/or feeding for this critical time period. Therefore the use of sarolaner in a tick-control program should reduce the risk of dogs becoming infected with tick-borne diseases as well as minimize the deleterious health effects associated with tick infestation.

5. Conclusions

In this controlled laboratory study, a single oral dose of sarolaner (Simparica™) successfully prevented the transmission of *B. burgdorferi* and *A. phagocytophilum* from infected wild-caught *I. scapularis* to dogs for 28 days.

Conflict of interest

The study reported here was funded by Zoetis, Florham Park, NJ. Nicole A. Honsberger, Robert H. Six, Thomas J. Heinz, Angela Weber, Sean P. Mahabir, and Thomas C. Berg are current employees of Zoetis. All authors assisted with the design and conduct of the study, interpretation of the data and manuscript review. There were no conflicting interests that could have influenced the conduct and reporting of this study.

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