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Efficacy of a novel oral formulation of sarolaner (Simparica™) against four common tick species infesting dogs in Europe

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ABSTRACT

The efficacy of single oral treatment of sarolaner (Simparica™, Zoetis), a novel isoxazoline compound, was evaluated against four tick species known to commonly infest dogs in Europe. Eight laboratory studies were conducted using adult purpose-bred Beagle dogs. In each study, 16 animals were randomly allocated to one of two treatment groups based on pre-treatment host-suitability tick counts. Dogs were infested with 50 unfed adult *Dermacentor reticulatus* (two studies), *Ixodes hexagonus* (three studies), *Ixodes ricinus* (two studies) or *Rhipicephalus sanguineus* (one study) ticks on Days −2, 5, 12, 19, 26 and 33. On Day 0, dogs were treated orally with placebo or sarolaner tablets providing the minimum dose of 2.0 mg/kg bodyweight and tick counts were conducted 48 h after treatment and after each subsequent weekly re-infestation. There were no treatment-related adverse reactions in any of the studies. Dogs in the placebo-treated group maintained tick infestations throughout the studies. Geometric mean live tick counts were significantly ($P \leq 0.0001$) lower in the sarolaner-treated group compared to the tick counts in the placebo group at all time-points. A single oral administration of sarolaner resulted in 100% efficacy against existing infestations of all tick species except *R. sanguineus,* for which the efficacy was 99.7%, within 48 h. Efficacy against weekly re-infestations was ≥97.5% for all tick species for 35 days.

Thus, a single dose of sarolaner administered orally at the minimum dosage of 2 mg/kg, resulted in ≥99.7% efficacy within 48 h against existing tick infestations, and in ≥97.5% efficacy against weekly re-infestations, for at least 35 days after treatment. These studies confirmed that administration of the minimum dose of sarolaner will provide treatment of existing infestations and give at least one month of control against re-infestation by the common tick species affecting dogs in Europe.

1. Introduction

Ticks are one of the most common ectoparasites infesting dogs. Tick infestation can lead to nuisance, alopecia and skin irritation. Heavy infestations can even lead to anemia (Dryden and Payne, 2004). Canine tick infestations are thus of direct veterinary importance to the animal, but are also important in the distribution of tick-borne diseases (Needham and Teel, 1991; Beugnet and Marié, 2009). Ticks are responsible for the transmission of a number of disease agents, of which some are zoonotic and some cause serious, even life-threatening conditions (Dryden and Payne, 2004). Zoonotic infections include Lyme disease caused by *Borrelia burgdorferi*, which is transmitted by *Ixodes* species (Beugnet and Marié, 2009). Other tick-borne pathogens cause predominantly dog-specific infections, such as *Babesia canis,* primarily transmitted by *Dermacentor* spp., and *Ehrlichia canis*, primarily transmitted by *Rhipicephalus sanguineus* (Chomel, 2011; Dantas-Torres et al., 2012).

Tick prevention has historically been based on the monthly use of acaricidal compounds applied as topical formulations (Dryden and Payne, 2004; Rust, 2005) but recently two isoxazoline compounds for use in dogs have been introduced in Europe that provide treatment and prevention of flea and tick infestations after oral treatment (Robertson-Plouch et al., 2008; Rohdich et al., 2014;

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Shoop et al., 2014). Sarolaner (Simparica™, Zoetis) is a novel isoxazoline with potent activity against ectoparasites (McTier et al., 2016). The objective of this series of studies was to evaluate the efficacy of sarolaner against four tick species of major importance in Europe, each of which has potential to transmit disease organisms (Beugnet and Marié, 2009). Eight laboratory studies were conducted to evaluate the efficacy of sarolaner against existing tick infestations and against re-infestations for a period of five weeks after treatment.

2. Materials and methods

The eight laboratory studies were conducted to evaluate against the following tick species commonly infesting dogs in Europe: *Dermacentor reticulatus* (ornate dog tick; two studies), *Ixodes hexagonus* (hedgehog tick; three studies), *Ixodes ricinus* (castor bean tick; two studies), and *R. sanguineus* (brown dog tick; one study). 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 (VICH guideline GL9, 2000). All studies were approved by the Zoetis Ethical Review Committee and by the study site's Ethical Review Committee.

2.1. Animals

All dogs used in these studies had not been treated with an ectoparasiticide for at least 60 days, had demonstrated good tick retention prior to treatment, and were in good health at enrollment. Sixteen different purpose-bred Beagles, including both sexes, were enrolled in each study. Dogs ranged in age from 12 months to 7 years, and weighed between 8.3 kg and 32.0 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. Study 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. For tick infestations, a pre-counted aliquot of approximately 50 adult unfed ticks were placed onto the hair coat and allowed to disperse on the dog. Ticks were applied in an approximate 1:1 sex ratio, with the exception of one *I. ricinus* study in which a 3:2 female to male ratio was used as this was indicated per previous use for that tick strain.

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 (movement and reaction to CO_2 stimulation) and the numbers of live ticks was quantified.

At least 16 animals arrived into the housing facilities on or before Day −7. 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 −9 and Day −7. The live attached ticks present on each dog were counted and removed at 48 (±2) hours after infestation. The 16 dogs with the highest counts were selected for inclusion, 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 placebo (Simparica™ formulation without active ingredient sarolaner) 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 a minimum of 1 min 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. In all studies except two of the three conducted against *I. hexagonus*, animals were subsequently re-infested with ticks on Days 5, 12, 19, 26 and 33. In one *I. hexagonus* study dogs were re-infested only on Days 19 and 26, and in one study only on Day 33. Dogs were examined, combed and parasites counted 48 (±2) hours after each infestation.

Ticks were sourced from multiple laboratory maintained colonies with isolates all originating from Europe. These ticks were originally isolated from the field, and new ticks had been introduced into each colony within the previous ten years.

2.3. 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 8.2, 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. Testing was two-sided at the significance level $\alpha = 0.05$. Percent efficacy was calculated using Abbott's formula:

$$\%\text{reduction} = 100 \times \frac{\text{meancount(placebo)} - \text{meancount(treated)}}{\text{meancount(placebo)}}$$

3. Results

3.1. Efficacy

Dogs in the placebo-treated group maintained tick infestations throughout the studies (Tables 1–4).

For *D. reticulatus*, efficacy against existing infestations was 100% at 48 h after treatment in both studies. Against subsequent weekly re-infestations, efficacy 48 h after infestation was ≥99.0% in one study and 100% in the second study through 35 days post treatment (Table 1). Efficacy against existing infestations of *I. hexagonus*

Table 1
Geometric (arithmetic) mean live *Dermacentor reticulatus* (ornate 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.

Tick Strain Origin		Placebo		Sarolaner		
	Day	Mean	Range	Mean	Range	% Efficacy
Europe (various countries)	2	23.4 (26.5)	9– 44	0.0* (0.0)	0–0	100
	7	19.1 (20.5)	10–34	0.0* (0.0)	0–0	100
	14	11.1 (12.1)	4–20	0.0* (0.0)	0–0	100
	21	24.5 (25.1)	14–31	0.0* (0.0)	0–0	100
	28	16.4 (18.0)	6–37	0.0* (0.0)	0–0	100
	35	16.7 (18.8)	6–38	0.0* (0.0)	0–0	100
Ireland/The Netherlands	2	19.5 (21.3)	10–35	0.0* (0.0)	0–0	100
	7	20.9 (23.3)	8–41	0.0* (0.0)	0–0	100
	14	19.4 (20.4)	13–34	0.2* (0.4)	0–3	99.0 (98.2)
	21	18.7 (20.8)	10–37	0.0* (0.0)	0–0	100
	28	18.0 (18.9)	12–31	0.1* (0.1)	0–1	99.5 (99.3)
	35	19.7 (21.3)	7–32	0.1* (0.1)	0–1	99.5 (99.4)

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

Tick Strain Origin		Placebo		Sarolaner		
	Day	Mean	Range	Mean	Range	% Efficacy
UK	2	13.2 (14.3)	7–23	0.0* (0.0)	0– 0	100
	21	11.4 (13.0)	3–23	0.0* (0.0)	0–0	100
	28	12.9 (13.8)	6– 20	0.1* (0.1)	0–1	99.3 (99.1)
UK	2	11.5 (11.9)	7– 15	0.0* (0.0)	0–0	100
	35	8.9 (9.1)	6– 12	0.0* (0.0)	0–0	100
The Netherlands	2	13.5 (14.1)	7–23	0.0* (0.0)	0–0	100
	7	16.5 (17.1)	11–26	0.0* (0.0)	0–0	100
	14	17.1 (17.8)	11–26	0.0* (0.0)	0–0	100
	21	13.7 (14.5)	8–26	0.0* (0.0)	0–0	100
	28	10.5 (14.3)	0–25	0.0* (0.0)	0–0	100
	35	12.2 (14.1)	4– 22	0.0* (0.0)	0– 0	100

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

Tick Strain Origin		Placebo		Sarolaner		
	Day	Mean	Range	Mean	Range	% Efficacy
Germany/Slovakia	2	23.1 (23.6)	15–31	0.0* (0.0)	0–0	100
	7	23.6 (24.0)	16–29	0.0* (0.0)	0–0	100
	14	20.1 (20.3)	17–25	0.0* (0.0)	0–0	100
	21	16.6 (17.6)	8–24	0.0* (0.0)	0–0	100
	28	12.6 (13.6)	7–23	0.2* (0.4)	0–3	98.5 (97.2)
	35	15.4 (15.8)	9–21	0.0* (0.0)	0–0	100
Germany	2	17.3 (17.4)	14–20	0.0* (0.0)	0–0	100
	7	16.8 (16.9)	13–19	0.0* (0.0)	0–0	100
	14	15.5 (15.6)	11–19	0.0* (0.0)	0–0	100
	21	16.2 (16.5)	12–20	0.1* (0.1)	0–1	99.4 (99.2)
	28	16.0 (16.1)	12–19	0.2* (0.3)	0–1	98.8 (98.4)
	35	16.2 (16.4)	14–22	0.4* (0.8)	0–4	97.5 (95.4)

Table 4
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 a laboratory study.

Tick Strain Origin		Placebo		Sarolaner		
	Day	Mean	Range	Mean	Range	% Efficacy
Germany	2	28.0 (29.4)	12–39	0.1* (0.1)	0–1	99.7 (99.6)
	7	25.9 (27.0)	17–40	0.0* (0.0)	0–0	100
	14	23.4 (24.1)	16–34	0.0* (0.0)	0–0	100
	21	24.6 (25.5)	15–37	0.1* (0.1)	0–1	99.6 (99.5)
	28	18.6 (19.9)	12–39	0.1* (0.1)	0–1	99.5 (99.4)
	35	24.1 (25.0)	16–33	0.1* (0.1)	0–1	99.6 (99.5)

was 100% at 48 h after treatment in all three studies. Efficacy 48 h after subsequent weekly re-infestations was 100% through 35 days post-treatment, except on Day 28 in one study when the efficacy was 99.3% (Table 2). For *I. ricinus*, efficacy was 100% against existing infestations at 48 h after treatment in both studies. After subsequent weekly re-infestations, efficacy was ≥97.5% at 48 h after infestation through 35 days post treatment in both studies (Table 3). Efficacy against existing infestations of *R. sanguineus* was 99.7% at 48 h after treatment and ≥99.5% at 48 h after subsequent weekly re-infestations through 35 days post treatment (Table 4). For all tick species, the mean live 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 observed in any study.

4. Discussion

The recent development of oral formulations of the isoxazoline class compounds for the treatment and prevention of tick infestations provides animal owners with an alternative to topically applied treatments, which can be messy to apply. Oral treatments provide dosing convenience, and for the dog to be handled immediately after treatment without the risk of being exposed to the drug. Furthermore, orally administered products are not affected by external factors such as bathing, rain or skin disease that might affect the uptake or efficacy of topically applied products, which is an added benefit for sporting dogs and those with outdoor lifestyles and that may be more at risk of exposure to ticks that can carry disease.

Sarolaner is a new member of the isoxazoline class that was developed to provide efficacy against existing tick infestations as well as persistent acaricidal efficacy for one month (McTier et al., 2016). Thus, it was expected that a single oral treatment with sarolaner at a minimum dosage of 2 mg/kg would provide consistent efficacy against the main ticks infesting dogs in the EU for at least one month.

In the eight laboratory studies presented here, a single oral treatment with the chewable formulation of sarolaner provided a 100% efficacy against existing infestations of *D. reticulatus, I. hexagonus*, and *I. ricinus*, and 99.7% efficacy against *R. sanguineus* within 48 h of dosing. Following weekly re-infestations of all four species, the single treatment resulted in > 99% reductions in live ticks for 35 days.

Sarolaner is the only isoxazoline with demonstrated efficacy against *I. hexagonus*. Although less prevalent compared to *I. ricinus* and less frequently evaluated, the hedgehog tick has been reported in 8.8–22.6% of examined dogs in recent studies (Smith et al., 2011; Claerebout et al., 2013; Beck et al., 2014). Furthermore, *I. hexagonus* seems to be as frequently infected with common tick-borne diseases as *I. ricinus* (Schreiber et al., 2014; Claerebout et al., 2013), underlining the importance of having a high and persistent efficacy against this tick species. The importance of efficacy until the end of the claimed treatment interval is not only driven by the direct clinical effects of tick infestations, but is also important in light of the risk of transmission of tick-borne diseases. The current studies have demonstrated that sarolaner provides a consistent efficacy for at least five weeks against the four major European tick species.

5. Conclusions

The consistent efficacy of a single oral treatment of sarolaner (Simparica™), at the proposed minimum dose of 2.0 mg/kg, against the four major EU tick species was demonstrated against existing infestations and weekly re-infestations for at least 5 weeks. Efficacy of ≥ 99.7% was achieved versus existing infestations within 48 h after treatment. Efficacy was maintained at greater than 97.5% within 48 h after infestation for the 35 day duration of all studies.

Conflict of interest

The studies reported here were funded by Zoetis. TG, CB, SPM, NS, AL and RHS are employees of Zoetis. SG, CS, PD, VD, JL, and AM 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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