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Efficacy and safety of a novel oral isoxazoline, sarolaner (Simparica™) in the treatment of naturally occurring flea and tick infestations in dogs presented as veterinary patients in Europe

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ABSTRACT

Two randomised, blinded, multi-centered field studies were conducted in Europe to demonstrate the efficacy and safety of three monthly oral doses of sarolaner (Simparica™, Zoetis) administered at a minimum dosage of 2.0 mg/kg (range 2–4 mg/kg) against natural flea or tick infestation of dogs presented as veterinary patients. In the flea study, the improvement in clinical signs associated with flea allergy dermatitis (FAD) was also investigated. The palatability of the sarolaner chewable tablet formulation was evaluated in both studies. Spinosad (Comfortis® Chewable Tablets, Elanco) and fipronil (Frontline® Spot on, Merial) were used as positive controls in the flea and tick study, respectively. Treatments were administered on Days 0, 30 and 60. Efficacy was calculated based on the mean percent reduction of live parasite counts on post-treatment days 14, 30, 60 and 90 versus the pre-treatment count on Day 0. Non-inferiority of sarolaner to the control products was assessed at each time-point using a margin of 15% at the one-sided 0.025 significance level.

Dogs were enrolled in a 2:1 ratio (sarolaner:comparator); 285 flea- and 181 tick-infested dogs were assessed for efficacy and safety, and 137 and 48 dogs were assessed for safety only, in the flea and tick study, respectively. There were no treatment-related adverse events.

Efficacy against fleas was 98.8%, 99.4%, >99.9% and >99.9% in the sarolaner-treated group and 98.9%, 93.7%, 96.8% and 95.1% in the spinosad-treated group on Days 14, 30, 60 and 90, respectively. Sarolaner was non-inferior to spinosad at all time-points and was superior on Day 30. For the 42 dogs identified as having FAD at enrolment, the clinical signs of FAD improved in all dogs and the incidence was markedly reduced by the end of the study.

Efficacy against ticks was 97.4%, 97.6%, 99.8% and 100% in the sarolaner-treated group and 94.1%, 88.5%, 89.9% and 98.1% in the fipronil-treated group on Days 14, 30, 60 and 90, respectively. Sarolaner was non-inferior to fipronil at all time-points, and was superior on Days 30 and 60. Sarolaner tablets were voluntarily and fully consumed within one minute in 93% of the 1280 occasions offered.

Sarolaner administered orally at monthly intervals at a minimum dosage of 2 mg/kg was safe and highly effective against natural infestations of fleas and ticks on dogs. In addition, clinical signs FAD improved in dogs treated with sarolaner, and the flavored, chewable tablets were highly palatable.

1. Introduction

Treatment and prevention of ectoparasite infestations on companion animals is an integral part of general veterinary practice around the world. It has been reported that flea infestations account for over 50% of the dermatological cases presented to veterinarians and dealing with flea infestations comprises 35% of their total case load (Bevier-Tournay, 1989; Kwochka, 1987). In recent studies, the prevalence of flea infestations in canine veterinary patients was found to be up to 27.1% in Hungary, 40.3% in Greece, 17.9% in Italy and 30.3% in Mexico (Koutinas et al., 1995; Cruz-Vazquez et al., 2001; Rinaldi et al., 2007; Farkas et al., 2009). The economic impact

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Table 1
Demographic characteristics of dogs presented as veterinary patients and dosed with sarolaner or spinosad tablets administered orally, or treated with fipronil topically once a month for three months.

	Tick study		Flea study	
	Sarolaner	Fipronil	Sarolaner	Spinosad
Number of dogs enrolled	150	79	287	141
Purebred	68.0%	62.0%	65.9%	64.5%
Non-purebred	32.0%	38.0%	34.1%	35.5%
Live indoors and outdoors	47.3%	49.4%	33.4%	38.3%
Live mostly indoors	4.7%	3.8%	12.5%	5.7%
Live mostly outdoors	48.0%	46.8%	54.0%	56.0%
Male	46.7%	50.6%	46.7%	52.5%
Female	53.3%	49.4%	53.3%	47.5%
Long hair	20.7%	27.8%	15.3%	14.2%
Medium length hair	41.3%	38.0%	41.5%	48.2%
Short hair	38.0%	34.2%	43.2%	37.6%
Receiving concurrent medication*	5.3%	7.6%	5.2%	6.4%
Not receiving concurrent medication	94.7%	92.4%	94.8%	93.6%

*In the sarolaner-treated group dogs were receiving the following concurrent medication at enrolment: doxycycline, meloxicam, vaccines, firocoxib, dexamethasone, benzylpenicillin, dihydrostreptomycin sulfate, moxidectin, ivermectin, carprofen, benazepril, amoxicillin in combination with clavulanic acid, medetomidine, cephalexine, pyrantel + ivermectin, spiramycine + metronidazole, phenobarbital, pentosan polysulphate.

of flea treatments is also high; it has been estimated globally that pet owners spend more than 2 billion USD annually on flea products alone (Conniff, 1995; Krämer and Mencke, 2001; Rust 2005).

Tick infestations are similarly a common problem in dogs. The median frequency of dogs carrying ticks in Great Britain was found to be 14.9% (Smith et al., 2011). Ticks are one of the most important vectors of diseases caused by pathogenic protozoa (e.g. *Babesia* spp.), viruses (e.g. tick-borne encephalitis virus), rickettsia, and bacteria (e.g. *Borrelia burgdorferi* s.l., *Ehrlichia* spp., *Francisella tularensis*, *Anaplasma* spp.) in dogs, many of which are zoonotic.

Due to the prevalence of flea and tick infestations and the potential severity of tick-borne diseases there is high need for safe and efficacious products to treat flea and tick infestations on dogs. Sarolaner (Simparica™) is the latest addition to the isoxazoline class of oral ectoparasiticides. Two clinical field studies were conducted in Europe to evaluate its efficacy and safety against natural flea and tick infestations in canine veterinary patients.

2. Materials and methods

Two randomised, blinded, positive-controlled clinical field studies were conducted at veterinary clinics in Belgium, Hungary, Italy, France and the United Kingdom enrolling dogs presenting with flea and/or tick infestations. All personnel (e.g., the Examining Veterinarian) involved with the collection of efficacy and safety data were blinded to treatment All treatments were dispensed to the Owners by separate study personnel (Dispenser), who were not involved in any other study activities. The studies were conducted in compliance with Good Clinical Practice, (VICH guideline GL9, EMEA, 2000) and the study protocols were reviewed and approved by the Zoetis Ethics Review Assessment team.

2.1. Animals

Enrolment was limited to households with three or fewer dogs. One dog in each household was allowed to be enrolled as the primary patient and only that dog received efficacy evaluations. Other dogs living in the same household as the primary dog were enrolled as supplementary patients and received the same treatment but were only evaluated for safety and palatability, not for efficacy. The primary patient had to harbor ≥5 live fleas or ≥3 live attached ticks at enrolment. Within each clinic the primary dogs were randomly allocated to the two treatment groups (separately in each study) in a ratio of 2:1, so that for every two patients that received sarolaner, one patient received the positive control product (spinosad in the flea study and fipronil in the tick study). Supplementary dogs in the flea study had to be enrolled and treated, while in the tick study, the enrolment and treatment of the supplementary dogs was optional for the Owners. Dogs that were pregnant, lactating or intended for breeding were excluded from the studies. All dogs received a physical examination by a veterinarian at study inclusion. The minimum age for enrolment was eight weeks in the tick study and 14 weeks (due to the age restrictions of the comparator product) in the flea study. Each dog was enrolled with the written informed consent of its owner.

2.2. Treatment administration

Dogs received three consecutive monthly treatments on study days 0, 30 and 60. For the follow up treatments and evaluations on Days 30 and 60 these could be conducted ± 3 days of the target date, but these are reported as Days 30 and 60. All treatments were dispensed according to a randomization plan that was provided for each clinic before study start. Treatment dispensing was based upon the body weights recorded on Day 0, 30 and 60, and treatments were administered by the Owner in the home environment after the clinic visits. The dogs' owners were not blinded to treatment allocation. Animals were dosed with the appropriate strength sarolaner tablet (Simparica™, Zoetis) to provide the

Table 2
Efficacy against fleas: Number of dogs, arithmetic mean live flea counts (all species), ranges and percent efficacies relative to pre-treatment counts for dogs presented as veterinary patients and dosed with sarolaner or spinosad tablets administered orally once a month for three months.

Study Day	Treatment group	Number of dogs	Flea counts		Efficacy[a] (%)	
			Arithmetic mean	Range	Arithmetic mean	95% Confidence interval
−1 to 0	Sarolaner	189	23.1	5 to 1029	–	–
	Spinosad	95	18.7	5 to 173	N/A	–
14 ± 3	Sarolaner	184	0.2	0 to 10	98.8	97.8 – 99.8
	Spinosad	88	0.2	0 to 4	98.9	98.1 – 99.8
30 ± 3	Sarolaner	186	0.1	0 to 8	99.4	98.8 – 99.9
	Spinosad	89	1.3	0 to 38	93.7	89.0 – 98.6
60 ± 3	Sarolaner	186	<0.1	0 to 1	>99.9	99.7 – 100.1
	Spinosad	87	0.3	0 to 17	96.8	91.3 – 102.7
90 ± 3	Sarolaner	182	<0.1	0 to 1	>99.9	99.7 – 100.1
	Spinosad	92	0.4	0 to 19	95.1	87.0 – 103.3

[a] Efficacy is the arithmetic mean of the percent reduction relative to pre-treatment calculated for each dog individually.

Table 3
Statistical comparison of flea efficacy (all species) for dogs presented as veterinary patients and dosed with sarolaner or spinosad tablets administered orally once a month for three months.

Study Day	Difference	95% Confidence interval	Non-Inferior?[a]	Superior?
14 ± 3	−0.1	−1.4 to 1.2	Yes	No
30 ± 3	5.6	0.8 to 10.4	Yes	Yes[b]
60 ± 3	2.9	−2.8 to 8.6	Yes	No
90 ± 3	4.7	−3.4 to 12.9	Yes	No

[a] sarolaner is non-inferior to spinosad at 2.5% alpha if the lower limit of the 95% confidence interval is greater than −15.
[b] On Day 30 ± 3 the lower limit of the 95% confidence interval is greater than 0, indicating superiority of sarolaner over spinosad.

recommended minimum dosage of 2 mg/kg (range 2–4 mg/kg). In the flea study the positive control product (Comfortis® Chewable Tablets; Elanco) was dosed following manufacturer's recommendations to deliver 45–70 mg/kg spinosad. In the tick study the positive control product (Frontline® Spot on, Merial) was dosed following manufacturer's recommendations to deliver 7.5–15 mg/kg fipronil and it was applied topically, directly to the skin. There were no restrictions regarding the prandial state at the time of sarolaner administration, therefore tablets could be administered with or without food. Spinosad was administered with the main meal of the dog in order to comply with the approved dosing directions for that product.

2.3. Efficacy assessment

Parasite counts on primary dogs were conducted prior to treatment on Day 0, and on post-treatment Days 14, 30, 60 and 90 (the post-treatment evaluations could be conducted ± 3 days of the target day). Dogs were thoroughly examined (and combed using flea combs) for at least 10 min by blinded study personnel (e.g., Examining Veterinarian or Technician) until all fleas and/or ticks were removed. The collected parasites were counted and stored in alcohol solution (at least 70%) at room temperature. The species and gender of the fleas, and the species, developmental status and gender of the adult ticks collected from the primary dogs were determined at a single central parasitology laboratory (Faculty of Veterinary Science, Budapest) under a stereomicroscope using identification keys (Szabó, 1975; Hillyard, 1996; Estrada-Pena et al., 2004). Engorgement status of the ticks was determined by visual inspection of the alloscutum.

In the flea study, each primary dog was also thoroughly examined for clinical signs of flea allergy dermatitis (FAD) including but not limited to pruritus, erythema, scaling, alopecia, and dermatitis/pyodermatitis, prior to treatment on Day 0, and on Days 14, 30, 60 and 90. The Examining Veterinarian assessed the severity of the clinical signs on a four level scale as absent, mild, moderate or severe.

2.4. Safety assessment

All dogs (primary and supplementary) that received at least one treatment were included in the safety assessment. All dogs received a physical examination by the veterinarian prior to treatment on Day 0, and on Days 30, 60 and 90. Primary dogs received an additional physical examination by the veterinarian on Day 14. All abnormal health events observed during the physical examinations by the veterinarian or observed by the owner between visits, were recorded.

2.5. Palatability assessment

The voluntary acceptance and consumption of tablets was evaluated by the dog's owner at each administration for all orally-dosed dogs. The dogs were allowed one minute to accept and consume the offered tablets for the palatability assessment. Tablets or tablet fragments that were not voluntarily consumed within this period were administered in food or by pilling to ensure complete dosing.

2.6. Data analysis

The animal (primary dog per household) was the experimental unit. Efficacy was calculated at each post-treatment visit day (Day 14, 30, 60 and 90) as the percentage reduction in live parasite counts compared to the pre-treatment counts (recorded on Day 0) for each animal using the following formula:

$$\%\text{efficacy post} - \text{treatment} = 100 \times \frac{\text{count (Day0)} - \text{count (post} - \text{treatment)}}{\text{count (Day0)}}$$

Efficacy was calculated across all flea and tick species in the flea and tick study, respectively. Additionally, efficacy was calculated for each flea and tick species separately if at least five dogs in each treatment group had parasites of a given species at enrolment.

Statistical comparison of efficacy between sarolaner and the positive control product was done by non-inferiority analysis at each visit using a margin of 15% at the one-sided, $\alpha = 0.025$ significance level. If the lower limit of the 95% confidence interval of the difference in efficacy between sarolaner and the positive control product was greater than −15% then sarolaner was non-inferior to the positive control product at that time point. If the lower limit of the 95% confidence interval was greater than 0, sarolaner was superior to the positive control product.

The improvement in clinical signs of FAD was assessed for primary dogs that were identified by the Examining Veterinarian as having FAD. The numbers and percentages of dogs with each of

Table 4
Numbers of dogs infested with the cat flea (*Ctenocephalides felis*) or with the dog flea (*Ctenocephalides canis*), and the maximum flea count for each species for dogs presented as veterinary patients and dosed with sarolaner or spinosad tablets administered orally once a month for three months.

			C. felis		*C. canis*	
Treatment group	Study day	Dogs	Dogs with fleas (%)	Maximum flea count	Dogs with fleas (%)	Maximum flea count
Sarolaner	−1 to 0	189	142 (75.1)	1316	50 (26.5)	249
	14 ± 3	184	7 (3.8)	5	6 (3.3)	10
	30 ± 3	186	8 (4.3)	8	1 (0.5)	1
	60 ± 3	186	1 (0.5)	1	1 (0.5)	1
	90 ± 3	182	1 (0.5)	1	1 (0.5)	1
Spinosad	−1 to 0	95	72 (75.8)	151	24 (25.3)	102
	14 ± 3	88	9 (10.2)	4	1 (1.1)	1
	30 ± 3	89	10 (11.2)	39	4 (4.4)	17
	60 ± 3	87	2 (2.3)	17	1 (1.1)	1
	90 ± 3	92	4 (4.3)	19	0 (0.0)	0

Table 5
Clinical signs of flea allergy dermatitis: Number and percent of dogs with clinical signs at enrolment and at study completion for dogs presented as veterinary patients and dosed with sarolaner or spinosad tablets administered orally once a month for three months.

Clinical Sign	Sarolaner				Spinosad			
	Enrolment n = 30		Completion n = 27		Enrolment n = 12		Completion n = 12	
	n	%	n	%	n	%	n	%
Alopecia	20	66.7	1	3.7	12	100	1	8.3
Dermatitis/Pyodermatitis	24	80.0	1	3.7	6	50.0	1	8.3
Erythema	25	83.3	0	0.0	11	91.7	0	0.0
Pruritus	29	96.7	2	7.4	12	100	0	0.0
Scaling	21	70.0	2	7.4	9	75.0	0	0.0

Table 6
Efficacy against ticks: Number of dogs, arithmetic mean live tick counts (all species), ranges and percent efficacies relative to pre-treatment counts for dogs presented as veterinary patients and dosed with sarolaner administered orally or treated with fipronil topically once a month for three months.

Study Day	Treatment group	Number of dogs	Tick counts		Efficacy[a] (%)	
			Arithmetic mean	Range	Arithmetic mean	95% Confidence interval
−1 to 0	Sarolaner	121	12.5	3 to 516	–	–
	Fipronil	59	10.9	3 to 81	–	–
14 ± 3	Sarolaner	119	0.2	0 to 10	97.4	94.8– 100.1
	Fipronil	58	1.6	0 to 34	94.1	90.3–97.9
30 ± 3	Sarolaner	118	0.4	0 to 32	97.6	93.4– 101.9
	Fipronil	57	2.5	0 to 53	88.5	81.9–94.1
60 ± 3	Sarolaner	119	0.0	0 to 2	99.8	96.1–103.5
	Fipronil	54	4.4	0 to 146	89.9	84.3–95.2
90 ± 3	Sarolaner	117	0.0	0	100	98.7–101.2
	Fipronil	54	0.4	0 to 10	98.1	96.2–99.9

[a] Efficacy is the arithmetic mean of the percent reduction relative to pretreatment calculated for each dog individually.

the clinical signs of FAD were calculated by severity category. Dogs that received concomitant medications that could have potentially affected the clinical signs of FAD were excluded from this analysis. To assess palatability, the percent of treatment administrations in which the whole prescribed dose was consumed voluntarily within one minute was calculated.

3. Results

3.1. Animals

In the flea study, 285 primary (189 sarolaner- and 96 spinosad-treated) and 137 supplementary (93 sarolaner- and 44 spinosad-treated) dogs were enrolled and treated. Dogs in the sarolaner group had a mean age of 5.5 years (range 14 weeks to 15 years) and mean weight of 19.6 kg (range 3.9–60.0 kg). Dogs in the spinosad group had a mean age of 5.7 years (range 14 weeks to 15 years) and mean weight of 18.8 kg (range 4.0–65.2 kg). Seven dogs in the sarolaner group and 4 dogs in the spinosad group did not complete the study. In the sarolaner group one dog was withdrawn because of suspected pregnancy, one primary dogs run away from home triggering the withdrawal of the 2 supplementary dogs living in the same household, one supplementary dog was hit by car and died, one supplementary dog was withdrawn due to concomitant disease (acute peritonitis due to pyometra) and one dog was withdrawn because of Owner non-compliance. In the spinosad-treated group one supplementary dog was withdrawn due to concomitant disease (pyometra), two dogs because of Owner non-compliance and one primary dog because the supplementary dog did not meet inclusion criteria. Thus 279 primary patients (186 sarolaner- and 93 spinosad-treated) and 132 supplementary patients (89 sarolaner- and 43 spinosad-treated) completed the flea study.

In the tick study, 181 primary (122 sarolaner- and 59 fipronil-treated) and 48 supplementary (28 sarolaner- and 20 fipronil-treated) dogs were enrolled and treated. Dogs in the sarolaner group had a mean age of 4.9 years (range 10 weeks to 14 years) and mean weight of 22.2 kg (range 2.9–66.5 kg). Dogs in the fipronil-treated group had a mean age of 4.9 years (range 10 weeks to 17 years) and mean weight of 21.7 kg (range 4.5–53.0 kg). Four dogs in each group did not complete the study. During the study one dog in the sarolaner group was withdrawn because of suspected pregnancy, two dogs run away from home and one supplementary dog was withdrawn because it was added to the household after enrolment of primary dog. In the fipronil-treated group one dog was withdrawn because of lack of efficacy, one due to concomitant disease (babesiosis) and two dogs because of Owner non-compliance. Thus 175 primary patients (119 sarolaner- and 56 fipronil-treated) and 46 supplementary patients (27 sarolaner- and 19 fipronil-treated) completed the tick study.

The demographic characteristics of the dogs at enrolment were similar in both treatment groups in both studies (Table 1).

3.2. Flea efficacy

3.2.1. Overall flea efficacy

The results for all flea species combined are summarized in Table 2. Arithmetic mean of live flea counts at enrolment were 23.1 (range 5–1,029) in the sarolaner-treated group and 18.7 (range 5–173) in the spinosad-treated group. On post-treatment Days 14, 30, 60 and 90, efficacy was 98.8, 99.4, >99.9 and >99.9%, respectively in the sarolaner-treated group, and 98.9, 93.7, 96.8 and 95.1%, respectively in the spinosad-treated group. Only single fleas were recovered from sarolaner-treated dogs on Days 60 and 90, while at the same time points, individual dogs in the spinosad-treated group harbored up to 17 and 19 fleas, respectively. Sarolaner was non-inferior to spinosad at all time-points and was superior on Day 30 (Table 3).

Table 7
Statistical comparison of tick efficacy (all species) for dogs presented as veterinary patients and dosed with sarolaner tablets administered orally or treated with fipronil topically once a month for three months.

Study Day	Difference	95% Confidence interval	Non-Inferior?[a]	Superior?
14 ± 3	3.4	−1.2 to 8.0	Yes	No
30 ± 3	9.7	2.3 to 17.1	Yes	Yes[b]
60 ± 3	10.0	3.5 to 16.6	Yes	Yes[b]
90 ± 3	1.9	−0.3 to 4.2	Yes	No

[a] Sarolaner is non-inferior to fipronil at 2.5% alpha if the lower limit of the 95% confidence interval is greater than −15.
[b] On Day 30 ± 3 and 60 ± 3 the lower limit of the 95% confidence interval is greater than 0, indicating superiority of sarolaner over fipronil.

3.2.2. Flea species

At enrolment 214 dogs (75.1%) were infested with *Ctenocephalides felis* (cat flea) and 74 dogs (26.0%) with *Ctenocephalides canis* (dog flea). Other fleas identified from less than five dogs at enrolment were a single *Nosopsyllus fasciatus* (northern rat flea) and two damaged fleas collected from two dogs could only be identified to the genus *Ctenocephalides*. The numbers and proportions of dogs infested with *C. felis* and *C. canis* and the maximum flea counts for each species are summarized in Table 4.

For *C. felis,* treatment with sarolaner resulted in a large decrease in the incidence of infestations with greater than 95% of dogs being flea-free on all post-treatment time-points, the maximum number of fleas recovered from any dog was eight on Day 30, and only single fleas were found on Days 60 and 90. Incidence of infestation with *C. felis* was higher for spinosad-treated dogs with over 10% of dogs having up to four or 39 fleas on Days 14 and 30, respectively (<90% of dogs were flea–free), and 2.3% and 4.3% of dogs with up to 17 and 19 fleas on Days 60 and 90, respectively.

For *C. canis*, the incidence of infestation in sarolaner-treated dogs also dropped sharply with only a single dog with a single flea found from Day 30 onward. A similar reduction in the incidence of *C. canis* infestation was observed in dogs treated with spinosad.

3.2.3. Flea allergy dermatitis

Thirty primary dogs in the sarolaner-treated group and 12 in the spinosad-treated group were identified as having FAD at enrolment. The clinical signs of flea allergy dermatitis (alopecia, dermatitis/pyodermatitis, erythema, pruritus and scaling) improved in all dogs following treatment administration in both groups (Table 5). At enrolment, incidence of any one of the clinical signs of FAD ranged from 50% to 100%; by the end of the study, the incidence of these signs had declined to 0–8.3% in both groups.

3.3. Tick efficacy

3.3.1. Overall tick efficacy

The efficacy against all tick species combined is summarized in Table 6. Arithmetic mean live tick counts at enrolment were 12.5 (range 3–516) in the sarolaner-treated group and 10.9 (range 3–78) in the fipronil-treated group. Efficacy on post-treatment Days 14, 30, 60 and 90 was 97.4, 97.6, 99.8 and 100% in the sarolaner-treated group, and 94.1, 88.5, 89.9 and 98.1 in the fipronil group. Sarolaner was determined to be non-inferior to fipronil at all time-points, and superior to fipronil on Days 30 and 60 (Table 7).

3.3.2. Tick species

At enrolment 95 dogs (52.8%) were infested with *Ixodes ricinus*, 74 (41.1%) with *Rhipicephalus sanguineus*, 43 (23.9%) with *Dermacentor reticulatus* and 12 (6.7%) with *Ixodes hexagonus*. Some dogs harbored mixed infestations of more than one tick species. The numbers and proportions of dogs infested with any of the four tick species and the maximum tick counts for each species are summarized in Table 8. Overall the initial incidence of each species was similar for each treatment group. A small number of damaged ticks were recovered from a few dogs during the study which could not be identified.

Treatment with sarolaner resulted in a large decrease in the incidence of infestations for all tick species by Day 14 (efficacy was 97.4% for *I. ricinus*, 99.7% for *I. hexagonus*, 97.9% for *R. sanguineus* and 100% for *D. reticulatus*). Tick infestations declined further at subsequent exams and all dogs were tick free by Day 90. Interestingly, while *I. hexagonus* had the lowest incidence on dogs at enrolment (5.0%), this species had the highest numbers of ticks infesting individual dogs (516 on one dog allocated to the sarolaner group). Ten and 32 *I. hexagonus* were collected from single sarolaner-treated dogs on Days 14 and 30, respectively, but none were found on Days 60 and 90. The fipronil-treated dogs also showed a reduction in all four tick species but for the majority of species, this reduction was markedly lower than for sarolaner and even after three treatments, three of the four tick species were found on one or two dogs. In contrast to the sarolaner-treated dogs, the dogs that harbored ticks in the fipronil-treated group tended to have high numbers of live ticks. This was most notable for *I. ricinus* where fipronil-treated dogs were infested with up to 53 and 59 ticks on Days 30 and 60, respectively, which was similar to the pre-treatment maximum of 64 ticks. This was also the case for *I. hexagonus*, where fipronil-treated dogs were infested with up to 145 ticks on Day 60, which was higher than the pre-treatment maximum of 77 ticks (Table 8).

3.4. Safety

There were no treatment-related adverse events in sarolaner-treated dogs. The overall incidence of adverse events was low. These occurred in 19 sarolaner-treated dogs (4.3%), in 9 spinosad-treated (6.4%) and 2 fipronil-treated dogs (2.5%). The majority of observed clinical signs were sporadic occurrences of conditions commonly observed in the general dog population. Dermatological conditions were the only adverse events that occurred in >1.0% of sarolaner-treated dogs in either study. These observations included single dogs diagnosed with a hot-spot on study Day 19, a bacterial skin infection on Day 9, skin necrosis following FAD on Day 8, an interdigital pyoderma on Day 38, an unidentified lump on the skin on Day 40 and alopecia of suspected endocrine origin on Day 8. Severe adverse events occurred in three dogs: in the sarolaner group, one dog died as a result of septicaemia, and one as a result of acute peritonitis secondary to pyometra; in the spinosad group, one dog developed pyometra and was euthanized. These abnormal health events were considered unlikely to be related to treatment administration.

3.5. Palatability

In the two studies, sarolaner chewable tablets were voluntarily and fully consumed within one minute on 93% of the 1280 occasions they were offered without food. Spinosad chewable tablets were voluntarily and fully consumed within one minute on 84.2% of the 411 occasions offered.

4. Discussion

Sarolaner (Simparica™) administered orally at the recommended minimum dosage of 2 mg/kg at monthly intervals was safe and highly effective against natural flea and tick infestations of dogs. The efficacy of sarolaner against fleas was superior to the commercial comparator spinosad at the end of the first treatment period (Day 30) and non-inferior at all other time points. Similarly, sarolaner had superior efficacy against ticks compared to fipronil

Table 8
Numbers of dogs infested with the ticks, *Ixodes ricinus, Rhipicephalus sanguineus*, *Dermacentor reticulatus* or *Ixodes. hexagonus*, and the maximum tick count for each species for dogs presented as veterinary patients and dosed with sarolaner administered orally or treated with fipronil topically once a month for three months.

		I. ricinus			*R. sanguineus*		*D. reticulatus*		*I. hexagonus*	
Treatment group	Study day	Dogs	Dogs with ticks (%)	Max. tick count	Dogs with ticks (%)	Max. tick count	Dogs with ticks (%)	Max. tick count	Dogs with ticks (%)	Max. tick count
Sarolaner	−1 to 0	121	66 (54.5)	43	49 (40.5)	63	31 (25.6)	7	6 (5.0)	516
	14 ± 3	119	3 (2.5)	5	2 (1.7)	5	1 (0.8)	4	1 (0.8)	10
	30 ± 3	118	2 (1.7)	2	2 (1.7)	10	1 (0.8)	1	1 (0.8)	32
	60 ± 3	119	0 (0.0)	0	1 (0.8)	1	0 (0.0)	0	0 (0.0)	0
	90 ± 3	117	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Fipronil	−1 to 0	59	29 (49.2)	64	25 (42.4)	81	12 (20.3)	10	6 (10.2)	77
	14 ± 3	58	3 (5.2)	34	4 (6.9)	5	1 (1.7)	3	3 (5.2)	14
	30 ± 3	57	6 (10.5)	53	3 (5.3)	23	0 (0.0)	0	3 (5.3)	26
	60 ± 3	54	5 (9.3)	59	2 (3.7)	10	0 (0.0)	0	3 (5.6)	145
	90 ± 3	54	2 (3.7)	10	1 (1.9)	1	0 (0.0)	0	1 (3.7)	8

at the end of the first and second treatment periods (Days 30 and 60) and was non-inferior on Day 90.

Under field conditions, dogs are continuously exposed to re-infestations by fleas and ticks from the environment. Therefore, ectoparasiticides should not only provide rapid immediate efficacy, but also sustained persistent efficacy after a single administration until the end of the treatment period to protect the dogs from re-infestation. Sarolaner provided >99% and >97% efficacy against fleas and ticks, respectively, at 30 days after the first administration and this efficacy was superior to both spinosad and fipronil. This sustained efficacy provided by treatment with sarolaner was even higher 30 days after the second and third monthly treatments.

Against fleas, sarolaner provided >99% efficacy at 30 days after each monthly treatment. In contrast, spinosad resulted in only 93.7% efficacy 30 days after the first treatment and efficacy remained <97% following the two subsequent monthly treatments.

The clinical signs of FAD were markedly reduced through the course of the study in sarolaner-treated dogs, with only a few dogs exhibiting any signs at the end of the study (Table 5). As no concomitant medications were administered to directly treat these signs, resolution was a result of the rapid and persistent elimination of adult fleas provided by sarolaner (Six et al., 2016).

Sarolaner was also highly efficacious against all tick species found on the enrolled dogs with efficacy >97% at all assessments and which was superior to fipronil at Days 30 and 60. In particular, sarolaner's efficacy against *D. reticulatus* during the study period is of interest, as this species is the main vector of *Babesia canis* that can cause lethal disease in dogs. Sarolaner also provided high efficacy against *I. hexagonus*, and *I. ricinus* the latter being the most prevalent tick infesting dogs in Europe and the main vectors of *B. burgdorferi s. l.* causing Lyme disease in dogs and people. At 30 days after the second treatment, neither of these species was found on any sarolaner-treated dogs and no live ticks at all were found after the third treatment. In contrast, at these time-points, fipronil had relatively poor efficacy against both *Ixodes* species, with up to 145 *I. hexagonus* recovered from almost 6% of fironil-treated dogs and up to 59 *I. ricinus* recovered from nearly 10% of fipronil-treated dogs at Day 60, and up to 10 live ticks found on over 6% of dogs after the third treatment.

Palatability of sarolaner chewable tablets was evaluated in this study in a large number of dogs of various breeds and sizes. The overall rate of 93% voluntary full consumption in this study demonstrates that sarolaner (Simparica™) was highly palatable. In comparison, spinosad (Comfortis®) was voluntarily and fully consumed within one minute on 84.2% of all occasions in this study. Another oral ectoparasiticde for dogs containing fluralaner (Bravecto® Merck) was reported to have only 74.4% acceptance within one minute of offerings by owners in the home environment, i.e. under similar conditions as in the current study (Meadows et al., 2014). Sarolaner chewable tablets may be administered with or without food. Thus, the high palatability and ease of administration of sarolaner chewable tablets should enhance owner compliance, which is a key factor for the success of ectoparasite treatments in companion animals (Halos et al., 2014).

5. Conclusions

Sarolaner administered orally at monthly intervals at a minimum dosage of 2 mg/kg (range 2–4 mg/kg) was safe and highly effective against natural infestations of fleas and ticks on dogs presented as veterinary patients. Specifically under conditions when dogs were continuously exposed to natural re-infestations with ticks and fleas, sarolaner provided high efficacy that was sustained until the end of the monthly dosing interval. Sarolaner improved the clinical signs of flea allergy dermatitis, due to rapid flea killing (Six et al., 2016). Simparica™ Chewable Tablets were highly palatable, thus providing a convenient and easy treatment option to owners for the treatment and control of flea and tick infestations on dogs.

Conflict of interest

The studies reported here were funded by Zoetis, Florham Park, NJ. RF was in independent investor and conducted the species identification of all fleas and ticks. All other authors were current employees of Zoetis. 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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