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Evaluation of the effectiveness of a novel oral formulation of sarolaner (Simparica™) for the treatment and control of fleas on dogs

Robert H. Six[a,*], Thomas Geurden[b], Raj Packianathan[c], Sally Colgan[d], William R. Everett[e], Sarah Grace[f], Andrew Hodge[c], Sean P. Mahabir[a], Melanie R. Myers[a], Nathalie Slootmans[b], Kylie Davis[c]

[a] Zoetis, Veterinary Medicine Research and Development, 333 Portage St. Kalamazoo, MI 49007, USA
[b] Zoetis, Veterinary Medicine Research and Development, Hoge Wei 10, B-1930 Zaventem, Belgium
[c] Zoetis, Veterinary Medicine Research and Development, 45 Poplar Road, Parkville, VIC 3052, Australia
[d] SCEC Pty Ltd., P.O. Box 211, Northbridge, NSW 1560, Australia
[e] BerTek, Inc., P.O. Box 606, Greenbrier, AR 72058, USA
[f] Charles River Laboratories, Pre-Clinical Services, Glenamoy, Co., Mayo, Ireland

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ABSTRACT

The efficacy of a single oral dose of a novel isoxazoline, sarolaner (Simparica™, Zoetis), for the treatment and control of flea infestations on dogs was confirmed in five laboratory studies. The studies were conducted using adult purpose-bred Beagles and/or mixed breed dogs. All animals were individually identified and housed, and were allocated randomly to treatment with either placebo or sarolaner (eight to 10 per group) based on pretreatment parasite counts. Three studies used cat flea (*Ctenocephalides felis felis*) strains recently isolated from the field from the US, EU, or Australia; in the fourth study a laboratory strain (KS1) with documented tolerance to a number of insecticides such as fipronil, imidacloprid, and permethrin was used. In the fifth study, dogs were infested with dog fleas, *Ctenocephalides canis*. Dogs were treated orally on Day 0 with a placebo or a sarolaner tablet providing a minimum dose of 2 mg/kg. Dogs were infested with approximately 100 unfed, adult fleas prior to treatment and at weekly intervals post-treatment. Comb counts were conducted to determine the numbers of viable fleas at 24 h after treatment and after each subsequent infestation. Efficacy against *C. felis* and *C. canis* was 99.8–100% from treatment through Day 35. In all five studies, elimination of existing infestations was achieved within 24 h after dosing, with only a single live *C. felis* found on one dog on Day 1. Similarly, control of flea challenges was achieved within 24 h after infestation throughout the 35 day study periods, with only single live *C. felis* found on two dogs on Day 28 in one study, and on a single dog on Day 35 in another study. There were no adverse reactions to treatment with sarolaner. These studies confirmed that a single oral dose of sarolaner at 2 mg/kg provided highly effective treatment of existing *C. felis* infestations and persistent control of *C. felis* on dogs for 35 days after treatment. Efficacy equivalent to that seen with *C. felis* was confirmed against *C. canis* and a known insecticide-tolerant strain of *C. felis*.

1. Introduction

Flea infestations have been recognized for decades as a common burden for companion animals and their owners worldwide. *Ctenocephalides felis felis* (cat flea) is considered to be the most common ectoparasite of companion animals and has a worldwide distribution (Rust and Dryden, 1997). *Ctenocephalides canis* (dog flea) has a similar distribution, host spectrum, and biology to *C. felis* (Krämer and Menke, 2001) but is generally less commonly encountered. They are recognized as a major cause of pruritus in companion animals, are intermediate hosts for the dog tapeworm, *Dipylidium caninum*, and can transmit a number of pathogens including *Bartonella henselae* (Kwochka, 1987; Foil et al., 1998; Breitschwerdt and Kordick, 2000; Krämer and Menke, 2001; Breitschwerdt et al, 2010), *Bartonella clarridgeae* and *Bartonella koehlerae* (Chomel and Kasten, 2010), and *Rickettsia felis* (Horta et al., 2014). Adult fleas are blood feeders, penetrating the skin with their sucking mouthparts and injecting salivary antigens as they feed, and when present in

* Corresponding author.
E-mail address: robert.six@Zoetis.com (R.H. Six).

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large numbers are capable of causing anemia (Dryden, 1989). Given the high prevalence and the pathogenic and zoonotic potential of fleas (Beugnet and Franc, 2012), flea control should be a high priority in wellness programs targeted at maintaining the health of dogs and cats over their lifetime. Key objectives for flea-control programs include rapid speed of kill for existing infestations on the animal, prevention of re-infestation of the pet with on-going exposure, and rapid elimination of adult fleas prior to egg production (Carlotti and Jacobs, 2000). Successful control and prevention enhances the human-companion animal bond by preventing these irritating and debilitating parasites, controlling signs of flea allergy dermatitis in allergic animals, and helping protect pets and their owners from exposure to vector-borne pathogens by minimizing vector exposure.

Control of fleas depends upon chemical parasiticides and on-animal treatments generally applied as monthly spot-on applications have been the standard accepted method (Dryden and Payne, 2004; Rust, 2005). Spot-on treatments include active ingredients from a number of chemical classes, including phenyl pyrazoles, fipronil; neonicotinoids, imidacloprid; the pyrethroids, permethrin and phenothrin; and selamectin, an avermectin (Rust, 2005). There are also products such as the insect growth regulators, lufenuron, pyriproxyfen, and methoprene, that control fleas by disrupting the development of eggs and larvae. Fleas have developed resistance to a number of insecticides; pest management strategies to reduce further development of resistance have been proposed (Bossard et al., 1998; Ross et al., 1998; Rust, 2005) to actively manage the use of these products to attempt to preserve the effectiveness of these older active ingredients. Newer classes of oral formulations have been introduced and have gained acceptance for their convenience and consistent efficacy as these are unaffected by environmental factors (sun, bathing, swimming, *etc.*) and owner application variation that can impact efficacy of topical products. These oral products include spinosad (for fleas only) and the recently introduced isoxazoline compounds that provide control of both fleas and ticks (Robertson-Plouch et al., 2008; Rohdich et al., 2014; Shoop et al., 2014).

Sarolaner is an isoxazoline ectoparasiticide with insecticide/acaricide activity that has excellent efficacy against fleas on dogs following oral administration at a minimum dose of 2 mg/kg (McTier et al., 2016). Here we report a series of laboratory studies conducted to confirm the efficacy of sarolaner (Simparica™, Zoetis) given as a single oral dose to dogs for the treatment of existing flea infestations, and the persistent control of fleas for one month. These studies assessed efficacy *versus C. felis* strains representative of current field populations from the US, Europe, and Australia, and a European strain of *C. canis*. Further, efficacy of sarolaner was evaluated against a known insecticide resistant strain of *C. felis*, KS1, (Dryden, 1998; Payne et al., 2001; Bossard et al., 2002; Rust et al., 2002; Dryden et al., 2005, 2008).

2. Materials and methods

Five studies were conducted to confirm the efficacy of the proposed minimum label dose of 2 mg sarolaner/kg against existing flea infestations and weekly challenges up to 35 days after a single oral dose. Four studies evaluated *C. felis*, and in the fifth study efficacy was confirmed against *C. canis* (Table 1).

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) and complied with Good Clinical Practices (VICH guideline GL9) (EMEA, 2000). Study protocols were reviewed and approved by the local and/or Zoetis Institutional Animal Care and Use Committee.

2.1. Animals

The studies used purpose bred Beagle and/or mixed breed dogs of both sexes, ranging in age from 9 to 96 months and weighing from 6.0 to 26.0 kg (Table 1). All dogs had demonstrated good flea retention prior to treatment, were in good health at enrolment, and had not been treated with an ectoparasiticide for at least 60 days. Dogs were individually identified and housed in enclosures that 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*.

2.2. Experimental design and methods

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 by personnel qualified through training and experience. A physical exam was performed on each dog by a veterinarian to determine health and suitability prior to inclusion in the study. For infestations, dogs were held and approximately 100 fleas were applied directly to the dogs and allowed to disperse into the hair coat. Flea counts were performed by personnel trained in the standard procedures in use at the test facilities. Protective gloves and clothing were changed between dogs and personnel conducting parasite or other observations were masked to treatment assignments. The dogs were thoroughly combed to remove fleas for counting. Fleas able to stand upright and/or move in a coordinated manner were considered live. Commercial fine-toothed flea combs were used. Dogs were systematically combed using repeated strokes while standing, starting from the head and proceeding caudally along the dorsum. The dog was then placed in lateral recumbency, followed by dorsal recumbency, for combing of the sides and ventral surfaces. Each animal was examined for a minimum of 10 min. Any animal on which fleas were found in the last five minutes was combed for an additional five minutes (one minute in Study 3).

Approximately one week prior to treatment, 20–22 dogs were infested with fleas. These dogs were then combed to count fleas 24 ± 2 h later. The 16 dogs (20 dogs in study 3) with the highest live flea counts were selected for inclusion in each study. The dogs were ranked by flea count and then randomly assigned to either placebo control or treatment with sarolaner.

Day 0 for each study was the day on which dogs were administered study treatment. On Day-1, all dogs were infested with fleas. On Day 0, the dogs were offered their normal ration of food ~20 min prior to dosing (one hour in Study 4). In Studies 1–5, dogs treated with sarolaner were administered a single or combination of tablets from strengths of 5, 10, 20 or 40 mg to achieve the appropriate dose (as close as possible to 2.0 mg sarolaner/kg without under dosing). Placebo-control dogs were dosed with similarly sized placebo tablets. In Study 4, 40 mg sarolaner tablets were individually shaved and/or sanded to deliver the minimum dosage of 2.0 mg/kg and control dogs received a single placebo tablet. To ensure complete dosing in these laboratory studies, all dogs were hand-pilled. Each dog was observed for several minutes to make sure the dose was swallowed and for any adverse events associated with administration, and then periodically for up to two hours 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 0, then at least once daily for the remainder of the study.

On Day 1, approximately 24 h after treatment, each dog was examined and combed to count and remove fleas. Subsequently,

Table 1
Study locations, age and weight ranges of dogs, and parasite details for five studies evaluating the efficacy of sarolaner against fleas.

Study		Dogs			Parasite	
#	Location	Number	Age (months)	Weight (kg)	Species	Source and History
1	Arkansas, USA	16	9–66	6.0–14.6	*Ctenocephalides felis*	Lab colony (NC), infusion of field fleas (AR) 2 years prior to study
2	Ireland	16	13–69	9.2–7.5	*Ctenocephalides felis*	Lab colony (UK), infusion of field fleas (EU) 9 years prior to study
3	New South Wales,Australia	20	36–96	7.5–26.0	*Ctenocephalides felis*	Lab colony (NSW), infusion of field fleas (NSW) 2 years prior to study
4	Arkansas, USA	16	20–22	6.8–10.6	*Ctenocephalides felis*	KS1 strain, KS, maintained as a closed colony since 1990
5	Ireland	16	15–85	9.0–15.9	*Ctenocephalides canis*	Lab colony established with field fleas 4 years prior to study

all animals were infested with fleas on Days 6, 13, 20, 26 (27 in Study 3) and 34. All dogs were examined and combed to count and remove fleas 24 h after each infestation.

2.3. Parasites

Fleas for all studies were obtained from local laboratory colonies and with the exception of Study 4 (KS1 strain) these colonies had all been infused with additional field collected fleas within two to nine years of the study (Table 1). The KS1 strain of *C. felis* was obtained from Kansas State University, Manhattan, KS. This colony was established in 1990 and has since been maintained as a closed colony. This colony has some level of resistance or reduced susceptibility to carbaryl, imidacloprid, fipronil, permethrin, and pyrethrins (Dryden, 1998; Payne et al., 2001; Bossard et al., 2002; Rust et al., 2002; Dryden et al., 2005). This *C. felis* strain has been repeatedly evaluated for its susceptibility to various insecticides and has been used in several evaluations of flea products (Dryden, 1998; Payne et al., 2001; Dryden et al., 2001, 2002, 2005, 2008; Bossard et al., 2002; Rust et al., 2002; Bass et al., 2004a,b).

2.4. Data analysis

The individual dog was the experimental unit and the primary endpoint was the live flea count. Flea counts were transformed by the $\log_e$ (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 block, the interaction between block and treatment, and error. Testing was two-sided at the significance level α=0.05. Percent efficacy relative to the control group was calculated as follows:

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

3. Results

3.1. Efficacy

Across all five studies treatment with a single oral dose of sarolaner was highly effective in controlling existing infestations and subsequent weekly re-infestations of *C. felis* and *C. canis* on dogs for 35 days (Table 2).

In Study 1, placebo-treated dogs maintained *C. felis* infestations throughout the study with post-treatment geometric mean counts ranging from 62.7 to 96.3*C. felis*/dog. Individual dog counts ranged from 11 to 100, and at least six of eight dogs had >70*C. felis* on each count day. No live *C. felis* were recovered from sarolaner-treated dogs at any post-treatment count. Thus efficacy was 100% from Day 1 through Day 35 after a single oral dose. Live *C. felis* counts were significantly lower for the sarolaner-treated dogs than placebo at all time-points ($P < 0.0001$).

In Study 2, dogs in the placebo group maintained *C. felis* infestations throughout the study with post-treatment geometric mean counts ranging from 62.4 to 85.8*C. felis*/dog. Individual dog counts ranged from 16 to 99, and at least six of eight dogs had >55 *C. felis* on each count day. No live *C. felis* were recovered from sarolaner-treated dogs at any post-treatment count up to Day 28; a single live *C. felis* was found on one dog on Day 35. Thus efficacy was 100% from Day 1 through Day 28, and 99.9% on Day 35 after a single oral dose. Live *C. felis* counts were significantly lower for the sarolaner-treated dogs than placebo at all time-points ($P < 0.0001$).

In Study 3, placebo-treated dogs had post-treatment geometric mean counts ranging from 59.2 to 83.3*C. felis*/dog. Individual dog counts ranged from 19 to 106, and at least seven of 10 dogs had >60*C. felis* on each count day. No live *C. felis* were recovered from sarolaner-treated dogs on Days 7, 14, 21 and 35. Single *C. felis* were found on one dog on Day 1, and on two dogs on Day 28. Thus, after a single oral dose, efficacy was 99.9% on Day 1, 99.8% on Day 28, and 100% on all other count days. Live *C. felis* counts were significantly lower for the sarolaner-treated dogs than placebo at all time-points ($P < 0.0001$).

In Study 4, placebo-treated dogs maintained good *C. felis* infestations throughout the study with post-treatment geometric mean counts ranging from 69.0 to 94.4*C. felis*/dog. Individual dog counts ranged from 56 to 101, and at least six of eight dogs had >70*C. felis* on each count day. No live *C. felis* were recovered from sarolaner-treated dogs at any time post-treatment. Thus efficacy was 100% from Day 1 day through Day 35 after a single oral dose. Live *C. felis* counts were significantly lower for the sarolaner-treated dogs than placebo at all time-points ($P < 0.0001$).

In Study 5, dogs in the placebo group maintained *C. canis* infestations throughout the study with post-treatment geometric mean counts ranging from 70.9 to 95.4*C. canis*/dog. Individual dog counts ranged from 47 to 102, and at least six of eight dogs had >65*C. canis* on each count day. No live *C. canis* were recovered from sarolaner-treated dogs at any post-treatment count through Day 35. Thus efficacy was 100% from Day 1 through Day 35 after a single oral dose. Live *C. canis* counts were significantly lower for the sarolaner-treated dogs than placebo at all time-points ($P < 0.0001$).

3.2. Dose acceptance

All animals were dosed completely; no tablets were expelled and no evidence of emesis of tablets was seen in any study. Two dogs in one study were noted to either gag or cough following the administration of water immediately following dosing.

3.3. Health observations

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

Table 2
Geometric mean flea counts for placebo dogs and percent efficacy relative to placebo at 24 h after treatment and weekly infestations for dogs treated orally with sarolaner tablets at 2 mg/kg.

Day	Study 1 (*C. felis*, AR)		Study 2 (*C. felis*, Ireland)		Study 3 (*C. felis*, NSW)		Study 4 (*C. felis*, KS1)		Study 5 (*C. canis*, Ireland)	
	Placebo	Sarolaner	Placebo	Sarolaner	Placebo	Sarolaner	Placebo	Sarolaner	Placebo	Sarolaner
1	92.3	0.0* (100)	63.9	0.0* (100)	59.2	0.1*(99.9)	69.0	0.0*(100)	74.4	0.0* (100)
7	96.3	0.0* (100)	70.2	0.0* (100)	70.7	0.0* (100)	90.5	0.0* (100)	95.4	0.0* (100)
14	79.5	0.0* (100)	66.5	0.0* (100)	83.3	0.0* (100)	84.9	0.0* (100)	84.3	0.0* (100)
21	71.9	0.0* (100)	62.4	0.0* (100)	82.3	0.0* (100)	94.0	0.0* (100)	85.4	0.0* (100)
28	62.7	0.0* (100)	79.1	0.0* (100)	79.6	0.2* (99.8)	94.4	0.0* (100)	75.8	0.0* (100)
35	75.9	0.0* (100)	85.5	0.1* (99.9)	74.9	0.0* (100)	87.1	0.0* (100)	70.9	0.0* (100)

* Geometric mean counts are significantly lower than placebo; $P \leq 0.0001$. Percent efficacy is given in parentheses.

4. Discussion

Over all five studies, sarolaner at the minimum oral dose of 2 mg/kg was highly effective for the treatment and control of *C. felis* and *C. canis* infestations for 35 days on dogs and there were no adverse reactions to treatment. Treatment of existing infestations was achieved within 24 h after dosing with efficacy ≥99.9% and only a single live *C. felis* found on one dog across all five studies on Day 1. Similarly, control of post-treatment *C. felis* and *C. canis* challenges was achieved within 24 h after infestation throughout the 35 day study periods, with only single live *C. felis* found on two dogs on Day 28 in one study, and a single dog on Day 35 in another study. Thus, the persistent efficacy was ≥99.8% within 24 h after challenges for 35 days after a single oral dose of sarolaner. Efficacy of 100% for five weeks was also achieved against the KS1 strain; a flea strain with demonstrated reduced susceptibility to many of the older topical parasiticides used for flea control.

Although *C. felis* is considered the predominate flea species infesting dogs globally, confirmation that sarolaner has similar excellent efficacy against *C. canis* is also clinically important, as this species has been shown to represent a significant proportion of the fleas infesting dogs in the United States and Europe (Franc et al., 1998; Durden, et al., 2005; Beck et al., 2006; Gracia et al., 2007).

Orally dosed flea control products need to be absorbed by the dog and the fleas need to feed in order to ingest an effective dose. While these dose confirmation studies did not directly address speed of kill, it is clear that sarolaner was rapidly effective as virtually all fleas in the existing infestations were killed in less than 24 h after treatment. Similarly, for up to five weeks after the single treatment, new flea infestations were almost entirely eradicated in less than 24 h after infestation. In separate studies, it was confirmed that sarolaner started killing fleas within three to four hours, and provided >95% control within eight hours for five weeks (Six et al., 2016). This rapid onset of efficacy also means that treatment will break the flea life cycle, as consistent control of fleas in less than 24 h means that adult fleas are killed before they can mature and lay eggs (Dryden et al., 2007; Six et al., 2016). This rapid and consistent control of fleas has been confirmed in the field (Cherni et al., 2016; Becskei et al., 2016) and combined with convenient oral dosing in a flavored, chewable formulation will make sarolaner (Simparica™) a valuable tool for veterinarians and pet owners in the treatment and control of flea infestations on dogs.

5. Conclusions

These five laboratory studies confirmed that a single oral dose providing a minimum of 2 mg sarolaner/kg provided highly effective treatment of existing *C. felis* infestations and persistent control of *C. felis* on dogs for 35 days after treatment. Equivalent excellent efficacy was confirmed against *C. canis*, and a known insecticide tolerant strain of *C. felis*.

Conflict of interest

The studies reported here were funded by Zoetis, Florham Park, NJ. RHS, TG, RP, AH, SPM, MRM, NS and KD are current employees of Zoetis. SC, WRE and SG were 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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References

Bass, C., Schroeder, I., Turberg, A., Field, L.M., Williamson, M.S., 2004a. Identification of the Rdl mutation in laboratory and field strains of the cat flea: *ctenocephalides felis* (Siphonaptera: pulicidae). Pest Manag. Sci. 60, 1157–1162.

Bass, C., Schroeder, I., Turberg, A., Field, L.M., Williamson, M.S., 2004b. Identification of mutations associated with pyrethroid resistance in the para-type sodium channel of the cat flea *Ctenocephalides felis*. Insect Biochem. Mol. Biol. 34, 1305–1313.

Beck, W., Boch, K., Mackensen, H., Wiegand, B., Pfister, K., 2006. Qualitative and quantitative observations on the flea population dynamics of dogs and cats in several areas of Germany. Vet. Parasitol. 137, 130–136.

Becskei, C., De Bock, F., Illambas, J., Mahabir, S.P., Six, R.H., 2016. 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. Vet. Parasitol. 222, 49–55.

Beugnet, F., Franc, M., 2012. Insecticide and acaricide moleculesand/or combinations to prevent pet infestation by ectoparasites. Trends Parasitol. 28, 267–279.

Bossard, R.L., Hinckle, N.C., Rust, M.K., 1998. Review of insecticide resistance in cat fleas (Siphonaptera: Pulicidae). J. Med. Entomol. 35, 415–422.

Bossard, R.L., Dryden, M.W., Broce, A.B., 2002. Insecticide susceptibilities of cat fleas (Siphonaptera: Pulicidae) from several regions of the United States. J. Med. Entomol. 39, 742–746.

Breitschwerdt, E.B., Kordick, D.L., 2000. Bartonella infection in animals: carriership reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin. Microbiol. Rev. 13, 428–438.

Breitschwerdt, E.B., Maggi, R.G., Chomel, B.B., Lappin, M.R., 2010. Bartonellosis: an emerging infectious disease of zoonotic importance to animals and human beings. J. Vet. Emerg. Crit. Care 20, 8–30.

Carlotti, D.N., Jacobs, D.E., 2000. Therapy: control and prevention of flea allergy dermatitis in dogs and cats. Vet. Dermatol. 11, 83–98.

Cherni, J.A., Mahabir, S.P., Six, R.H., 2016. Efficacy and safety of sarolaner (Simparica™) for the treatment and prevention of natural flea infestations on client-owned dogs in the US. Vet. Parasitol. 222, 43–48.

Chomel, B.B., Kasten, R.W., 2010. Bartonellosis, an increasingly recognized zoonosis. J. Appl. Microbiol. 109 (3), 743–750.

Dryden, M.W., Payne, P.A., 2004. Biology and control of ticks infesting dogs and cats in North America. Vet. Therap. 26, 2–16.

Dryden, M.W., McCoy, C.M., Payne, P.A., 2001. Rate of kill of nitenpyram tablets: imidacloprid spot-on and fipronil spot-on against flea infestations on dogs. Comp. Cont. Educ. Pract. Vet. 23, 24–27.

Dryden, M.W., Payne, P.A., Blagburn, B.L., Bledsoe, D.L., Denholm, I., Hansen, O., Hopkins, T., Jacobs, D.E., Mehlhorn, H., Mencke, N., Rust, M.K., Vaughn, M.B., 2002. Establishment of susceptibility profiles of cat fleas to imidacloprid and development of a program to monitor for imidacloprid susceptibility among cat flea populations—a 2002 Update. Comp. Cont. Educ. Pract. Vet 24, 14–16.

Dryden, M.W., Smith, V., Payne, P.A., McTier, T.L., 2005. Comparative speed of kill of selamectin imidacloprid, and fipronil-(S)-methoprene spot-on formulations against fleas on cats. Vet. Ther. 6, 28–236.

Dryden, M.W., Payne, P.A., Lowe, A., Mailen, S., Smith, V., Rugg, D., 2007. Efficacy of a topically applied formulation of metaflumizone on cats against the adult cat flea, flea egg production and hatch, and adult flea emergence. Vet. Parasitol. 150, 263–267.

Dryden, M.W., Payne, P.A., Lowe, A., Mailen, S., Smith, V., Rugg, D., 2008. Efficacy of a topically applied spot-on formulation of a novel insecticide metaflumizone, applied to cats against a flea strain (KS1) with documented reduced susceptibility to various insecticides. Vet. Parasitol. 151, 74–79.

Dryden, M.W., 1989. Host association: on-host longevity and egg production of *Ctenocephalides felis*. Vet. Parasitol. 34, 117–122.

Dryden, M.W., 1998. Laboratory evaluations of topical flea control products. Proc. Br. Vet. Dermatol. Study Group, 14–17.

Durden, L.A., Judy, T.N., Martin, J.E., Spedding, L.S., 2005. Fleas parasitizing domestic dogs in Georgia, USA : Species composition and seasonal abundance. Vet. Parasitol. 130, 157–162.

EMEA, 2000. Guideline on good clinical practice. VICH Topic GL9. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004343.pdf.

Foil, L., Andress, E., Freeland, R., Roy, A., Rutledge, R., Triche, P., O'Reilly, K., 1998. Experimental infection of domestic cats with *Bartonella henselae* by inoculation of *Ctenocephalides felis* (Siphonoptera: Pulicidae) feces. J. Med. Emtomol. 35, 625–828.

Franc, M., Choquart, P., Cadiergues, M.C., 1998. Répartition des espècesde puces rencontrées chez le chien en France. Rev. Med. Vet. 149, 135–140.

Gracia, M.J., Calvete, C., Estrada, R., Castillo, J.A., Peribanez, M.A., Lucientes, J., 2007. Fleas parasiting domestic dogs in Spain. Vet. Parasitol. 151, 312–319.

Horta, M.C., Ogrzewalska, M., Azevedo, M.C., Costa, F.B., Ferreira, F., Labruna, M.B., 2014. *Rickettsia felis* in *Ctenocephalides felis felis* from five geographic regions of Brazil. Am. J. Trop. Med. Hygiene 91 (1), 96–100.

Krämer, F., Menke, N., 2001. Flea Biology and Control. Springer, Berlin, pp. 192 pp.

Kwochka, K., 1987. Fleas and related disease. Vet. Clin. North Am.: Small Anim. Pract. 17, 1235–1262.

Marchiondo, A.A., Holdsworth, P.A., Fourie, L.J., Rugg, D., Hellmann, K., Snyder, D.E., Dryden, M.W., 2013. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition: guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats. Vet. Parasitol. 194, 84–97.

McTier, T.L., Six, R., Becskei, C., Fourie, J.J., Pullins, A., Hedges, L., Mahabir, S., Myers, M.R., Slootmans, N., 2016. Determination of the effective dose of a novel oral formulation of sarolaner for the treatment and month-long control of fleas and ticks on dogs. Vet. Parasitol. 222, 12–17.

Payne, P.A., Dryden, M.W., Smith, V., Ridley, R.K., 2001. Effect of 0.29% w/w fipronil spray on adult flea mortality and egg production of three different cat flea, *Ctenocephalides felis* (Bouche), strains infesting cats. Vet. Parasitol. 102, 331–340.

Robertson-Plouch, C., Baker, K.A., Hozak, R.R., Zimmermann, A.G., Parks, S.C., Herr, C., Hart, L.M., Jay, J., Hutchens, D.E., Snyder, D.E., 2008. Clinical field study of the safety and efficacy of spinosad chewable tablets for controlling fleas on dogs. Vet. Ther. 9, 26–36.

Rohdich, N., Roepke, R.K.A., Zschiesche, E., 2014. A randomized, blinded, controlled and multi-centered field study comparing the efficacy and safety of Bravecto™ (fluralaner) against Frontline™ (fipronil) in flea- and tick-infested dogs. Parasit. Vectors 7, 83.

Ross, D.H., Young, D.R., Young, R., Pennington, R.G., 1998. Topical pyriproxyfen for control of the cat flea and management of insecticide resistance. Feline Pract. 26, 16–22.

Rust, M.K., Dryden, M.W., 1997. The biology ecology, and management of the cat flea. Ann. Rev. Entomol. 42, 451–473.

Rust, M.K., Waggoner, M., Hinkle, N.C., Mencke, N., Hansen, O., Vaughn, M., Dryden, M.W., Payne, P.A., Blagburn, B.L., Jacobs, D.E., Bach, T., Bledsoe, D., Hopkins, T., Mehlhorn, H., Denholm, I., 2002. Development of a larval bioassay for susceptibility of cat fleas (Siphonaptera: Pulicidae) to imidacloprid. J. Med. Entomol. 39, 671–674.

Rust, M.K., 2005. Advances in the control of *Ctenocephalides felis felis* (cat flea) on cats and dogs. Trends Parasitol. 21, 232–236.

Shoop, W.L., Hartline, E.J., Gould, B.R., Waddell, M.E., McDowell, R.G., Kinney, J.B., Lahm, G.P., Long, J.K., Xu, M., Wagerle, T., Jones, G.S., Dietrich, R.F., Cordova, D., Schroeder, M.E., Rhoades, D.F., Benner, E.A., Confalone, P.N., 2014. Discovery and mode of action of afoxolaner: a new isoxazoline parasiticide for dogs. Vet. Parasitol. 201, 179–189.

Six, R.H., Becskei, C., Carter, L., Gale, B., Young, D.R., Mahabir, S.P., Chapin, S., Myers, M.R., 2016. Evaluation of the speed of kill, effects on reproduction, and effectiveness under a simulated infested-home environment of sarolaner (Simparica™) against fleas on dogs. Vet. Parasitol. 222, 23–27.