Veterinary Parasitology 238 (2017) S18–S21

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Efficacy and speed of kill of a new spot-on formulation of selamectin plus sarolaner against flea infestations in cats

Csilla Becskei[a,*], Judith A. Cherni[b], Adriano F. Vatta[b], Vickie L. King[b], Dan Lin[a], Douglas Rugg[b]

[a] Zoetis, Veterinary Medicine Research and Development, Mercuriusstraat 20, Zaventem B-1930, Belgium
[b] Zoetis, Veterinary Medicine Research and Development, 333 Portage Street, Kalamazoo, MI 49007, USA

ARTICLE INFO

Article history:
Received 10 January 2017
Received in revised form 7 March 2017
Accepted 8 March 2017

Keywords:
Sarolaner
Selamectin
Flea
Cat
Stronghold
Revolution
Speed of kill

ABSTRACT

The efficacy of a new spot-on formulation of selamectin plus sarolaner against induced flea infestations in cats was confirmed in three placebo-controlled, blinded studies. Purpose-bred adult cats (n = 8/group) were blocked by pre-treatment flea counts and randomly allocated to treatment with either a placebo or with the spot-on formulation at the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight. Treatments were applied topically once on Day 0. All cats were infested with approximately 100 unfed, adult *Ctenocephalides felis* prior to treatment and at weekly intervals for 5 weeks. In Studies 1 and 2 comb counts were conducted to determine the numbers of viable fleas 24 h after treatment and subsequent weekly infestations. In Study 3, flea counts were conducted at 6, 12, 24 and 48 h after treatment and 3, 6, 12 and 24 h after subsequent weekly infestations to evaluate the speed of kill against fleas. Cats in the placebo-treated groups maintained flea infestations throughout all studies. In Study 1, no live fleas were found on any of the treated cats, resulting in 100% efficacy for 5 weeks after a single treatment ($P \leq 0.0001$). In Study 2, selamectin/sarolaner reduced flea counts by 92.4% immediately after treatment and by 97.7%–100% after re-infestations for five weeks ($P \leq 0.0001$). In the speed of kill study, selamectin/sarolaner started killing fleas within 12 h after treatment administration and within 6 h following re-infestation for at least 28 days. Efficacy was 98.1% by 24 h after treatment and 100% within 24 h after re-infestations for 5 weeks. A single topical administration of a new spot-on formulation of selamectin plus sarolaner at the minimum dose rapidly and consistently kills fleas on cats for at least 5 weeks.

1. Introduction

Flea infestations are very common in cats, although they may only be recognized as a nuisance by pet owners particularly when they are accompanied by pruritus or when infestations become particularly heavy. Severe flea infestations may cause anaemia, particularly in young cats (Dryden, 1989). Cats with access to the outdoors are particularly at risk of flea infestations because of their more frequent contact with feral animals that are reservoirs for fleas (Rust, 2005). While flea infestations are known to peak during the warmer summer months, adult fleas also survive and reproduce in the colder months in home environments, where immature stages may survive for several months. Year-round flea control that provides efficacy against the immature stages of fleas should therefore be considered for pets in most geographic areas (Rust and Dryden, 1997). In addition, fleas are the intermediate hosts for the tapeworm, *Dipylidium caninum*, and can transmit a number of pathogens, including zoonotic pathogens such as *Rickettsia felis* (Pérez-Osorio et al., 2008) and *Bartonella henselae* (Chomel et al., 1996; Breitschwerdt and Kordick, 2000). Given the high prevalence and the pathogenic potential and zoonotic relevance of fleas (Beugnet and Franc, 2012), effective flea control is a concern for pet owners and veterinarians.

Most parasiticides have adulticidal activity (e.g. nitenpyram, spinosad, fipronil) or adulticidal and larvicidal activity, but are not effective against flea eggs (e.g. imidacloprid, indoxacarb). Selamectin is the only molecule with efficacy against the adult and larval stages of fleas and against flea eggs (McTier et al., 2000b; Dryden et al., 2007). Selamectin is also unique with its broad spectrum of activity, not only providing protection against flea infestations, but also against ear mites, lice, heartworm, and gastrointestinal nematode infections in cats (Fisher et al., 2007).

* Corresponding author.
E-mail address: csilla.becskei@zoetis.com (C. Becskei).

http://dx.doi.org/10.1016/j.vetpar.2017.03.010

Sarolaner is a novel isoxazoline with potent activity against a wide range of ectoparasites (McTier et al., 2016), and broadens the efficacy spectrum to include ticks.

A series of laboratory studies were conducted to confirm the efficacy of the new spot-on formulation of selamectin plus sarolaner, administered at the minimal dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight in the treatment and control of cats for one month.

2. Materials and methods

Three placebo-controlled, blinded and randomized studies were conducted. 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 Practice and 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 adult purpose-bred cats of both sexes, ranging in age from 9 months to 8 years. All cats were in good health at enrolment as confirmed by a physical examination by a veterinarian, and had undergone a wash-out period sufficient to ensure that no residual efficacy remained from any previously administered compounds. Cats were housed individually in enclosures with no physical contact between them that conformed to accepted animal welfare guidelines. Cats were acclimatized to the facilities for at least a week prior to treatment. Cats received an appropriate maintenance ration of a commercial feed for the duration of the study. Water was available ad libitum.

2.2. Experimental design

All cats were observed for general health at least twice daily throughout the studies.

To determine host suitability, cats were infested with approximately 100 fleas prior to treatment. The fleas were removed and counted 24 h after infestation. Cats with the highest live flea counts were selected for inclusion. In Studies 1 and 2, one placebo-treated group and one selamectin/sarolaner-treated group were enrolled (n = 8/group). In Study 3 (speed of kill), eight groups of cats were utilized (n = 8/group); four groups received placebo and four groups received selamectin/sarolaner. In each study, the cats were blocked by flea count and then randomly assigned to either placebo treatment or treatment with selamectin/sarolaner in a randomised complete block design.

2.3. Treatment administration

Treatments were administered topically directly to the skin in a single spot at the base of the neck in front of the shoulder blades on Day 0. All treatments were administered at a dose volume of 0.1 mL per kg bodyweight providing the minimum dose of 6.0 mg selamectin and 1.0 mg sarolaner per kg bodyweight in the treated groups. The placebo formulation was identical to the selamection plus sarolaner combination product, but only contained the excipients. Clinical observations were conducted pre-treatment and at 1, 3, 6 and 24 h after treatment.

2.4. Flea infestations and flea counts

Each cat was infested with approximately 100 unfed viable adult *Ctenocephalides felis* on Days −1, 6, 13, 20, 27 and 34 in Studies 1 and 2 and on Days −1, 7, 14, 21, 28, and 35 in Study 3. Infestations were performed by applying the fleas directly to the fur while the cats were restrained for a few minutes until the fleas dispersed into the hair coat. Fleas for all studies were obtained from local laboratory colonies that had all been genetically enriched with fleas within 1–2 years of the study. Study 1 and 3 used the same flea strain that originated from the USA, Study 2 used a separate flea strain that originated from Europe.

Flea counts were conducted by systematically combing the hair coat of each cat with a fine-toothed flea comb for at least 10 min and removing all fleas. Any animal on which fleas were found in the last 5 min was combed for an additional 5 min. All live fleas, including those incapable of maintaining upright orientation and/or coordinated movement at the time of their removal from the cats were counted. Protective clothing was changed between animals. In Studies 1 and 2, flea counts were conducted 24 h after treatment and after weekly re-infestations. In the third study, flea counts were conducted 6, 12, 24 and 48 h after treatment and 3, 6, 12 and 24 h after weekly re-infestations in separate pairs of placebo-treated and selamectin/sarolaner-treated groups. All personnel conducting parasite or other observations were blinded to treatment allocation.

2.5. Data analysis

Flea counts of individual cats were ln(count + 1) transformed prior to analysis in order to stabilize the variance and normalize the data. Transformed counts were analyzed using a general linear mixed model for repeated measures (SAS 9.3, Cary NC). 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. Hypothesis testing was two-sided at the significance level of 0.05. Percent efficacy relative to the control group was calculated using the Abbott formula: $[(C - T)/C] \times 100$, where C = arithmetic or geometric mean flea count for the control group and T = arithmetic or geometric mean flea count for the treated group.

3. Results

3.1. Efficacy

The results of the efficacy assessments (Studies 1 and 2) are summarized in Table 1. In Study 1, placebo-treated cats maintained flea infestations throughout the study with arithmetic mean counts between 89.4 and 94.6 fleas. No live fleas were recovered from any selamectin/sarolaner-treated cat at any post-treatment count. Efficacy was thus 100% through Day 35 after a single treatment. Live flea counts were significantly lower for the selamectin/sarolaner-treated animals compared to placebo at all time-points ($P < 0.0001$).

In Study 2, cats in the placebo-treated group maintained flea infestations throughout the study with arithmetic mean counts between 65.8 and 95.4 fleas. In the selamectin/sarolaner-treated group, efficacy based on arithmetic mean flea counts was 92.4%, 99.9%, 100.0%, 99.9%, 97.7% and 97.7% on Days 1, 7, 14, 21, 28 and 35, respectively. Live flea counts were significantly lower for the selamectin/sarolaner-treated cats compared with placebo at all time-points ($P < 0.0001$).

In the speed of kill study (Study 3), all placebo-treated groups maintained flea infestations throughout the study, with arithmetic mean counts between 72.0 and 90.9 fleas (Table 2). A significant reduction in flea counts was observed within 12 h after treat-