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Research paper

The efficacy of a novel topical formulation of selamectin plus sarolaner (Revolution® Plus/Stronghold® Plus) in preventing the development of *Dirofilaria immitis* in cats

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ABSTRACT

Three controlled studies were conducted to investigate the efficacy of selamectin plus sarolaner (Revolution® Plus/Stronghold® Plus) in preventing feline heartworm disease in cats. In all studies, cats were inoculated with 100 *Dirofilaria immitis* third stage larvae on Day -30. In the first study, cats were treated with selamectin plus sarolaner as a single dose on Day 0 or as three consecutive monthly doses on Days 0, 28 and 56. In the second and third studies, cats were treated with either sarolaner alone on Day 0, selamectin plus sarolaner on Day 0 or selamectin plus sarolaner as three consecutive monthly doses on Days 0, 28 and 56. In all three studies, dosages were 6 mg/kg selamectin plus 1 mg/kg sarolaner or 1 mg/kg sarolaner alone. Control cats were given a placebo containing inert formulation ingredients (vehicle). All treatments were administered at a single site topically to the skin cranial to the scapulae. Cats were humanely euthanized on Day 145/146 (i.e., 175/176 post-inoculation), and adult *D. immitis* worms were recovered and enumerated. Across the three studies, adult heartworms were recovered from 87 to 100% of control cats, with geometric mean worm counts ranging from 2.1 to 5.4. No adult *D. immitis* worms were recovered from cats treated with selamectin plus sarolaner. Cats treated with sarolaner alone were not protected against *D. immitis* infection, showing geometric mean worm counts of 1.9 to 2.4. In these studies, selamectin (6 mg/kg) plus sarolaner (1 mg/kg) was 100% effective in preventing heartworm development in cats when administered topically as one dose 30 days after inoculation or as three consecutive monthly doses starting 30 days post-inoculation. These studies demonstrated that a single topical administration of selamectin plus sarolaner at the recommended dosage was completely effective in preventing the development of *D. immitis* in cats.

1. Introduction

Within the past twenty years there has been an increasing awareness of the need for effective preventives for feline heartworm disease (Arther et al., 2003; Lee and Atkins, 2010; Baker et al., 2014). This recognition has been followed by the registration of ivermectin (Heartgard®, Merial, GA) in 1996, followed by selamectin (Revolution®/Stronghold®, Zoetis, NJ) in 1999, moxidectin (Advantage Multi®, Bayer Animal Health, KS) in 2007 and eprinomectin in the European Union (EU) in 2014 (Broadline™, Merial, GA) and in United States (USA) in 2018 (Centragard™, Merial, GA). Although cats are not natural definitive hosts for *Dirofilaria immitis*, they are susceptible to heartworm infection via mosquito transmission and through artificial means (McTier et al., 1992a, b; Mansour et al., 1995; Genchi et al., 2008; Levy et al., 2017). However, many infections are truncated due to the host immune response and few worms develop to patent adult infections, making it difficult to diagnose and accurately estimate feline heartworm prevalence using currently available serological tests. In addition, cats with low numbers of adult heartworms or single sex infections may have adult antigen that is bound in immune complexes and thus undetectable, also making underestimation of prevalence an issue in these cats, especially when cats have younger infections (Gruntmeir et al., 2017). Dogs are the definitive host for the parasite, and canine heartworm infection has been recorded from all states of the USA and many countries worldwide (Bowman et al., 2009; Simon et al., 2009; Venco et al., 2011; Morchon et al., 2012). Prevalence of infection in dogs

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Table 1
Study design and efficacy of a single dose or three monthly unit doses of selamectin plus sarolaner combination against *Dirofilaria immitis* in cats (Study 1).

Group[1] (n = 10)	Treatment	Dosage (mg/kg)	Day(s) of Treatment	No. of Cats with Worms	Adult *D. immitis* Worm Counts[2]		
					Individual Worm Counts	Geometric Mean[3]	Percentage Reduction
T01	Vehicle	0	0, 28, 56	9	0, 1, 1, 4, 5, 6, 16, 17, 19, 21	5.4[a]	–
T02	Selamectin + Sarolaner	6 + 1	0[4]	0	0	0[b]	100
T03	Selamectin + Sarolaner	6 + 1	0, 28, 56	0	0	0[b]	100

[1] All cats were inoculated with 100 *D. immitis* L3 (ZoeKy isolate) at Day -30.
[2] All cats were necropsied for recovery and enumeration of adult heartworms on Day 145 (175 days post-inoculation).
[3] Different superscripts indicate groups with significantly different geometric means ($p < 0.001$) from T01.
[4] Cats received a treatment with the vehicle on Days 28 and 56 to preserve masking.

varies depending on geographical location, environmental conditions and owner compliance, with estimates as high as 29% in Europe and approaching 50% in some parts of the USA (Bowman et al., 2009b; Levy et al., 2011; Venco et al., 2011; Little et al., 2014a). Necropsy surveys of shelter cats suggest the USA prevalence of feline heartworm disease is between 5% and 18% of that observed in dogs within a given geographic locale (Guerrero et al., 1992a,b; Ryan and Newcomb, 1995; Litster and Atwell, 2008; Venco et al., 2011), and global prevalence rates have been reported to be as high as 24% in endemic areas of the world (Courtney et al., 1989; Genchi et al., 1992; Guerrero et al., 1992a,b; Magi et al., 2002; Montoya-Alonso et al., 2015).

Cats infected with *D. immitis* show a wide range of clinical manifestations, including coughing, vomiting, lethargy, anorexia, dyspnea, convulsions and even sudden death (Litster and Atwell, 2008; Lee and Atkins, 2010). One or two adult worms are sufficient to induce these symptoms, which result from the physical presence of these large worms in the cat's pulmonary vasculature as well as from the strong host immune response mounted by the cat in response to their presence. However, cats do not have to develop mature adult *D. immitis* infections to be at risk of disease from larval heartworm exposure. A significant condition, known as heartworm-associated respiratory disease (HARD), can occur when migrating *D. immitis* larvae die before reaching the heart, either due to innate immune mechanisms or preventive treatments (Blagburn and Dillon, 2007; Dillon et al., 2007). The strong host immune response mounted by the cat to the dead/dying worms can result in respiratory disease and long-lasting pulmonary pathology (Dillon et al., 2014, 2017a, b).

The same general immune responsiveness of cats that contributes to HARD also makes adulticidal heartworm treatment potentially dangerous. Melarsomine dihydrochlorine, the only approved and available adulticide for dogs, is not approved for use in felines and is actually contraindicated for cats because it can lead to rapid worm death followed by an acute host response and subsequent death of the cat. The lack of adequate treatment options combined with the feline immune response leaves chemoprophylaxis as the only real option for protecting cats against both parasite- and host-induced harm (Baker et al., 2014).

Macrocyclic lactones (MLs) have a long history of safety and efficacy in companion animals, and selamectin is known to protect cats against infection with *D. immitis* and other endo- and ectoparasites (Jernigan et al., 2000; McTier et al., 2000a, b,c,d). However, selamectin has limited efficacy against ticks (Jernigan et al., 2000); for this reason, selamectin was combined with the isoxazoline, sarolaner, which has been shown to have excellent efficacy against ticks (McTier et al., 2016). The three studies reported in this paper evaluated the efficacy of a novel combination of selamectin and sarolaner (Revolution® Plus/Stonghold® Plus) in preventing the establishment and development of heartworms in cats. The selamectin plus sarolaner combination was administered as either a monthly dose or as three consecutive monthly doses in cats experimentally infected with *D. immitis* third stage larvae (L3). To demonstrate that the addition of sarolaner did not interfere with the efficacy of selamectin within the combination product, groups treated with sarolaner alone were included in two of the studies. In all studies, worm counts at necropsy were used to assess efficacy.

2. Materials and methods

Three separate studies were conducted in the USA to investigate the efficacy of a product designed to deliver selamectin (6 mg/kg) in combination with sarolaner (1 mg/kg) to prevent the development of induced *D. immitis* infections in cats. Generally, two studies are required by USA regulatory authorities to obtain approval for this type of heartworm prevention claim. However, a third study was necessary as one of the initial two studies did not meet the requirement of adequate infection in the control group as defined by the Food and Drug Administration Center for Veterinary Medicine (FDA-CVM) (that is, 2 or more worms in 60% of the control cats). Studies were conducted at either TRS Labs, Inc (GA, USA) or Zoetis (MI, USA) in accordance with the CVM Guidance for Industry #85, Good Clinical Practices (VICH guideline GL9) (CVM, 2001) and CVM Guidance for Industry #113, Efficacy of Anthelmintics-Specific Recommendations for Felines (VICH guideline GL20) (CVM, 2002). Study protocols were reviewed and approved by the TRS Labs and/or Zoetis Institutional Animal Care and Use Committees, and all studies were conducted according to relevant state, national, and international regulations regarding animal welfare.

2.1. Study design

Studies were designed to evaluate the efficacy of selamectin plus sarolaner administered topically as either a single dose or three consecutive monthly doses in preventing heartworm disease in cats. Following inoculation with 100 *D. immitis* L3 (Day -30), cats were treated with either a placebo containing inert formulation ingredients (vehicle), a combination product containing selamectin plus sarolaner, or sarolaner alone on either Day 0 only (single dose) or on Days 0, 28 and 56 (three consecutive monthly doses), according to the study objective and design (see Tables 1–3). Preventive efficacy was evaluated at Day 145/146 (175/176 days post-inoculation) following necropsy and adult worm recovery and enumeration.

2.2. Animals

Individually identified, purpose-bred domestic shorted-haired cats were acclimated to the facilities for at least 7 days prior to *D. immitis* inoculation. At the time of study initiation all cats were 5–9 months of age, 2.5–5.7 kg and confirmed by a veterinarian to be in good health. Animals were housed individually, due to the constraints from the topical treatment, in indoor cages but were housed in the same room in a manner that allowed cats to view other cats. All housing conformed to accepted animal welfare guidelines, and animals were fed an appropriate maintenance diet of a commercial dry feline ration, and had access to water ad libitum. Standard accepted environmental conditions were maintained, and environmental enrichment and social interactions were provided. Thirty cats were used in Study 1 and 40 cats were used in each of Studies 2 and 3. Each treatment group (n = 10)

Table 2
Study design and efficacy of a single dose or three monthly unit doses of selamectin plus sarolaner or a single dose of sarolaner against *Dirofilaria immitis* in cats (Study 2).

Group[1] (n = 10)	Treatment	Dosage (mg/kg)	Day(s) of Treatment	No. of Cats with Worms	Adult *D. immitis* Worm Counts[2]		
					Individual Worm Counts	Geometric Mean[3]	Percentage Reduction
T01	Vehicle	0	0, 28, 56	7	0, 0, 0, 1, 1, 2, 2, 8, 13, 15	2.1 [a]	–
T02	Selamectin + Sarolaner	6 + 1	0[4]	0	0	0 [b]	100.0
T03	Sarolaner	1	0[4]	6	0, 0, 0, 0, 1, 2, 4, 7, 11, 12	1.9 [a]	9.5
T04	Selamectin + Sarolaner	6 + 1	0, 28, 56	0	0	0 [b]	100.0

[1] All cats were inoculated with 100 *D. immitis* L3 (GCFL-01 isolate) at Day -30.
[2] All cats were necropsied for recovery and enumeration of adult heartworms on Day 146 (176 days post-inoculation).
[3] Different superscripts indicate groups with significantly different geometric means ($p < 0.001$) from T01.
[4] Cats received a treatment with the vehicle on Days 28 and 56 to preserve masking.

consisted of approximately equal numbers of male (5–6) cats and female (4–5) cats.

No cat was treated with a ML-containing product within 90 days prior to the start of the study. All animals received a physical examination by a veterinarian to determine health status and suitability for inclusion in the study and were observed for general health at least once (and generally twice) daily while on the study. Cats were determined to be free of heartworm infection via serological analyses 1–5 days prior to L3 inoculation. The absence of circulating microfilariae was verified using a modified Knott's test, and commercially available tests were used according to the manufacturers' instructions to confirm a lack of circulating adult *D. immitis* antigen (DiroCHEK®, Zoetis) and antibodies to *D. immitis* (Solo Step® Feline Heartworm Antibody Test, Heska, CO). Sera for the antigen test were heat-treated to 103 °C or 104 °C for 10 min in a heat block, according to Little et al. (2014a, b). To identify any infections not previously detectable, additional blood samples were collected on Day 64 (Study 1), Day 87 (Study 2) or Day 69 (Study 3). All samples were tested for adult *D. immitis* antigen and, in Studies 2 and 3, the samples were also examined for microfilariae of *D. immitis*. For the antigen tests, samples were heat fixed as described above. Within each study, cats were ranked by Day -2 or Day -3 body weight and allocated to treatments and cages according to a randomized complete block design with a one-way treatment structure. Blocking was based on cage location and pre-treatment body weight. Vehicle or test formulations were administered at a single site topically to the skin cranial to the scapulae. Personnel changed protective gloves and clothing between handling each cat and applying treatments. Clinical observations and administration site evaluations were made on all animals prior to and at 1, 3, 6, and 24 h after the administration of vehicle or test product on Days 0, 28 and 56. Additionally, administration sites were evaluated 3 days after each treatment and on Day 84 in each study. In Studies 1 and 3, evaluations of administration sites were also made 5 days after treatment. All personnel conducting observations were masked to treatment allocations. Cats were humanely euthanized on Days 145 or 146 for recovery of adult heartworms.

2.3. Heartworm isolates

The heartworm isolates used in these studies were recent isolates taken from naturally infected dogs from the southeastern USA. Study 1 used the ZoeKy isolate, whereas Studies 2 and 3 used the GCFL-01 isolate.

ZoeKY isolate: The ZoeKY isolate was collected from a dog originally from Slayersville, KY on 4 March 2013.

GCFL-01 isolate: the GCFL-01 isolate was collected from a 3-year-old mixed breed from the Fort Myers, FL area on 4 September 2014. Both of these isolates have been characterized as not resistant to MLs (McTier et al., 2017).

2.4. Inoculation with D. immitis

On Day -30, each cat was inoculated with 100 *D. immitis* L3 in RPMI balanced salt solution by subcutaneous inoculation in the inguinal area. Larvae were harvested from infected *Aedes aegypti* mosquitoes reared and maintained at Zoetis (MI, USA) (Study 1) or at TRS Labs (GA, USA) (Studies 2 and 3), as previously described (McCall et al., 1980).

2.5. Treatments

Placebo treatments consisted of the vehicle only. Sarolaner, selamectin plus sarolaner and placebo treatments were administered using a 1-mL syringe. All doses were calculated according to body weights collected within 24–72 h of treatment. The selamectin plus sarolaner combination product was formulated at 60 mg/mL for selamectin and 10 mg/mL for sarolaner, and sarolaner alone was formulated at 10 mg/mL. Both control and selamectin plus sarolaner combination formulations were applied at a volumetric ratio of 0.1 mL/kg. This resulted in the cats receiving an effective dose of 6 mg/kg selamectin and/or 1 mg/kg sarolaner, as appropriate (see Tables 1–3 for study specific treatment groups). The vehicle control was administered at the same volumetric ratio of 0.1 mL/kg.

Table 3
Study design and efficacy of a single dose or three monthly unit doses of selamectin plus sarolaner or a single dose of sarolaner against *Dirofilaria immitis* in cats (Study 3).

Group[1] (n = 10)	Treatment	Dosage (mg/kg)	Day(s) of Treatment	No. of Cats with Worms	Adult *D. immitis* Worm Counts[2]		
					Individual Worm Counts	Geometric Mean[3]	Percentage Reduction
T01	Vehicle	0	0, 28, 56	10	1, 1, 1, 3, 3, 3, 3, 9, 15, 20	3.8 [a]	–
T02	Selamectin + Sarolaner	6 + 1	0[4]	0	0	0 [b]	100.0
T03	Sarolaner	1	0[4]	8	0, 0, 1, 2, 3, 3, 3, 4, 10, 10	2.4 [a]	36.2
T04	Selamectin + Sarolaner	6 + 1	0, 28, 56	0	0	0 [b]	100.0

[1] All cats were inoculated with 100 *D. immitis* L3 (GCFL-01 isolate) at Day -30.
[2] All cats were necropsied for recovery and enumeration of adult heartworms on Day 146 (176 days post-inoculation).
[3] Different superscripts indicate groups with significantly different geometric means ($p < 0.001$) from T01.
[4] Cats received a treatment with the vehicle on Days 28 and 56 to preserve masking.

2.6. Necropsy and parasite recovery

Cats were euthanized via an intravenous injection of heparin and an approved euthanasia solution on Day 145 or 146 (i.e., 175 or 176 days post-inoculation). The inoculation site (Studies 2 and 3) and the pleural and peritoneal cavities (all studies) were examined for adult *D. immitis* worms. The posterior and anterior venae cavae were clamped, and the heart, lungs and liver were removed. The precaval, right atrium, right ventricle, pulmonary arteries and blood vessels of the liver (Studies 2 and 3) were dissected, and any adult worms present were recovered. Worms recovered from a cat were classified as male or female and as either dead (worms abnormal in both appearance and motility) or alive (all other worms), according to Holmes et al. (1986). Cats were randomized to order of necropsy with blocking structure maintained so that necropsy order for both block and individual animal within block was random.

2.7. Data analysis

The experimental unit for treatment was animal. Within each study, the numbers of adult *D. immitis* recovered during post mortem examinations were summarized for each treatment group. Natural log (x + 1) transformation was applied to all counts prior to analysis, and the geometric means (back-transformed least squares means were calculated.

Percent efficacy, relative to the control group and based on geometric means, was calculated as follows:

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

Treatments with prevention rates of 100% were considered efficacious. Treatment differences were assessed between control and treated groups using contrasts in a general linear mixed model analysis of natural logarithm transformed worm counts and a significance level of $p < 0.05$. All analyses were carried out using SAS/STAT Release 9.3 or 9.4 (SAS Institute, Cary, NC).

3. Results

Serological analyses conducted 1–5 days prior to L3 inoculation were negative for all cats included in the three studies. None of the cats tested positive for heartworm on antigen testing on Day 64 (97 days PI) in Study 1. However, one cat tested positive on antigen testing on Day 69 (99 days PI) in Study 3 and 5 cats tested positive on Day 87 (117 days PI) in Study 2. When the positive cats were re-tested using the same samples but without heat treatment, the results were negative. Heating the samples prior to testing was considered to have increased the sensitivity of the test to the extent that the infections resulting from experimental inoculation were being detected; consequently, the animals were retained on study. This decision was supported by a lack of microfilariae being noted in any of the samples tested on Day 69 or Day 87. More importantly, all worms subsequently recovered at necropsy were of a similar size and maturity. All animals were thus considered to have been heartworm-free prior to experimental inoculation with *D. immitis* L3 on Day -30.

3.1. Efficacy

An adequate infection was achieved in Study 1 using the ZoeKy isolate of *D. immitis*, with nine of the ten cats in the vehicle control group (Group T01) having adult worms at necropsy (Table 1). Worm counts in infected control cats ranged from 1 to 21, with seven cats having four or more worms. Compared to the control group, cats treated with either a single (Group T02) or three consecutive monthly doses (Group T03) of selamectin plus sarolaner showed a 100% reduction in the geometric mean number of worms recovered ($p < 0.001$) for both Group T02 and Group T03 versus Group T01 (Table 1). No live or dead adult *D. immitis* worms were recovered from any of the selamectin plus sarolaner combination-treated cats.

In Studies 2 and 3, the *D. immitis* GCFL-01 isolate established infections in cats, with seven out of 10 (Study 2) and 10 out of 10 (Study 3) animals in the control groups (T01) having at least one adult *D. immitis* worm (Tables 2 and 3). However, in Study 2, the FDA-CVM defined criteria for an adequate infection (2 or more heartworms in 60% of the cats in the control group) was not met because only five of the 10 cats had two or more worms. Similar results were seen for the sarolaner-alone group (Group T03), with five of the 10 cats having two or more heartworms. Because Study 2 did not meet the FDA-CVM criteria for adequate infection, a third study was required to support substantial evidence of effectiveness for the prevention of heartworm disease indication. For Study 3, all cats in the control group were infected with at least one worm, and seven cats had three or more worms. In the sarolaner-alone group, eight cats had one or more worms, seven cats had two or more and six cats had three or more heartworms. As in Study 1, no live or dead adult *D. immitis* worms were recovered from cats treated with either a single dose (Group T02) or three consecutive monthly doses (Group T04) of selamectin plus sarolaner in either Study 2 or Study 3. Within each study, the reduction in geometric mean worm counts was significant for both Group T02 and Group T04 ($p < 0.001$ for both groups versus Group T01 in Study 2; $p < 0.001$ for both groups versus Group T01 in Study 3). Cats treated with a single dose of sarolaner (Group T03 for Studies 2 and 3) did not show significantly reduced worm counts compared to controls.

3.2. Health observations

There were no mortalities among any animals in all three studies. No cats showed persistent cosmetic changes at the test administration site in any of the three studies. Some cats in Study 1 showed a transient greasiness of fur at the administration site; this change was absent within 24 h of product administration.

Various abnormal health observations (e.g., emesis, abrasions, mild diarrhea, ocular discharge, and conjunctivitis) were made during these studies and occurred across all treatment groups. The abnormal health signs were not unexpected for cats housed for studies of six months duration. In Study 3, one case of regurgitation 24 h after Day 28 treatment in a cat from Group T04 was determined to be possibly related to treatment; however, no similar regurgitation by the cat was noted following subsequent test product administration on Day 56. All other reports of abnormal health were not likely to be related to treatment administration.

4. Discussion

Administration of a selamectin plus sarolaner combination product at the recommended minimum dosage (6 mg/kg selamectin plus 1 mg/kg sarolaner) was effective in preventing the development of *D. immitis* in all selamectin plus sarolaner-treated cats in all three studies. While the authors acknowledge the limitations inherent even in carefully executed laboratory studies and in how the data from these studies may be analyzed as recently identified by Vidyashankar et al. (2017), in the three studies presented here selamectin plus sarolaner was 100% effective in preventing the development of *D. immitis* in cats receiving either a single dose or three consecutive monthly doses.

Control cats in the same studies showed robust infections, with an average infection rate of 87% and geometric mean adult worm counts ranging from 2.1 to 5.4. These data reflect those reported for control cats in similar studies (McTier et al., 1992b, 2000b; Arther et al., 2005; Baker et al., 2014). With regard to single worm infections, these are very common in cats, even with induced infections of up to 100 L3s (McTier et al., 1992b; Stewart et al., 1992; Genchi et al., 2004; Arther et al., 2005; Baker et al., 2014; Little et al., 2015). The authors therefore

believe that single-worm infections should be considered adequate infections in control cats for efficacy evaluation (provided that they do not represent the majority of infected cats in the control group) and that they are sufficient to satisfy the VICH guidelines for adequacy of infection (VICH GL20) (CVM, 2002). This would apply to Study 2 in this case, in which 2 of the 10 control cats had single-worm infections and 5 of the cats had more than 2 worms. This is especially true as 100% effectiveness is required to obtain product approval for heartworm preventive products in laboratory studies, and this was demonstrated in Study 2. In addition, mean differences between control and treated groups in this study were statistically significant ($p < 0.001$). The authors recommend that feline heartworm experts re-examine these aspects of feline laboratory study design to ensure appropriate and realistic infection levels in control animals to enable reliable efficacy evaluation. In addition, the authors suggest that these recommendations be communicated in an appropriate manner (i.e., American Heartworm Society's: *Prevention, Diagnosis, and Management of Heartworm (Dirofilaria immitis) Infection in Cats* or in other widely distributed publications) highlighting these recommendations.

Heat treatment of serum samples prior to antigen testing increases the sensitivity of the test and allow for earlier detection of heartworm-positive cats and dogs (Little et al., 2014a, b; Carmichael et al., 2017). Heating sera is thought to disrupt antigen-antibody complexes or other factors that inhibit the detection of parasite antigen. In our studies, *D. immitis* antigen was detected in one cat at 99 days post infection (Day 69, Study 3) and in five cats at 117 days post infection (Day 87, Study 2). Given all cats were purpose-bred and housed in mosquito-proof enclosures from birth to necropsy and given that all worms recovered at necropsy were adults of similar size and maturity, accidental heartworm infection is unlikely to have occurred. Additionally, when re-tested without prior heat treatment, the same sera samples were negative for *D. immitis* antigen. It is likely, then, that heat treatment of the samples increased the sensitivity of the antigen tests such that the current experimental infections were detected.

The need for effective heartworm protection for cats continues to be a growing concern in the USA and around the world. Despite the considerable prevalence of heartworm in cats in many areas and the known pathology associated with feline *D. immitis* infection, many at-risk cats still do not receive regular heartworm preventive (Gates and Nolan, 2010; Lee and Atkins, 2010; Venco et al., 2011). In conjunction with ongoing educational programs aimed at both veterinarians and pet owners, continued emphasis on the availability of safe and effective preventive products is essential. Multiple approved products are currently available for the prevention of *D. immitis* infection in cats, including the ML, selamectin. Previous studies have shown that a single treatment with selamectin is 100% efficacious in preventing heartworm development in cats when administered topically at a minimum dose of 6 mg/kg (Bishop et al., 2000; McTier et al., 2000b). To ensure the addition of sarolaner did not inhibit the activity of selamectin against *D. immitis*, all three of the current studies contained cats treated with the selamectin plus sarolaner combination, and the dose of selamectin (administered as part of the combination) was 6 mg/kg. Some cats were treated with the combination as a single dose to replicate the typical efficacy of selamectin against *D. immitis*. Other cats were treated for three consecutive months with the combination product to assess the effectiveness of multiple monthly treatments in the unlikely event that a single dose of the combination product proved less effective than selamectin alone. In all three studies, the selamectin plus sarolaner combination was completely effective in preventing heartworm development in cats after a single dose, demonstrating that the required 100% efficacy of selamectin was retained in the combination product. These results compare favorably to those obtained in cats using other approved preventives, such as the MLs ivermectin, milbemycin oxime, moxidectin and eprinomectin (McTier et al., 1992b; Stewart et al., 1992; Genchi et al., 2004; Arther et al., 2005; Baker et al., 2014). The ZoeKY and the GCFL-01 isolates used in these studies provide a background of genetic diversity for the testing of this combination product, being isolated a year apart from infected dogs within Kentucky and Florida, respectively. Both isolates had also been maintained in the laboratory for less than four years at the time of inoculation.

It was assumed for many years that cats were only rarely infected with *D. immitis*, but this erroneous view continues to be amended with our increasing knowledge of feline heartworm disease. Whereas some cats do develop mature *D. immitis* infections, most L3 do not develop into adult worms in cats but die at around 3 months post-infection, prior to reaching the heart. It is not surprising, then, that recent studies suggest the prevalence of heartworm infections in cats is much higher than necropsy and adult worm recovery data suggest (Levy et al., 2017), as the early death of migrating larvae masks the true prevalence of feline *D. immitis* infection. The accuracy of active heartworm disease diagnosis in cats is also affected by the early death of immature worms. Dying/dead *D. immitis* larvae release by-products that activate feline intravascular macrophages and other pro-inflammatory mediators, predisposing some infected cats to develop HARD (Blagburn and Dillon, 2007; Dillon et al., 2007). The clinical signs and pulmonary pathology of HARD reflect those observed for other feline bronchial diseases (Browne et al., 2005; Blagburn and Dillon, 2007; Dillon et al., 2007, 2017a, b; Bowman and Atkins, 2009), which can confound an accurate diagnosis of heartworm disease. Additionally, currently available serological tests, when used according to manufacturer's instructions, are only able to consistently and reliably detect mature heartworms, which often do not develop and are not always the primary cause of disease in cats. The combination of common clinical symptoms and inability to detect *D. immitis* larvae make it reasonable to assume that some immature feline heartworm infections are mis-diagnosed as respiratory disease, particularly in areas endemic for heartworm (Blagburn and Dillon, 2007). Unfortunately, the subsequent lung pathology and clinical disease development in cats with HARD is significant and can lead to death (Dillon et al., 2007, 2008; Holmes, 1993). Animals that survive the initial acute immune response may still develop chronic pulmonary inflammation, and studies have shown that lung pathology may be present in cats many years after *D. immitis* infection (Robertson-Plouch et al., 2000; Browne et al., 2005). However, although HARD in cats can be debilitating and potentially fatal, it is completely avoidable. Previous studies have described the use of selamectin in preventing the development of HARD in cats, with treatment with selamectin prior to heartworm inoculation yielding the best results (Dillon et al., 2014, 2017a, b). Thus, the regular use of efficacious heartworm preventives, such as selamectin and the selamectin plus sarolaner product described here, provides cats protection against the development of *D. immitis* and consequently against the development of HARD.

More than 30% of households in the USA (AVMA, 2012) are estimated to own domestic cats, and recent evidence suggests hundreds of thousands of cats are likely currently infected with *D. immitis* in the USA (Levy et al., 2017). It is unsurprising, then, that interest in combination heartworm preventatives with safe, broad-spectrum efficacy against other common parasites of cats and a convenient topical formulation continues to grow (Abongwa et al., 2016; Otranto and Little, 2017). In addition to preventing heartworm infection in cats, selamectin treats and prevents the infestation of fleas, and treats and controls lice, ear mites, roundworms, and hookworms (McTier et al., 2000a, b, c; Shanks et al., 2000, 2003), making it a practical choice for pet owners. Our studies confirm sarolaner has no activity against heartworms in cats, as cats treated with sarolaner alone (Group T03 in Studies 2 and 3) were not protected against *D. immitis* infection. However, sarolaner is effective in protecting dogs against ticks, fleas and mites (Becskei et al., 2016; Six et al., 2016a, b), and combining it with selamectin has broadened the original efficacy of Revolution® to include the protection of cats against tick infestation (Becskei et al., 2017b; Geurden et al., 2017a; Vatta et al., 2018a, b). The new selamectin plus sarolaner product also retains the efficacy seen with Revolution® against feline hookworm, roundworm, fleas and ear mites (Becskei et al., 2017a, c;

Geurden et al., 2017b; Vatta et al., 2017). This extended activity makes the selamectin plus sarolaner combination product one of the broadest-acting feline heartworm preventives on the market and provides pet owners with a convenient, safe and comprehensive parasiticide.

5. Conclusions

In these three controlled studies, 6 mg/kg selamectin plus 1 mg/kg sarolaner was 100% effective in preventing the development of *D. immitis* in cats when applied as a single, topical dose 30 days after inoculation of L3. The addition of sarolaner did not inhibit the efficacy of selamectin against heartworm in cats. Topical application of selamectin plus sarolaner is an effective and convenient means of preventing heartworm disease in cats while also providing protection against additional endo- and ectoparasites.

Conflict of interest

The studies reported here were funded by Zoetis, Kalamazoo, MI, USA. TM, AP, SC, MvR, VK, JR and AV are current or former 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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