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Efficacy of a single dose of a novel topical combination product containing eprinomectin to prevent heartworm infection in cats

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ABSTRACT

Cats may be infected by heartworm, *Dirofilaria immitis*, through mosquito bites. They can develop severe heartworm disease when infective *D. immitis* larvae migrate and develop into adults in the pulmonary vasculature or other tissues. As there is no curative treatment for feline heartworm infection, the monthly administration of preventative treatment is recommended in endemic areas. Three controlled, blinded laboratory studies were conducted to evaluate the preventative efficacy of BROADLINE®, a novel combination of fipronil, (S)-methoprene, eprinomectin, and praziquantel against *D. immitis* in cats. In each study, 28 cats were inoculated with approximately 100 (studies 1 and 2) or 40 (study 3) infective third stage *D. immitis* larvae by subcutaneous injection, thirty days prior to treatment. The larvae were from recent field isolates from naturally infected dogs from three distinct geographic areas (two in the USA and one in Europe). In each study, the cats were allocated randomly to two study groups of 14 cats each. The control group remained untreated. On Day 0, each cat in the treated group received one topical application of the novel topical formulation, delivering the minimum intended dose of 0.5 mg of eprinomectin per kilogram of body weight. At 6 months after infection, all cats were humanely euthanized and examined for adult *D. immitis*. Across all three studies, 28 (68%) of the 41 untreated cats harbored one or more heartworms, while 100% of the 42 treated cats remained free of heartworm infection, demonstrating the 100% preventive efficacy of BROADLINE® against *D. immitis* in cats. The treatment was well tolerated and no health abnormality was observed in any treated cat.

1. Introduction

As observed in domestic dogs (Genchi et al., 1991; Guerrero et al., 1992), coyotes (Willingham and Gehrt, 2010), or within mosquito populations, the incidence of *Dirofilaria immitis* (heartworm) infection is highest in the tropical and sub-tropical regions of the world where high

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density populations of reservoir and intermediate hosts coexist and perpetuate the life cycle. Even if less receptive than dogs, cats can also be infected by *D. immitis* in the same endemic areas (Patton and McCracken, 1991; Ryan and Newcomb, 1995; Miller et al., 2000; Levy et al., 2003). Because they are not the natural host for this parasite, they are less susceptible to patent infection than dogs and their infection rate has been estimated to be approximately 9–18% that of dogs in the same endemic areas (Venco et al., 2011). The presence of *D. immitis* in cats results in marked immunological reactions that will often kill the parasite before or after it reaches its final destination in the pulmonary vessels. This strong immunological reaction may also cause severe pathology in the host, especially in response to the death of the adult pulmonary stage of *D. immitis*. Heartworm disease in cats may present with episodic, chronic or acute clinical signs that may include coughing, vomiting, dyspnea, lethargy, anorexia or weight loss, or severe respiratory distress, convulsions and sudden death in the most severe cases, which may occur even in cats harboring a single heartworm (McCall et al., 1992; Jacobs et al., 1994; Dillon, 1995; Atkins et al., 2000; Litster and Atwell, 2008; McCall et al., 2008). High risk surgical removal is the only potential treatment of pulmonary stages of *D. immitis* in cats. The severity of heartworm disease in cats leaves chemoprophylaxis as the only safe and effective option to protect cats from the adverse effects of adult heartworms.

Eprinomectin is a macrocyclic lactone that has proved to be highly effective as a topical formulation against internal and external parasites of cattle (Shoop et al., 1996; Holste et al., 1997; Pitt et al., 1997; Williams et al., 1997; Holste et al., 1998; Watson and Forbes, 2000; Rehbein et al., 2005). Preliminary studies confirmed the excellent bioavailability and systemic activity of eprinomectin in cats following topical application (Kvaternick et al., 2014). The present studies were conducted to evaluate the efficacy of a single topical treatment of eprinomectin at the minimum intended dose of 0.5 mg/kg body weight in a novel topical combination with fipronil, (S)-methoprene and praziquantel, Broadline®, to prevent heartworm disease in cats.

2. Materials and methods

2.1. Study design and randomization

Three controlled, blinded laboratory studies were conducted, two in the United States and one in France, with similar designs, as summarized in Table 1. The 3 studies were designed in accordance with the "International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products" (VICH) guideline (GL) 7, "Efficacy of Anthelmintics: General Requirements" (Vercruysse et al., 2001); VICH GL 20, "Efficacy of Anthelmintics: Specific Recommendations for Felines" (Vercruysse et al., 2002); as well as with the "World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics for dogs and cats" (Jacobs et al., 1994). All studies were also conducted in compliance with local Institutional Animal Care and Use Committee requirements, and with International laws and ethics.

Twenty-eight cats (14 females and 14 males) were included in each study. Fourteen cats were assigned to each control and treatment group using a randomized block design based on body weight within sex in studies 1 and 2 and on sex in study 3. All personnel collecting animal health and efficacy data were blinded to the treatment.

2.2. Study animals

All study subjects were healthy purpose-bred laboratory cats, born and raised in a mosquito-protected environment. For completeness, all cats were tested for the presence of *D. immitis* antigens before infection and on Day 89 or 90 using an ELISA test (DiroCHECK®, Synbiotic Corporation, San Diego, CA, USA in studies 1 and 2; SNAP 4Dx, IDEXX Laboratories, Portland, Maine, USA in study 3). Any positive test result up to Day 90 (4 months after infection) would indicate that the infection had been acquired before the study. The cats had never been treated with macrocyclic lactones and had not been reared by queens treated with macrocyclic lactones.

2.3. Animal health

All animals had a physical examination conducted by a veterinarian to detect any health abnormality prior to infection. Health observations were conducted, at hourly intervals for four hours after treatment, and daily throughout the studies to detect the presence or absence of any health abnormality or adverse treatment reaction.

2.4. D. immitis inoculations

On Study Day −30, all cats were inoculated by subcutaneous injection of approximately 100 (study 1 and 2) or 40 (study 3) infective third stage larvae (L_3). For studies 1 and 2, the inoculation was performed immediately after the *D. immitis* larvae had been processed from mosquitoes infected with laboratory isolates originated in the USA (Georgia and Michigan, respectively). For study 3, the larvae were processed from mosquitoes in Italy and transferred to the study site under refrigerated conditions for approximately 5 h before infections were conducted.

2.5. Treatment

Control cats were not treated. On Study Day 0, each cat in the treated group received a single topical application of the novel combination product at the minimum intended dose of 0.12 mL/kg body weight (bw), delivering 10 mg/kg bw fipronil, 12 mg/kg bw (S)-methoprene, 0.5 mg/kg bw eprinomectin and 10 mg/kg bw praziquantel.

2.6. Efficacy evaluation: heartworm counts

At approximately 6 months after infection, based on local logistic availability of personel, on Day 150 (studies 1 and 2) or Day 159 (study 3), all cats were humanely