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Safety and efficacy of 10% imidacloprid + 2.5% moxidectin for the treatment of *Dirofilaria immitis* circulating microfilariae in experimentally infected dogs

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ABSTRACT

A controlled laboratory study was conducted to establish the safety and efficacy of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage Multi® for Dogs, Bayer HealthCare, Shawnee, KS) for the treatment of circulating *Dirofilaria immitis* microfilariae in dogs. Twenty beagles were experimentally infected with *D. immitis* via surgical implantation of 10 pairs of adult worms (Pepper strain, TRS Labs) from donor dogs on Day −82. Between Days −7 and −1, physical examinations were performed, chest radiographs were taken, and blood and urine samples were collected for microfilariae counts, serum chemistry, complete blood counts, and urinalysis. Each dog was required to have a mean pretreatment count of at least 300 mf/ml of blood. On Day −1, all 20 dogs were randomized by mean pretreatment microfilarial counts to two study groups (10 animals/group). Animals in Group 1 were treated on Days 0 and 28 with 10% imidacloprid + 2.5% moxidectin topical solution at the minimum label dose of 0.1 ml/kg. Group 2 animals served as negative controls and were treated on Days 0 and 28 with mineral oil at an equivalent volume as for the study solution. All dogs were observed hourly for 8 h after treatment, again at 12 h, and then once daily on all other study days. Blood samples for microfilarial counts were collected daily for 3 days after treatment and then weekly for 6 weeks. The percentage reduction in microfilariae was determined by comparing the geometric mean number of circulating microfilariae remaining in Group 1 dogs with the mean counts remaining in control dogs. Group 1 mean microfilarial counts were reduced 93.1% three days following the first treatment and by >99% on Days 14 through 42. Group 1 had significantly fewer ($p < 0.05$) microfilariae compared with Group 2 counts on Days 28 and 42. In addition, log-transformed geometric mean microfilarial counts were significantly different between the two groups ($p < 0.05$) using separate repeated measures analysis of covariance for Days 2, 3, 7, 14, 21, 28, 35, and 42. No adverse events related to treatment were reported during the study. The results of this study demonstrate that 10% imidacloprid + 2.5% moxidectin topical solution is efficacious for treatment of circulating *D. immitis* microfilariae in heartworm-positive dogs with no treatment-related adverse events observed.

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1. Introduction

Advantage Multi® for Dogs (Bayer HealthCare, Shawnee, KS) containing 10% (w/v) imidacloprid plus 2.5% (w/v) moxidectin was developed for the prevention and treatment of internal and external parasites of dogs (Arther et al., 2005). This topically applied product kills adult fleas and is indicated for the treatment and control of nematode infections, including fourth-stage larvae, immature adults, and adult stages of *Ancyclostoma caninum* and *Uncinaria stenocephala*, fourth-stage larvae and adult *Toxocara canis* and adult *Toxascaris leonina* and *Trichuris vulpis*. The label also includes monthly use for preventing the development of *Dirofilaria immitis* and the subsequent development of canine heartworm disease (NADA 141-251, approved December 2006). The moxidectin component of the product has also been shown to have activity against older larvae (McCall et al., 1992; McTier et al., 1992), immature adults (juvenile) (McCall et al., 2001b; McCall, 2005), and microfilariae of *D. immitis* (Hendrix et al., 1992; McCall et al., 2001b; McCall, 2005).

The removal of circulating *D. immitis* from dogs in conjunction with or following adulticidal therapy is important for the clinical condition of the dog, as well as for controlling the spread of heartworm by decreasing the reservoir of infection in the dog population. Historically, several drugs, including stibophen, fenthion, dithiazanine iodide, and levamisole, have been used as microfilaricides, but only fenthion and dithiazinine iodide, were approved by the Food and Drug Administration (FDA) for this purpose. Various macrocyclic lactones labeled for use as heartworm preventive and high doses of ivermectin have routinely been used off-label for this purpose (American Heartworm Society, 2014).

The objectives of this study were to establish the safety and efficacy of 10% imidacloprid plus 2.5% moxidectin for the treatment of dogs with circulating microfilariae of *D. immitis* and to provide pivotal efficacy data for this additional label indication to be approved for the commercial 10% imidacloprid plus 2.5% moxidectin product.[1,2]

2. Materials and methods

2.1. Animals

Twenty beagle dogs (10 males and 10 female), approximately 1.2 years of age, were purchased from a commercial supplier (Ridglan Farms Inc., Mt. Horeb, WI) and delivered to the test facility. During the study, the dogs were housed individually in indoor runs with temperature controlled by forced heat or air conditioning as needed and were fed a standard ration daily in quantities sufficient for growth and maintenance (Harlan Teklad Diet 8755, Madison, WI). Water was provided from the local municipality via individual automatic water dispensers in each run. Lighting was provided by overhead fluorescent lamps. Animals were exposed to approximately 12 h of light and 12 h of darkness. Lighting was controlled by an automatic timer. General health observations were conducted daily beginning on Day −7. The animals were maintained with due regard for their welfare and in accordance with applicable laws, regulations and guidelines. The protocol was approved by the Institutional Care and Use Committee prior to initiation of the study.

2.2. Study design

This study was conducted as a well-controlled, blinded, laboratory efficacy study.[1] On Day −82, each dog was anesthetized and implanted with 10 pairs of adult male and female *D. immitis* (Pepper Strain, TRS Labs) removed earlier from experimentally infected donor dogs via the left jugular vein (Dzimianski et al., 1989). All animals recovered uneventfully from anesthesia and the surgery.

From Days −7 to −5, blood and urine samples were collected from each dog for serum chemistry, complete blood counts, and urinalysis; and three blood samples were collected for quantification of circulating *D. immitis* microfilarial counts using the modified Knott Test. Chest radiographs were conducted on Days −5 and −4. Body weights were recorded on Day −5 and physical examinations were performed on Day −4. These tests and examinations were used to determine the heartworm disease classification of each dog (Di Sacco and Vezzoni, 1992; Merial, 2010; Louisiana State University School of Veterinary Medicine, 2014). Only dogs with classification of 1, 2, or 3 were eligible for inclusion. To qualify for the study each dog was required to have a pretreatment mean microfilarial count $\geq 300\ ml^{-1}$ of blood. On Day −1, dogs meeting inclusion criteria were ranked from highest to lowest mean pretreatment microfilarial counts. The first two dogs with the highest counts were assigned to set 1, the next 2 dogs were assigned to set 2, etc. until the final two dogs were assigned to set 10. Within sets, one dog was randomized to receive treatment with Advantage Multi® for Dogs (10% imidacloprid + 2.5% moxidectin topical solution at 0.1 ml/kg, which provides 10 mg imidacloprid/kg + 2.5 mg moxidectin/kg) (Group 1) and the second dog received an equivalent volume of mineral oil (Group 2). All dogs were treated on Day 0. For both treatment groups, the hair along the dorsal midline between the shoulder blades of the dog was parted and the liquid was applied topically to the skin surface. The dogs were observed hourly for 8 h and at 12 h (±15 min) after treatment. The dogs were re-weighed on Day 27, and treatments were repeated on Day 28, based on the current body weight. Post-treatment observations were recorded on Day 28 at the same intervals as recorded on Day 0. The dogs were observed once daily on all other post-treatment study days. Post-treatment blood samples for microfilarial counts were collected on Days 1, 2, 3, 7, 14, 21, 28, and 42.

2.3. Microfilarial counting

At each blood collection, a 20-μl sample was removed to make a Giemsa-stained preparation (Schneider et al.,

[1] Effectiveness of anthelmintics: special recommendations for Canine VICH GL 19 (June 2001).

[2] Supplemental approval for Advantage Multi® for Dogs Topical Solution, NADA 141-251, labeled for the treatment of *D. immitis* circulating microfilariae in dogs was approved by the Center for Veterinary Medicine (FDA) on 24 October 2013.