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# Efficacy of a single oral administration of milbemycin oxime against natural infections of *Ancylostoma braziliense* in dogs

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ABSTRACT

The objective of this randomized, blinded, placebo controlled laboratory study was to confirm the efficacy of a single oral administration of two marketed formulations of milbemycin oxime (Interceptor® Flavor Tabs® and Sentinel® Flavor Tabs®) at a minimum dose of 0.5 mg/kg (0.23 mg/lb) against natural infections of *Ancylostoma braziliense* in dogs. Thirty-six hookworm infected dogs, a minimum of 10 weeks of age and of various breeds and genders were used. Fecal egg counts were done on three separate days prior to treatment for randomization purposes. Dogs were ranked by descending order of the fecal egg count arithmetic means and randomly assigned to either the two milbemycin treatment groups or the placebo control group in blocks of three dogs each, 12 dogs per group. Dogs were dosed according to the product label with blinding maintained by separation of function. Worm counts were done at necropsy 7 days after treatment. Reduction in *A. braziliense* worm counts in the milbemycin groups were compared to the placebo control group using analysis of variance of the *A. braziliense* logarithmic mean worm counts and percent efficacy was based on geometric means. Efficacy was defined as the ability of the test products to significantly ($p \leq 0.05$) reduce parasite load by 90% or greater in treated dogs when compared to adequately infected placebo control dogs. The placebo control group had a geometric mean worm count of 19.2. The Interceptor treated group had a geometric mean worm count of 0.38 representing a 98% reduction in parasite load and the Sentinel treated group had a geometric mean worm count of 0.98 representing a 95% reduction in parasite load. Both reductions were highly significant ($p < 0.0001$). In this study, milbemycin oxime, when administered as two marketed formulations at a minimum dose of 0.5 mg/kg (0.23 mg/lb), was efficacious for removing adult *A. braziliense* in naturally infected dogs.



## 1. Introduction

*Ancylostoma braziliense* is a pathogenic hookworm parasite of dogs most generally associated with warm and moist climates. Although not as pathogenic as *Ancylostoma caninum*, heavy infections in young dogs may result in blood loss (Rep, 1980) and hypoproteinemia (Miller, 1968). However, the most significant concern with *A. braziliense* is its ability to cause cutaneous larva migrans in both dogs (Vetter and Leegwater-vd Linden, 1977; Vetter and van der Linden, 1977a,b; Bowman et al., 2010) and humans (Brenner and Patel, 2003; Patel et al., 2008; Purdy et al., 2011). Of the hookworm larvae, *A. braziliense* tends to be more invasive by cutaneous penetration and shows the greatest enzyme activity for breaking down structures of the skin (Hotez et al., 1992), thus allowing the larvae to enter by direct contact with intact skin and mucous membranes. Not only has *A. braziliense* been associated with cutaneous larva migrans in humans, but also migration to the lungs (Butland and Coulson, 1985) and oral mucosa

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(Damante et al., 2011). Even though the parasite is more common in the tropical regions of the world, it has also been reported in non tropical settings (Herbener and Borak, 1988), suggesting the need for control outside the areas typically considered endemic. Therefore, effective control of *A. braziliense* in dogs is important because of its potential pathogenicity in dogs and zoonotic potential for cutaneous larva migrans in humans.

Milbemycin oxime is a macrocyclic lactone that is efficacious against infections of *A. caninum* (Blagburn et al., 1992; Niamatali et al., 1992), but no studies have been done specifically investigating effectiveness against *A. braziliense*. We hypothesized that milbemycin oxime would be over 90% efficacious when administered as a single treatment to dogs infected with *A. braziliense*.

## 2. Materials and methods

The study was a randomized, blinded, placebo controlled laboratory study using naturally infected dogs conducted in compliance with GCP (VICH GL9), South African animal welfare regulations, as stipulated in the "National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs and Related Substances in South Africa". The protocol was submitted to the ClinVet Animal Ethics Committee (CAEC), the composition of which was in compliance with the National Code, for approval. In addition, the protocol was reviewed and approved by the Novartis Animal Health US, Inc. Institutional Animal Care and Use Committee.

### 2.1. Animals

Thirty-six hookworm infected dogs (21 males and 15 females), a minimum of 10 weeks of age and of any pure or mixed breed were randomly assigned to cages at the beginning of acclimation. Animals were purchased from owners who were fully informed of the nature of the study. Each dog was identified by a unique number on a collar tag. All purchase contracts indicated each animal's origin and procurement records traceable to each animal by identification number. Individual dog pens were marked with corresponding animal identification numbers. Potential infection with *A. braziliense* was assessed by fecal egg counts (presence of hookworm eggs) for three consecutive days prior to inclusion in the study. A sample of dogs from a specific source were acquired for necropsy purposes to confirm the presence of *A. braziliense*. Other than intestinal parasitism, dogs were clinically healthy as determined by a general physical examination and clinical pathology review. Dogs were acclimated at least 7 days prior to treatment and were observed at least once daily up to the time of treatment.

### 2.2. Housing

Dogs were housed individually. Room temperature was monitored and exercise was conducted according to facility standard operating procedures, as appropriate. Animals were acclimated at least seven days prior to treatment and were observed at least once daily up to the time of treatment and then twice daily until euthanasia. A commercially available high quality complete canine diet, which provided the nutritional requirements for the age of dog used in this study was offered *ad libitum* to each dog. Water was also available *ad libitum*.

### 2.3. Randomization

Fecal egg counts were done using the McMaster technique (Henriksen and Aagaard, 1976) on three separate days prior to treatment for randomization purposes. Dogs were ranked by descending order of the fecal egg count arithmetic means and randomly assigned to treatment in blocks of three dogs each (one dog to each treatment group), 12 dogs per group. Randomizations were performed in two replicates (phases), with each replicate group containing 18 dogs. The randomization process was applied independently within each replicate. Counts were recorded as hookworm eggs per gram of feces (epg) with subsequent determination of arithmetic means by a statistician.

### 2.4. Blinding

Blinding was accomplished by separation of function. Individuals responsible for general health observations, clinical observations, or examination of dogs at necropsy for adult *A. braziliense* worms did not know the allocation of dogs to treatment. Only the statistician and individuals responsible for drug administration were aware of treatment assignments until analyses.

### 2.5. Procedures

All dogs were fasted overnight before the day of treatment to encourage food intake prior to dosing and treatment was administered within approximately 30 min after feeding. Dogs received either a single oral administration of the marketed formulations of milbemycin oxime (Interceptor® Flavor Tabs® or Sentinel® Flavor Tabs®; Novartis Animal Health US, Inc.) according to the label at a minimum dose of 0.5 mg/kg (0.23 mg/lb) or a placebo (Pet-Tabs®; Virbac Animal Health, Inc.). Observations for adverse clinical signs were done on each dog within 1 h (±15 min) prior to treatment and again at 1, 4, 8 h (±15 min) and 24 h (±1 h) after treatment. Seven days after treatment each dog was administered a lethal intravenous dose of euthanasia solution (Euthapent®; Kyron Laboratories [Pty] Ltd) in the same order as treatment group assignment for assessing adult worm counts in the intestinal tract. The dose of euthanasia solution was based on the body weight determined on the day of euthanasia. Dogs were fasted 12 h immediately prior to euthanasia in order to decrease the amount of material in the intestinal tract. The abdominal cavity was opened along the ventral midline and double ligatures were placed at the cardia of the stomach and at the distal rectum prior to removal of the gastrointestinal tract from the abdominal cavity. The stomach was opened along the greater curvature and the contents collected in a suitable container. The mucosa was