Short Communication

Assessing the speed of kill of hookworms, *Ancylostoma caninum*, by Advantage Multi® for Dogs using endoscopic methods

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A B S T R A C T

Endoscopic capsules and endoscopy were used to assess the speed of kill and the clearance of hookworms in dogs experimentally infected with *Ancylostoma caninum*. A total of four adult dogs were inoculated in two separate cohorts comprised of two 4-year-old females and two 7-year-old males. Dogs were treated topically with Advantage Multi® for Dogs 13 days (Cohort 1) or 16 days (Cohort 2) after infection. Endoscopic imaging of the small intestine was carried out both pre- and post-treatment. Examination of the first cohort revealed that the worms had been cleared and the hookworm-induced lacerations were markedly diminished within 48 h of treatment. In the second cohort, endoscopic capsules were given the day of, the day after, and two days after treatment; within 24 h of product administration, the worms had been removed with a concurrent reduction in observed lesions. Topical application of Advantage Multi® for Dogs rapidly removed worms from the small intestine of the dogs in this study as early as 24 h post-treatment, with a marked reduction in the number of mucosal lesions seen.

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1. Introduction

In the Freedom of Information summary of the New Animal Drug Application (NADA) made to the Food and Drug Administration (FDA) for the approval of Advantage Multi® for Dogs, the time from treatment to necropsy in the efficacy trials against intestinal helminths (*Ancylostoma caninum, Uncinaria stenocephala, Toxocara canis, Toxascaris leonina,* and *Trichuris vulpis*) is 10 days (NADA 141-251). FDA Guidance Document 111 for the effectiveness of anthelmintics in canines (VICH GL19) states that "with the majority of parasites seven days is a sufficient time period from the termination of treatment until the animals are necropsied," but exceptions are presented for other helminths, including the gastrointestinal dwelling species of *Physaloptera, Spirocerca, Echinococcus, Taenia, Dipyilidium,* and *Mesocestodes* where the time is extended to 10 days. For approval purposes, because Advantage Multi® for Dogs is applied topically and takes 9 days to reach maximum plasma concentrations (NADA 141-251), 10 days post-treatment was a logical time point to assess efficacy based on postmortem worm counts.

The development of endoscopy, and particularly endoscopic camera capsules, has now provided a means to examine intestinal helminths in vivo at different time points after infection (Lee et al., 2011, 2013; Liotta et al., 2012). This would also potentially allow for assessment of the rate of worm clearance after treatment and, in the case of hookworms, the resultant effects on the lesions caused by feeding adult worms. Blood lost to feeding hookworms
has been reported to occur in two peaks after infection: one during the very rapid growth of the maturing adult worms (10–15 days after infection) and one at 20 days after infection when maximal egg output begins (Miller, 1966). Advantage Multi® for Dogs is labeled for the treatment of fourth-stage larvae and both immature and mature adult worms, and thus, treatment was applied in this study earlier than the routinely used time point of >21 days post-infection to minimize blood loss by the dogs. This work documented the effects of anthelmintic treatment on populations of young *A. caninum* and subsequent resolution of hookworm-induced intestinal lesions.

2. Materials and methods

2.1. Animals

All work was performed under a protocol approved by Cornell University’s Institutional Animal Care and Use Committee. The four Beagle dogs used in this study had been acquired from an approved Class A dealer (Marshall BioResources, North Rose, NY) and had resided in Cornell’s research facility for a number of years as part of an unrelated investigation into canine herpesvirus-associated ocular disease. The work was performed in two cohorts with two 4-year-old females in Cohort 1 and two 7-year-old males in Cohort 2. At the beginning of the trial utilizing Cohort 1, all dogs were transferred from the room in which they had been housed to a different room within the same facility. The female dogs were housed together, but due to between-dog aggressive tendencies, the male dogs were housed individually. Although transferred at the same time, dogs were monitored for the study such that there was a 4 day (Cohort 1) or 7 day (Cohort 2) acclimation period to the new room before the study began, i.e., the day on which the dogs received their hookworm infections (Day 0). Dogs received initial physical examinations between Days −5 and −2; all were clinically healthy. The study ended on Day 22 (Cohort 1) or 25 (Cohort 2) and, this being a non-terminal study, at study completion the dogs were transferred back to the previous investigator.

2.2. Infection and treatments administered

On Day 0 for each cohort, the dogs were orally inoculated with 500 third-stage larvae of *A. caninum* that had been grown in charcoal cultures (Bowman et al., 1991). The source of the larvae was a fecal sample from a random source dog supplied by Cheri-Hill Kennel and Supply, Inc., Stanwood, MI. The larvae were grown in culture for 6 days prior to harvest and were stored in water at room temperature in T-25 vented tissue culture flasks until used. Aliquot counts were made for preparing the doses to infect the dogs in Cohorts 1 and 2, respectively. The dogs were treated with Advantage Multi® for Dogs at the dose band recommended by the manufacturer for their weight. For Cohort 1, treatment (equivalent to 6.8 mg/kg moxidectin) was administered on Day 13 of the trial, and for Cohort 2, treatment (4.6–5.5 mg/kg moxidectin) was applied on Day 16. Fecal samples from these dogs were examined by sucrose centrifugal flotation on Days 19 and 24 for Cohorts 1 and 2, respectively.

2.3. Endoscopic examination

Dogs were fasted overnight prior to each endoscopic examination, then fed after completion of the procedure. Capsule endoscopy was performed according to the method previously described (Lee et al., 2011). Briefly, dogs were instrumented with external antennae along the ventral body wall, fitted with a jacket and pouch holding the image recording device, and the endoscopic capsule was then given by mouth. After image acquisition, instruments were removed and the dog’s feces were checked for capsule expulsion. Stored images were downloaded onto a computer for review. For conventional endoscopy, dogs were premedicated with a butorphanol–midazolam mixture, induced with propofol, and maintained under general anesthesia with isoflurane inhalant gas. Examination of the duodenum was carried out as per standard endoscopic protocols. Dogs were monitored until fully recovered from anesthesia. In Cohort 1, the dogs were examined with endoscopic capsules on Days 7, 9, 12, and 15, and with a conventional endoscope on Days 11 and 16. The dogs in Cohort 2 were examined by capsule endoscopy on Days 9, 12, and 15–18, and by conventional endoscopy on days 14 and 19.

3. Results

In the examination of the images collected from Cohort 1, hookworm-induced lacerations were observable as early as Day 7 (Fig. 1A), the first day that endoscopic capsules were given to the dogs. Worms were seen on Day 9 (Fig. 1B), and this was verified with endoscopy on Day 11 (Fig. 1C and D; see also Video 1 online). Worms were larger and more numerous on Day 12, with mucosal hemorrhages still being present as would be expected (Fig. 1E and F). Cohort 1 dogs were treated on Day 13, and the endoscopic capsule images captured on Day 15 (2 days post-treatment, DPT) showed the presence of healing scars only (Fig. 1G); this was supported by the endoscopy views collected on Day 16 (3 DPT) (Fig. 1H and I). Centrifugal flotation of feces collected on Day 19 was negative for hookworm eggs.

In the case of Cohort 2, worms and lesions were obvious from the images collected from Day 9 (Fig. 2A) and Day 12 (Fig. 2B) through Day 14 (Fig. 2C and D; see Video 2). These dogs were treated on Day 16, and images collected by capsule endoscopy on this day showed that worms and lesions were abundant (Fig. 2E and F; see Video 3). However, by Day 17 (1 DPT), worms were no longer present in the small intestine and lesions were few in number (Fig. 2G). Again, this was verified by endoscopy performed on Day 19 (3 DPT), which showed only a few small lesions remaining as compared to the extensive lesions present before treatment (Fig. 2H and I; see Video 4). Fecal examination on Day 24 revealed no hookworm eggs.
4. Discussion

The discovery that the worms and associated hemorrhage in dogs of Cohort 1 had been cleared by the second day post-treatment prompted a modification of the schedule of events for the next set of dogs. Thus, in Cohort 2, two additional endoscopic capsules were given: one on the day of treatment and another on the following day. Images from Cohort 2 clearly demonstrated that as early as 24 h after topical treatment with Advantage Multi®, *A. caninum* worms were removed and lesions were reduced in number. Another difference between the two cohorts was the delaying of treatment for Cohort 2 to Day 16; this three-day postponement permitted the development of extensive and severe lesions by Day 14 (Fig. 2C) that were not present in Cohort 1 (Fig. 1G–I). The speed with which the worms were removed from the intestine after treatment was much more rapid than had been anticipated when the study was designed. It is possible, however, that a few worms may have still been present in the colon 24 h after treatment, because this segment of the intestinal tract is not examined by the small bowel endoscopic capsule, and colonoscopies were not performed.

Fecal examinations were not performed on these dogs except for the purpose of verifying that they were egg-negative before being returned to the care of their original investigator. Because the worms were young, eggs may not have been present in the feces of the dogs in Cohort 1 that were treated on Day 13 post-infection, and the females would not have yet reached their maximal egg production.
until four weeks after infection (Bowman et al., 1991). After the treatment with Advantage Multi® of dogs with experimentally induced *Ancylostoma ceylanicum* infections, daily eggs counts were negative for all treated dogs four days after treatment (Taweethavonsawat et al., 2010). The rapid clearing of the eggs in these dogs would suggest that, as was seen in the present study, there was likely removal of the worms as a source of eggs within a day of treatment.

The endoscopic camera capsules have the capacity to image the entire small intestinal tract, and it would be of interest to know when other helminths – and perhaps most interestingly, tapeworms – are actually killed by treatment. It would also be of interest to see the response of larger helminths to treatment; this was not examined in this case. *A. caninum* grows rapidly, but the worms were still quite small at the time this study was performed. According to the careful work by Matsusaki et al. (1965), the respective lengths in millimeters of male and female *A. caninum* at different days post-infection are 3.0–4.1, 3.3–3.8 (6 days); 5.0–6.5, 5.0–7.6 (10 days); 7.5–10.0, 8.0–11.5 (14 days); 8.1–10.1, 11.0–14.0 (18 days); 10.9–11.6, 13.5–14.8 (25 days); 10.2–12.2, 14.1–19.1 (35 days); and 11.0–12.9, 18.4–19.4 (45 days). Thus, most of the worms seen in this study were smaller than half (3.3–14.0 mm) of what their lengths would have been (10.9–14.8 mm) at the typical time of necropsy 28–30 days after infection, and still much smaller than they would have been (11.0–19.4 mm) when fully mature at one-and-a-half months of age.
Role of funding source

The sponsor had no role in study design, implementation, or study report preparation.

Conflict of interest

Dr. Bowman has been paid by Bayer HealthCare LLC for work relative to consultancies and honoraria for speaking; Cornell has received funds for research and contractual work from Bayer with Dr. Bowman as the principal investigator.

Dr. Lee has received compensation for educational articles written for Bayer.

Dr. Hostetler is employed by Bayer HealthCare LLC.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetpar.2014.05.028.

References


