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Research paper

Determination of anthelmintic efficacy against *Toxocara canis* in dogs by use of capsule endoscopy

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ABSTRACT

Industry guidelines for anthelmintic testing call for postmortem inspection of animals to verify treatment efficacy. A previous study showed that capsule endoscopy (CE) can be performed on dogs in vivo to quantify hookworms in the small intestine. Adoption of a minimally invasive procedure such as this could reduce the need for necropsy in efficacy trials. The present study employed CE to enumerate *Toxocara canis* in dogs, with two main goals: to determine if multiple capsule examinations improves the accuracy of worm counts compared to a single examination, and to establish if the efficacy of an anthelmintic compound is the same whether calculated using CE or necropsy data. To avoid needless animal sacrifice, the study was carried out on beagle dogs already in a product development trial with a planned terminal endpoint. Dogs were infected by oral inoculation with *T. canis* eggs. Untreated control dogs ($n = 8$) were evaluated by CE three times while dogs treated with test compounds (3 groups of 4) were examined only once. Utilizing either the average count or just the last complete capsule examination, a robust correlation was found between CE and postmortem numbers ($r = 0.94$, $p < 0.001$). Calculated anthelmintic efficacy was essentially identical for the two enumeration methods, ranging from 94% to 100% for the three research compounds. CE may therefore be a viable alternative to necropsy for *T. canis* parasiticide trials.

1. Introduction

Current anthelmintic testing guidelines call for postmortem examination of test animals to prove that target helminths are successfully eliminated by treatment. This translates into hundreds of euthanasias for each product that reaches market. Recently, gastrointestinal endoscopy has been investigated for its potential to assess anthelmintic drug efficacy in living animals, including dogs in a research setting and European shags (*Phalacrocorax aristotelis*) in the field (Burthe et al., 2013; Lee et al., 2013). Although conventional endoscopy allows for observation of helminths in the upper and lower gastrointestinal tract, it does not provide a good representation of worm burdens in the small intestine (SI) due to a lack of jejunal access (Lee et al., 2013). Capsule endoscopy (CE)—the use of wireless ingestible cameras—has been tested in dogs as a means of overcoming this limitation. Pilot work demonstrated the utility of CE for detection and enumeration of *Ancylostoma caninum* in the SI, showing a clear but imperfect correlation to worm counts acquired by necropsy (Lee et al., 2011).

The present study applied CE to another common SI nematode of dogs, *Toxocara canis*. Objectives were twofold: (1) Assess whether averaging the counts from three capsule passages in the same dog would better reflect true worm burden compared to a single count; and (2) Determine if the anthelmintic efficacy calculated using CE versus necropsy data is comparable. The latter is important because countries that adhere to the VICH guidelines for anthelmintic testing (VICH, 2001), including the United States and members of the European Union, require a minimum efficacy of 90% before a drug label claim can be made. Any method intended to replace gold-standard necropsy in the testing process must therefore produce similar results in terms of efficacy evaluation. In order to avoid sacrificing dogs expressly for this investigation, CE was incorporated into an existing product development trial that had a terminal endpoint as per the industry norm.

2. Materials and methods

The study protocol was approved by the institutional animal care and use committees of Cornell University and Novartis Ani-

Abbreviations: CE, capsule endoscopy; SI, small intestine; VICH, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.

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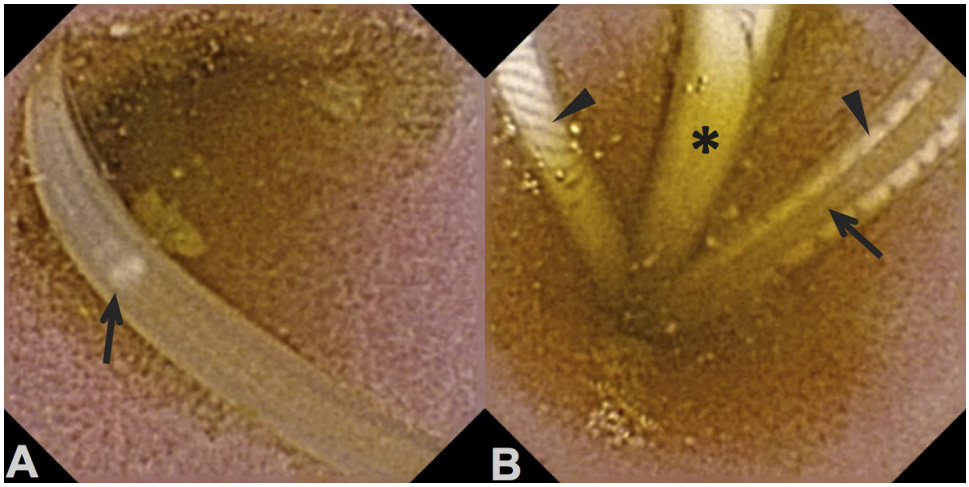

Fig. 1. Morphological features of *T. canis* visible on capsule endoscopy. (A) Anterior end of a worm with a conspicuous, white-colored ventriculus (arrow). (B) Clearly discernible within the bodies of the centrally located female and two flanking males are the uterus (asterisk), testis (arrowheads), and intestine (arrow).

mal Health in St-Aubin, Switzerland. Four-month-old beagle dogs were orally inoculated with eggs cultured from the feces of a dog naturally infected with *Trichuris vulpis* and *T. canis*. The inoculum volume was chosen based on the main target species of the anthelmintic trial, *T. vulpis*; *T. canis* eggs were present but not quantified, so the exact dose administered to the dogs was unknown. Three months after inoculation, dogs were allocated based on *T. vulpis* fecal egg counts into a control group ($n = 8$) or one of three treatment groups ($n = 4$ each), all comprised of an equal number of males and females. All dogs were verified to be shedding *T. canis* eggs.

Over a period of 15 days, control dogs were examined by CE (Endo Capsule System, Olympus America Inc., Center Valley, PA) on three separate occasions and treatment dogs were examined once. The CE protocol has been described elsewhere (Lee et al., 2011). Briefly, dogs were fasted the day before CE, instrumented with the external antennae and recorder, and given the capsule by mouth. Instruments were removed once the real-time monitoring device showed the capsule was in the colon or when the capsule battery was exhausted. Recorded images were downloaded and analyzed. Capsules were expelled in fecal matter within 1–2 days and were discarded. Postmortem examinations were carried out 5–7 days after treatment groups were administered research compound A, B, or C. Dogs were sedated with an intramuscular injection of medetomidine, butorphanol, and ketamine, followed by intravenous delivery of 400 mg/kg pentobarbital. The intestinal contents and mucosa were rinsed through a 50 μm steel mesh sieve. *T. canis* worms recovered were counted. There was a 5-day interval between the last CE performed on each control dog and the day on which it was euthanized. For treatment dogs, this period was 1–3 days. All CE images were reviewed by a single endoscopist blinded to the necropsy results.

Anthelmintic efficacy for the three compounds was calculated as follows: 100% × (geometric mean of control–geometric mean of treated)/geometric mean of control. Pearson correlation coefficient was determined using GraphPad Prism v.6 (GraphPad Software, La Jolla, CA) with significance set at $p < 0.05$. Incomplete CE examinations (i.e., lack of SI images as a consequence of protracted gastric transit of the capsule) were excluded from analysis. For evaluation of single CE counts in control dogs, incomplete examinations were replaced with data from the next available complete CE for that dog. No substitutions were made when taking the average across three capsule passages.

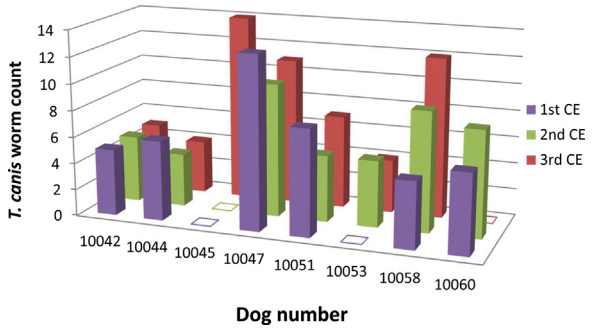

Fig. 2. Intra-dog variability in capsule endoscopy counts for *T. canis*. The number of worms observed during each capsule passage in the 8 control dogs is presented. Open squares correspond to incomplete examinations wherein the capsule remained in the stomach until the battery was depleted and so no images of the small intestine were available for review. Within-dog counts tended to be similar, though a marked difference was observed between the first and third CE for dog no. 10058.

3. Results

Due to a scheduling error relative to the timing of CE, two dogs each from treatment groups A and C were excluded from analysis. Fifteen dogs in total—8 control and 7 treated—had at least one complete CE. A combined 66 worms were observed in the last complete CE performed prior to necropsy, i.e., the third CE for most control dogs. Of these, 62 (93.9%) were located in the first half of the SI images. *T. canis* adults were easily detected by CE due to their large size. Additionally, various morphological features such as the digestive and reproductive tracts could be identified (Fig. 1, Supplementary Video 1 online). In the control group, repeated CEs revealed only slight variability in worm numbers measured within the same animal aside from dog no. 10058, which showed a distinct increase in the count between the first and third capsule passage (Fig. 2). The median and range for the first, second, and third CE counts were 6 (5–13), 5 (4–10), and 7 (4–14), respectively; for necropsy it was 8.5 (4–18). In the treated groups, one dog had a single worm that was detected by both CE and necropsy (Fig. 3). The median and range were thus the same for the two methods: 0.0 (0–1).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetpar.2015.08.013.