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Research paper

Diagnostic strategies to reveal covert infections with intestinal helminths in dogs

Chris Adolph^{a,b}, Sharon Barnett^c, Melissa Beall^d, Jason Drake^e, David Elsemore^d, Jennifer Thomas^a, Susan Little^{a,*}^a Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA^b Zoetis, Kalamazoo, MI, USA^c Animal Health, Greensboro, NC, USA^d IDEXX Laboratories, Inc., Westbrook, ME, USA^e Elanco, Greenfield, IN, USA

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ABSTRACT

Intestinal helminths are common in dogs in the United States, particularly non-treated dogs in animal shelters, but surveys by fecal flotation may underestimate their prevalence. To determine the prevalence of intestinal helminths and evaluate the ability of fecal flotation and detection of nematode antigen to identify those infections, contents of the entire gastrointestinal tract of 97 adult (> 1 year) dogs previously identified for humane euthanasia at two animal control shelters in northeastern Oklahoma, USA, were screened. All helminths recovered were washed in saline and fixed prior to enumeration and identification to genus and species. Fecal samples from each dog were examined by passive sodium nitrate (SG 1.33) and centrifugal sugar solution (SG 1.25) flotation. Fecal antigen detection assays were used to confirm the presence of nematode antigen in frozen fecal samples from 92 dogs. Necropsy examination revealed *Ancylostoma caninum* in 45/97 (46.4%), *Toxocara canis* in 11/97 (11.3%), *Trichuris vulpis* in 38/97 (39.2%), *Dipylidium caninum* in 48/97 (49.5%), and *Taenia* sp. in 7/97 (7.2%) dogs. Passive fecal flotation identified 38/45 (84.4%) *A. caninum*, 6/11 (54.5%) *T. canis*, 26/38 (68.4%) *T. vulpis*, 2/48 (4.2%) *D. caninum*, and 1/7 (14.3%) *Taenia* sp. infections, while centrifugal flotation combined with antigen detection assays identified *A. caninum* in 97.7% (43/44), *T. canis* in 77.8% (7/9), and *T. vulpis* in 83.3% (30/36) of infected dogs based on necropsy recovery of nematodes. Taken together, these data indicate that detection of nematode antigen is a useful adjunct to microscopic examination of fecal samples for parasite eggs, and that this approach can improve diagnostic sensitivity for intestinal nematode infections in dogs.

1. Introduction

Intestinal nematodes and cestodes are commonly identified in dogs in North America (Nolan and Smith, 1995; Blagburn et al., 1996; Gates and Nolan, 2009; Villeneuve et al., 2015). However, surveys conducted by fecal flotation alone underestimate the true prevalence of these infections. Even when conducted using best practices of centrifugation, fecal flotation lacks perfect sensitivity and is unable to detect prepatent and single-sex infections (Epe, 2009). Fecal flotation is particularly poorly suited for detecting the presence of cestodes, which pass dense eggs or egg packets only intermittently and often in discrete proglottids (Burrows and Lillis, 1960; David and Lindquist, 1982; Dryden et al., 2005).

Surveys to determine the prevalence of intestinal helminths in dogs have largely been conducted using fecal flotation (Jordan et al., 1993; Blagburn et al., 1996; Gates and Nolan, 2009; Little et al., 2009). Results vary dramatically according to population of dog considered, technique used, and geographic area. When surveys are conducted by fecal flotation alone, more nematodes than cestodes are identified (Jordan et al., 1993; Blagburn et al., 1996; Gates and Nolan, 2009). Historic surveys in North America have estimated prevalence ranging from 0.8% to 39% for hookworms, 3.1% to 8% for ascarids, 0.8% to 12.3% for whipworms, 0.09% to 4.0% for *Dipylidium caninum*, and 0.6% to 4.6% for *Taenia* spp. although a decrease in prevalence in pet dogs is thought to be associated with the advent of modern parasite control products (Lillis, 1967; Amin, 1980; Kirkpatrick, 1988; Jordan et al.,

* Corresponding author at: Room 250 McElroy Hall, Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA.

E-mail address: susan.little@okstate.edu (S. Little).

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1993; Hackett and Lappin, 2003; Nolan and Smith, 1995; Blagburn et al., 1996; Gates and Nolan, 2009).

Left untreated, nematode infections in dogs can lead to clinical signs including gastrointestinal disease, anemia, dermatitis, and decreased body condition. Some canine intestinal parasites are also zoonotic (Chomel and Sun, 2011; Bowman, 2014). *Toxocara canis* and *A. caninum* cause visceral/ocular and cutaneous larval migrans, respectively. Indeed, nearly 1 in 7 people in the United States have antibodies to *Toxocara* sp., indicating zoonotic infections commonly occur (Won et al., 2008; Bowman, 2014).

Accurate detection of canine helminth infections allows early, appropriate treatment and is a key component of basic wellness care for dogs. In addition, both the Companion Animal Parasite Council (capvet.org) and the European Scientific Counsel on Companion Animal Parasites (escap.org) recommend routine parasite control for dogs to limit environmental contamination with zoonotic parasites. Antigens excreted or secreted by helminths are available for detection in the feces of an infected dog, and recent work has documented strategies to detect fecal antigen of the common canine intestinal nematodes (Elsemore et al., 2014; Elsemore et al., 2017). To determine the degree to which currently available diagnostic assays accurately identify helminth infections in dogs, we evaluated dogs from municipal animal shelters in northeastern Oklahoma for helminth infections by necropsy examination, passive fecal flotation with sodium nitrate, centrifugal fecal flotation with sugar, and detection of fecal antigen by enzyme linked immunosorbent assay (ELISA).

2. Materials and methods

2.1. Sample collection

All data were collected via examination at necropsy from 97 adult (> 1 year) dogs (59 male and 38 female) previously selected for humane euthanasia as the standard protocol at two municipal animal shelters in northeastern Oklahoma, USA. Shelter administrators reviewed and approved the objective and design of the study. Post mortem physical examination was performed, and estimated age, sex, neuter status, and body condition score were recorded. An external parasite examination was performed via use of thumb counts as previously described (Marchiondo et al., 2007). Fleas collected were placed in 70% ethanol for later identification by comparison to standard keys (Pratt, 1956) and proglottids were placed on slides for microscopic examination and identification by embryo morphology (Zajac and Conboy, 2012). The entire gastrointestinal tract was collected and examined grossly from the gastroesophageal sphincter to the distal colon. All visible gastrointestinal helminths present were collected with forceps, rinsed in phosphate buffered saline, and placed in Beltsville fixative (Lichtenfels, 1984) for later identification. All fecal material in the colon was collected and stored at 4 °C for flotation or frozen at –20 °C for antigen assays. The gastrointestinal tract and all ingesta were then placed in a large bowl containing saline, the entire mucosa was scraped, and the contents of the bowl filtered through progressive sieves to recover smaller helminths.

2.2. Identification and enumeration of helminths

Helminths were examined microscopically and identified using standard keys (Khalil et al., 1994; Anderson et al., 2009). Because cestodes may break during handling, only individual scolices were included in the enumeration.

2.3. Fecal flotation

Fecal samples from each dog were examined for helminth eggs by passive flotation using sodium nitrate solution and by centrifugal flotation as previously described (Zajac and Conboy, 2012); 4 g of feces

were used in each flotation procedure. Briefly, for passive flotation, feces were mixed with a saturated sodium nitrate solution (SG 1.33), strained to remove larger debris, allowed to sit for 15 min, and then a coverslip used to transfer the surface of the flotation to a slide for examination. For centrifugal flotation, the preferred technique for standard qualitative fecal flotation was used (Zajac and Conboy, 2012). Briefly, feces were mixed with Sheather's sugar solution (SG 1.25) in a small disposal cup, the mixture strained into a second cup, the fecal suspension transferred to a 15 ml centrifuge tube creating a slight positive meniscus, and a cover slip placed on top. The tubes were spun at 500 G for 10 min and the cover slip was removed and placed on a slide for examination. For both methods the entire area under the coverslip was systematically examined with a compound microscope using the 10X objective (100X magnification), and all parasitic eggs present identified. Quantitative evaluations (eggs per gram) were not performed.

2.4. Fecal antigen ELISA

Three previously described microtiter plate ELISA tests were used to detect distinct antigens from each of the following intestinal nematodes: *A. caninum*, *T. canis*, and *T. vulpis* (Elsemore et al., 2014; Elsemore et al., 2017). In this study, the fecal extract was prepared as described previously (Elsemore et al., 2014). Briefly, 1 g of fecal material was suspended in 4 ml of a previously described sample buffer comprised of Tris buffer [pH 7.2] supplemented with Tween-20 and mouse serum (Elsemore et al., 2014). Silica beads were added and the material was homogenized using a vortex. Following centrifugation of the fecal mixture, 100 µl supernatant was tested in each of the 3 microtiter plate ELISAs (Elsemore et al., 2014; Elsemore et al., 2017).

As previously described, each ELISA used a unique pair of antibodies to specifically capture the nematode antigen from the sample (Elsemore et al., 2014; Elsemore et al., 2017). Samples were incubated on the microtiter plate for 1 h at room temperature. The plates were washed 5 times with phosphate buffered saline containing 0.05% Tween-20 (PBST). The paired detection antibody for each assay was conjugated to horseradish peroxidase and 100 µl was added to each well. Following a 30 min incubation at room temperature, the plates were washed 5 times with (PBST). The substrate, which was 3,3',5,5'-tetramethylbenzidine (TMB) solution containing 0.2 g/l TMB and 0.01% hydrogen peroxide, was prepared and 50 µl was added to each well. After incubating for 10 min at room temperature, the reaction was stopped by the addition of 50 µl of 0.1% sodium dodecyl sulfate solution. Plates were read at a wavelength of 650 nm and interpreted using an ELISA cutoff of 0.100 (Elsemore et al., 2014; Elsemore et al., 2017).

2.5. Data analysis

Infection with each identified helminth was compared to age, sex, neuter status, body condition score, proglottid recovery, egg identification by fecal flotation, and presence of flea infestation using Chi-square contingency analysis with significance assigned at $P < 0.05$. Sensitivity and specificity were calculated for all methods relative to necropsy using exact binomial probabilities to determine the 95% confidence intervals. Positive and negative percent agreement was used to compare centrifugal flotation with nematode antigen testing. Correlation between the number of worms recovered and the antigen ELISA optical density was evaluated by the Spearman correlation coefficient (r). A P -value of < 0.05 was considered statistically significant. Analyses were performed using standard applications (Microsoft Excel (2011), Microsoft, Redmond, WA and GraphPad Prism version 6.0 for Mac OS, GraphPad Software, La Jolla, CA, USA).

3. Results

Dogs included in this study were estimated by dentition and

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