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Emergence of canine hookworm treatment resistance: Novel detection of *Ancylostoma caninum* anthelmintic resistance markers by fecal PCR in 11 dogs from Canada

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OBJECTIVE
To describe dogs with detected *Ancylostoma caninum* anthelmintic treatment resistance markers in Canada.

ANIMALS
11 client-owned dogs with fecal quantitative PCR (qPCR) assay detected *A caninum* with benzimidazole (BZ) resistance genotypic markers.

METHODS
Signalment, presenting concern, duration of clinical signs, fecal testing, treatment, and outcomes were obtained. Where available, follow-up data were collected via telephone or email with the primary veterinarian.

RESULTS
Ancylostoma spp was detected from 184/32,205 dog fecal samples by reference laboratory qPCR surveillance, between May 15, 2022, and April 26, 2023. 11 of these 184 samples had *A caninum* with genetic BZ F167Y resistance marker detection. 4 dogs had not traveled outside Canada, 6 had been imported from the US, and the travel history was unclear in 1 dog.

7 of the dogs had gastro-intestinal signs (diarrhea or soft stool) on initial presentation. Clinical improvement was reported in 6 of these dogs (resolution of diarrhea and soft stool), with 1 dog lost to follow-up. All 11 dogs received anthelmintic treatment (varied drugs and duration).

CLINICAL RELEVANCE
Identification of genetic markers of BZ resistance raises concerns about the potential animal and human impacts of resistant hookworms. 4 dogs lacked an origin from or travel history to the US, indicating true emergence and/or novel spread within Canada, not just importation from an area where resistance has been reported. Fecal surveillance was performed with a qPCR test incorporating treatment (BZ) resistance markers. There is a need to raise clinician awareness around treatment-resistant hookworm in dogs and the capability of fecal surveillance for genotypic and phenotypic resistance.

Keywords: hookworm, PCR, anthelmintic resistance, *Ancylostoma caninum*

Canine hookworm, *Ancylostoma caninum*, is one of the most important, and common, gastrointestinal (GI) parasites.[1–3] Infection is most concerning for young animals, with the degree of clinical signs related to hookworm burden and worm feeding. Severe disease and negative clinical outcomes, typically caused by anemia, can be fatal in puppies and kittens. A recent North American publication reported a rise in annual hookworm prevalence of 47%, from 2012 to 2018.[2]

Dogs infected with *A caninum* can pose several veterinary management challenges. This is due to the risk of dog infection (or re-infection) from L3 larvae present in the environment, for example, dog skin penetration **(Figure 1)**. A further hookworm challenge is "larval leak," whereby larvae in the arrested development stage (typically the dog's somatic tissues), are re-activated after anthelmintic treatment or during pregnancy **(Figure 2)**. For these reasons, current endoparasite guidelines (Companion

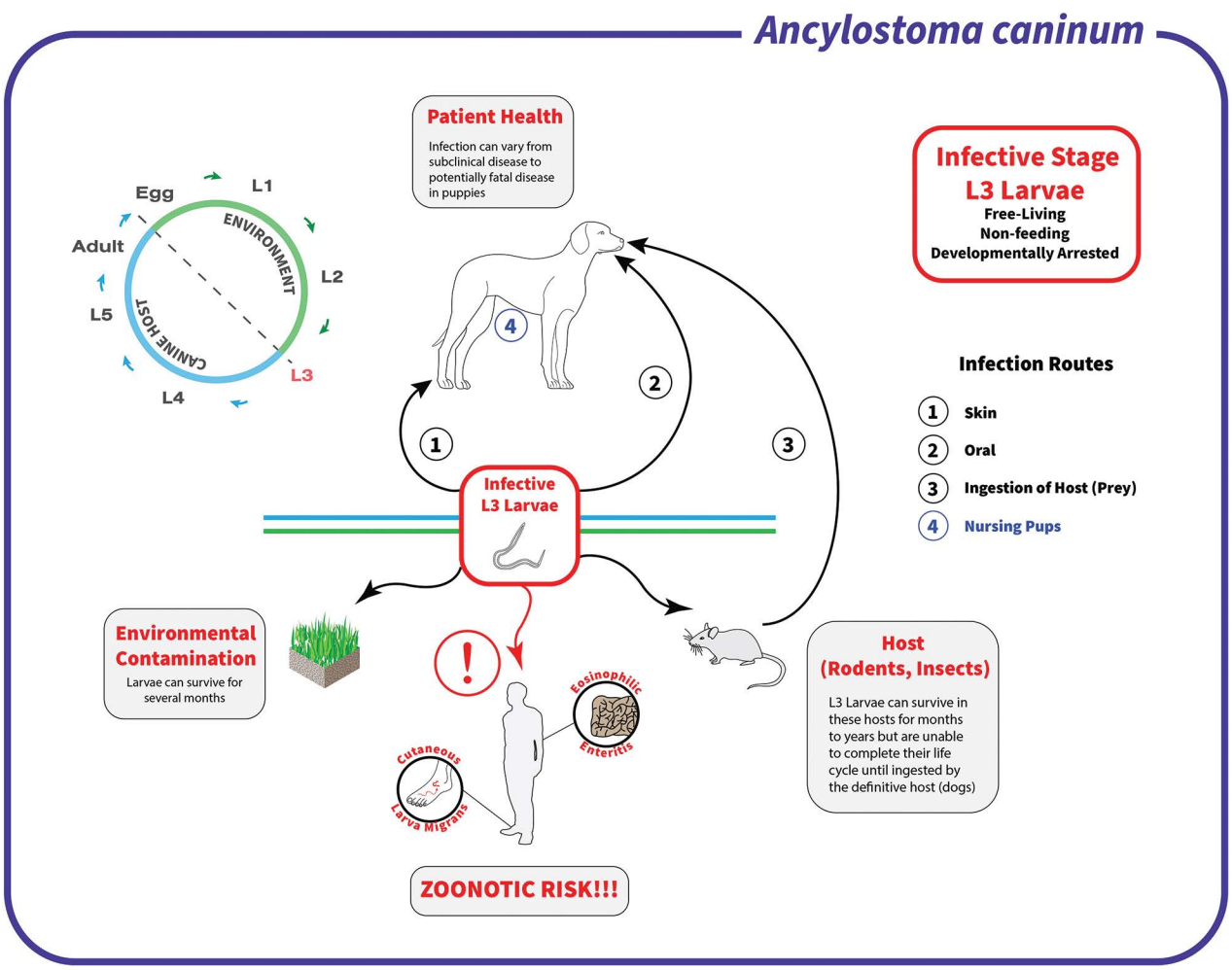

Figure 1—Life cycle of canine hookworm, *Ancylostoma caninum*, image reproduced with permission of MARS Pet Care/Antech Diagnostics, Education, the copyright holder, all rights reserved. Individuals wishing to reproduce the image should contact the corresponding author.

Animal Parasite Council [CAPC], Canadian Parasite Expert Panel [CPEP], and European Scientific Council of Companion Animal Parasites [ESCCAP]) advise routine fecal testing and deworming in puppies, adult dogs, and the pregnant bitch, together with ongoing hookworm (and overall parasite) preventive care.[4–6]

A further challenge, hookworm treatment resistance, has emerged as a rapidly evolving clinical concern in the US, with detection reported in a variety of regions, and dog breeds, beyond the original concern in racing Greyhounds facilities.[7–14] At present, *A caninum* multi-anthelmintic drug resistance has been reported, including resistance to benzimidazoles (BZ) like fenbendazole and febantel, macrocyclic lactones, avermectin/milbemycin, and tetrahydropyrimidine drug classes.[7–15] Multi-anthelmintic drug-resistant hookworms have been speculated to be of concern in Canada from the importation of dogs and selection pressure from widespread anthelmintic use.[16,17] While it is currently unknown whether hookworm treatment resistance is a concern for dogs outside the US, the criteria of factors for "the perfect storm" of emergence has been theorized to exist.[17]Additionally, as hookworms are zoonotic (eg, cutaneous larval migrans) and BZ are commonly used for treatment of infections in humans, this is a larger *One Health* antimicrobial resistance issue, with potential impacts on animals, humans, and the environment.

Here, we report a novel case series of dogs outside the US with detected *A caninum* anthelmintic treatment resistance markers. Our study also includes the first reports of dogs in Canada with resistance markers lacking an importation history (US to Canada). Broad fecal surveillance with a qPCR test (KeyScreen™ GI Parasite PCR)[14] was used to detect *Ancylostoma* spp dog samples, with concurrent BZ resistance markers, and obtain frequency data. This research describes a newly recognized change in local resistance patterns (Canada) and highlights global risk factors for resistance (canine importation), together with the provision of dog clinical case outcome information.

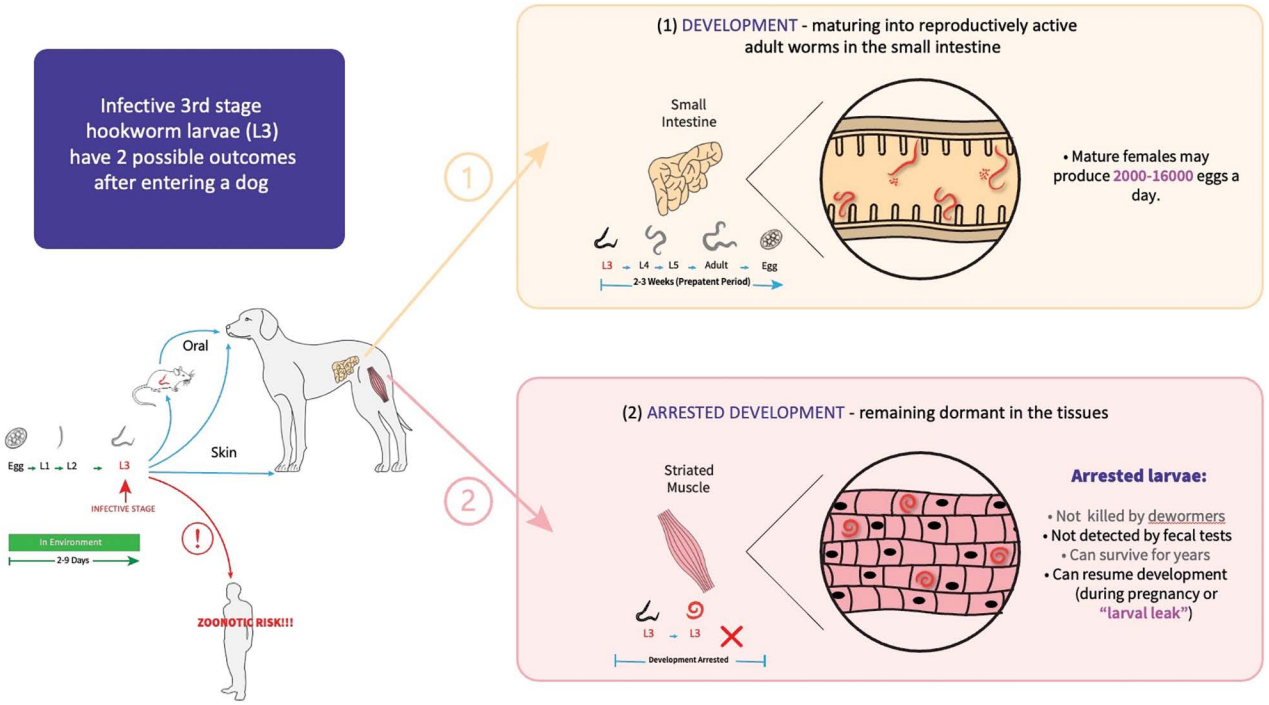

Figure 2—Overview of hookworm "Larval leak," image reproduced with permission of MARS Pet Care/Antech Diagnostics, Education, the copyright holder, all rights reserved. Individuals wishing to reproduce the image should contact the corresponding author.

Methods

Data were collected retrospectively from clinical submissions to Antech Laboratories between May 15, 2022, and April 26, 2023, inclusive. Samples submitted by Canadian veterinary clinics from dogs for analysis (n = 32,205) with the fecal qPCR test were eligible for inclusion. The real-time fecal qPCR test (KeyScreen™ GI Parasite PCR) has been validated for *Ancylostoma* spp and *Uncinaria stenocephala*.[14] This test also contains an allele-specific real-time fecal qPCR established and validated to detect the BZ resistance polymorphism at codon F167Y in the *Ancylostoma caninum* β-tubulin isotype-1gene.[14] All fecal qPCR tests were performed at Antech Diagnostics Canada.

Dogs from which *Ancylostoma* spp with concurrent genetic BZ 167Y resistance marker detection was reported were analyzed. All cases were confirmed as *A caninum* by ITS-2 gene Sanger sequencing. The presence of the 167Y mutation on the beta-tubulin gene was confirmed by Sanger sequencing. All positive results were confirmed to have been from different dogs.

Case information included dog location, travel history, signalment (age, reproductive status, and breed), presenting clinical concern (health status), duration of clinical signs, fecal testing, treatments, and outcome/follow-up data (short and longer term, where available). These were collected via telephone or email with the primary veterinarians.

Statistical analysis

Descriptive statistics (range, count, and categorical variables) were calculated as indicated.

Results

Ancylostoma spp was detected by qPCR from 184/32,205 (0.57%) samples. Eleven (5.9%) of these *Ancylostoma* spp detected samples (n = 184) harbored the BZ resistance polymorphism at codon F167Y in the *A caninum* β-tubulin isotype-1gene.

Dog signalment (age, reproductive status, and breed), travel history, presenting clinical concern (health status), and duration of signs are summarized **(Supplementary Table S1)**, with dog location depicted **(Figures 3 and 4)**. Dogs were a range of breeds, with most (9, 82%) being medium to large breeds. Eight dogs (73%) were from Ontario (ON), 1 (9%) was from British Columbia, and 2 (18%) were from Alberta (AB). Four (36%) of the dogs had not traveled outside of Canada (ON, AB), while 6 (54%) were imported to Canada from the US. Three (27%) of these were former racing Greyhounds. Another dog was suspected to be imported, but this could not be definitively confirmed.

All dogs were presented to general practitioners in Canada, with 1 dog later being referred for additional specialist assessment (dog 7). Most of the dogs (7, 64%) presented to their primary veterinarian with GI signs, consisting of diarrhea or soft stool

Figure 3—Canadian locations of dogs identified with benzimidazole resistant *Ancylostoma* spp (n = 11), mapped based on forward sortation areas.

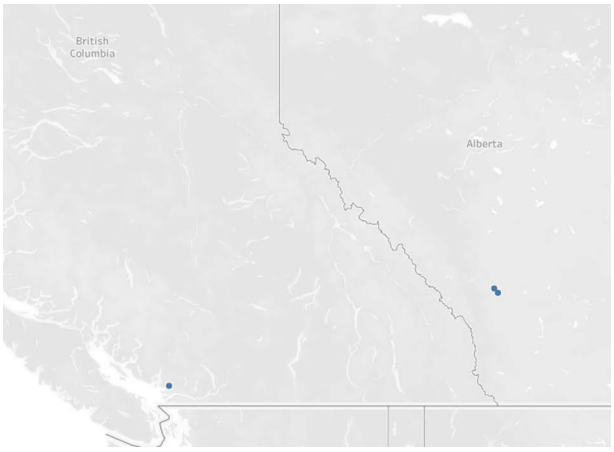

Figure 4—Western Canada locations of dogs identified with benzimidazole resistant *Ancylostoma* spp (n = 11), mapped based on forward sortation areas.

(4, 36%), reduced appetite (2, 18%), or a combination of the 2 (1). Duration of clinical signs varied, with 1 dog rapidly improving (<1 week) and another having persistent GI signs (>1 year, December 2021 to January 2023). The remaining 5 dogs had persistent or intermittent GI signs, ranging from 3 to 9 months, with a median of 5.75 months. Detailed clinical data to determine the relevance of *A caninum* in GI signs was lacking. Four dogs (36%) were clinically normal at the time of sampling. Co-infection with *Giardia duodenalis* was detected by qPCR in 6 dogs (54%).

Fecal testing, treatments, and outcome (follow-up data) where available, are summarized **(Supplementary Table S2)**. Six dogs (54%) had additional fecal testing performed, centrifugation-flotation (3 dogs) and 3 dogs had fecal egg count reduction tests (FECRT) without a follow-up FECRT being performed, that is, single FECRT.

Treatment (anthelmintic and duration) varied for all 11 dogs, with 7 (64%) of the dogs receiving treatment with a BZ (fenbendazole, febantel). Clinical improvement (resolution of GI signs) was reported in 6 of the 7 dogs that had GI signs at presentation, with 1 dog lost to follow-up.

Discussion

Our work represents a novel case series, with outcome information, on dogs outside of the US (Canada) with detected hookworm, *A caninum*, anthelmintic resistance markers. Before our study, there had been a single case of a dog in Canada with reported *A caninum* treatment resistance, and that dog was imported from the US.[16] While there may be a tendency to focus on imported dogs, particularly greyhounds, as the main or sole risk group, these data suggest that BZ resistance is endemic at some degree in Canada, and that risk is not just from greyhounds.

The 11 dogs that were identified with the *A caninum* BZ F167Y genetic marker for hookworm treatment resistance were tested through broad reference laboratory surveillance of Canadian dog fecal samples (n = 32,205) utilizing a rapid fecal PCR test (KeyScreen™ GI Parasite PCR).[14] This commercially available fecal qPCR test detects twenty GI parasites, including hookworms, *A caninum*, and BZ treatment resistance and zoonotic potential *Giardia* markers.[14] The study timeframe (1 year's worth of test data) was purposefully selected to provide a baseline for regional hookworm (0.6%) and *A caninum* BZ F167Y resistance mutation frequency (6.0% of dogs with hookworm infections), which will allow for further (ongoing) fecal qPCR surveillance. This baseline is also important as the fecal qPCR test has recently been evolved, considering recent hookworm

treatment resistance research, to include an additional BZ mutation Q134H marker.[15]

In dogs, hookworm diagnosis is typically made through clinical history and signs (usually GI) or as a finding on routine fecal screening (subclinical dogs), and confirmed with fecal testing (eg, centrifugal flotation, antigen ELISA, or PCR). As described in this case series (history, clinical signs, duration, and fecal tests), hookworm treatment resistance (in subclinical and clinically ill dogs) should be suspected when fecal testing reveals that hookworms have not been killed by routine deworming, and persistent infection is unlikely to be related to larval leak or environmental re-infection. In these cases, further fecal testing (either through a pre- and post-treatment fecal egg count reduction test (FECRT), molecular (PCR) testing for resistance, or in vitro drug bioassays) is critical to assess dogs for hookworm treatment resistance, a rapidly growing concern in the US.[7–15] and now reported in Canada. This case series had limited information regarding FECRT testing and dog response to treatment. The FECRT (when performed as a serial test, ie, FECRT followed by a later FECRT) can be important for the assessment of anthelmintic resistance and to help differentiate resistance from larval leak, when a dog has persistent positive test results.

Clinically, and as reported for many of the dogs in our case series (treatments, duration, and outcomes), once hookworm treatment resistance is diagnosed, there is often no simple or easy answer for veterinary management. Initially, long-term triple-drug combination treatment, together with strict environmental hygiene (to avoid re-infection), was successful and may still be advised in some dogs.[8,10,12] Unfortunately, evolving hookworm treatment resistance has likely occurred. Triple drug combinations have failed in some dogs, with an emodepside/praziquantel product (not currently authorized for use in dogs in North America) now being used for its potential efficacy (emodepside) against multi-anthelmintic drug resistance cases in the US.[12] A variety of anthelmintics (including the BZ, fenbendazole, and febantel), and treatment durations were used in the dogs in our case series. This finding highlights the challenges of clinical management, the need for awareness of hookworm treatment resistance in Canada, and potentially beyond, and ideally subsequent practitioner outreach to infectious disease consultants/parasitologists for prompt assistance upon detection of these cases. Emerging hookworm treatment resistance is so important that the American Association of Veterinary Parasitologists (AAVP) has formed a task force "to address multi-anthelmintic drug-resistant *A caninum*."[13] Careful considerations of anthelmintic use (ie, antimicrobial stewardship) in emerging regions, like Canada, is key, as once hookworm treatment resistance has occurred for a drug class, treatment utility is minimized. At the veterinary clinic level, efforts to quickly identify resistance through routine fecal screening with commercially available rapid and affordable molecular tests may also assist in limiting further environmental contamination (ie, emphasis on pick up poop messaging), subsequent zoonotic risk, and aid in antimicrobial use (AMU) for these cases.

Globally, and in addition to the dogs in our Canadian series, reports of hookworm treatment resistance have emerged from Nigeria and Brazil.[18,19] Due to the ongoing international dog movement (ie, importation), the global prevalence of hookworms, *A caninum*, antimicrobial use, and selection pressure, hookworm treatment resistance has the potential to be reported in additional countries. At the broader level, following endoparasite prevention guidelines (CAPC, CPEP, and ESCCAP) for testing in Canada, the US, and globally, may provide needed parasite frequency and other data, such as reported in our study for Canada. Routine fecal qPCR can provide this broad surveillance and serve to identify hookworm treatment resistance "hot spots," for the veterinary and human medical communities, which may help reduce parasite abundance, limit further spread, and alert to zoonotic risk.

Limitations of our work include the small number of dog cases (11), limited follow-up in some dogs, and the retrospective nature of the study. Further hookworm treatment resistance research is indicated in Canada and likely globally, and we agree with recommendations for widespread fecal surveillance and consideration of testing of dogs imported to Canada, and potentially more broadly (ie, in countries outside the US) to detect growing hookworm anthelmintic treatment resistance.[17]

Hookworm prevalence is increasing in the US,[2] and may also be in Canada,[3,16,17] and there is a need to raise clinician awareness around parasite frequency, and the emergence of cases of hookworm treatment-resistant *A caninum*. This novel canine case series highlights the need for clinical adherence to CAPC, CPEP, and ESCCAP guidelines for puppy and adult dog endoparasite detection and prevention (fecal, deworming), together with judicious antimicrobial (anthelmintic) use, and stewardship, for this evolving *One Health* concern. Our study provides new information on dog clinical outcomes, and changes in local resistance patterns (Canada), and highlights global risk factors for resistance (canine importation).

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Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.