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# Parasite prevalence in fecal samples from shelter dogs and cats across the Canadian provinces

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## Abstract

**Background:** In Canada, surveys of enteric parasites in dogs and cats have been reported sporadically over the past 40 years, mostly focusing on a specific region. The present work was performed to determine the current prevalence of various parasites in fecal samples from shelter dogs and cats across the Canadian provinces.

**Methods:** A total of 1086 dog and 636 cat fecal samples from 26 shelters were analysed using a sugar solution double centrifugal flotation technique. Prevalences (national, regional, provincial, age and parasite-specific), were calculated and compared using the Fisher-Exact test. A multiplex PCR was performed to distinguish *Taenia* spp, *Echinococcus granulosus* and *E. multilocularis* on samples positive for taeniid eggs.

**Results:** Overall, 33.9% of dogs and 31.8% of cats were positive for at least one parasite. *Toxocara canis* and *T. cati* were the most prevalent parasite present in fecal samples followed by *Cystoisospora* spp. Prevalence in dogs was similar across the Atlantic, East, West and Pacific regions, while prevalence in cats varied regionally. Eggs of *E. granulosus*/*E. canadensis* were detected in samples from dogs from BC, AB, and ON.

**Conclusions:** Data from this study will help in the development of strategies, based on the level of risk per geographic location for the prevention and response to these parasites in pets and free-roaming and shelter animals in Canada.

**Keywords:** Parasite, Prevalence, Dog, Cat, Canada, Canadian province

## Background

For zoonotic enteric parasites of dogs and cats, meaningful assessment of their possible impacts on companion animal and human health, as well as the design of optimal protocols for parasite control, depend significantly on robust prevalence data in animals and in people. In Canada, of particular concern are *Toxocara* species, *Baylisascaris procyonis*, *Echinococcus granulosus* (*E. canadensis*) and *E. multilocularis*, *Cryptosporidium* and *Giardia* species, and *Toxoplasma gondii*. These parasites also occur in other domestic animals and/or wildlife hosts in Canada, which in some circumstances can be important sources of human infection.

In Canada, surveys of enteric parasites in dogs and cats have been reported sporadically over the past

40 years, based primarily on fecal examinations. For example, these include studies of dogs in St. John's, Newfoundland and Labrador (NL) [1], on the island of St. Pierre (off the south coast of NL and technically part of France) [2], in aboriginal communities in Alberta (AB), Saskatchewan (SK) and the Northwest Territories (NT) [3-5], in Saskatoon (SK) [6,7], in Calgary (AB) [8], and visiting veterinary hospitals in Ontario (ON) [9]. Cats have been surveyed in and around Saskatoon [10,11], and dogs and cats in Calgary [12], Halifax, Nova Scotia (NS) [13], Montreal, Quebec (QC) [14,15], and Ottawa (ON) [15], as well as in other communities in ON [16]. Only a few surveys detected *Cryptosporidium* in dogs or cats [5,11,16-18].

Studies in Canada that focused on individual parasites have detected *E. granulosus* in dogs in British Columbia (BC) [19], and *E. multilocularis* in cats in Saskatoon [20]. There is no published record for adult cestodes of *E. multilocularis* in dogs, however, morphological

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differentiation between adults of *E. granulosus* and *E. multilocularis* is not always simple. The larval stage of *E. multilocularis* was recently detected in the liver of a dog from central BC [21], and further cases were recently diagnosed in the Niagara region of ON [22,23] and in dogs from AB, SK, and Manitoba (MB) (E. Jenkins, K. Gesy, unpublished observations). A survey for *Giardia* in dogs found positive animals in New Brunswick (NB), QC, ON, MB, AB and BC [24]. There is only a single published record of *Giardia* in cats [16]. Serological surveys for *T. gondii* infection in cats in Canada have been published [25,26], as well as one of farm dogs in QC [27]. Eggs of *B. procyonis* were detected during routine flotation in feces from a dog on Prince Edward Island (PEI), but were considered a result of the ingestion of feces from an infected raccoon, the parasite's usual host [28].

Assessment of the risks to human health associated with zoonotic enteric parasites of pets is impeded in many jurisdictions by the absence of long-term, large scale surveillance of infection and disease associated with the parasites in people, in part because of the low incidence of disease in the human populations. There are, however, exceptions. In Canada there is continuing surveillance for *Cryptosporidium* and *Giardia*, which are nationally notifiable infections in people, as well as regional surveillance for toxoplasmosis in five provinces and Nunavut (NU), and tapeworms, primarily diphyllobothriid cestodes and, much less commonly, echinococcosis, which are notifiable in the NT and NU. Serosurveys for *T. canis* have also been reported for veterinary clinic personnel in ON [29], for children in Halifax, (NS) [30], for people in Nunavik (QC), James Bay (QC), Inuvialuit (NT), Nunatsiavut (NL) [31], and for indigenous communities in SK [32,33]. Cases of visceral and ocular larva migrans linked to *Toxocara* spp. have been described from Montreal [34] and Toronto [35]. Two cases of human infection with larvae of *B. procyonis* have been reported in ON, one with ocular involvement [36,37]. Human cases of autochthonous cystic hydatid disease, caused by the larval stage of *E. granulosus*, have also been reported and continue to be reported in western and northern Canada, often in aboriginal people [38-43]. Only one endemically acquired human case of alveolar hydatid disease (the larval stage of *E. multilocularis*) has been reported in Canada [44].

*Giardia*, and to a lesser extent *Cryptosporidium*, remain important human pathogens in Canada causing individual cases and outbreaks of disease, the latter often resulting from the presence of the parasites in water, although such outbreaks may not be of animal origin [45]. For *T. gondii* in people, there are published reports of serological surveys and outbreaks in several provinces [32,33,46-52]. These outbreaks have been associated with the consumption of meat from wildlife by aboriginal

people in QC [53], and with oocysts in municipal drinking water in Victoria (BC) [54].

The study reported here is the largest of its kind ever in Canada. The purpose was to determine the prevalence of intestinal parasites in shelter dogs and cats in all Canadian provinces. Data generated from this study will help veterinarians and physicians to better educate their clients and patients about parasite prevalence and help guide parasite diagnostic and preventive programs.

## Methods

### Study design

Using sample size estimates based on the parasite prevalence previously reported in other studies in Canada, the goal of this study was to collect fecal samples from 1200 dogs and 500 cats from shelters in every province across the country. Shelter populations were selected to more accurately reflect the degree of parasite contamination in the environment and of infection in hosts (definitive, intermediate and paratenic) than would client-owned animals in which routine deworming is believed to occur more commonly.

The size of the pet population in Canada was assumed to correlate closely with that of the human population, and the number of samples to be collected in each province was based on the 2008 Canadian Census Records [55], with a minimum of 50 samples from each animal shelter participating in the study [56]. The number of samples from each province was rounded upward or downward to the nearest 50 specimens.

Shelter selection was based on their geographic location (the majority of those selected were in urban centres), monthly rates of dog and cat arrivals, ability to identify a contact person within the shelter, staff availability and expertise for implementing the sampling protocol, and willingness of the shelter staff to participate. Each shelter was assigned a unique ID, and was given strict written protocols for sample collection and shipping, targets for the number of samples to be collected from dogs and cats, and a list of characteristics to be recorded for each animal sampled. Samples were collected only from newly admitted stray or surrendered dogs and cats. A stray animal was defined as one that was found and brought to the shelter. The animals tested were categorized by age group:  $\leq 1$  year old and  $> 1$  year old. When unknown, age was based on the presence of deciduous or permanent dentition. Animals known to have been dewormed within 5 months prior to sampling were not included in the study. However, deworming history prior to shelter arrival was not always available.

### Sampling and shipping

For each sample submitted, species, breed, gender, reproductive status, age and origin (stray or surrender),

and date of sampling were recorded. When possible, a complete bowel movement was collected and transferred into a Ziploc-type bag for submission. Samples were refrigerated at 4°C, placed in styrofoam boxes or insulated metallic envelopes, and shipped within 24 hours to the Parasitology Laboratory of the University of Montreal in St-Hyacinthe (QC). Samples were collected from May 2009 to November 2010.

### Fecal examination

Upon receipt in the laboratory, samples were stored at 4°C and processed within 5 days from the time of sampling. Five grams of each fecal sample were analyzed using a sugar solution (SG 1.28) double centrifugal flotation technique [57]. The entire coverslip was examined using a 10X objective, and 50 fields were systematically checked using the 40X objective of a compound microscope. Parasites were identified based on morphology to the family, genus or, when possible, species level. In dogs, *T. canis* and *T. cati* were distinguished by morphology based on egg measurements [58].

Taeniid cestode eggs were recovered from positive fecal samples using a flotation method similar to that described above [57]. DNA was extracted from the eggs recovered from individual samples using the FASTDNA® kit (MP Biomedicals, Santa Ana, California, USA). A multiplex PCR was performed using primers to distinguish *Taenia* spp., *E. granulosus*, and *E. multilocularis* on the basis of band position as visualized by agarose gel electrophoresis [59].

### Statistical analysis

Two datasets were constructed, one for dogs and one for cats. For each dataset, subsets were defined as male, female, unknown; age ≤ 1 year, or > 1 year, or unknown; stray, surrendered; and one of the ten Canadian provinces; as well as combinations of these (e.g., males of age ≤ 1 year). The overall prevalence of infection with any parasite species and the prevalence for each parasite species were calculated for the entire dataset and for each data subset. Prevalence was the number of positive samples/total number of samples × 100%. A 95% confidence interval (CI) was calculated using the formula for the binomial distribution. When there was at least one positive sample in a data subset, both the upper and lower confidence limits were non-zero. When there was no single positive sample, only the lower confidence limit was equal to zero, whereas the upper confidence limit was non-zero; this upper confidence limit increased as the sample size decreased. Any non-overlapping subsets of the dataset were compared using the Fisher-Exact test. The dataset was split into four regions and defined as follows: Atlantic (NL, NS, NB, PEI), East (QC, ON), West (MB, SK, AB) and Pacific (BC). For pairwise comparison of the four regions and to detect

differences among provinces, a Bonferroni correction was applied to the p-values, in order to avoid inflation of type-I error. All calculations were carried out on the AH Development Biostatistics IT infrastructure, PC AHCHBS-L13418, using the software SAS®, Version 9.2.2.

### Results

Samples were obtained from 26 shelters across the country. Of the samples submitted, 1086 were from dogs and 636 were from cats (Table 1). The overall prevalence of gastrointestinal parasites in dogs and cats was 33.9% (CI 31.1 – 36.8) and 31.8% (CI 28.2 – 35.5), respectively (Table 1). Eleven different species of parasites were identified in dogs and eight in cats. Of the dogs that tested positive for any parasite on fecal analysis, 67% were infected with a single species of parasite and 33% with multiple species. Seventy-three percent of positive cats were infected with a single species of parasite and 27% with multiple species.

*Toxocara canis* was the most prevalent parasite in fecal samples from dogs (12.7%, CI 10.8-14.8) followed by *Cystoisospora* spp. (10.4%, CI 8.7 – 12.4). Total prevalence of ascarid infection (*T. canis* and *Toxascaris leonina*) was 14.6% (CI 12.6 – 16.9). The most prevalent parasite in cats was *T. cati* (16.5%, CI 13.7 – 19.6) followed by *Cystoisospora* spp. (14%, CI 11.4 – 16.9).

The prevalence of any parasites was higher in dogs ≤ 1 yr of age than in dogs > 1 yr of age ( $p = < 0.0001$ ). *Toxocara canis* ( $p = < 0.0001$ ), *T. leonina* ( $p = 0.0040$ ), *Uncinaria stenocephala* ( $p = 0.0469$ ), *Giardia* ( $p = 0.0004$ ), *Cystoisospora* ( $p = 0.0170$ ) and *Cryptosporidium* ( $p = 0.0003$ ) were the parasites that contributed most to this result. In cats, differences in parasite prevalence between the age groups produced significant results only for *T. cati* ( $p = < 0.0001$ ).

Parasite prevalences are listed by region (Table 2) and by province (Table 3). The overall prevalence of intestinal parasitism in dogs was similar across the Atlantic, East, West and Pacific regions with a prevalence of 31.7% ( $n = 101$ ), 32.8% ( $n = 622$ ), 38.2% ( $n = 228$ ) and 33.3% ( $n = 135$ ), respectively. Comparisons between regions were not statistically significant. The overall prevalence in cats did vary regionally. Prevalence for Atlantic, East, West and Pacific were 32.1% ( $n = 81$ ), 36.8% ( $n = 285$ ), 29.1% ( $n = 175$ ) and 21.1% ( $n = 95$ ), respectively. Comparison between East and Pacific regions showed a statistically significant difference ( $p = 0.0321$ ).

The prevalence of individual parasites varied among regions. Although not statistically significant, ascarids were most frequently found in canine samples from the Pacific region (20%,  $n = 135$ ) and in feline samples from the Atlantic (23.5%,  $n = 81$ ). In cats, *Ancylostoma tubaeforme* was diagnosed only in the East region (4.6%,  $n = 285$ ). *Trichuris vulpis* infection was more frequently diagnosed in

**Table 1 Prevalence of intestinal parasites (%) in fecal samples from shelter dogs and cats by age group**

Parasite	All Dogs (n = 1086) <sup>a</sup>	All Cats (n = 636) <sup>a</sup>	Dogs ≤ 1 yr (n = 546)	Cats ≤ 1 yr (n = 328)	Dogs >1 yr (n = 539)	Cats >1 yr (n = 306)
	Prevalence (95% CI)	Prevalence (95% CI)	Prevalence (95% CI)	Prevalence (95% CI)	Prevalence (95% CI)	Prevalence (95% CI)
<i>Toxocara canis</i>	12.7 (10.8-14.8)	n/a	20.9 (17.5-24.5)	n/a	4.5 (2.9-6.6)	n/a
<i>Toxocara cati</i>	n/a	16.5 (13.7-19.6)	n/a	23.5 (19.0-28.4)	n/a	8.8 (5.9-12.6)
<i>Toxascaris leonina</i>	3.0 (2.1-4.2)	n/a	4.6 (3.0-6.7)	n/a	1.5 (0.6-2.9)	n/a
<i>Ancylostoma caninum</i>	3.1 (2.2-4.3)	n/a	3.3 (2.0-5.2)	n/a	3.0 (1.7-4.8)	n/a
<i>Uncinaria stenocephala</i>	2.9 (2.0-4.1)	n/a	4.0 (2.5-6.0)	n/a	1.9 (0.9-3.4)	n/a
<i>Ancylostoma tubaeforme</i>	n/a	2.0 (1.1-3.5)	n/a	2.1 (0.9-4.3)	n/a	2.0 (0.7-4.2)
Taeniid <sup>b</sup>	1.6 (0.9-2.5)	4.4 (2.9-6.3)	1.1 (0.4-2.4)	2.7 (1.3-5.1)	2.0 (1.0-3.6)	5.9 (3.5-9.1)
<i>Trichuris vulpis</i>	4.4 (3.3-5.8)	n/a	4.2 (2.7-6.3)	n/a	4.6 (3.0-6.8)	n/a
Capillarid eggs	0.7 (0.3-1.4)	2.5 (1.4-4.1)	1.1 (0.4-2.4)	1.2 (0.3-3.1)	0.4 (0.0-1.3)	3.9 (2.0-6.7)
<i>Cystoisospora</i>	10.4 (8.7-12.4)	14.0 (11.4-16.9)	12.6 (10.0-15.7)	16.8 (12.9-21.3)	8.2 (6.0-10.8)	11.1 (7.8-15.2)
<i>Giardia</i>	3.5 (2.5-4.8)	1.4 (0.6-2.7)	5.5 (3.7-7.8)	2.1 (0.9-4.3)	1.5 (0.6-2.9)	0.7 (0.1-2.3)
<i>Cryptosporidium</i>	3.0 (2.1-4.2)	1.3 (0.5-2.5)	4.9 (3.3-7.1)	2.1 (0.9-4.3)	1.1 (0.4-2.4)	0.3 (0.0-1.8)
<i>Sarcocystis</i>	4.5 (3.4-5.9)	0.2 (0.0-0.9)	4.4 (2.8-6.5)	0.0 (0.0-1.1)	4.6 (3.0-6.8)	0.3 (0.0-1.8)
All parasites <sup>a</sup>	33.9 (31.1-36.8)	31.8 (28.2-35.5)	43.8 (39.6-48.1)	39.0 (33.7-44.5)	23.9 (20.4-27.8)	23.9 (19.2-29.0)

<sup>a</sup>All parasites include many cases of multiple infections. <sup>b</sup>taeniid-type eggs are produced by *Taenia* and *Echinococcus* cestode species, and cannot be identified to genus level by morphology alone.

n/a: Species not an usual host or parasite not identified in the species.

dogs in the East region (6.1%, n = 622). This result reached significance in comparison with the West region (p = 0.009). *Cystoisospora* spp. oocysts were most frequently identified in dogs from the Pacific region (16.3%, n = 135) and in cats from the East (19.3%, n = 285). The prevalence of *U. stenocephala* in dogs was significantly higher from samples collected from June to November compared to

those collected from December to May (p = 0.0155). No significant seasonal difference was detected for any of the parasites found in cats.

Taeniid cestode eggs from 9 canine and 22 feline positive fecal samples were further characterized using molecular techniques. Eggs of *E. granulosus*/*E. canadensis* were detected in fecal samples from 4 dogs (all of which were

**Table 2 Prevalence of intestinal parasites (%) in fecal samples from shelter dogs and cats from different regions of Canada**

Parasite	Atlantic - Dogs	Atlantic - Cats	East - Dogs	East - Cats	West - Dogs	West - Cats	Pacific - Dogs	Pacific - Cats
n	101	81	622	285	228	175	135	95
<i>Toxocara canis</i>	9.9	n/a	12.2	n/a	11.8	n/a	18.5	n/a
<i>Toxocara cati</i>	n/a	23.5	n/a	18.6	n/a	13.7	n/a	9.5
<i>Toxascaris leonina</i>	0.0 <sup>a</sup>	n/a	1.6 <sup>b,c</sup>	n/a	6.6 <sup>a,b</sup>	n/a	5.9 <sup>c</sup>	n/a
<i>Ancylostoma caninum</i>	3.0	n/a	4.0	n/a	1.8	n/a	1.5	n/a
<i>Uncinaria stenocephala</i>	3.0	n/a	2.9	n/a	4.4	n/a	0.7	n/a
<i>Ancylostoma tubaeforme</i>	n/a	0.0	n/a	4.6 <sup>d</sup>	n/a	0.0 <sup>d</sup>	n/a	0.0
Taeniid	0.0	7.4	1.4	3.3	3.1	6.3	0.7	2.1
<i>Trichuris vulpis</i>	4.0	n/a	6.1 <sup>e</sup>	n/a	0.9 <sup>e</sup>	n/a	3.0	n/a
Capillarid eggs	1.0	3.7	0.6	2.8	0.4	1.7	1.5	2.1
<i>Cystoisospora</i>	8.9	7.4	8.7	19.3	12.3	10.3	16.3	10.5
<i>Giardia</i>	3.0	0.0	3.9	2.1	3.5	1.7	2.2	0.0
<i>Cryptosporidium</i>	5.0	0.0	3.4	0.4	2.2	2.9	1.5	2.1
<i>Sarcocystis</i>	4.0	0.0	2.3 <sup>f</sup>	0.0	11.8 <sup>f,g</sup>	0.0	3.0 <sup>f</sup>	1.1
All parasites <sup>i</sup>	31.7	32.1	32.8	36.8 <sup>h</sup>	38.2	29.1	33.3	21.1 <sup>h</sup>

<sup>a</sup>p = 0.0428; <sup>b</sup>p = 0.024; <sup>c</sup>p = 0.0445; <sup>d</sup>p = 0.0382 <sup>e</sup>p = 0.009; <sup>f</sup>p < 0.0001; <sup>g</sup>p = 0.0185; <sup>h</sup>p = 0.0321. <sup>i</sup>All parasites include many cases of multiple infections, p values relate to shelter dog and shelter cat populations separately. n/a: Species not an usual host or parasite not identified in the species.

**Table 3 Prevalence of intestinal parasites (%) in fecal samples from shelter dogs and cats from each Canadian province**

Parasite	Dogs NL	Cats NL	Dogs NS	Cats NS	Dogs NB	Cats NB	Dogs PE	Cats PE	Dogs QC	Cats QC	Dogs ON	Cats ON	Dogs MB	Cats MB	Dogs SK	Cats SK	Dogs AB	Cats AB	Dogs BC	Cats BC	Dogs Total	Cats Total
<i>n</i>	18	28	25	6	35	16	23	31	270	114	352	171	60	35	46	34	122	106	135	95	1086	636
<i>Toxocara canis</i>	0.0	n/a	16.0	n/a	5.7	n/a	17.4	n/a	12.6	n/a	11.9	n/a	11.7	n/a	13.0	n/a	11.5	n/a	18.5	n/a	12.7	n/a
<i>Toxocara cati</i>	n/a	14.3	n/a	16.7	n/a	43.8	n/a	22.6	n/a	12.3	n/a	22.8	n/a	22.9	n/a	8.8	n/a	12.3	n/a	9.5	n/a	16.5
<i>Toxascaris leonina</i>	0.0	n/a	0.0	n/a	0.0	n/a	0.0	n/a	3.0	n/a	0.6 <sup>a</sup>	n/a	3.3	n/a	6.5	n/a	8.2 <sup>b</sup>	n/a	5.9	n/a	3.0	n/a
<i>Ancylostoma caninum</i>	0.0	n/a	4.0	n/a	5.7	n/a	0.0	n/a	3.0	n/a	4.8	n/a	3.3	n/a	2.2	n/a	0.8	n/a	1.5	n/a	3.1	n/a
<i>Uncinaria stenocephala</i>	5.6	n/a	8.0	n/a	0.0	n/a	0.0	n/a	0.7	n/a	4.5	n/a	6.7	n/a	4.3	n/a	3.3	n/a	0.7	n/a	2.9	n/a
<i>Ancylostoma tubaeforme</i>	n/a	0.0	n/a	0.0	n/a	0.0	n/a	0.0	n/a	3.5	n/a	5.3 <sup>b</sup>	n/a	0.0	n/a	0.0	n/a	0.0	n/a	0.0	n/a	2.0
Taeniid	0.0	3.6	0.0	0.0	0.0	18.8	0.0	6.5	0.0	2.6	2.6	3.5	3.3	8.6	0.0	5.9	4.1	5.7	0.7	2.1	1.6	4.4
<i>Trichuris vulpis</i>	5.6	n/a	8.0	n/a	0.0	n/a	4.3	n/a	0.7 <sup>b</sup>	n/a	10.2 <sup>c</sup>	n/a	1.7	n/a	0.0	n/a	0.8	n/a	3.0	n/a	4.4	n/a
Capillarid eggs	0.0	0.0	0.0	0.0	0.0	18.8	4.3	0.0	0.0	2.6	1.1	2.9	0.0	2.9	0.0	5.9	0.8	0.0	1.5	2.1	0.7	2.5
<i>Cystoisospora</i>	5.6	7.1	0.0	0.0	17.1	12.5	8.7	6.5	4.8 <sup>a</sup>	9.6	11.6	25.7 <sup>c</sup>	10.0	11.4	15.2	11.8	12.3	9.4	16.3	10.5	10.4	14.0
<i>Giardia</i>	0.0	0.0	4.0	0.0	5.7	0.0	0.0	0.0	5.2	2.6	2.8	1.8	3.3	0.0	4.3	0.0	3.3	2.8	2.2	0.0	3.5	1.4
<i>Cryptosporidium</i>	5.6	0.0	0.0	0.0	8.6	0.0	4.3	0.0	3.3	0.0	3.4	0.6	1.7	0.0	2.2	0.0	2.5	4.7 <sup>b</sup>	1.5	2.1	3.0	1.3
<i>Sarcocystis</i>	5.6	0.0	0.0	0.0	5.7	0.0	4.3	0.0	1.9	0.0	2.6	0.0	8.3	0.0	4.3	0.0	16.4 <sup>c</sup>	0.0	3.0	1.1	4.5	0.2
All parasites <sup>d</sup>	16.7	21.4	28.0	16.7	37.1	56.3	39.1	32.3	27.8	27.2	36.6	43.3 <sup>a</sup>	35.0	37.1	39.1	32.4	39.3	25.5	33.3	21.1	33.9	31.8

<sup>a</sup> =  $p < 0.01$ ; <sup>b</sup> =  $p < 0.05$ ; <sup>c</sup> =  $p < 0.0001$ ; <sup>d</sup>All parasites include many cases of multiple infections.

p values relate to shelter dog and shelter cat populations separately. n/a: Species not a usual host or parasite, not identified in the species.



strays, 1-5 years old, and medium to large breed) from BC, AB and ON. Eggs of *Taenia* spp. were detected in 2 dogs from AB and MB, and 9 cats from BC, AB, SK, MB, ON, NB, and PEI. Eggs from 3 dogs and 13 cats failed to amplify on the multiplex PCR. Eggs of *E. multilocularis* were not detected in any of the canine or feline samples. No mixed infections (i.e. a single sample with eggs of both *Taenia* and *Echinococcus* spp.) were detected.

## Discussion

This study is the largest national companion animal parasite prevalence survey performed in Canada. Results were based on a single fecal analysis. It is likely that the prevalence of some organisms was higher than detected due to intermittent shedding of ova and the likelihood that some infections may have been prepatent at the time of sample collection. Fecal specimen collection occurred at the shelter prior to the administration of anthelmintics; however, deworming history prior to shelter arrival was not always available. Finally, even though a wide range of parasites infecting multiple organ systems may be detected by centrifugal fecal flotation examination, the detection sensitivity of this technique is poor for protozoan trophozoites, operculate cestode and trematode eggs, spirurid eggs and first-stage nematode larvae [58].

### Ascarid roundworms

The most common parasites identified nationally in both dogs and cats were *Toxocara* spp., with prevalences of 12.7% for *T. canis* and 16.5% for *T. cati*. This is consistent with previous Canadian studies published since 1999 [4,10,12,15,16,18,60,61]. Although not statistically significant, the highest prevalence of ascarid infection in this study was in the Pacific region. Similar results in client-owned dogs have been reported from the western United States (US) [62]. The match between these results might be partly due to the similarity of the climate in BC to that some of the US West region.

There was a higher prevalence of *T. canis* and *T. cati* relative to other parasites. The eggs of these species can also persist in the environment for many years and serve as an important source of infection. In addition, coprophagy is another potential transmission route in dogs (but is not generally thought to occur in cats [63]). *Toxocara* infections in dogs could be false positive as they represent shedding of swallowed *T. cati* eggs. However, we differentiated the 2 species using egg measurements and did not find *T. cati* in dogs.

Although *T. canis* is better recognized as a cause of human toxocarosis, *T. cati* migrating larvae can also cause visceral larva migrans (VLM) and ocular larva migrans (OLM) in people [64-67]. Both syndromes can compromise health, especially in children. Seroprevalence surveys do not differentiate between *T. canis* and

*T. cati* antibodies, potentially underestimating the zoonotic importance of feline ascarid infection [64,65]. Current prevalence of human infection with these ascarids in Canada remains unknown, and published reports of either clinical syndrome are rare [9].

Nationally, prevalence of *Toxascaris leonina* in dogs in the current study was 3%. It was reported at a higher level in the western provinces of AB (8.2%,  $p = 0.0216$ ), BC (5.9%,  $n = 135$ ) and SK (6.5%,  $n = 46$ ) compared to the eastern provinces. These prevalences are lower than those reported in the present study for *T. canis*. These results differ from those of a previous survey in Calgary [11] in which these two species had similar prevalences in dogs. Coyotes in AB are commonly infected with *T. leonina* and parklands in the Calgary region are shared habitats in which dogs and coyotes congregate. This interaction may explain the level of *T. leonina* in dogs in this region [68]. *Toxascaris leonina* was not reported in cats in this study, and appears to be rare (0-4% infected) in cats in all Canadian studies [12].

No eggs of *B. procyonis* were found in dogs or cats in this study, although they have been recovered from the feces of 14 dogs in QC between 2009-2013 [60], from 2 dogs in PEI [61], and from 1 cat in AB [11]. The majority of these reports might result from coprophagia rather than patent infections; however, cats are less likely than dogs to be coprophagic. Unlike raccoons, which defecate in latrines, carrier dogs could disperse these eggs broadly in environments shared with people, increasing the potential of zoonotic transmission.

### *Ancylostoma* and *Uncinaria*

Hookworms were the second most frequent intestinal helminth found in this study, with prevalences of 5.6% in dogs and 2.0% in cats. In dogs, we found both *U. stenocephala* (considered non-zoonotic) and *A. caninum* (potentially zoonotic) with the latter more common in the eastern provinces. *A. tubaeforme* was detected only in cats in the East. *Uncinaria*, also has a more north-western distribution in Canada than *Ancylostoma* [69]. *Ancylostoma caninum* is a well-documented zoonotic infection causing CLM in people, although this has not been reported in Canada, probably due to environment and behavioural practices. The relationship between *U. stenocephala* and CLM remains unclear [58,70].

### Cestodes

We detected taeniid eggs (eggs of *Taenia* or *Echinococcus* spp.) in fecal samples from 1.6% of dogs and 4.4% of cats by fecal centrifugation. This prevalence may be artificially low; in general, coproscopy underestimates helminth prevalence, especially for cestodes, which shed segments, compared to necropsy examination [71-73]. For example, in a comparison of the two techniques, taeniids were

found in more than 50% of dogs (n = 97) and cats (n = 116) examined by necropsy, but taeniid eggs were found in fecal samples from only 7.2% of the dogs and 6.9% of the cats [71,73].

Unlike other common parasites, cestode infection rates in the current study were higher in adult dogs and cats than in younger animals. This might be explained by age differences and opportunities to consume intermediate hosts (e.g. small mammals and fleas) prior to their arrival at the shelter.

We detected eggs of zoonotic *E. granulosus*/*E. canadensis* in dogs in BC, AB, and ON. In Canada, this parasite is most likely *E. canadensis* (G8 and/or G10 genotypes of the *E. granulosus* species complex) [74,75]. Dogs become infected through consumption of hydatid cysts in the organs of infected cervids. People are most often infected with this parasite through inadvertent consumption of eggs shed in feces of dogs or of wild canids. It is reassuring that *E. multilocularis* was not detected in the current study. This parasite can cause severe human disease and has been identified in wildlife in Canada and the US [75,76]. The detection of *E. granulosus*/*E. canadensis* in dogs in the current study, and the detection of alveolar hydatid stages of *E. multilocularis* in dogs [21-23] (E. Jenkins, K. Gesy, unpublished observations) and adult cestodes in cats [20] emphasizes the need for cestocidal treatment of owned, shelter, and surrendered animals, especially where there is risk of human exposure.

Limitations of the multiplex PCR used in this study are evidenced by the failure to generate amplicons from eggs from 13 cats and 3 dogs, which may reflect difficulty in extracting DNA from a small number of eggs, as well as the fact that the PCR primers used may not be optimized for taeniid species and genotypes in North America.

#### Trichuridae

*Trichuris vulpis* prevalence observed in this study was 4.4%. This is higher than previously reported in other Canadian studies [5,7,8,12,15,16,43,60,61]. The West region had the lowest prevalence at 0.9% with AB at 0.8% (n = 122). In a survey in Calgary, whipworm eggs were not detected in any fecal samples from dogs [12]. In Colorado, *T. vulpis* eggs were found in a fecal sample from only one of 130 dogs examined [77]. A lower rate of whipworm infection has also been reported in the West region of the US [62]. Additional research is required to define the zoonotic potential of this parasite [78,79].

A range of factors may complicate diagnosis of this infection. Whipworm eggs are dense and have a specific gravity greater than that of the other nematode eggs in dog feces. A double centrifugal flotation using a sugar solution with a specific gravity of 1.28 has been used for maximal recovery of whipworm eggs [79]. False negative results could also have occurred due to the long

prepatent period of *T. vulpis* and intermittent shedding of eggs.

#### Protozoans

The most common protozoan identified in both dogs and cats was *Cystoisospora* spp., with a total prevalence of 10.4% and 14.0%, respectively. Previously published Canadian surveys indicated similar results (0.4-16.3% in dogs and 1-12.8% in cats) [11,12,15,16,18,60,61]. Although not statistically significant, *Cystoisospora* spp. oocysts were more frequently identified in dogs from the Pacific region and in cats from the East. In dogs, this result is similar to that found in the West and Midwest regions of the US [62]. Although not known to be of zoonotic significance, clinical coccidiosis can be severe in young animals and transmission can be a pervasive problem in shelter and kennel environments [80].

In this survey 3.5% of dogs and 1.4% of cats were diagnosed with *Giardia*. It is probable that we underestimated the prevalence for this parasite. The addition of immunofluorescent assay to fecal centrifugation sucrose flotation has been shown to increase diagnostic sensitivity tremendously for *Giardia* and *Cryptosporidium* [11]. Another limitation of this study is that assemblage identification was not performed to determine whether the *Giardia* detected were host specific for dogs (Assemblage C), cats (Assemblage F), both (Assemblage D), or for dogs, cats and humans (Assemblages A and B) [81-83].

The majority of Canadian studies have shown the prevalence of canine and feline *Giardia* infection to be under 10% [4,7,11,12,15,16,18,24,60,61], except in dogs from northern communities where prevalence can be 21-60% [4,5,76], and in rural cats from AB and free-roaming cats from SK where prevalences of 11% and 16%, respectively, have been reported [11]. The primary genotype found in these dog and cat populations is Assemblage A, which is potentially zoonotic [4,5,11,76]. In contrast, in shelter and kennel environments, non-zoonotic (i.e., host-specific) genotypes tend to dominate [83-85]. While transmission from dogs and cats to humans appears to be uncommon, owners of infected pets should be advised of the risk.

*Cryptosporidium* was also most likely underestimated [11] in the current study: it was detected in fecal samples from 33 dogs (3%) and from 8 cats (1.3%). In the Niagara region of ON, Shukla et al. [16] reported the prevalence of *Cryptosporidium* spp. by antigen detection in 7.4% and 7.3% in dogs and cats, respectively. Based on a sucrose gradient isolation and immunofluorescent assay, Hoopes et al. [11] reported oocysts in 2.3% and 7% of client-owned and free-roaming cats. Most infections in dogs and cats are caused by host-specific *C. canis* and *C. felis*, respectively and they have been responsible for only a small number of human cases,

usually in immunocompromised people. Pet ownership has not been found to be a significant zoonotic risk for people to develop cryptosporidiosis [86].

*Sarcocystis* was found in 49 dog fecal samples (4.5%) and in only one cat (0.2%). In a recent study the prevalence of *Sarcocystis* spp. was 0.3% in dogs from Calgary [8]. However, it is a common finding in dogs from northern and rural communities and is often found in co-infections with *Taenia* and *Echinococcus* spp. [4] (J. Schurer, E. Jenkins unpublished observations). Clinical disease in dogs and cats associated with *Sarcocystis* appears to be rare and *Sarcocystis* spp. in dogs and cats are generally considered not transmissible to humans [87,88].

This study provides current information on the prevalence of canine and feline intestinal parasites in provincial shelters across Canada, and some guidance regarding regionally-appropriate parasiticide treatments and the risk of human infections with zoonotic species and genotypes. The application of these data, however, requires considerable caution. First, the data are based on a single sample examined with a globally-used technique of essentially variable sensitivities over a range of parasites [72,89-91]. Second, shelter animals were the subject of our study, and because of the low likelihood of parasiticide treatment relative to client-owned animals, they do represent the potential for pet exposure to parasites. Third, while the parasites occurred in dogs and cats across the Canadian provinces, transmission is regionally variable and depends on a particular sequence of events that leads to infection. This is among the reasons why the local knowledge of veterinarians and physicians about parasite occurrence and risk factors for infection is so important.

## Conclusions

The parasite prevalence levels reported in this study reinforce the need to monitor pets across Canada, for intestinal parasites and to treat infected animals promptly and correctly with effective parasiticides. Animals adopted from shelters with untreated, or ineffectively treated, parasite infections pose ongoing risks for animal and human health. This reinforces the importance of strategies for prevention, which depend in part on shelter management and owner awareness of the sources and management options for parasites in their pets. This awareness can be greatly enhanced by veterinarians and their staff.

Veterinarians are an important source of information for pet owners and play a critical role in the initiation of education programs emphasizing the importance of preventive measures in reducing the risks of environmental contamination and zoonotic transmission [92]. In addition, periodic fecal monitoring of pets allows determination of the efficacy of the products being used, compliance with the recommended administration schedules and

re-assessment of the therapeutic approach based on current patient health status.

The animal surveillance data from this study will help in the development of strategies, based on risk per geographic location for the prevention and response to endoparasites in pets and free-roaming and shelter animals in Canada.

## Consent to publish

Consent to publish results of the fecal analysis conducted in this study was obtained from the participating shelters organizations.

## Competing interests

France Gagné, Donald Benoit and Wolfgang Seewald are employees of Novartis Animal Health. Funding for fecal sample collection and analysis, as well as article processing fees was provided by Novartis Animal Health.

## Authors' contributions

The study design and protocol were prepared by AV, LP, JG, GC, SK and FG. FG coordinated the study and collection of the fecal samples at the participating shelters. Fecal sample analyses were conducted at the Parasitology Laboratory of the University of Montreal in St-Hyacinthe (QC) under AV supervision. EJ and JS carried out the multiplex PCR on samples positive for taeniid eggs. WS completed the statistical report. LP drafted the background of the manuscript. EJ summarized data and drafted the cestode section. AV, FG and DB drafted all remaining sections of the manuscript. All authors revised and approved the final version.

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