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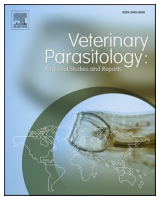

Short communication

Assessing the potential for infections of *Echinococcus multilocularis* in dogs in a hotspot of human alveolar echinococcosis infections in North America

Emilie Porter [a], M. Alexis Seguin [b], Marko Estrada [b], Donald Szlosek [b], Alessandro Massolo [c,d,e], Darcy R. Visscher [a,f,g,*]

[a] Department of Biology, The King's University, 9125 50 Street NW, Edmonton, AB T6B 2H3, Canada
[b] IDEXX Reference Laboratories, Inc, Westbrook, ME 04092, United States of America
[c] Ethology Unit, Department of Biology, University of Pisa, Via Volta 6, 56126 Pisa, Italy
[d] Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, 2500 University Dr NW, Calgary, Alberta T2N 1N4, Canada
[e] UMR CNRS 6249 Chrono-environnement, Université Bourgogne Franche-Comté, 16 Route de Gray, 25030 Besançon, France
[f] Biological Sciences, University of Alberta, CW405 Biological Sciences Bldg., Edmonton, AB T6G 2E9, Canada
[g] Naturalis Biodiversity Center, Darwinweg 2, 2333CR Leiden, the Netherlands

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ABSTRACT

Echinococcus multilocularis is a zoonotic tapeworm, whose metacestode larval stage is the etiological agent for alveolar echinococcosis in humans and is a parasite of emerging concern according to the World Health Organization which is difficult to diagnose and has a case mortality rate of >90% when left untreated. *Echinococcus multilocularis* requires two mammalian hosts to complete its lifecycle: wild and domestic canids as definitive hosts, and small mammals (mostly rodents) as intermediate ones. Because of their close relations with humans, domestic dogs have been indicated as a mean of infection to people. Human alveolar echinococcosis has historically been rare in North America, however, since 2013, at least seventeen diagnoses have been confirmed in Alberta, Canada. Because of this unprecedented series of cases, assessing the frequency of infections in dogs in Alberta is key to estimate risk for dog owners and animal health professionals. This study was carried out in Edmonton to determine the frequency of *E. multilocularis* infection in domestic dogs and potential risk factors. Fecal samples and corresponding behavior risk surveys were collected from 775 dogs in seven urban off-leash parks within Edmonton city limits during the summer of 2020. A quantitative PCR fecal test was used to diagnose *E. multilocularis* infection. We found a single case of *E. multilocularis* infection (1/775) and determined that the overall true prevalence was 0.2% (95% CrI: 0.0–0.7%) corrected for detection sensitivity and specificity. Overall, these findings confirm the presence of *E. multilocularis* infection in domestic dogs in Edmonton although further work is required to fully understand the risk factors that may contribute to infection and potential transmission to humans.

1. Introduction

Increased urbanization has the potential to facilitate the transmission of zoonoses to humans through increased contact with synanthropic wildlife (Bradley and Altizer, 2007; Jones et al., 2008; Hassell et al., 2017). The *Echinococcus* genus includes trophically transmitted parasitic tapeworms, of which *E. multilocularis* is an emerging zoonotic parasite of concern and is considered to be amongst food-borne parasites with the greatest global impacts (Eckert et al., 2001). *Echinococcus multilocularis* is the etiological agent for alveolar echinococcosis in humans, which is difficult to diagnose and has a case mortality rate of >90% when untreated. While benzimidazole chemotherapy has significantly improved survival rates, chronic cases represent a significant economic loss globally, which could be reduced with early detection. In order to better assess the potential risk that these tapeworms pose to humans, it is imperative to examine the ecological and behavioural factors that facilitate transmission of these parasites to domestic dogs which may be involved in transmission to humans as accidental hosts (Houston et al., 2021; Polish et al., 2021; Torgerson et al., 2020).

Echinococcus multilocularis requires two mammalian hosts to complete its lifecycle and is frequently transmitted by ingestion through a predator and prey system (Eckert et al., 2001; Romig et al., 2017). The

* Corresponding author at: Department of Biology, The King's University, 9125 50 Street NW, Edmonton, AB T6B 2H3, Canada.
E-mail address: darcy.visscher@kingsu.ca (D.R. Visscher).

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E. multilocularis lifecycle consists of a small rodent as intermediate hosts, whereas wild or domestic canids (e.g., foxes, coyotes, dogs), which prey upon these mammals, serve as definitive hosts. Wild canids indirectly and incidentally interact with humans and domestic dogs, potentially facilitating the transmission of these parasites to human-dominated environments (Bradley and Altizer, 2007; Deplazes et al., 2004; Liccioli et al., 2015). Human infection may occur through the accidental ingestion of eggs normally excreted in infected canid feces, either directly or through contaminated soil (Torgerson et al., 2020). Dog ownership in endemic areas is considered as a risk factor in the development of human alveolar echinococcosis (Conraths et al., 2017; Kern et al., 2004). However, domestic dog studies have only been conducted in a few countries with a known distribution of *Echinococcus* spp., and most of these studies focused on strays or dogs in rural areas (Toews et al., 2021). While dogs with *E. multilocularis* infections tend to have a lower worm burden than the wild canid hosts (Kapel et al., 2006), infections in pet dogs could increase the likelihood of human alveolar echinococcosis, due to their close association with humans (Houston et al., 2021; Toews et al., 2021; Torgerson et al., 2020).

Alveolar echinococcosis was considered rare in North America until recently (Massolo et al., 2014). However, since 2013, at least seventeen diagnoses have been confirmed by hospitals in Alberta, Canada (Houston et al., 2021; Massolo et al., 2019). Therefore, it is essential to assess the prevalence of *E. multilocularis* in urban domestic dogs to determine the role they play in human infections. Specifically, Edmonton is considered a hotspot for human AE cases and warrants attention to better understand the risk to human health. Therefore, in this study we estimated the frequency of *E. multilocularis* infections in domestic dogs that frequent off-leash dog parks in Edmonton.

2. Materials and methods

2.1. Sampling design

We recruited participants in Edmonton, Alberta, Canada in seven urban off-leash dog parks dispersed throughout the city. A small number of participants (about 6%) were recruited with an online survey distributed via social media and at smaller residential off-leash parks. Surveys were collected on at least three consecutive days at each park location and all locations were visited once in May or June and then once again in July and August, except for one park. We used an intercept approach with dog owners being opportunistically approached, asked to participate in the study, and to provide a fecal sample from their dog.

We used a modified form of the survey tool employed by Smith et al. (2014) which was divided into different sections (Smith et al., 2014; Toews et al., 2021). The first section included participant screening questions for inclusion in the survey, which included owning the dog in question and residing in the greater Edmonton region, the owner being over the age of 18, and the dog being over 3 months in age. The second section focused on dog demographic details such as gender, breed, age class, spay/neuter status as well as veterinary care and deworming practices. The following sections were specific to dog owner behavior including dog walking routines and levels of off leash activity. We included questions about the dog's behavior on walks including questions to understand the prey drive and scavenging activity of the dogs. We used a Likert type ranked 6-point scale to record the frequency of walking behavior at different types of location as well as the frequency of off-leash activity at those locations (Smith et al., 2014). To be included in the study, participants had to answer yes to the screening questions, complete the entire survey, and provide a fecal sample of their dog. This project had ethics approval by The King's University ethics board (2017–08-DRV).

2.2. Fecal collection and analysis

Participants were asked to collect a fresh fecal sample and leave it with the researcher after completing their walk. Fecal samples were stored in coolers until the end of the day and were subsampled into 1.5 mL centrifuge tubes and frozen at −18 °C. Following the summer collection period, all samples were frozen at −80 °C for 96 h in order to kill any virulent *Echinococcus* eggs that could be present in the samples (Veit et al., 1995). Samples were then stored at −18 °C until they were analyzed. Fecal samples for PCR analysis were submersed in 3 mL guanidinium thiocyanate based lysis solution (Boom et al., 1990), vortexed to facilitate organism detachment and rapid protein denaturation and incubated at room temperature for 15 min. The lysate was then used to extract total nucleic acid on a MagMax 96 Flex (Life Technologies, Valencia, CA) with magnetic beads (Roche, Indianapolis, IN) using manufacturer's guidelines. Total nucleic acid was eluted in 200 uL of PCR-grade nuclease-free water (Fisher) and 5 uL amplified in subsequent single plex real-time PCR reactions. Analysis was performed on a Roche LightCycler 480 (Roche Applied Science, Indianapolis (IN) and raw data analyzed using the 2nd derivative maximum method with the 'high sensitivity' setting to generate crossing points (CP values).

Commercially available real-time polymerase chain reaction (qPCR) assays (IDEXX Laboratories, *Echinococcus* RealPCR™ Panel) targeting both *Echinococcus* spp. and *E. multilocularis* were used to detect the presence of *E. multilocularis* in the fecal samples. Real-time PCR was performed with proprietary forward and reverse primers and hydrolysis probes. The *Echinococcus* spp. and *E. multilocularis* qPCR assays each target ribosomal RNA sequences between the Cox 1 and Cox 2 genes. Real-time PCR was run with seven quality controls including PCR positive controls, PCR negative controls, negative extraction controls, DNA pre-analytical quality control targeting the host ssr rRNA (18S rRNA) gene complex, RNA pre-analytical quality control targeting the host ssr rRNA gene complex, an internal positive control spiked into the lysis solution, and an environmental contamination monitoring control. To be considered as a positive test it had to both test positive for the *Echinococcus* spp. and the *E. multilocularis* test. A full description of real time PCR protocol can be found in Kopper et al. (2021).

2.3. Data analysis

Survey data were collated, and descriptive statistics were used to present results for each section. To estimate the true prevalence of *E. multilocularis*, we used a Bayesian approach (Flor et al., 2020; Speybroeck et al., 2013; Toews et al., 2021) using the R package '*prevalence*' (version 0.4.0). We modelled true prevalence using uniform priors for both sensitivity (80.0–99.9) and specificity (90.0–99.9) that captured the published values for this test (Isaksson et al., 2014; Slovis et al., 2014). The prevalence model used two chains containing 10,000 "burn-in" samples and 10,000 samples that were retained. We used a multivariate Brooks-Gelman-Rubin statistic to ensure model convergence. The true prevalence estimate is reported along with 95% credibility intervals.

3. Results

Between May and August 2020, a total of 804 surveys were administered, however 29 (3.6%) were excluded because they did not meet the inclusion criteria (under 18 years old or were walking a dog they did not own), did not live in Edmonton, or failed to provide a fecal sample. Therefore, a total of 775 fecal samples and corresponding surveys were included in the analysis (Fig. 1). We found a single positive case of *E. multilocularis*, corresponding to true prevalence of 0.2% with a 95% credibility interval ranging from 0.0 to 0.7% (multivariate BGR = 1.001).

The dogs sampled in the study were evenly split between males (52.0%) and females (48.0%), and nearly all had been spayed or neutered (90.1%). The dog age ranged from less than 1 year to 16 years of age, with a median age of 4 years old. Most dogs (58.6%) were of mixed breed. The remainder (41.4%) were considered purebred dogs of various

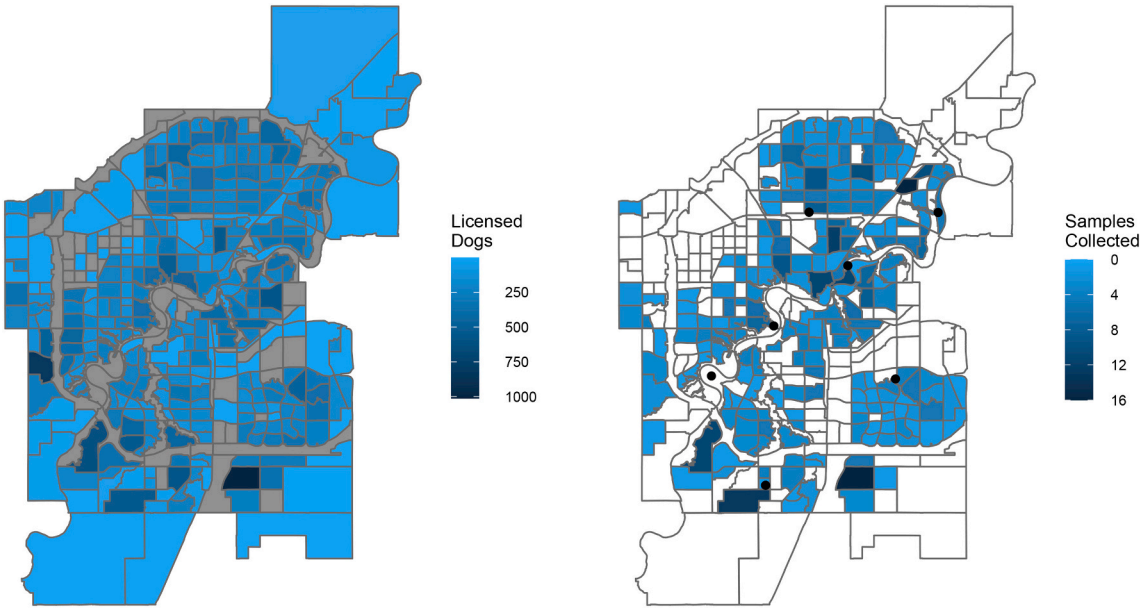

Fig. 1. A choropleth map of the neighborhoods in Edmonton, Alberta (Canada), with the number of licenced dogs (left panel) based on city records, and the number of fecal samples and corresponding surveys that were collected (right) during a study conducted in summer 2020 ($N_{total} = 775$). The black points indicate the main dog parks where collections occurred. Large blank areas tend to correspond to the light industrial or business districts in Edmonton. Note that the color scale in each map is different.

American Kennel Club classifications. Based on owners' responses, nearly all dogs had been to a veterinarian within the past year (93.4%), however, only 63.6% had been dewormed in the past year. The 60.3% of owners also reported that, given the opportunity, their dogs would chase after small prey species like rodents, although they also ranked their dogs as "unlikely" to be successful in capturing and eating these prey species. Most dog owners (85.2%) reported that their dogs would scavenge (often grass and feces) from the ground. Most owners were walking their dogs near daily at designated off leash dog parks (81.5%) and most allowed their dogs off-leash at those locations (78.8%; Fig. 2).

4. Discussion

Despite Edmonton being a known hotspot for human alveolar echinococcosis (Houston et al., 2021; Massolo et al., 2019) and having a high prevalence (up to 70%) of *E. multilocularis* in resident coyotes (Catalano et al., 2012; Luong et al., 2020; Steckler, 2021), we found a low prevalence (0.2%, 95% CrI 0.0–0.8%) of *E. multilocularis* in dogs that frequent off leash dog parks as assessed by fecal qPCR. Our results are comparable to studies determining prevalence of *E. multilocularis* completed in southern Ontario and Winnipeg, Manitoba, both of which found no cases of *E. multilocularis* in dogs (Kotwa et al., 2021; Tse et al.,

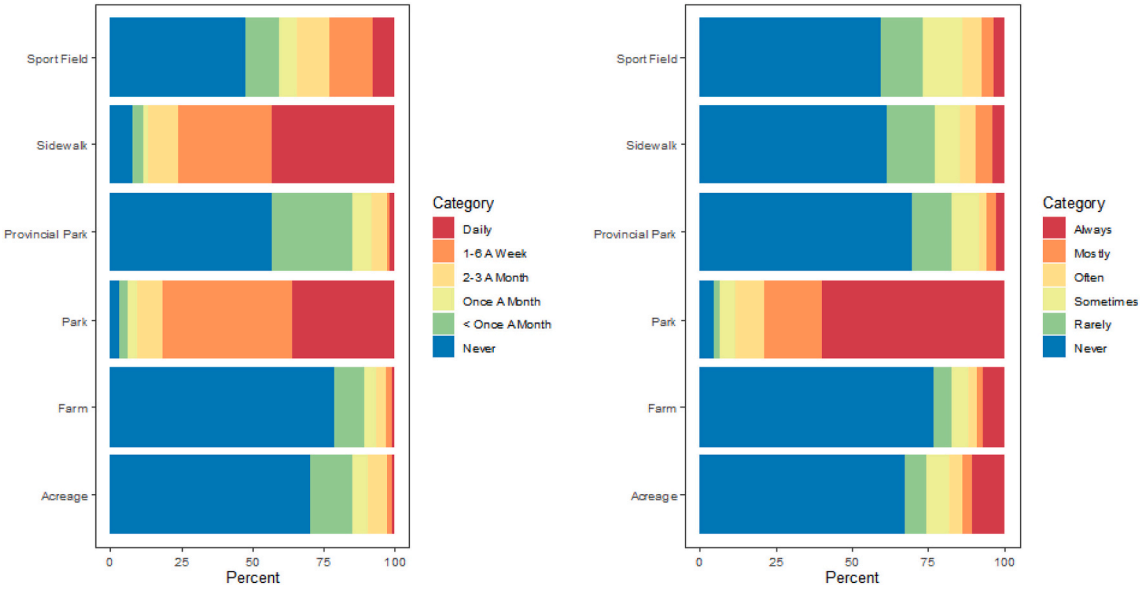

Fig. 2. Dog owners and their pets' walking behaviors in Edmonton, Alberta (Canada), during a study conducted in summer 2020. The left panel describes the locations that dog walkers frequented, ranging from daily (red) to never (blue). The right panel describes the frequency of off-leash behavior in these same locations, ranging from always off-leash (red) to never off-leash (blue). Park refers specifically to designated off-leash parks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2019). Conversely, a recent study in Calgary found 13 cases of *E. multilocularis* infection based on reassessment of 696 fecal samples collected in 2012 (Toews, 2020). Similar to other studies, we suggest that our results do not rule out the risk of intestinal *E. multilocularis* infection in dog populations in Edmonton (Kotwa et al., 2021; Tse et al., 2019), or being transmitted to humans (Houston et al., 2021; Massolo et al., 2019), but suggest that more work is needed to elucidate the pathways associated with transmission to other populations of dogs (rural dogs, hunting dogs, etc.) and, potentially, humans.

The risk of infection may vary with environmental conditions. We specifically elected to sample dogs at off-leash parks as we hypothesized that this group may be at higher risk due to their potential for unsupervised interaction with rodent intermediate hosts on the landscape (Kotwa et al., 2021, Toews et al., 2021). The city of Edmonton contains one of the largest urban park systems in North America and has adopted a policy of naturalization of green spaces (City of Edmonton, 2008; 2021a,b; Steckler, 2021). This policy is meant to promote biodiversity; however, it may simultaneously increase the abundance and diversity of habitat for competent natural host species (rodents and coyotes) and could provide opportunities for exposure and transmission to people recreating with their pets. Similarly, backyard conditions and unsupervised dogs, while not investigated in this study, may increase the risk of infection (Toews et al., 2021). In particular, backyard compost piles may be a potential attractant for intermediate hosts and have been implicated in hotspots of infection in urban coyotes in Edmonton and should be investigated for dogs (Murray et al., 2016). Further work should focus on rural dog populations where unsupervised access to locations harboring potential intermediate hosts may exceed urban populations that frequent off-leash parks.

The risk of infection may also relate to dog age and breed, as well as behavior of owners. Dogs that have not been infected before seem to have the severest infection and carry the highest worm burden, whereas individual dogs with repeated infections seem to get an acquired immunity, as has been noted in some fox populations (Kouguchi et al., 2016; Sweet et al., 2021; Torgerson, 2006). Indeed, our one positive sample came from a dog under the age of one, however, our inclusion criteria, requiring a dog over 3 months in age, may have underrepresented puppies and as a result the true overall parasite prevalence could be higher. Recent work seems to indicate that breed could have an effect on infection risk and may be related to prey drive; specifically higher rates of infection in breeds within the hound and terrier categories (Mehrkam and Wynne, 2014; Toews, 2020). However contrasting reports in the literature suggest it is less clear if purebred or mixed breed dogs are at greater risk for parasitic infection (Bridger and Whitney, 2009; da Ferreira et al., 2016; Toews, 2020). Our sample of off-leash dogs in Edmonton contained 57 individuals (7.4%) in the hound or terrier class, however the only positive case we identified was a herding class mixed breed individual.

Although we found only one case of *E. multilocularis* infection, there remains a potential risk to humans. Based on City of Edmonton records (Edmonton, 2021c; Government of Canada, 2017), there are approximately 60,000 dogs registered within the city, and an average household size of 2.6 people per dwelling. Assuming, for simplification, the mean and high limit of estimated prevalence (0.2 and 0.7%, respectively) and a single dog per house, our data suggests that approximately from 312 to 1092 individuals in summer 2020 might have been exposed to dogs shedding *E. multilocularis* eggs, with an increased risk of alveolar echinococcosis. Further work should expand testing beyond the summer season to ensure there is not a seasonal bias in infections as has been noted in other endoparasites and *E. multilocularis* in coyotes, where summer prevalence was lowest of all seasons (Liccioli et al., 2014; Drake and Carey, 2019).

While the low prevalence in our study precludes us from further elucidating patterns of infection, walking behaviors, levels of off-leash activity, and regularity of veterinary visits may be indicators of the risk of *E. multilocularis* infection. Veterinary care and, in particular, the regular treatment of dogs with anthelminthic drugs (eg. praziquantel) are important steps in the management of infection in domestic populations (Deplazes et al., 2011; Kotwa et al., 2019). Indeed, preventative actions, including regular tapeworm deworming of dogs, rodent proof compost bins, picking up after pets, and public education, are essential in limiting the infection potential of *E. multilocularis* in urban areas (Deplazes et al., 2011). As landscapes steadily urbanize, a "one health" approach may be increasingly important for managing risks to public health at the interface of wildlife, domestic animals and people (Webster et al., 2016).

Declaration of Competing Interest

The authors Emilie Porter, Alessandro Massolo and Darcy R. Visscher declare no potential conflict of interest with respect to the research, authorship and/or publication of this article. Authors M. Alexis Seguin, Marko Estrada and Donald Szlosek are employed at IDEXX Laboratories, Inc. This laboratory offers the Echinococcus RealPCR™ Panel on a commercial basis and performed the testing in this study. IDEXX played no role in the study design, in the collection and interpretation of data, or in the decision to submit the manuscript for publication.

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