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Echinococcus multilocularis Infections in Domestic Dogs

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by

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ABSTRACT

Echinococcus multilocularis, a parasitic helminth of the northern hemisphere, normally cycles through definitive (coyotes, foxes, wolves, raccoon dogs) and intermediate hosts (rodents and some lagomorphs). In urban areas, domestic dogs can also become hosts for this parasite, maintaining the *E. multilocularis* population in areas with less wild canids. Occasionally, this zoonotic parasite infects humans, resulting in alveolar echinococcosis, which is often lethal. *Echinococcus multilocularis* has been well-studied in wild definitive hosts throughout its known global distribution, but further investigation into the prevalence of this parasite in dogs is needed. I conducted a literature review and meta-analysis estimating the true prevalence and risk factors associated with *E. multilocularis* infections in dogs globally. Then, I attempted to fill gaps in the literature by investigating *E. multilocularis* in dogs living near several parks in Calgary, Alberta. Using qPCR on fecal samples collected in 2012, I determined the true prevalence of *E. multilocularis* in Calgary dogs. Associated risk factors were assessed using dog behaviour questionnaires submitted by owners. While intestinal *E. multilocularis* has not previously been found in dog population studies in Canada, I found the prevalence in Calgary in 2012 to be similar to Europe and Asia. Some risk factors for infection were confirmed but more work is warranted. Therefore, I also conducted a pilot study directed at Calgary vet clinics to provide an updated estimate of intestinal *E. multilocularis* presence and also, for the first time, alveolar echinococcosis prevalence in dogs. This study design and dog behaviour questionnaire are suitable for assessing risk factors for both types of infections and is intended to be applied to a larger future study. Understanding the role of dogs in both the maintenance of the *E. multilocularis* lifecycle and the transmission of the infection to humans is paramount as urbanization drives humans and their pets closer to wildlife populations.

THESIS STRUCTURE

This thesis is composed of three separate manuscripts (Chapters 2 to 4), with an introductory and a concluding chapter. The work in Chapter 2 and 3is the result of a multi-author collaboration.

Chapter 2 has been published in the International Journal for Parasitology. Chapter 3 is also intended for publication and will be submitted to Emerging Infectious Diseases this year. The candidate contribution in each chapter is described below.

Chapter 2 has been accepted for publication by the International Journal for Parasitology. I am the first author on this paper, followed by Marco Musiani, Sylvia Checkley, Darcy Visscher, and Alessandro Massolo. I performed the literature search and meta-analysis under direction from SC and AM. I also provided most statistical analyses with the help of AM, MM, and DV. The Bayesian estimation of true prevalence was performed by DV. The beta-regression model was provided by AM and Dr. Dimitri Giunchi.

Chapter 3 is also a multi-author collaboration that will be submitted to the journal of Emerging Infectious Diseases. I am the first author on this paper, followed by Anya Smith, MM, SC, DV, and AM. Specifically, all physical samples and questionnaires were initially collected by AS, who designed the original study in 2012. However, I performed all laboratory procedures involved in the *E. multilocularis* testing and genotyping under the supervision of AM and MM. I also performed all statistical analyses with guidance and collaboration with AM and DV.

Lastly, **Chapter 4** is another multi-author collaboration, authored by the same individuals as in Chapter 3, but it is not intended for publication. Sylvia Checkley recruited the veterinary clinics to our study and, along with AM and me, designed the study and sampling procedure. I adapted the dog behaviour questionnaire from AS's 2012 study, and she reviewed and approved the current

survey. I performed all lab work and statistical analysis under the direction and supervision of AM and MM.

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This Master's thesis has been a long journey that I have been proud to see through to completion. It has been a struggle and a great learning experience that I will always cherish. It will be incredibly difficult to move on from this project, although I know it has prepared me for a great research career which I am excited now to begin. In the completion of this endeavour, I have many people to thank for keeping me sane and focused.

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I wish to extend a special thanks to Anya Smith. Without her, I would not have had access to the vast amounts of samples and data from the 2012 dog population. Especially in the beginning of my degree, Anya has been invaluable in teaching me about survey design and analysis and I would like to acknowledge all the hard work she did in her PhD which made my own degree possible.

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much I value your wisdom. To my grandparents, Wylie and Emily, and the rest of my family, thank-you for your endless support. Lastly, a great thanks to the dogs, cats, and critters belonging to my family who were always quick to cuddle and play. My love for animals was the inspiration for this thesis, and it was all the love from these animals that helped me to complete it.

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LIST OF ABBREVIATIONS

AB	Alberta
ABVMA	Alberta Veterinary Medical Association
AE	Alveolar Echinococcosis
ANOVA	Analysis of Variance
AP	Apparent Prevalence
ATP	Re-assessed True Prevalence
BC	British Columbia
BM	Bowmont Park
CI	Confidence Interval
CKC	Canadian Kennel Club
CrI	Credible Interval
Ct	Cycle of Quantification
DH	Definitive Host
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
Epg	Eggs per Gram
FCPP	Fish Creek Provincial Park
GLMM	Generalized Linear Mixed Model
IAC	Internal Amplification Control
IH	Intermediate Host
IQR	Interquartile Range
IST	Intestinal Scraping Technique
LCA	Latent Class Analysis
NHP	Nosehill Park
PCR	Polymerase Chain Reactions
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
qPCR	Quantitative Polymerase Chain Reactions
RP	River Park
SCT	Sedimentation and Counting Technique

Se	Sensitivity
SEM	Standard Error of the Mean
SL	Southland Lowlands
Sp	Specificity
SPSS	Statistical Package for Social Sciences
TP	True Prevalence
WHO	World Health Organization
WSH	Weaselhead Park

EPIGRAPH

"There was no other wanderer on that road, yet I was not alone, for his tracks went with me, each pawprint as familiar as the print of my own hand. I followed them, and I knew each thing that he had done, each move that he had made, each thought that had been his; for so it is with two who live one life together."

Farley Mowat, The Dog Who Wouldn't Be

CHAPTER 1: GENERAL INTRODUCTION

ALVEOLAR ECHINOCOCCOSIS AS AN EMERGENT DISEASE

Alveolar echinococcosis (AE here after) is a primarily hepatic infection, caused by the larval stage of the zoonotic tapeworm, *Echinococcus multilocularis*, upon ingestion of infective eggs shed by definitive hosts (4, 5). Human AE is characterized by a long, asymptomatic incubation period (5-15 years) (6, 7) during which the infection can metastasize, spreading to other organs through the bloodstream (7). Lesions that form on the liver during this time can cause abdominal pain, jaundice, and liver failure as well as other symptoms (8), and, if left untreated, can be deadly (7).

Alveolar echinococcosis is an emerging disease that is endemic to most countries of the northern hemisphere (9, 10). In 2010, it was estimated that a median of 18,235 cases of human AE occur globally each year, with the majority occurring in China, and most others being scattered through Europe (4). *E. multilocularis* has been placed third on the World Health Organization's (WHO) list of the most important food-borne parasites worldwide in 2014 (11) and of highest importance in Europe in 2016 (12). Previously, few locally-occurring human AE cases have been reported in North America (13-16). More recently, a new case was documented in Alberta (AB), Canada in 2013 (17) and since then, incidence in AB has been increasing, now similar to some areas of Europe (18).

ECHINOCOCCUS MULTILOCULARIS BIOLOGY AND ECOLOGY

Echinococcus multilocularis, the aetiological agent of AE, employs a two-host lifecycle which relies on predator-prey trophic interactions (19) (*Figure 1*). Adult worms attach to the intestinal lining of definitive hosts (DH hereafter; mainly wild coyotes, foxes, wolves, and others, globally (20), but also domestic dogs (21)) via the protoscolex (*Figure 1*). During this intestinal (or enteric) infection, mature worms produce eggs which, once fecundated, are shed into the environment with

the feces of the DH (22), enduring harsh conditions (23) until accidental ingestion by intermediate hosts (IH hereafter; small mammals (20)) or by people, resulting in AE. Larvae (oncospheres) are released from the eggs during digestion, entering the blood stream through the intestinal lining to infect the liver, and subsequently other organs (4). The asexual proliferation and metastasis of these parasitic larvae (4) result in lesions on the target organs, which develop protoscoleces (*Figure 1*), becoming infective (22). After predation of the IH, the ingested protoscoleces attach to the intestinal wall of the IH, where they develop into adults (24) (*Figure 1*).

By preying upon or scavenging infected IHs, domestic dogs can contract intestinal *E. multilocularis* infection and contribute to the normal lifecycle as DHs (21, 25), especially in urban areas where dogs are much more prevalent than wild DHs (25, 26). Similarly, infected companion dogs can shed eggs which can be accidentally ingested by their owners, resulting in human AE (27). As aberrant hosts, dogs can contract AE themselves through ingestion of infected eggs present in the environment (28), or, potentially, in their own feces (28, 29). Dogs have also been recorded as being infected with both hepatic and intestinal infections at the same time (30).

URBAN DOMESTIC DOGS - RESERVOIRS AND SENTINELS FOR ZOONOTIC DISEASE

Dogs frequently become infected by zoonotic diseases by spillover from urban wildlife, especially in areas of high population density where wildlife is also present (31). Specifically, urban *E. multilocularis* is thought to have arisen from synanthropic coyote populations (32) but can be maintained by dogs (32) due to the high biotic potential of *E. multilocularis* infection in these domestic hosts (21). Even though domestic dog *E. multilocularis* prevalence may or may not be quite low (an important unknown addressed in this Thesis), this is compensated for by the extremely large pet dog population in urban and peri-urban areas (25, 26). For example, the dog population reported in census data for Calgary, AB, Canada in 2016 was above 135,000 - more than one dog per 10 residents. As well, any prevalence of *E. multilocularis* in companion dogs can be a

significant risk factor to humans due to their close proximity (27, 33). The habitat and habits of human owners and their dogs constantly overlap, with many dogs even sleeping in their owner's bed, increasing the potential risk of transmission of zoonoses from dog to human (34) by the transfer of infective eggs attached to dog fur to human living areas (35).

While the close relationship between pet dogs and their owners (36) increases risk for human AE, it also exemplifies how the health of the dog often mirrors the health of the owner (34, 37). Therefore, when dogs and other *E. multilocularis* hosts are sympatric, especially those intermediate hosts which could be preyed upon by dogs, a dog-owner may have a similar risk of infection as their dog (38). Dogs can thus act as effective sentinels of high environmental contamination of *E. multilocularis* (37, 39). Even though companion dogs represent an efficient and easily-sampled sentinel population (39) which would allow for effective surveillance of the entire range of a pathogen (37), they are currently underutilized as sentinels of zoonotic disease (40).

A VETERINARY PRACTITIONER'S PERSPECTIVE ON *ECHINOCOCCUS MULTILOCULARIS*

Veterinarians and other animal health professionals may be more likely than the general public to contract zoonoses such as *E. multilocularis* due to their occupation and the potential for infection in companion animals (41, 42). In 2006, 28% (105/371) of veterinarians in a Washington county reported that they had contracted zoonoses during their practice (42). Not only are veterinary and animal health practitioners at higher risk for infection, but they also provide the front line of defense against zoonotic diseases that can be transmitted by companion animals (42). When veterinarians are involved in screening for zoonoses, the benefits are three-fold: (1) sampling can be much more efficient, where *E. multilocularis* screening could be performed with other routine tests or on pre-existing samples (40); (2) increased awareness in veterinary clinics could lead to better prevention or increased vaccinating and de-worming procedures which could limit the number of potential DHs in urban areas; and (3) the health of veterinary practitioners is

emphasized, providing greater protections for a sub-population of people that may be at greater risk for infection by zoonoses (41, 42). These outcomes are the reason we chose to test client-owned dogs rather than the general population of domestic dogs in the pilot study outlined in Chapter 4. Overall, animal and human health are inextricably linked, so a One Health approach involving both veterinary and human health professionals and practitioners should be adapted to adequately deal with zoonoses such as *E. multilocularis* (40, 42).

OVERALL AIM

The overall aim of this thesis was to investigate infections of *Echinococcus multilocularis* in domestic dogs to infer their potential role in zoonotic transmission.

SPECIFIC RESEARCH QUESTIONS

Specifically, this thesis aimed to: (1) review the data available globally on both alveolar and hepatic *E. multilocularis* infections in domestic dogs; (2) assess the prevalence of hepatic *E. multilocularis* and risk factors for infection in domestic dogs in a metropolitan area in North America; and finally (3) develop a protocol to investigate both intestinal and hepatic *E. multilocularis* infections in client owned dogs.

OVERVIEW OF CHAPTERS

This thesis is comprised of five chapters. Chapter 1 is an introductory chapter. Chapters 2 and 3 are research papers. Chapter 4 is a pilot study which is intended to be repeated at a larger scale in the future. The fifth chapter is a general conclusion on the data presented in the prior chapters.

In **Chapter 2**, I conducted a literature review and meta-analysis on the global prevalence of *E. multilocularis* in domestic dogs and factors influencing the risk of infection in these dogs. Peer-reviewed research articles were collected by searching specified terms in several databases and

were then analyzed for information on study design, sample size, diagnostic tests used, and prevalence and risk factor results. Using these data, I estimated the true prevalence of *E. multilocularis* for each study using Bayesian techniques to account for imperfect testing. These results were used to graphically demonstrate the distribution of *E. multilocularis* infections in dogs across the world. I also investigated risk factors identified by each study by pooling odds ratios for the four most common intrinsic and extrinsic factors that significantly influenced likelihood of infection in dogs.

Chapter 3 is an investigation into the prevalence of intestinal *E. multilocularis* in dogs living near dog parks in Calgary, AB and the risk factors influencing these infections. Fecal samples collected from dogs in 2012 were tested for the presence of *E. multilocularis* DNA via qPCR. From these, I estimated the true prevalence of *E. multilocularis* in Calgary dogs. Using a dog behaviour questionnaire that was administered to the owners of these dogs, I also assessed potential risk factors for infection including both intrinsic (*e.g.* breed, age, gender) and extrinsic (*e.g.* time spent walking in dog parks and other areas, time spent off-leash in these areas, time spent in the backyard) characteristics.

In **Chapter 4**, I conducted a pilot study to assess the feasibility of a study estimating true prevalence and risk factors for both intestinal and hepatic *E. multilocularis* infections in dogs in urban areas of AB (Calgary and Edmonton). This study was performed on a small scale, involving four veterinary clinics in Calgary, and approximately ten dogs sampled per clinic, from which we took blood and fecal samples and dog behaviour questionnaires from owners. In this study, we outlined two different sampling designs for use in clinics which will aid in the administration of a future study to fully analyze the presence of dog infections by *E. multilocularis* in AB.

Lastly, **Chapter 5** briefly summarized the results of the above three chapters, synthesizing overall conclusions on the importance of studying *E. multilocularis* infections in domestic dogs. I

also discussed several limitations of my study and outline future directions for *E. multilocularis* research in dogs.

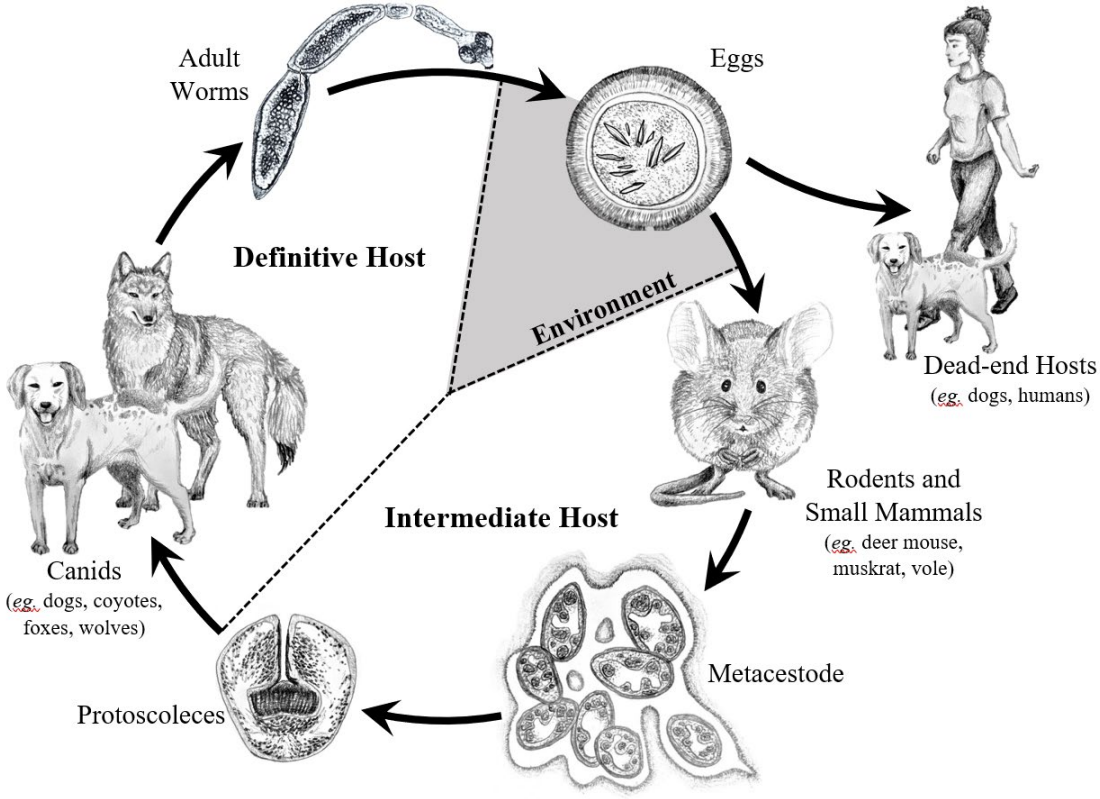


Figure 1: Lifecycle of *Echinococcus multilocularis*

CHAPTER 2: A GLOBAL ASSESSMENT OF *ECHINOCOCCUS MULTILOCULARIS* INFECTIONS IN DOMESTIC DOGS: PROPOSING A FRAMEWORK TO OVERCOME PAST METHODOLOGICAL HETEROGENEITY

ABSTRACT

Echinococcus multilocularis, the aetiological agent of human Alveolar Echinococcosis (AE), is transmitted between small mammals and wild or domestic canids. Dogs infected with *E. multilocularis* can transmit this infection to humans and can themselves be infected with canine AE as dead-end hosts. Whereas *E. multilocularis* infections in wild hosts and humans have been well-studied in recent decades, infections in domestic dogs are sparsely reported. This literature review and meta-analysis highlighted gaps in the available data and provided a re-assessment of the global distribution of domestic dog *E. multilocularis* infections. We found 46 published articles documenting the prevalence of *E. multilocularis* in domestic dogs from 21 countries across Europe, Asia and North America. Apparent prevalence estimates ranged from 0.00% (0.00-0.33%) in Germany to 55.50% (26.67-81.12%) in China. Most studies were conducted in areas of high human AE. By accounting for reassessed diagnostic sensitivity and specificity, we estimated true prevalence in a subset of studies, which varied between 0.00% (0.00-12.42%) and 41.09% (21.12-65.81%), as these true prevalence estimates were seldom reported in the articles themselves. Articles also showed a heavy emphasis on rural dogs, dismissing urban ones, which is concerning due to the role urbanization plays in the transmission of zoonotic diseases, especially those utilizing pets as definitive hosts. Lastly, population studies on canine AE were absent, highlighting the relative focus on human rather than animal health. We thus developed a framework for

investigating domestic dog *E. multilocularis* infections and performing risk assessment of dog-associated transmission to fill the gaps found in the literature.

INTRODUCTION

Alveolar Echinococcosis (AE) is a hepatic infection by *Echinococcus multilocularis*, a parasitic taeniid helminth. It causes cyst-like lesions in organs of intermediate (small mammals) and dead-end (dogs and humans) (7) hosts. Whereas most human AE cases (~18,000 per year) occur in China (4) due to the Asian strain (43), *E. multilocularis* (including the Asian, European and North American strains) is present in most of the cold and temperate regions of the northern hemisphere (9, 24). As this parasite was ranked by the World Health Organization as the third most important food-borne zoonotic parasite worldwide (44), and of highest importance in Europe (12), it is concerning that an outbreak of AE, likely caused by invasion of a European-like strain now endemic in North American wildlife, has recently been documented in North America (Alberta, Canada)(18).

To complete its lifecycle, *E. multilocularis* requires a complex two-host predator-prey system. Definitive hosts (DH hereafter; mostly wild canids as foxes, coyotes, wolves, and raccoon dogs, but also domestic dogs) (20, 21, 24, 45, 46) present intestinal *E. multilocularis* infection (also known as enteric infection) with adults worms producing eggs that, once fecundated, are shed with feces into the environment. These embryonated eggs can endure harsh conditions (22) until accidental ingestion by intermediate hosts (IH hereafter; small mammals) (8, 20, 24, 47) or, occasionally, by people. In the IH stomach, larvae (oncospheres) are released from eggs and enter the blood stream through the intestinal lining, infecting target organs (mostly the liver) (4, 22). Here, metacestode larvae mature and proliferate asexually (4) causing tumor-like lesions and developing protoscoleces, thereby becoming infective (22). When protoscoleces are ingested by DHs following predation upon infectious IHs, they attach to the DH intestinal wall and develop into adults (24).

Domestic dogs can host two stages of the *E. multilocularis* lifecycle – adult worms, when acting as DHs preying upon infected small mammals, and larval stages, when acting occasionally as dead-end IHs, where they do not contribute to the cycle, (24) developing liver lesions (*i.e.,* canine AE) often with fatal consequences (48, 49). It is still unknown whether canine AE occurs when dogs consume eggs present in the environment (28, 49) or by self-infection following intestinal *E. multilocularis* (49), or both.

Urbanization is an emergent phenomenon known to impact wildlife movements (50), behaviors (51), density (52), and distribution (53). Wild and domestic canids are regularly found living among people in such urban and suburban areas (54, 55). The spatial overlap between domestic dogs and wild hosts in these areas (56, 57) allows the *E. multilocularis* sylvatic lifecycle, once established by wild hosts, to be maintained by domestic dogs due to their high population density compared to wild DHs (58). In a similar manner, in rural environments, free-roaming domestic dogs in rural environments may become the primary DH for *E. multilocularis* (59, 60).

As DHs, dogs can contribute to *E. multilocularis* transmission to humans directly (*e.g.,* petting or handling) (35, 55), or indirectly (through fecal contamination of households) (56, 57). Although mainly listed as a food-borne disease, there is little evidence to support that food is the primary route of infection with *E. multilocularis*. Rather, it is likely that consumption of contaminated food (*e.g*., berries, vegetables) and accidental ingestion of *E. multilocularis* eggs possibly mediated by dogs both play important roles in *E. multilocularis* transmission to humans (44), with dog ownership possibly being a greater risk factor for human AE than consumption of unwashed, contaminated food (27, 61).

Several common methods have been used to diagnose *E. multilocularis* infections in various DHs, including domestic dogs (*Table 1*). For hepatic infections (canine AE), two main approaches are used: enzyme-linked immunosorbent assays (ELISA) to detect antibodies in blood (28, 29, 62), and polymerase chain reactions (PCR) to detect parasite DNA in biopsied lesions (5, 63). Conversely,

serological screening cannot be used for intestinal *E. multilocularis* as the presence of adult worms in the intestine and antibodies in the blood are not necessarily correlated (64). However, some ELISAs (65-67) have been developed to detect coproantigens in fecal samples of DHs, which are detectable only during the pre-patent and patent periods of the parasite and disappear just after the parasite has been eliminated from the host (68, 69). Often, genus-specific copro-ELISAs are used to detect *Echinococcus* species antibodies; in these cases, species characterization is confirmed through PCR. Development of various PCR assays has also aided the detection of *E. multilocularis* in the feces of live DHs and can be performed directly on fecal samples (70-72) or on concentrated egg solutions obtained through zinc chloride sedimentation analysis (73, 74). However, the sensitivity of copro-PCR depends largely on the worm burden of the infected host (73). In addition, arcoline purgation can be used to obtain purged worms from the DH small intestine (59) which can then be identified morphologically through microscopy or by using PCR. Lastly, a sieve and counting technique (SCT) on worms in the small intestine of necropsied animals (75) has traditionally been used to microscopically identify *E. multilocularis* in carcasses of definitive hosts including stray domestic dogs.

Despite the wide distribution of *E. multilocularis* (9, 24) and the potential role of dogs in its maintenance and transmission to people, a global systematic review of the prevalence of *E. multilocularis* infections in domestic dogs is still missing. Thus, we aimed to review the existing literature on both prevalence and risk factors for intestinal *E. multilocularis* and AE in domestic dogs worldwide, as well as the methodological approaches (sampling design and diagnostic techniques) used in these studies. Also, we aimed to obtain true prevalence estimates via a meta-analysis of available *E. multilocularis* prevalence data. Finally, we provided a framework for future epidemiological studies of intestinal and alveolar echinococcosis in domestic dogs to gain more comparable data on *E. multilocularis* infections in dogs, potentially high-risk carriers of this severe zoonosis.

MATERIALS AND METHODS

LITERATURE REVIEW

The literature search followed PRISMA guidelines for reporting systematic reviews and meta-analyses (3) and focused only on peer-reviewed papers, excluding grey-literature (*e.g.,* unpublished student theses, government reports). Scopus was selected as a database due to its ability to search through non-English journals, including those that do not use the Latin alphabet. In this way, non-English articles were included in our literature search. Combinations of keywords were searched in Web of Science, Scopus, PubMed, and Science Direct. Differing combinations of keywords and Boolean operators were tailored to each database (*Appendix A1*).

Two rounds of screening determined article eligibility. First, titles and/or abstracts were screened for relevance; specifically, they had to mention both "*Echinococcus* species" and "domestic dogs". Second, the full text for each article was screened for the presence of an *E. multilocularis* prevalence estimate in domestic dogs in order to determine eligible articles (*Appendix A2*). Studies reporting *Echinococcus* species prevalence in domestic dogs in areas where *E. multilocularis* is endemic were removed if they used only genus-specific diagnostic tests that could not confirm the presence of the *E. multilocularis* species. Case-studies, clinical papers, diagnostic test evaluations, and other literature not conducting a population study were also removed. Systematic and critical review articles were also removed if they did not report any new prevalence data, but their reference lists were screened for other literature that fit the criteria. For non-English articles, data were gleaned from abstracts and tables while methods and results were translated via Google translate.

EVALUATION OF STUDY DESIGNS

Included studies were characterized in terms of time, location, study methods, and results. Studies that included multiple countries were sorted into methods and results by country. Study methods were further characterized in terms of sampling design (*e.g*., statistical units, selection procedure), diagnostic techniques and parameters, sample size, target population (*e.g.*, owned, stray, urban, rural dogs), accounting for and quantification of risk factors for dog E. multilocularis infection (*e.g.*, demographics, dog walking habits), and if the parasite strain was assessed through genotyping. When diagnostic technique sensitivity and specificity were not directly reported by the authors, we retrieved them from the primary literature cited in the article. Finally, for each study we recorded the apparent and true prevalence estimates of *E. multilocularis*, if reported.

META-ANALYSIS

For each study we calculated the naïve apparent prevalence, the true prevalence, and an updated true prevalence based on a recent re-assessment of the sensitivity and specificity of common diagnostic test. We report the apparent prevalence, with the exact binomial confidence intervals, under the assumption of perfect sensitivity and specificity. To account for the drawbacks in the Rogan-Gladen estimator for prevalence we used a Bayesian approach (76, 77) implemented using the *R* package '*prevalence'* (version 0.4.0) to estimate true prevalence and its credible intervals. We first calculated the true prevalence of *E. multilocularis* using the sensitivity and specificity of diagnostic tests reported in each paper, if no sensitivity or specificity was given, we modelled true prevalence using a uniform distribution representing the range of sensitivities and specificities, respectively, reported across all studies (*Appendix A3*).

Subsequently, we calculated an updated true prevalence based on two recent re-assessments of common *Echinococcus* species diagnostic techniques (*Table 1*) (1, 2). This provided new sensitivity and specificity estimates for several *E. multilocularis* tests and enabled true prevalence calculation for studies in which these parameters were not reported (*Table 1*). The range of sensitivities and

specificities calculated in this re-assessment were used to bound their respective uniform distribution when we modelled re-assessed true prevalence. In all cases the prevalence model was implemented using two chains containing 10000 "burn-in" samples and 10000 samples that were retained, a multivariate Brooks-Gelman-Rubin statistic was inspected to ensure model convergence. For true and re-assessed prevalence estimates we report the 2.5% and 97.5% credible intervals; for studies that had 0 positive cases we report the 0% and 95% credible intervals. The modelled estimates for sensitivity and specificity, along with their credibility intervals are given in *Appendix A3*.

Re-assessed true prevalence and 95% confidence intervals for each country were then weighted by sample size, bootstrapped and mapped using *R* (78).

A risk factor meta-analysis was conducted using odds-ratios of known extrinsic risk factors for *E. multilocularis* infection in dogs (59) including: being used for hunting, living in a rural area, roaming untethered, and predating on rodents. Individual and pooled weighted odds-ratios were calculated using *MedCalc Statistical Software* (version 18.11.6) .

Chi-squared analysis comparing population characteristics (*i.e.,* proportions of owned, stray, rural, and urban dogs) across studies was performed in *R*. A direct comparison of prevalence estimates of intestinal *E. multilocularis* across continents could not be performed because too few studies were available for Europe and North America. To compare apparent and re-assessed true prevalence we formulated a model using a zero-inflated generalized linear mixed model with a beta distribution and a logit-link, with the type of prevalence (apparent or true) as a fixed effect, and the 'study' as random effect in both conditional and zero-inflated components (79). The model was formulated using the R package 'glmmTMB' version 1.0.2.1, and assumptions verified using '*DHARMa*', both run on the R Software version 4.0.2 (2020-06-22).

An ANOVA with Tukey's *post hoc* test was run to compare Log10 transformed data of study sample size across different ownership groups (owned, stray, mixed); both tests were performed in

in *SPSS v.25* (IBM®, Armonk, NY, US). Lastly, after checking for monotonicity, linear association between human AE incidence and the number of dog *E. multilocularis* studies performed in each country was tested using Spearman's *rho* in *SPSS.* Prevalence data are reported with their 95% confidence intervals, whereas other proportions and means are reported with their standard errors (SEM), unless otherwise stated.

RESULTS

LITERATURE SEARCH

The keyword combination "(alveolar OR multilocularis) AND echinococc* AND dog AND (prevalence OR population)" yielded the largest number of relevant hits when searched in the electronic databases (*Table 2, Appendix A1*) on July 21, 2020. From the resulting 695 articles (after removal of duplicates), 527 were excluded upon screening of the title and abstract. Subsequently, through the full-text screening, 122 more articles were removed. Of the 122 articles removed from the pool of eligible articles, exclusion most commonly occurred due to absence of original data (*e.g.,* critical and systematic reviews), the analysis of other helminth or *Echinococcus* species instead of *E. multilocularis*, and the absence of prevalence determination (*e.g.,* case-studies and experimental infection studies) (*Appendix A2*). Thus, 46 articles on enteric *E. multilocularis* were included in the review (*Figure 2*).

CANINE ALVEOLAR ECHINOCOCCOSIS

No articles provided an estimate of canine AE prevalence. However, four articles (3 from Canada, 2 from Switzerland, and 1 each from Belgium, the United States, and Germany) presented case studies on individual domestic dogs infected with AE. The breed of dogs infected with AE was not consistent across cases. The Canadian dogs were two boxers and a mixed-breed shih-tzu/bichon

fries, the Swiss dogs were a dachshund and Labrador retriever, the Belgian dog was also a dachshund, the German dog was a spaniel, and the American dog was a Labrador retriever.

INTESTINAL ECHINOCOCCUS MULTILOCULARIS INFECTION

Analysis of Study Design

Forty-six articles from the search ("articles" here-after) provided prevalence data for intestinal *E. multilocularis* in dogs. Many of these publications reported data on multiple dog populations; as a result, the 46 articles delivered 59 estimates of prevalence ("studies" here-after).

The 46 articles were published between 1960 and 2020 (*Appendix A4*); surveillance of *E. multilocularis* prevalence persisted across all seasons (and multiple years) in 22 of them (37.29%) (*Table 3*). Twenty-four studies (40.68%) spanned different combinations of seasons, which most commonly began in spring (*Table 3*). Thirteen studies (22.03%) did not specify the season in which sampling took place.

Studies were performed in 21 countries across Europe (28/59; 47.46%), Asia (28/59; 47.46%), and North America (3/59; 5.08%) (*Appendix A5*), and we detected a statistically significant relationship between the number of studies performed in each country and the global trend of AE incidence in humans (4) (Spearman's rho: r_s=0.605, df=37, p<0.01). Sample sizes in the studies ranged from 9 to 17,894 (650.97±308.94; median=156, IQR=392) (*Table 4*).

Article objectives focused on estimating the prevalence of intestinal *E. multilocularis* in targeted dog populations, more often in areas of high human AE incidence (21/59; 35.56%) and wildlife *E. multilocularis* prevalence (14/59; 23.73%) than in areas where *E. multilocularis* had not previously been studied (5/59; 8.47%). More specific objectives and target populations were occasionally identified (4/59; 6.78%) (*Table 3*), and objectives were not defined at all in 15 studies (25.42%).

Sampling methods were sporadically reported in the reviewed literature. Most studies (22/59; 37.73%) recruited dogs through local veterinary clinics, but sampling designs were generally not

described (35/59; 59.32%) (*Table 3*). Convenience sampling was used more often (14/59; 23.73%) than other methods including stratified (5/59; 8.48%), cluster (2/59; 3.39%), random (2/59; 3.39%), and systematic sampling (1/59; 1.69%) (*Table 3*, *Figure 3*).

Diagnostic techniques for intestinal *E. multilocularis* were also inconsistent across the articles. Nested PCR directly on fecal samples (70) was the most used diagnostic technique (15/59; 25.42%), followed by zinc chloride flotation/sedimentation analysis (73), and then PCR (74) (12/59; 20.34%). Other techniques included various copro-ELISA tests (6/59; 10.17%) (66, 67, 69, 80), qPCR (5/59; 8.47%) (81-83), arcoline purgation (5/59; 8.47%) (59), a post-mortem sedimentation and counting technique (SCT) (4/59; 6.78%) (75, 84), a magnetic bead capture PCR (1/59; 1.69%) (71), and two unknown diagnostic techniques following necropsy (3.39%).

Prevalence meta-analysis

The apparent intestinal *E. multilocularis* prevalence in domestic dogs ranged from 0% (0.0-0.3%) to 55.5% (21.2-86.3%) although it has not been investigated in all countries known to be endemic for the parasite (4) (*Figure 3*, *Figure 4*). Most studies (53/59; 89.83%) (*Table 4*) did not consider the diagnostic test performance in prevalence calculations, so these estimates (apparent prevalence) were potentially biased. Re-assessed true prevalence values ranged from 0% (0.0-0.0) to 56.1% (5.8-97.3) and were higher in Asian countries (4.76%; 95% CI: 2.33-7.28%) than European (0.19%; 95% CI: 0.05-0.51%) and North American (0.80%; 95% CI: 0.20-2.63%) countries, although North American countries were poorly represented.

Upon applying the reassessed sensitivity and specificity to the prevalence estimation (1, 2), feasible for almost all studies (58/59; 98.3%), the true prevalence was higher than the apparent prevalence in 46/58 (79.3%) studies, lower in 11/58 (19.0%) studies, and the same in 1/58 (1.7%) (*Table 4, Figure 3a*). In studies where re-assessed true prevalence was higher than apparent prevalence, the difference was, on average, less (55.2±5.7%) than when the apparent prevalence estimate was higher (105.8±44.1%). Overall, apparent and re-assessed true prevalence (ATP)

estimates significantly differed from each other across the studies (zero-inflated model component: (intercept) = -0.72, $ß_{(ATP)}$=-3.33, $ß_{(ATP)}$SE=1.05, $p_{(ATP\_Coeff)}$=0.0015; model X^2= 23.18, df=1, $p_{(model)}$< 0.0001); conditional model component: (intercept) = -3.76, $ß_{(ATP)}$=0.19, $ß_{(ATP)}$SE=0.063, $p_{(ATP\_coeff)}$=0.0024; model X^2= 8.003, df=1, $p_{(model)}$= 0.0047). The re-assessed true prevalence could not be estimated in one study (60) as not enough data were reported in literature (*Table 4*).

Risk factor analysis

Risk factors like dog ownership, locality, predation habits, and time spent roaming freely, were not addressed in an equal manner in the literature and questionnaires addressing these risk factors were distributed to owners in almost half of owned and mixed-ownership studies (20/44; 45.45%).

More studies focused exclusively on owned dogs (32/59, 54.24%) than stray dogs (10/59; 16.95%) or mixed ownership (12/59; 20.34%) (χ^2=16.44, *df*=2, p<0.001) and five studies did not determine dog ownership (8.47%). Studies on owned dog studies also tended to have a larger average sample size (1,018.34±563.74) than those on stray dogs (166.90±103.44), but not than those on mixed (260.92±62.22) (ANOVA, $F_{2,51}$=6.207, p=0.004; Tukey test, Stray vs Owned, Mean Diff=0.68, p=0.003). Similarly, more studies focused exclusively on rural dogs (31/59, 52.54%) than urban dogs (5/59, 8.47%) (χ^2=18.78, *df*=1, p<0.001). Eighteen studies (30.51%) did not distinguish between rural and urban dogs, and five (8.47%) did not specify this information.

Odds ratios revealed that hunting dogs were significantly more likely to become infected (Pooled odds ratio: pooled OR=4.02, 95% CI=2.31-7.02, z=4.91, p<0.001) (*Figure 5(a)*). Dogs that were untethered in their owner's yard were also more likely to become infected with intestinal *E. multilocularis* (pooled OR=12.37, 95% CI=5.35-28.61, z=5.88, p<0.001) (*Figure 5(b)*). Lastly, dogs from rural areas were more likely to be infected than dogs from urban areas (pooled OR=2.48, 95% CI=1.16-5.28, z=2.35, p=0.019) (*Figure 5(c)*). Unexpectedly, the analysis of pooled odds ratios did not support the hypothesis that dogs that prey upon rodents (pooled OR=4.61, 95% CI=0.89-23.73, z=1.83, p=0.068) were significantly more likely to be infected with intestinal *E. multilocularis*

(*Figure 5(d)*), although, given the *p* value close to the 0.05 threshold, we cannot exclude that this could be due to a low sample size (n=2).

DISCUSSION AND RECOMMENDATIONS

This critical review and meta-analysis highlighted four major inadequacies in literature regarding *E. multilocularis* infections in domestic dog populations. First, even though human AE is an emerging global issue, studies on domestic dogs are often only conducted in areas that already have high human AE prevalence and therefore do not reflect the actual geographic distribution of the parasite in dogs. Second, the reported prevalence of intestinal *E. multilocularis* in domestic dogs was underestimated, as most studies did not consider diagnostic sensitivity and specificity. Third, few studies addressed risk factors for intestinal *E. multilocularis* infections in dogs, limiting the possibility of risk and exposure assessment for dog owners. Lastly, no attempt of estimating the prevalence of canine AE has ever been reported.

Domestic dog intestinal *E. multilocularis* studies have been conducted in only a few countries within the known distribution of the parasite, not adequately quantifying the total spread of *E. multilocularis* in dogs (4) (*Figure 3*). The positive association between human AE incidence per country and the number of domestic dog intestinal *E. multilocularis* studies conducted in those same countries indicates two important concerns. First, the current approach to research appears reactive and misses the opportunity to prevent transmission from dogs to humans in areas of low human AE incidence and high dog intestinal *E. multilocularis* prevalence. Second, there seems to be little concern over *E. multilocularis* in dogs despite the potential development of AE in dogs too. Thus, other known endemic areas should be considered for population studies to determine the actual distribution of dog intestinal *E. multilocularis* infections (59) and also the role of dogs in perpetuating the lifecycle of *E. multilocularis* and potentially affecting humans (85).

Further spatial discrepancies exist in the representation of dogs from rural and urban areas. Literature on rural dog intestinal *E. multilocularis* is more prevalent due to higher human AE incidence in rural communities (4, 35, 59, 84). This lack of urban dog studies is concerning due to the increased density of infective feces in urban green spaces compared to rural areas (83) even though rural dogs may have a higher intestinal *E. multilocularis* prevalence (31) (*Figure 6(c)*). Therefore, *E. multilocularis* transmission from animals to humans in areas of higher population densities should be explored further, especially in urban green-spaces which are frequently visited by both humans and dogs.

Both the temporal spread of the literature and the seasons during which sampling occurred were sporadic and inconsistent. To accurately address the prevalence of *E. multilocularis* in an area, surveillance should occur over several years and cover all seasons (4). However, we found few studies that fulfill this attribute. The heavy occurrence of sampling during spring coincides with higher rates of predation on small mammals such as rodents and lagomorphs (47, 86), which are typical IHs for *E. multilocularis*. Even so, it is desirable for studies to be performed with several years of sampling and an equal focus across all seasons to report an accurate true prevalence estimate, accounting for seasonal fluctuations in rates of zoonotic disease transmission (86-89).

The primary methodological issue detected by this review was the lack of true prevalence estimates – which normally account for inadequate diagnostic techniques (90). By basing true prevalence calculations on diagnostic parameters, biases inherent of apparent prevalence estimates are reduced (91). However, uncertainty in true prevalence estimates increases when sample size, number of positives, or diagnostic parameters are too small (90). To account for this, we used a Bayesian approach to estimate true prevalence, which provided a more flexible method to account for uncertainties in sensitivity and specificity measurements and also the absence of these measurements in the calculation of the prevalence value (76, 77). Using this method, we were able to obtain re-assessed true prevalence estimates for almost all studies.

When re-assessed true prevalence could be estimated for a study, it was up to 489.47% different (64.8±9.6% on average) from apparent prevalence (Figure 4). This was likely due to both to the low sensitivity of the diagnostic tests that were used and also the uncertainty that accompanies estimating diseases of low prevalence in the population (77, 90). Differences between re-assessed true prevalence and apparent prevalence were greatest when *E. multilocularis* was less present in the population (under 2%). Overall, the traditional practice of reporting only apparent prevalence estimates in *E. multilocularis* studies has considerably underestimated the actual presence of the parasite. It is therefore necessary for researcher not only to focus on estimating true prevalence in *E. multilocularis* population studies, but also to use the best possible strategies for diagnosing these infections (eg. copro-PCR on fecal samples from live dogs, IST on necropsied dogs) especially in domestic dogs where worm burden may be lower than in other DHs (21), further lowering the sensitivity of most common diagnostic techniques.

The sampling designs used to recruit individuals to each study may also be a possible source of bias. Most commonly, these studies targeted dogs in areas of high human AE or wildlife *E. multilocularis* prevalence using convenience (opportunistic) sampling. To recruit individual dogs, veterinarians selected dogs based on owner volunteer, resulting in a bias due to potentially excluding dogs outside this clinic-attending group from the selection process. Owned dogs selected from a veterinarian's client list could be dewormed more often, carrying less parasites (*i.e.,* less likely to be infected with *E. multilocularis*) than those who are not associated with a veterinarian. However, veterinary involvement presents a convenient way to enroll domestic dogs into research (42) and therefore accounts for many of the studies in this review.

Potential risk factors for intestinal *E. multilocularis* in domestic dogs were infrequently considered with less than half of studies surveying the effect of intrinsic and extrinsic characteristics of dogs on likelihood of infection. Even fewer odds ratios on these risk factors were estimated.

The pooled odds ratios in this meta-analysis indicate that dogs have a higher probability of intestinal *E. multilocularis* infection if they roam untethered (*Figure 5(a)*) and if they live in rural areas (*Figure 5(c)*). Off-leash frequency has previously been indicated as a significant risk factor for gastrointestinal parasitic infection in park-attending dogs in Canada (92). Only three studies in this review (93-95) investigated the relationship between tethering and intestinal *E. multilocularis* infection and there is therefore a need to address this further.

Hunting dogs also had higher risk of intestinal *E. multilocularis* infection (*Figure 5(b)*). However, the hunted game was not specified. It is possible that hunting dogs have higher probability of infection due to their natural predatory role in the *E. multilocularis* lifecycle. Unexpectedly, dogs which preyed upon rodents were found not to be at increased risk for intestinal *E. multilocularis* infection, which conflicts with both the increased risk due to hunting and the role of dogs in the *E. multilocularis* cycle. These two risk factors should be further explored to determine their relationship to domestic dog intestinal *E. multilocularis* infection.

Perhaps the most obvious gap in this collection of literature is the absence of canine AE studies. As dual participants in the lifecycle of *E. multilocularis*, dogs can act both as definitive hosts contracting intestinal *E. multilocularis*, and dead-end intermediate hosts, developing liver infection and AE (24, 49). At the time of this literature review, no published studies on AE prevalence in domestic dogs existed, although several case studies have been reported. Potentially, these cases were underreported due to misdiagnosis stemming from a lack of awareness of dog AE, and a comparatively increased emphasis on human AE (96).

In newly endemic areas, human AE cases may not be present due to lower levels of *E. multilocularis* in the environment (97), or may not be detected because AE cases go unnoticed as the disease is not on the differential diagnosis list (98). However, our meta-analysis confirmed that the predominant focus on canine intestinal *E. multilocularis* in areas of high human AE has prevented determination of the actual distribution of *E. multilocularis* in domestic dogs (96). Given

the ability of dogs to both act as sentinels and contribute to the environmental contamination by *E. multilocularis* (34, 99, 100), surveillance of human AE must begin to focus on domestic dogs as potential indicators of high environmental contamination due to their convenient and efficient prospects as sentinels for zoonotic disease (37, 39).

The impact of urbanization on *E. multilocularis* infections in dogs must also be analyzed, as few population studies were conducted on dogs in urban areas, despite the known effect of urbanization on the transmission of *E. multilocularis* (55, 58). While dogs tend to have a lower worm burden than wild DHs, they shed, on average, greater number of eggs per adult worm through their feces than wild DHs and have thus been proven to be capable of perpetuating the spread of *E. multilocularis* (21, 59, 101). Similarly, pet dogs acting as DHs may provide opportunity for the sylvatic *E. multilocularis* cycle to spill over to domestic hosts (31). Therefore, increased *E. multilocularis* surveillance in domestic dogs - for both intestinal *E. multilocularis* and canine AE - is key for the management of human AE.

RECOMMENDED FRAMEWORK

Finally, to make future globally collected data comparable, we recommend a methodological framework and subsequent workflow (*Figure 6*) based on key recommendations: (1) clearly define the study objectives (surveillance, prevalence/risk assessment, trend); (2) identify the study population based on the objectives, geographical area, type of dogs, etc.; (3) carry out surveys in different seasons and years, if feasible, to account for seasonal and year to year fluctuations; (4) provide an assessment of diagnostic sensitivity and specificity and integrate these parameters into prevalence estimates, and (5) develop targeted questionnaires to collect ancillary data allowing for subsequent assessment of risk factors to be tested at broader scales. It would be desirable to apply such a framework also to areas where human AE is not reported yet, but *E. multilocularis* presence has been. Furthermore, samples should undergo genetic characterization of the strain of the

parasites which may provide important insights on emergence of newly endemic strains, infection sources, and potential risk for humans (18).

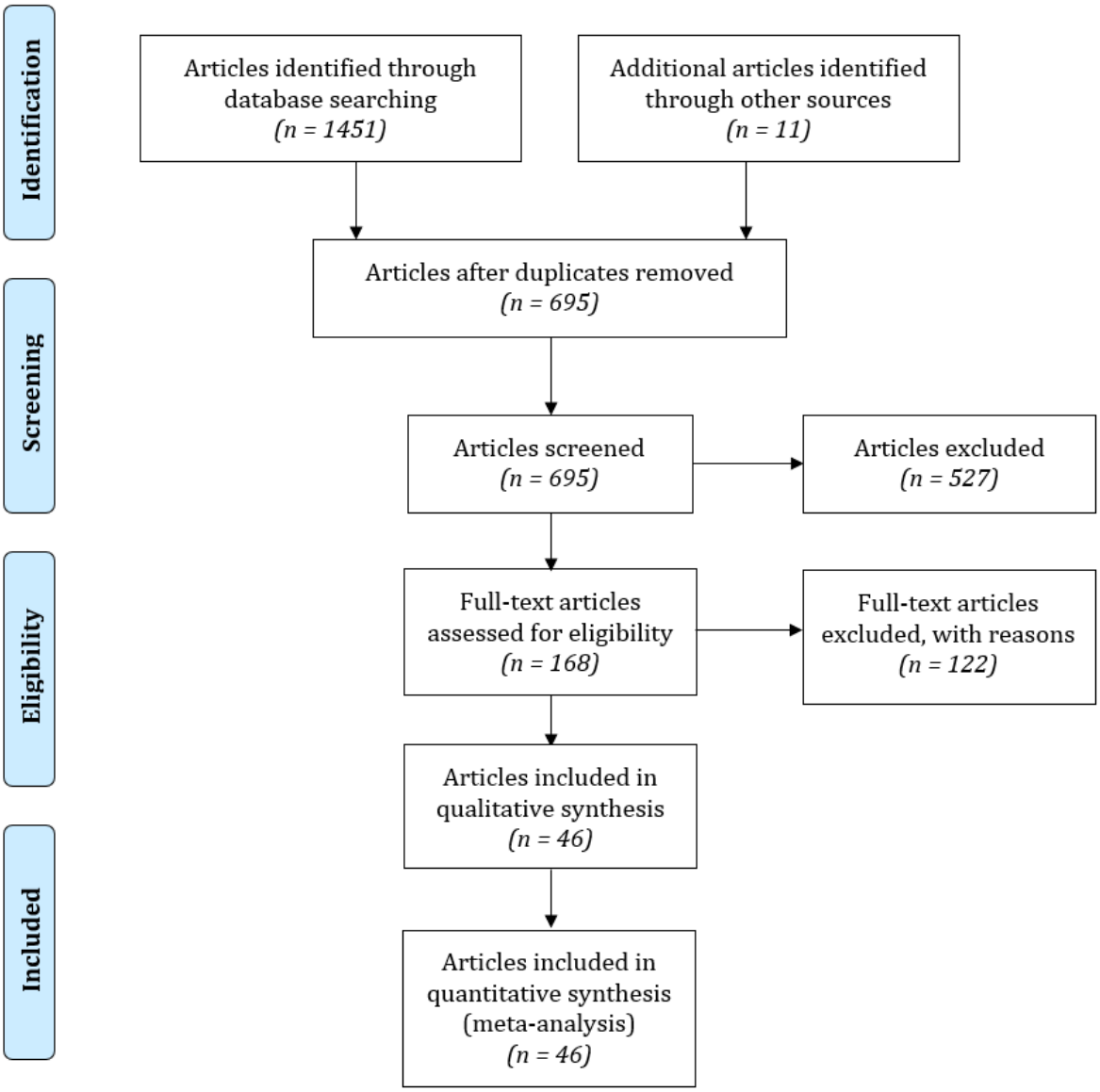


Figure 2: Process flowchart describing the outcome of the literature search and review of papers on *Echinococcus multilocularis* in dogs (completed on July 21, 2020) outlined using the PRISMA protocol (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (3)

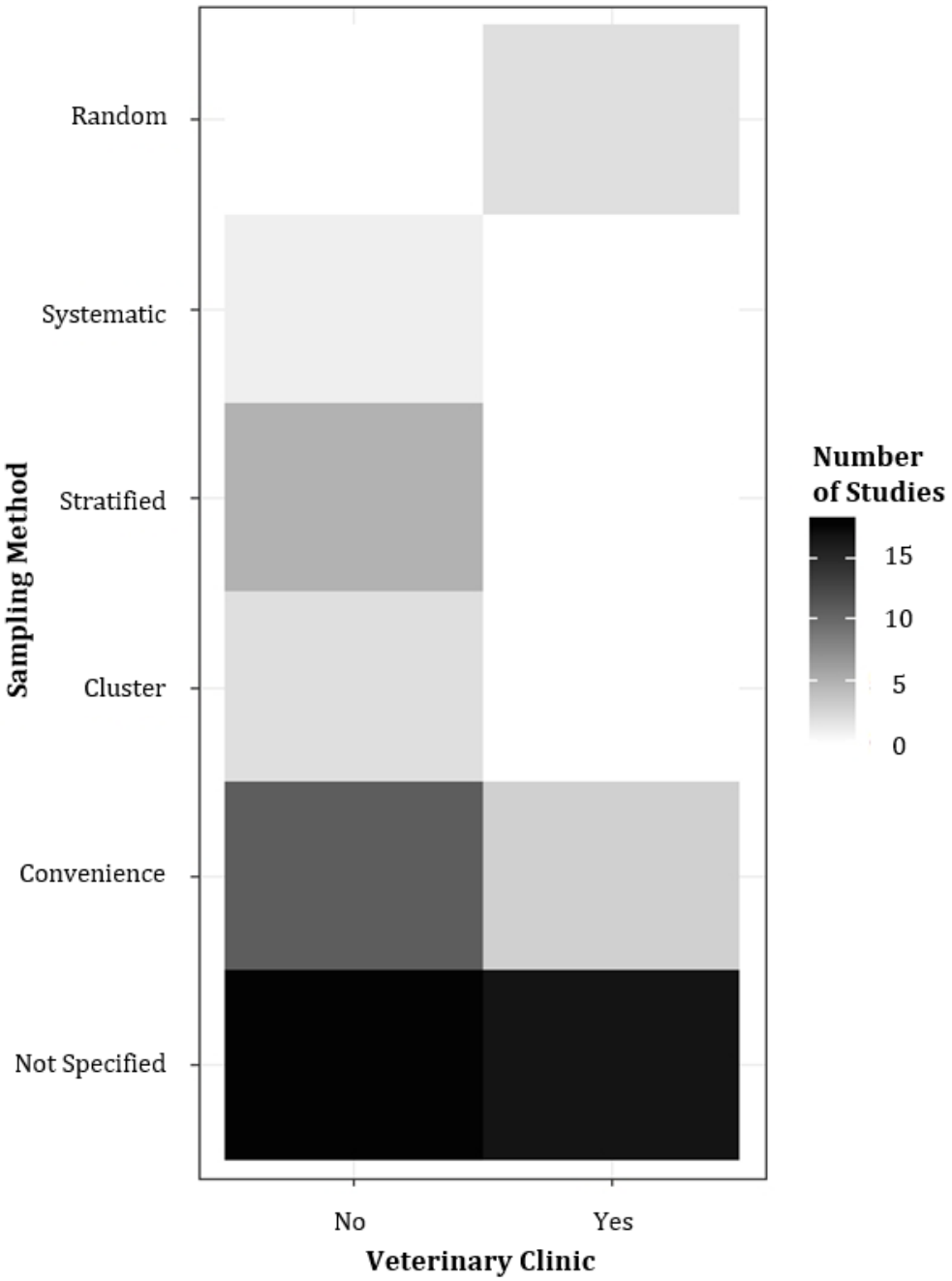
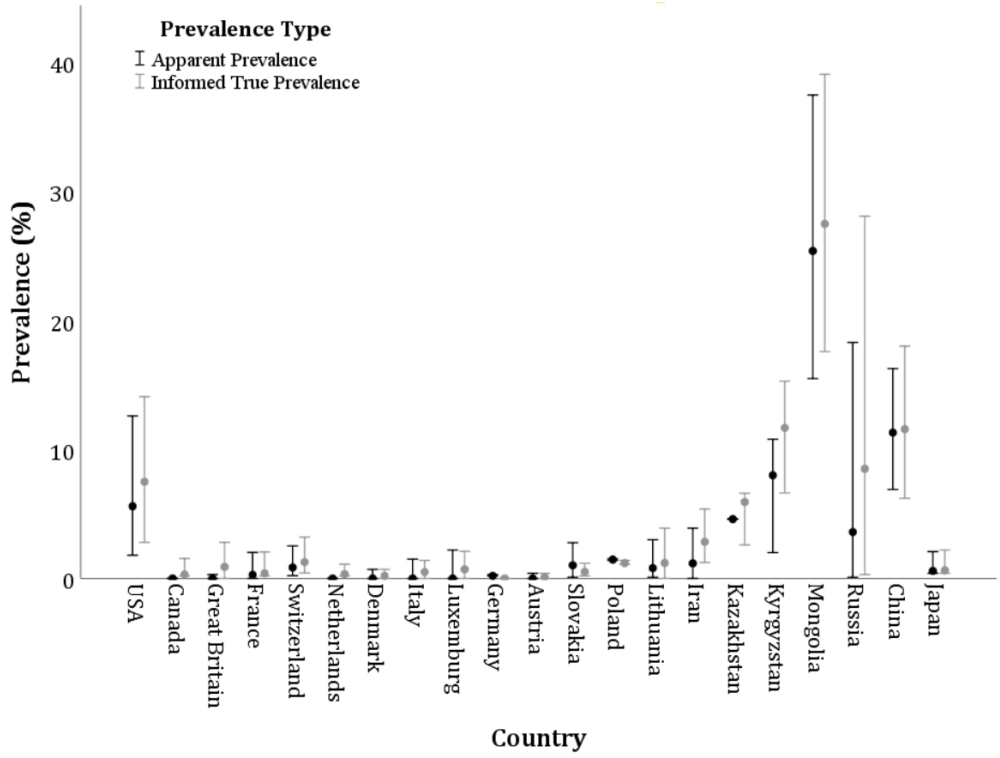


Figure 3: Characteristics of the sampling design (stratified, cluster, and random) and whether dogs were recruited by veterinary clinics in 59 studies on the prevalence of intestinal *Echinococcus multilocularis* in domestic dogs selected through a formal literature review completed July 21, 2020.

(a)



(b)

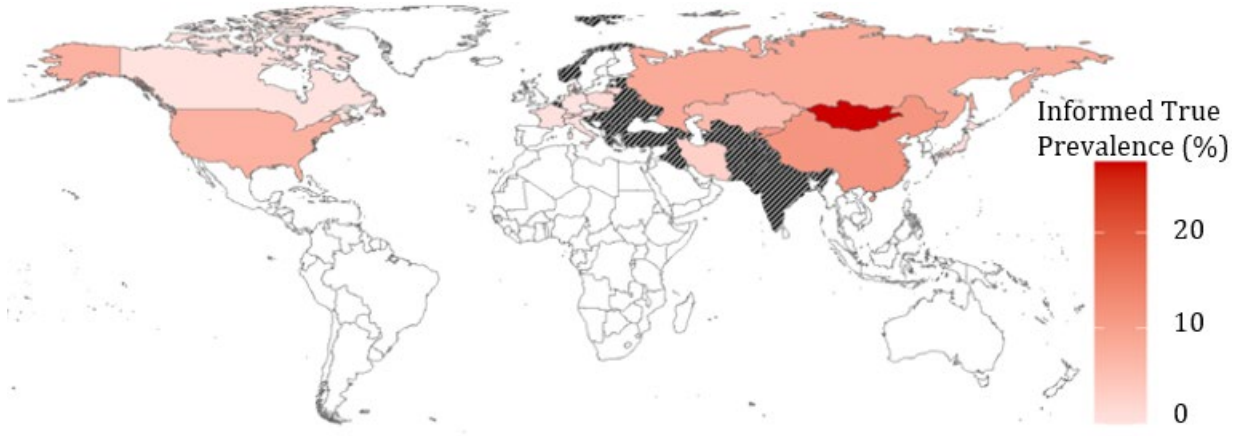


Figure 4: (a) Mean apparent prevalence ± 95% confidence intervals and re-assessed true prevalence ± credible intervals calculated via Bayesian methods accounting for diagnostic sensitivity and specificity (1, 2) and weighted by sample size for country means) of intestinal infection by *Echinococcus multilocularis* in domestic dogs in each country as reported in a selection of studies obtained through a formal literature review completed on of July 21, 2020. Confidence and credible intervals were obtained for each study and bootstrapped and weighted by sample size for country means). *(b)* A visual description of the present knowledge on *E. multilocularis* in domestic dogs globally. True prevalence (%) is displayed in a red scale. Data on intestinal *E. multilocularis* in dogs is unavailable for countries in light grey and black stripes even though they are known to be endemic for *E. multilocularis* (4).

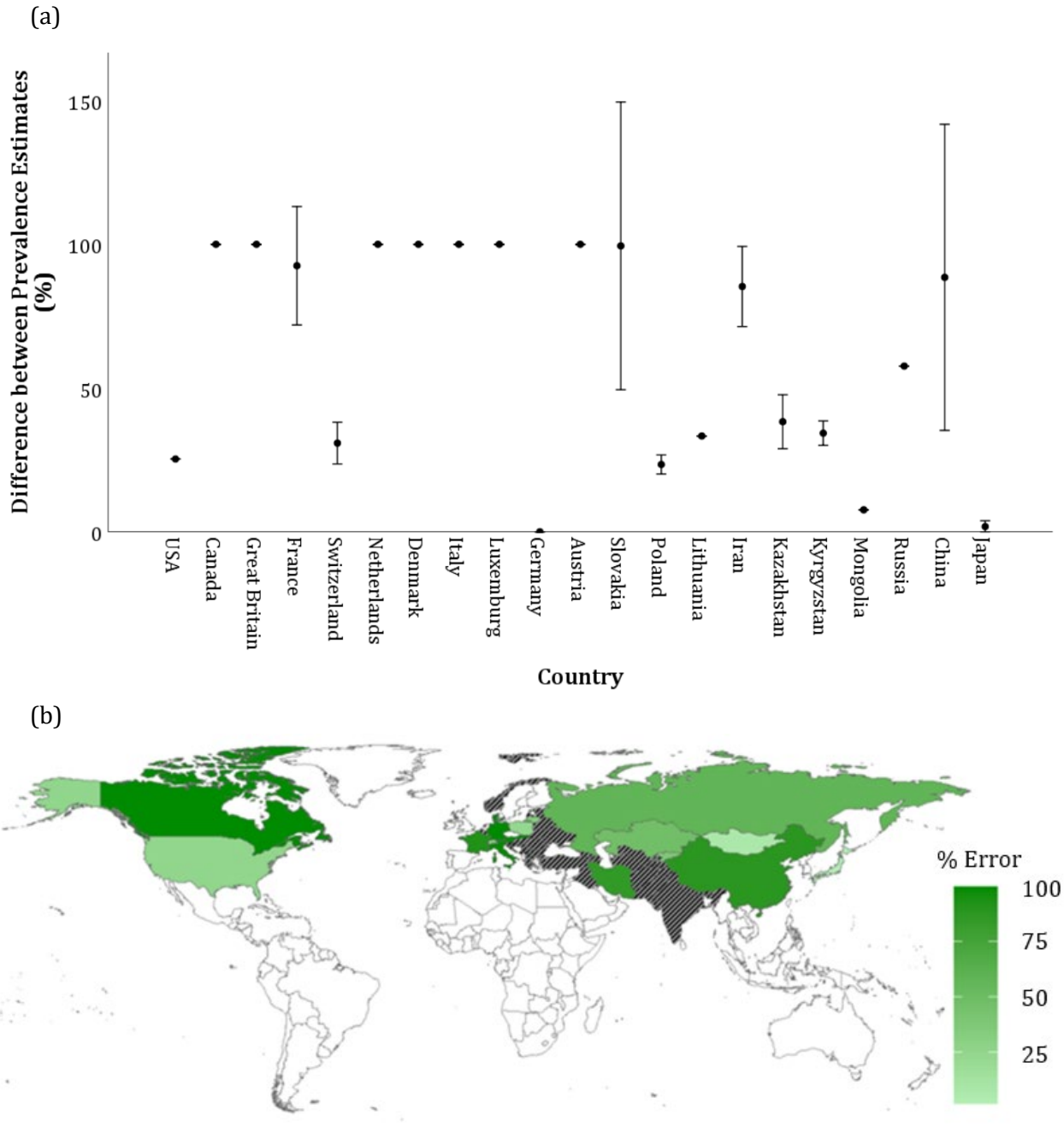


Figure 5: (a) Measurement differences (expressed as % of change in prevalence estimates when adjusted for diagnostic precision) ± SEM between re-assessed true prevalence and apparent prevalence of intestinal infection by *Echinococcus multilocularis* in domestic dogs globally as reported in a selection of studies obtained through a formal literature review completed on of July 21, 2020. *(b)* A visual description of this trend shows the difference between true prevalence and apparent prevalence (%) in a green scale. Several countries (light grey and black stripes) are known to be endemic for *E. multilocularis* (4) but do not have published data for *E. multilocularis* in domestic dogs.

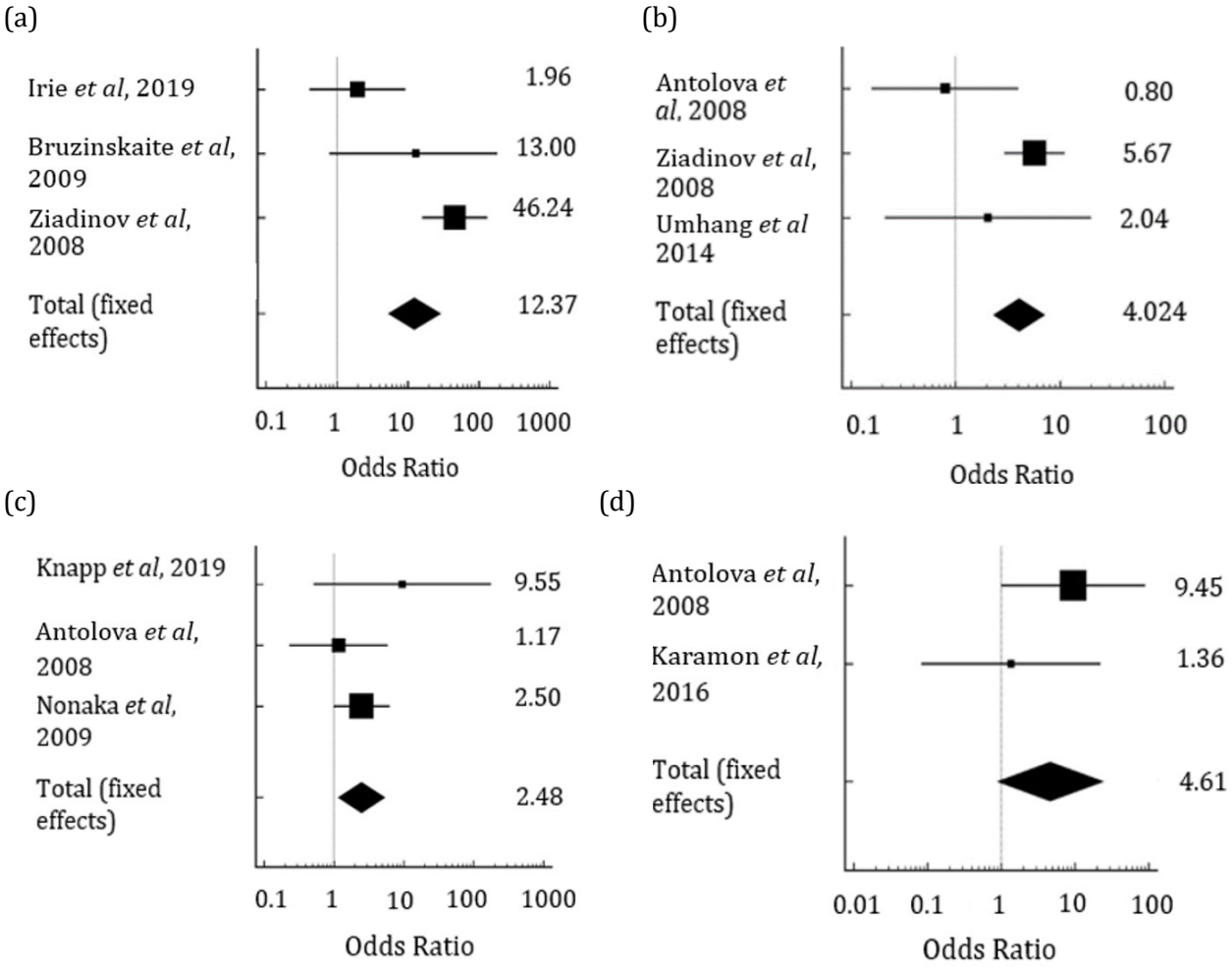


Figure 6: Pooled weighted log-odds ratios (and 95% CI) for intestinal infection by *Echinococcus multilocularis* in domestic dogs which *(a)* were not tethered to their owner's property, *(b)* were used for hunting, *(c)* lived in rural environments, and *(d)* preyed upon rodents, as reported in a selection of studies obtained through a formal literature review completed on of July 21, 2020. Box size is scaled with sample size and odds ratios are reported on the right-hand margin.

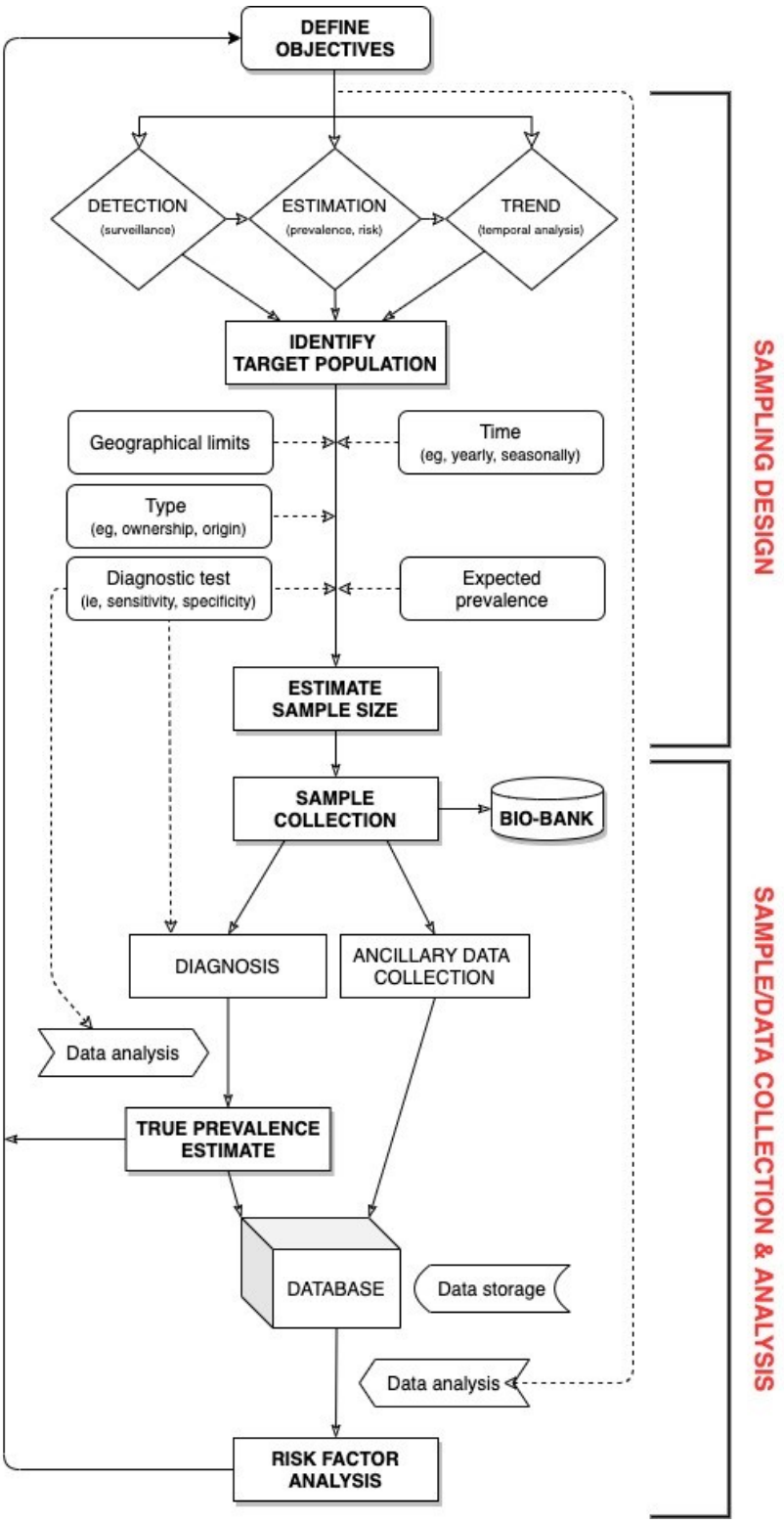


Figure 7: Recommended framework for future investigations into *Echinococcus multilocularis* infection in domestic dogs

Table 1: A priori sensitivity and specificity of common diagnostic techniques used to detect *Echinococcus multilocularis* enteric and hepatic infections in definitive hosts, compared to re-evaluated diagnostic parameters from recently published latent-class analyses (LCA).

Diagnostic test	Source sensitivity	LCA[a] sensitivity (95% CI)	Source specificity	LCA* specificity (95% CI)	LCA* source
Arcoline purgation (59)	na	0.758 (0.549-0.942)	1.00	1.00	(2)
Flotation-PCR (73, 74)	0.94	0.548 (0.485-0.610)	1.00	0.934 (0.873-0.991)	(1)
Nested PCR (70)	0.89	0.892 (0.789-0.963)	1.00	0.928(0.882-0.979)	(2)
pAb-ELISA (69)	0.836	0.56 (0.480-0.639)	0.995	0.659 (0.558-0.756)	(1)
mAb-ELISA (65)	0.94	0.632 (55.3-70.8)	1.00	0.700 (0.601-0.794)	(1)
Copro-ELISA (66, 80)	0.83	0.55 (0.408-0.689)	0.96	0.706 (0.653-0.767)	(2)
SCT/IST (75)	0.98	0.885 (0.827-0.934)	1.00	1.00	(1)

[a] LCA: Latent class analysis used to determine sensitivity and specificity

Table 2: The number of peer-reviewed articles obtained after searching keyword and vector combination "(alveolar OR *multilocularis*) AND *echinococc** AND dog AND (prevalence OR population)" in four major scientific databases. Last search was completed July 21, 2020. An asterisk was used to search for words with a similar prefix (in this case, *echinococc** was used to search for *echinococcus*, echinococci, and echinococcosis).

Name of database	Number of articles
Web of Science	277
PubMed	259
Scopus	246
Science Direct	672
Total:	1,451

Table 3: Sample design information on studies (literature search completed on July 21, 2020) on *Echinococcus multilocularis* in dogs. Dog ownership and demographic, the presence of risk factor analysis and sequencing, and diagnostic techniques are reported.

Country	Dates of study	Seasonality	Clinic	Sampling method	Ownership, locality	Risk factors	Sequenced	Source
Austria	2004-2005	All	Yes	not specified	Owned, Mixed	No	No	(102)
Canada	2009-2010	not specified	No	Stratified	Stray, Mixed	Yes	Yes	(103)
	2018	Spring, Summer	No	Convenience	Owned, Urban	No	No	(104)
China	2001-2007	Spring, Autumn	No	Stratified	Mixed, Rural	No	No	(60)
	2000	not specified	No	Convenience	Stray, Rural	No	No	(43)
	2000	Not specified	No	not specified	Stray, Rural	No	No	(105)
	2002-2003	Spring, Autumn	No	not specified	Owned, Rural	Yes	No	(59)
	2004-2005	Autumn, Winter, Spring	No	Convenience	Stray, Rural	No	Yes	(106)
	2006-2007	Spring	No	Stratified	Owned, Rural	No	No	(107)
	2006-2007	not specified	No	Convenience	Stray, Rural	No	No	(108)
	2004-2007	All	No	not specified	Mixed, Rural	No	No	(109)
	2006-2007	Spring, Summer, Autumn	No	not specified	Owned, Rural	No	No	(110)
	2006-2007	Spring, Summer, Autumn	No	not specified	Owned, Rural	No	No	(110)
	2012	not specified	No	Cluster	Owned, Rural	Yes	No	(82)
	2015	Spring	No	not specified	Owned, Rural	Yes	No	(111)
	2015-2017	Summer	No	Systematic	Owned, Rural	Yes	Yes	(101)
Denmark	2004-2005	All	Yes	not specified	Owned, Mixed	No	No	(102)
France	2008-2010	Spring, Summer	Yes	not specified	Owned, Mixed	Yes	No	(85)
	2008-2010	Spring, Summer	Yes	not specified	Owned, Mixed	Yes	No	(85)
	2006-2008	not specified	Yes	not specified	Owned, Mixed	Yes	Yes	(56)
	2004-2005	All	Yes	not specified	Owned, Mixed	No	No	(102)
	2011-2013	All	No	not specified	Unknown, Rural	No	No	(112)
	2012-2015	Winter, Spring	No	Convenience	Unknown, Mixed	No	Yes	(83)
Germany	2004-2005	All	Yes	not specified	Owned, Mixed	No	No	(102)
Great Britain	2004-2006	All	Yes	not specified	Owned, Mixed	No	No	(102)
Iran	not specified	not specified	No	Convenience	Stray, Unknown	No	No	(113)
	2009-2010	Winter	No	not specified	Mixed, Rural	No	Yes	(114)
	2013	All	No	not specified	Owned, Rural	No	No	(115)

	2013-2014	All	Yes	Random	Unknown, Rural	No	No	(116)
	not specified	not specified	No	not specified	Unknown, Rural	No	No	(117)
Italy	2004-2005	All	Yes	not specified	Owned, Mixed	No	No	(102)
Japan	1997-2007	All	Yes	not specified	Owned, Mixed	Yes	Yes	(57)
	2003-2004	not specified	No	not specified	Owned, Mixed	No	Yes	(118)
	2013-2017	All	No	not specified	Stray, Urban	No	No	(119)
	2018-2019	All	Yes	not specified	Owned, Rural	Yes	Yes	(93)
Kazakhstan	2002	Autumn	No	not specified	Mixed, Rural	No	Yes	(120)
	2003-2005	Summer, Autumn	No	Convenience	Mixed, Rural	Yes	No	(121)
Kyrgyzstan	2012	Spring	No	Stratified	Mixed, Rural	Yes	No	(122)
	2012	Spring	No	Stratified	Mixed, Rural	No	Yes	(122)
	2005	Summer, Autumn	No	Cluster	Mixed, Rural	Yes	Yes	(95)
Lithuania	2005-2006	Autumn, winter	No	Convenience	Mixed, Rural	Yes	Yes	(94)
Luxemburg	2004-2005	All	Yes	not specified	Owned, Mixed	No	No	(102)
Mongolia	not specified	not specified	No	Convenience	Stray, Rural	No	No	(123)
The Netherlands	2004-2005	All	Yes	not specified	Owned, Mixed	No	No	(102)
	2012-2013	All	Yes	Convenience	Owned, Urban	Yes	No	(81)
Poland	2015	Spring	Yes	Convenience	Owned, Rural	Yes	Yes	(124)
	2017-2018	All	Yes	not specified	Owned, Rural	Yes	Yes	(125)
	2017-2018	All	Yes	Convenience	Stray, Unknown	Yes	Yes	(125)
Russia	2017-2018	All	No	Convenience	Unknown, Rural	No	Yes	(126)
Slovakia	2006	Spring, Summer, Autumn	No	Convenience	Owned, Rural	Yes	Yes	(127)
	2002-2005	All	No	not specified	Mixed, Mixed	Yes	No	(128)
	2016-2019	All	No	not specified	Mixed, Mixed	Yes	Yes	(129)
Switzerland	1996-1997	All	Yes	not specified	Owned, Unknown	No	No	(5)
	2009-2010	Autumn, Winter	Yes	not specified	Owned, Unknown	No	No	(35)
	2009-2010	Autumn, Winter	No	not specified	Stray, Urban	No	No	(35)
	2009-2010	Autumn, Winter	Yes	not specified	Owned, Urban	No	No	(35)
	not specified	not specified	Yes	Random	Owned, Unknown	Yes	No	(130)
	not specified	not specified	No	not specified	Mixed, Mixed	No	No	(69)

United States	1951	not specified	No	not specified	Owned, Rural	No	No	(84)

Table 4: Sample size, diagnostic parameters, and results in studies on *Echinococcus multilocularis* in dogs as from the literature search completed on July 21, 2020. Apparent prevalence (AP) recorded by each study (in per-cent) was used to calculate the true prevalence (TP) using Bayesian methods, accounting for both the sensitivity (Se) and specificity (Sp) reported in each study. Similarly, the re-assessed true prevalence (ATP) was estimated relying on the updated sensitivity and specificity measure reported in Otero-Abad, 2017 and Hartnack *et al* 2013 (ATP Se and Sp).

Country	**TP reported**	**Methods of analysis**	**Se (%); Sp (%)**	**Sample size**	**Dogs infected**	**AP (%); CI**	**TP (%); CrI[c]**	**LCA[a] Se (%); Sp (%)**	**ATP (%); CrI[c]**	**Source**
Austria	No	Nested PCR	89; 100	812	0	0; 0-0.4	0.1; 0-0.4	89.2; 92.8	0.1; 0-0.4	(102)
Canada	No	Flotation-PCR	94; 100	1086	0	0; 0-0.3	0.1; 0-0.3	48.5-61; 87.3-99.1	0.2; 0-0.5	(103)
	No	Mag-PCR	88; 99.9	44	0	0; 0-8	2.9; 0-8.7		2.9; 0-8.5	(104)
China	No	Copro-PCR	no data; no data	228	no data	14.8; 10.38-19.62	NA			(60)
	No	SCT	98; 100	22	8	36.4; 17.2-59.3	38.3; 20.1-58.3	88.5; 100	42.7; 22.3-65.4	(131)
	No	SCT	98; 100	23	8	34.8; 16.4-57.3	36.9; 19-56.5	88.5; 100	40.8; 21.3-63.3	(105)
	Yes	Arcoline purgation	67; 92	371	45	12.1; 9-16	7.4; 2.1-13.2	75.8; 100	17.3; 11.4-25	(59)
	No	Nested PCR (modified)	89; 100	30	1	3.3; 0.1-17.2	7.1; 0.9-19.1	89.2; 92.8	5.2; 0.2-17	(106)
	No	Nested PCR	85; 100	142	32	22.5; 16-30.2	27; 19.3-35.5		27.6; 18.7-38.4	(107)
	No	unknown	no data, no data	9	5	55.5; 21.2-86.3	63.6; 27.2-95.8		56.1; 5.8-97.3	(108)
	No	Arcoline purgation	67; 92	74	4	5.4; 1.5-13.3	3.9; 0.1-12.5	75.8; 100	9.3; 3-19.3	(109)
	No	Copro-PCR	69; 100	276	31	11.2; 7.8-15.6	16.7; 11.6-22.4		1.9; 0-7.6	(110)
	No	Copro-PCR	69; 100	311	4	1.3; 0.3-3.3	2.3; 0.8-4.7		0.9; 0-3.7	(110)
	No	qPCR	no data; 100	750	106	14.1; 11.7-16.8	17.6; 13.3-22.8		12; 0.6-29	(82)
	No	qPCR	86; 93	256	0	0; 0-1.4	0.5; 0-1.4		0.5; 0-1.3	(111)
	No	Copro-PCR	69; 100	105	25	23.8; 16-33.1	35.3; 24.3-48		35.2; 24-47.7	(101)
Denmark	No	Nested PCR	89; 100	517	0	0; 0-0.7	0.2; 0-0.7	89.2; 92.8	0.2; 0-0.7	(102)
France	Yes	Flotation-PCR	94; 100	367	0	0; 0-1	0.3; 0-0.8	48.5-61; 87.3-99.1	0.5; 0-1.5	(85)

	Yes	Flotation-PCR	94; 100	493	0	0; 0-0.7	0.2; 0-0.6	48.5-61; 87.3-99.1	0.4; 0-1.1	(85)
	No	Flotation-PCR	94; 100	817	4	0.5; 0.1-1.2	0.7; 0.2-1.3	48.5-61; 87.3-99.1	0.4; 0-1.2	(56)
	No	Nested PCR	89; 100	980	0	0; 0-0.4	0.1; 0-0.3	89.2; 92.8	0.1; 0-0.4	(102)
	No	qPCR	86; 93	18	2	11.1; 1.4-34.7	11.8; 0.6-32.6		12.2; 0.6-34.1	(112)
	No	qPCR	86; 93	748	4	0.5; 0.1-1.4	0.2; 0-0.6		0.2; 0-0.6	(83)
Germany	No	Nested PCR	89; 100	17894	43	0.2; 0.2-0.3	0.3; 0.2-0.4	89.2; 92.8	0; 0-0	(102)
Great Britain	No	Nested PCR	89; 100	121	0	0; 0-0.3	0.3; 0.2-0.4	89.2; 92.8	0.9; 0-2.8	(102)
Iran	No	SCT	98; 100	29	0	0; 0-3	0.9; 0-2.7	88.5; 100	3.5; 0-10.4	(113)
	No	Flotation-PCR	94; 100	77	5	6.5; 2.1-14.5	8.1; 3-15	48.5-61; 87.3-99.1	7.9; 0.4-21.4	(132)
	No	Flotation-PCR	94; 100	100	0	0; 0-3.6	1; 0-3.1	48.5-61; 87.3-99.1	1.8; 0-5.4	(115)
	No	Flotation-PCR	94; 100	167	0	0; 0-2.2	0.6; 0-1.9	48.5-61; 87.3-99.1	1.1; 0-3.3	(116)
	No	Copro-ELISA[b]	80; 95	59	0	0; 0-6.1	2.1; 0-6.1		2.1; 0-6.3	(117)
Italy	No	Nested PCR	89; 100	249	0	0; 0-1.5	0.4; 0-1.3	89.2; 92.8	0.5; 0-1.4	(102)
Japan	No	Copro-ELISA	94.9; 100	4768	18	0.4; 0.2-0.6	0.4; 0.3-0.6		0.4; 0.3-0.6	(57)
	No	Nested PCR	89; 100	183	1	0.5; 0-3	1.2; 0.2-3.4	89.2; 92.8	0.7; 0-2.5	(118)
	No	Copro-PCR	no data, no data	156	3	2; 0.4-5.5	2; 0.1-5.8		2.6; 0.1-8	(119)
	No	Copro-PCR	no data, no data	98	7	7.1; 3-14.2	6.1; 0.4-14.9		7.6; 0.3-21.1	(93)
Kazakhstan	No	Nested PCR (modified)	89; 100	131	6	4.6; 1.7-9.7	5.9; 2.4-10.9	89.2; 92.8	2.6; 0.1-7.8	(120)
	No	Arcoline purgation	67; 92	632	29	4.6; 3.1-6.5	0.5; 0-1.8	75.8; 100	6.6; 4-10.1	(121)
Kyrgyzstan	No	Copro-PCR	69; 100	204	4	2; 0.5-4.9	3.6; 1.2-7.17		3.5; 1.2-7.1	(122)
	No	Arcoline purgation	67; 92	20	1	5; 0.11-24.9	9.8; 0.3-1.2	75.8; 100	12.9; 1.7-34.7	(122)
	Yes	Arcoline purgation	21; 100	466	50	10.8; 8.1-13.9	51.8; 39.1-65.7	75.8; 100	15.3; 10-22	(95)
Lithuania	No	Flotation-PCR	94; 100	240	2	0.8; 0.1-3	1.3; 0.3-3.1		1.2; 0-3.9	(94)
Luxemburg	No	Nested PCR	89; 100	165	0	0; 0-2.2	0.7; 0-2	89.2; 92.8	0.7; 0-2.1	(102)
Mongolia	No	Copro-ELISA	94; 100	67	17	25.4; 15.5-37.5	27.7; 17.5-39.1		27.5; 17.6-39.1	(123)
The Netherlands	No	Nested PCR	89; 100	734	0	0; 0-0.5	0.1; 0-0.4	89.2; 92.8	0.2; 0-0.5	(102)

	No	qPCR	no data, no data	142	0	0; 0-2.6	0.9; 0-2.6		1.1; 0-3.6	(81)
Poland	No	Nested PCR	89; 100	148	2	1.4; 0.2-4.8	2.2; 0.5-0.52	89.2; 92.8	1.1; 0-3.7	(124)
	Yes	Nested PCR	89; 100	145	2	1.4; 0.2-4.9	2.3; 0.5-5.5	89.2; 92.8	1.1; 0-3.9	(125)
	Yes	Nested PCR	89;100	123	2	1.6; 0.2-5.8	2.7; 0.6-6.5	89.2; 92.8	1.4; 0-4.7	(125)
Russia	No	Necropsy (unknown)	no data; no data	28	1	3.6; 0.1-18.3	6.5; 0.2-20.6		8.5; 0.3-28.1	(126)
Slovakia	No	Nested PCR	89; 100	752	1	0.1; 0-0.7	0.3; 0-0.8	89.2; 92.8	0.2; 0-0.6	(127)
	No	Copro-ELISA[b]	80, 95	289	8	2.8; 1.2-5.4	0.8; 0-2.8		0.8; 0-2.9	(128)
	No	Nested PCR	89, 100	110	3	2.7; 0.6-7.8	3.8; 1-8.2	89.2; 92.8	1.9; 0.1-6.4	(129)
Switzerland	No	Flotation-PCR	94; 100	86	6	7; 2.6-14.6	8.9; 3.7-16.3	48.5-61; 87.3-99.1	8.1; 0.4-21.2	(5)
	No	Flotation-PCR	94; 100	118	0	0; 0-3.02	0; 0-0	48.5-61; 87.3-99.1	1.5; 0-4.7	(35)
	No	Flotation-PCR	94; 100	124	3	2.4; 0.5-6.9	3.4; 0.9-7.3	48.5-61; 87.3-99.1	3.2; 0.1-10	(35)
	No	Flotation-PCR	94; 100	49	0	0; 0-7.3	2.1; 0-6.3	48.5-61; 87.3-99.1	3.5; 0-10.4	(35)
	No	pAb-copro-ELISA	84; 99.5	505	2	0.4; 0.1-1.4	0.4; 0-1.3	48.0-63.9; 55.8-75.6	0.5; 0-1.9	(130)
	No	pAb-copro-ELISA	96; 99.5	660	2	0.3; 0-1.1	0.2; 0-0.8	48.0-63.9; 55.8-75.6	0.4; 0-1.4	(69)
United States	No	SCT	98; 100	89	5	5.6; 1.8-12.6	6.7; 2.5-12.8	88.5; 100	7.5; 2.8-14.1	(84)

[a] LCA: Sensitivity and specificity of the test determined via latent-class analysis (*Table 1*)
[b] Copro-ELISA was not species-specific, but results were confirmed as *E. multilocularis* via PCR
[c] Credible intervals (CrI) used were 2.5% and 92.5% unless the number of positives was 0, in which case CrI were 0% and 95%

CHAPTER 3: *ECHINOCOCCUS MULTILOCULARIS* INTESTINAL INFECTIONS IN OWNED DOMESTIC DOGS IN A NORTH AMERICAN METROPOLIS

INTRODUCTION

Intestinal infections in dogs by *Echinococcus multilocularis* – a parasitic helminth of the Northern Hemisphere (9) – have been increasingly documented in Europe and Asia but have seldom been reported in North America (133). These studies predominantly occur in rural areas (133), where definitive hosts (dogs, coyotes, foxes, wolves, and others; DHs hereafter) (20, 24) and intermediate hosts (rodents and some lagomorphs; IHs hereafter) (20, 24, 47) are plentiful, *E. multilocularis* infections in urban environments should be investigated as well, because of the higher potential for human exposure in these settings. Wild DHs such as coyotes and foxes commonly inhabit urban and suburban areas, bringing zoonotic diseases into cities with them (31, 54). However, they occur in much lower numbers than domestic dogs, and it has already been demonstrated that dogs can perpetuate the *E. multilocularis* lifecycle after it has been established in their urban habitat by wild host species (21, 56, 57). While dogs may carry a lower worm burden than their wild counterparts, individual worms that infect dogs seem to shed more eggs than when infecting coyotes and foxes (21) and adult worms actually persisted longer in dogs than in coyotes and foxes (134), causing these DHs to have similar biotic potential. Therefore, it is possible that dogs in metropolitan areas such as Calgary, Alberta (AB) – which boasts a population of over 350,000 dogs (2016 census data) –could be paramount in maintaining an urban *E. multilocularis* population (59, 60).

Moreover, not only can dogs act as proficient components of the urban *E. multilocularis* lifecycle, but they also can be instrumental in transmitting the parasite to humans, resulting in human AE (44). Although AE is listed as a food-borne disease of extreme importance in Europe (12)

and worldwide (44), dog ownership may be an even greater risk factor for human AE (27, 61). Alveolar echinococcosis affects over 18,000 people worldwide each year, with 91% of cases occurring in China (4). North American AE cases, while rare in the continental areas, have been recently reported in AB, Canada (17, 18) with an unprecedented cluster of cases. More importantly, genotyping of the parasitic material from hepatic lesions of these patients indicated that the parasites causing this cluster were similar to a European strain of *E. multilocularis* now common in wildlife in AB where many infections have been confirmed to be caused by this European-like haplotype (labelled "ECA") (18). The increased proportion of ECA infections (versus the expected North American strain) could be explained by a possible difference in pathogenicity of strains (43) resulting in the ECA haplotype outcompeting other haplotypes in wildlife .The presence of the ECA haplotype in intestinal *E. multilocularis* infections of domestic dogs in AB has not yet been determined, but could provide further insight into the dominance of this newly endemic haplotype.

There are several factors which may influence the probability of intestinal echinococcosis in domestic dogs. Intrinsic and extrinsic risk factors have been analyzed in several previous studies and we have recently summarized those that are most common in the literature (133). Pooled odds ratios revealed that hunting dogs, dogs that are free-roaming, and rural dogs are at higher risk for intestinal infection with *E. multilocularis* (133). Guard dogs in rural China were also found to have higher levels of infection than dogs with other occupations and uses in the same area (82). Two studies also reported male dogs being more often infected than female dogs (59, 101). Lastly, dogs that were frequently fed offal from livestock were more likely to be infected by *E. multilocularis* (101). Other risk factors have also been investigated (*e.g.* age, range of roaming, time spent walking in rural areas), but results are inconclusive. Dog breed, and behaviours characteristic of different breeds, have never been analyzed as a potential intrinsic risk factor for intestinal infection by *E. multilocularis*.

The overall objective of the study was to investigate domestic dog intestinal infections by *E. multilocularis* in a large metropolitan area in AB, where this unprecedented human AE cluster of cases occurred. Specifically, we aimed to (1) estimate the prevalence of intestinal *E. multilocularis* in owned dogs living near city dog parks in Calgary, AB, Canada; (2) assess possible intrinsic and extrinsic risk factors for *E. multilocularis* infection in owned dogs in this setting; and finally, to (3) characterize the *E. multilocularis* strain infecting Calgary dogs, comparing it to the one responsible for the recent surge in human cases in AB, Canada.

MATERIALS AND METHODS

STUDY AREA

The study was carried out in Calgary (51°50'N, 114°55'W), a region with a population of >1.3 million that extends over 5107 km^2 in the grasslands of southern AB, Canada. Within Calgary city limits, the elevation ranges from 1060 meters above sea level (asl) in the two river valleys (Bow and Elbow) to 1240 meters asl in the surrounding hills. Several other creeks and water bodies are present within the city limits, providing much riparian habitat (135), often encompassed in city parks, natural areas, and golf courses, while the city is mainly surrounded by agricultural land. Calgary hosts many species of urban wildlife, including wild canids that can be hosts of *E. multilocularis* (mainly coyotes *Canis latrans,* but also fox *Vulpes vulpes*) and various species of rodents that are also potential hosts (136). The climate is highland continental, which includes long, variable winters and short, warm summers. According to Environment Canada (climate.weather.gc.ca), average daily temperatures in Calgary range from 16.5 °C (61.7 °F) in July to −6.8 °C (19.8 °F) in December.

Calgary was inhabited by 135,070 dogs in 2016 (2016 civic census data) which is an increase of 12,745 dogs since 2010. At the time of the last census in 2016, the Calgary dog population had more than doubled in the past decade. In the outer communities of Calgary, and especially in the

southwest and southeast quadrants, there are as many as one dog for every two or three households (2016 civic census data). The city center has a much lower density of dogs per household, with one dog being owned only for every five to seven dwellings (2016 civic census data). The northeast quadrant of Calgary also only has one dog owned for approximately every four households while there are slightly more dogs in the northwest – about one for every three households (2016 civic census data).

SAMPLING DESIGN

The target population was owned dogs living in postal-codes directly adjacent to any of six city parks: River Park (RP), Nosehill Park (NHP), Fish Creek Provincial Park (FCPP), Weaselhead Flats (WSH), Bowmont Park (BM), and Southland Lowlands (SL) (137).

Participants were recruited in a previous study (137) by randomly selecting 6000 dog-owners from the City of Calgary's 2011 dog license database, 1000 living in residential communities bordering each of these parks. The selected dog owners were sent a recruitment letter by ground mail by the City of Calgary, Animal Services and Bylaw Division in June 2012 as outlined in Smith *et al*, 2015 (137).

SURVEY DESIGN

All selected dog-owners were asked to complete a survey including 27 questions organized in seven sections (*Supplementary Material 1;*) titled: (A) "Screening", (B) "Questions about your Dog", (C) "Outside of Park: Dog Owner Recreational and other Activities", (D) "Within Park: Recreational Activities", (D) "Questions about You and Your Household", (E) "Request for Dog Fecal Sample", and (F) "Personal Information".

To be included in the study, participants had to adhere to the following conditions: answer "yes" to the screening questions, consent to provide a sample of their dogs' feces, and complete the survey. As outlined in Smith *et al*, 2015 (137), 1293 participants responded to the survey. Most

(1082) agreed to the sample collection, and 860 fecal samples were collected during August and September 2012.

Sample collection and processing

Prior to processing, all samples were frozen at -80°C for 72 hours to inactivate *E. multilocularis* eggs (23) in 2012 and then stored at -20°C until this study started in 2018 with *E. multilocularis* testing. Out of the 860 fecal samples, 696 were selected for this study, based on the remaining sample having approximately 2 grams of feces or more leftover from the previous study – *i.e.* enough sample had to be present to complete the required analyses described below.

DNA extraction

DNA was extracted from 200mg of each fecal sample using the Omega Mag-Bind® Universal Pathogen DNA extraction kit (#M4029-01) as per the manufacturer's instructions, with one amendment: we added five cycles of freezing with dry ice for 1 minute and heated at 70°C for 1 minute between the initial homogenization step and the addition of proteinase K to the sample to release DNA from the chitinous shell surrounding *E. multilocularis* eggs (138). Automated DNA extraction was then performed using a MagMAX™ Express 96 (Applied Biosystems).

qPCR detection of E. multilocularis

To detect presence of *E. multilocularis*, a duplex qPCR reaction of the mitochondrial gene *nad2* (139) and an internal amplification control (IAC)(140) was performed as described in Santa *et al*, 2018 (139) using a C1000™ Thermal Cycler Chassis with CFX96™ Optical Reaction Module (Bio-Rad) and visualized using the CFX Maestro™ Software. Nad234 primers were used to amplify *E. multilocularis* DNA while IAC was used to assess the presence of PCR inhibitors in the sample.

The analytic sensitivity and specificity of this test were 87.1% (70.2-96.4%) and 100% respectively (141). Samples negative for IAC amplification were diluted using a ten-fold dilution and re-run (139) again in duplicate using the same procedure to overcome PCR inhibition.

FLOTATION/SEDIMENTATION AND EGG HARVESTING

Parasite eggs were collected from samples that had tested positive for *E. multilocularis* DNA using qPCR. We carried out a $ZnCl_2$ flotation/sedimentation procedure as outlined in Liccioli *et al*, 2014 (142) and Davidson *et al*, 2008 (143) on 2g of fecal samples. In this way, Taeniid eggs were collected from each positive sample.

We performed microscopy on the resulting egg slurry using a compound light microscope under 10-40X magnification. A maximum of three 100µL aliquots of each sample were analyzed. Once Taeniid eggs were found in an aliquot, all Taeniid eggs on the microscope slide were counted to determine egg density per gram of feces, and no further aliquots were analyzed. After Taeniid eggs were counted, 1µL of egg slurry was diluted tenfold and analyzed under a stereomicroscope. Individual eggs – ten from each sample – were then isolated and harvested as described in Huttner *et al*, 2008 (144).

NESTED PCR

Individual Taeniid eggs were lysed in 0.02M NaOH at 95°C for 10 minutes. Subsequently, we performed a nested PCR on the mitochondrial *nad1* gene using external primers (Ex_Nad1_Fwd: TATTAAAAATATTGAGTTTGCGTC, Ex_Nad1_Rvs: TCTTGAAGTTAACAGCATCACGAT) and internal primers (Int_Nad1_Fwd: TGGAACTCAGTTTGAGCTTTACTA, Int_Nad1_Rvs: ATATCAAAGTAACCTGCTATGCAG) (144).

The 50µL external reaction was comprised of 25µL AccuStart™ II PCR SuperMix, 1µL of each 10µM external primer, 1µL template DNA, and 22µL H_2O as described by the manufacturer. The 50µL internal reaction was comprised of the same materials but used the internal primers instead

of external primers, and 1µL of external PCR product instead of template DNA. Each reaction was denatured at 94°C for 2 minutes followed by 35 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 60 seconds in a T100™ Thermal Cycler (Bio-Rad).

Results were visualized on a 3% agarose gel run at 80-150V and post-stained with GelRed® Nucleic Acid Gel Stain (Biotium) for 30 minutes. Successful nested PCR amplicons were cleaned up using the E.Z.N.A.® Cycle Pure Kit (Omega Bio-tek) prior to strain genotyping.

STRAIN GENOTYPING

The whole *nad1* gene (1072bp) was sequenced from single eggs which were successfully amplified by nested PCR. For each reaction, 50 to 100ng of template and 3.2pmol of the internal NAD1 forward primer were diluted with water and sequenced by the University of Calgary Core DNA Services (Calgary, AB). Obtained sequences were uploaded to *Sequence Scanner Software v2.0* (Thermofisher Scientific, Waltham, MA) and assessed for quality. Viable sequences were compared to existing *nad1* templates obtained from GenBank™ (http://www.ncbi.nlm.nih.gov/genbank/). BLAST® (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to align the obtained sequences to existing templates.

DATA ANALYSIS

Dog demographics

A descriptive analysis of survey data was performed to describe the distribution of various risk factors in the sample. We used Kruskal-Wallis tests to analyze differences in non-parametric Likert-scaled responses on walking behaviours such as the amount of time spent by dogs in different outdoor environments (*e.g.* dog parks, sidewalks and streets, school and sports fields, *etc.*) and the proportion of time dogs spent off-leash in these environments. Likert-scaled responses were summarized by their median and 25% to 75% interquartile range (IQR). Dog breeds were

categorized into classes based on Canadian Kennel Club (CKC) standards. In cases where mixed-breed dogs belonged to multiple CKC classes, they were categorized according to the breed with the higher approximate prey drive. Exact chi-square tests were used to test for differences in intrinsic (*e.g.* breed, gender) and extrinsic characteristics (*e.g.* time spent walking in dog parks and other areas, time spent off-leash in these areas, time spent in the backyard) between dogs sampled around the six dog parks and also to test for the difference in these same characteristic between dogs with and without intestinal echinococcosis. These analyses were all performed in *SPSS v.25* (IBM®, Armonk, NY, US). Throughout the text, means are reported along with Standard Error of the Mean (mean±SEM).

Assessing risk factors

We analyzed various risk factors that could be correlated with *E. multilocularis* positivity using Pearson chi-squared goodness-of-fit tests (for categorical data) and binary logistic regression (for ordinal and numeric data). Specifically, the extrinsic variables tested included: time spent walking in city parks and off-leash in these parks, time spent alone in the yard, known coyote *E. multilocularis* prevalence in the area. Intrinsic factors such as frequency of rodent predation, number of dogs in each household, dog breed and sex were also analyzed. Odds ratios and their corresponding 95% Confidence Intervals (CI) were obtained for all significant risk factors to determine likelihood of *E. multilocularis* infection for dogs with these intrinsic and extrinsic characteristics. Odds ratios and CIs were gleaned directly from the logistic regression with a logit link for numeric and ordinal data but were estimated separately *post hoc* for categorical data. All these statistical analyses were performed in *SPSS.*

True prevalence determination

True prevalence of *E. multilocularis* in dogs – overall, and also surrounding each park – was determined to account for the specificity estimate (100%) and the 95% confidence interval of the sensitivity of the qPCR (141) which were used in a Bayesian prevalence model where we

implemented the sensitivity distribution using two chains containing 10000 "burn-in" samples and 10000 samples that were retained (76). We used the package '*prevalence*' in R Software version 4.0.2 (2020-06-22) to estimate Bayesian true prevalence. In the same way, we also calculated the true prevalence of *E. multilocularis* infections in both coyotes and rodents in each Calgary park using data from previous studies (136). Bayesian true prevalence values are accompanied by 2.5 and 97.5% credible intervals which were provided by the model.

RESULTS

CHARACTERISTICS OF THE SAMPLED DOGS

This study revealed that between one and seven dogs were owned per household (median: 1, IQR: 1-2). Almost all dogs were spayed or neutered (667/695, 96.0%) and male and female dogs occurred equally (350/692, 50.6% and 342/692, 49.4% respectively) (*Table 5*). Ages ranged from pups under a year old to senior dogs of 17 years old (mean: 7.0±0.1 years). Most dogs were purebred (434/694; 62.5%) rather than of mixed breed (261/695; 37.6%) (χ^2=43.1, df=1, p<0.0001) and the most common breeds were Labrador retriever (57/694; 8.2%), terrier (general) (41/694; 5.9%), golden retriever (28/694; 4.0%), and bichon frise, border collie, and shi-tzu (19/694 each; 2.7% each). Of these dogs, most (457/692; 66.0%; N=692) were of breeds with high prey drive regarding rodents (χ^2=71.2, df=1, p<0.0001), based on descriptions of dog breeds by the CKC. Specifically, most dogs in this sample were of sporting breeds compared to all other breed classes (χ^2=149.2, df=6, p<0.0001) and the proportion of dogs belonging to each CKC class was consistent in the communities surrounding each park all (χ^2=35.3, df=35, p=0.4) (*Table 5*).

Activity levels of dogs also remained consistent across sample locations (χ^2=19.7, df=20, p=0.5). In most cases, dogs were regularly to always kept in the yard rather than the house while on their property (560/692; 80.9%) (*Table 6*). Overall, when away from the property, dogs were

mostly walked in dog parks, followed closely by sidewalks and streets (Chi-squared exact test of homogeneity: χ^2=824.2, df=4, p<0.0001) (*Table 6*).

However, dogs sampled near FCPP were more frequently walked on sidewalks and streets than in dog parks (*Table* 6), visiting parks less often than dogs living in other sampling locations (H=16.0, df=5, p=0.007). Mountains and acreages, ranches, and farms were rarely the most common areas for dogs to be walked (*Table 6*).

Characteristics of dogs positive for E. multilocularis

Thirteen of 696 dog fecal samples tested positive for the *nad2* gene of *E. multilocularis*.

The cycle of quantification (Ct) value for these samples ranged from 27.79-37.82 (average 34± 0.7) and samples where DNA was detected after the 38th cycle were considered negative due to likelihood of type II error. The thirteen positive dogs consisted of four neutered males and nine spayed females which were between two and 14 years old (average 7± 1 years) (*Table 8*). Most (7/13; 53%) were from single-dog households although four participants recorded owning two dogs and two more participants owned three and five dogs each. Eight (61%) of the infected dogs were purebred while only five (38%) were of mixed breed (*Table 8*). Specifically, dog breeds with traditionally high prey drive were well represented (11/13; 84%), reflecting the proportion this demographic was present in the entire sample (χ^2=2.0, df=1, p=0.2). The breed classes represented in the positive cases also reflected the breed distribution of the entire sample (χ^2=7.6, df=6, p=0.3). Most of the dogs positive for intestinal *E. multilocularis* were of active breeds such as those of sporting, hound, and terrier breed classes (9/13; 69%) although one herding and one working dog were also positive (*Table 8*). Two dogs of the non-sporting breed class were also among these positive cases (*Table 8*). No toy dogs were found to be infected with *E. multilocularis*.

All dogs that had intestinal echinococcosis were mostly walked on sidewalks and streets (6/13; 46%) or in dog parks (5/13; 38%), except for one dog which was more frequently walked in the

mountains and one dog for which no data were available (*Table 8*). Infected dogs were walked in dog parks approximately twice to six times per week (median=5, IQR=3-6) and were walked a similar amount on sidewalks and streets (median=5, IQR=4-6). These dogs were almost never walked in other areas (school and sports fields: median=1, IQR=1-3; mountains: median=1, IQR=1-3). Walking off-leash almost never occurred in any of these areas (dog parks: median=1, IQR=1-3; sidewalks and streets: median=1, IQR=1-1; school and sports fields: median=1, IQR=1-1; mountains: median=1, IQR=1-2).

TRUE PREVALENCE ESTIMATE OF E. MULTILOCULARIS

The Bayesian true prevalence of *E. multilocularis* in dogs living around Calgary dog parks was 2.4% (95% CrI: 1.3-4.0%), after accounting for the qPCR sensitivity and sensitivity. Even though the true prevalence of *E. multilocularis* infection was high in both hounds (9.6%; CrI: 2.6-20.7%) and terriers (5.0%; CrI: 1.4-10.9%), there was no overall difference between the number of dogs infected within each individual breed class (χ^2=7.6, *df*=6, *p*=0.3) (*Figure 8*).

The Bayesian true prevalence in previously sampled coyotes was 16.2% (95% CrI: 12.0-20.7%) with the highest prevalence recorded in BM (136) (*Figure 9*). For rodents sampled in the same study, the Bayesian true prevalence was calculated to be 1.0% (95% CrI: 0.4-1.9%) with a higher prevalence again occurring in BM (136) (*Figure 9*).

EUROPEAN-TYPE STRAIN GENOTYPING

Microscopy of egg sediment retrieved from the $ZnCl_2$ flotation/sedimentation analysis showed that all 13 positive dogs were actively shedding Taeniid species eggs at the time of sample collection. These dogs were shedding between 0.9 and 19.1 eggs per gram of feces (median: 7.2, IQR: 2.5-12.9) (*Table 8*).

Seven to nine eggs were isolated per fecal sample, except for one sample where only one egg was obtained. Thus, a total of 97 single Taeniid eggs were isolated from the 13. The *nad1* gene was successfully amplified in a total of 15 eggs from seven of the 13 samples from patent infections. Viable sequences were obtained for two of the 15 eggs which came from different samples. Both sequences had were identical to the *E. multilocularis* haplotype E (KF962559), a European-like haplotype found in coyotes and a dog from central B.C., Canada (145).

Risk Factors for Echinococcus multilocularis infections

Intrinsic factors

Only one intrinsic risk factor had a significant effect on likelihood of dog infection with *E. multilocularis*. Dogs who belonged to the hound breed class were 5.0 times more likely (95% OR: 1.3-20.1) to be infected (χ^2=5.1, df=1, p=0.02) (*Figure 10*). No other breed class showed this effect on probability of *E. multilocularis* intestinal infection.

Extrinsic factors

Several extrinsic factors also had important influence over the probability of infection with intestinal *E. multilocularis*. First, dogs kept on-leash at dog parks were 4.6 times (95% OR: 1.4-15.3) more likely to be infected with intestinal echinococcosis (OR: 4.6, z=2.5, p=0.01) (*Figure 10*). As well, a high proportion (5/13; 38.5%) of the infected dogs were always kept in a yard when at home (χ^2=7.1, df=2, p=0.03). Compared with dogs that were rarely or never kept in a yard at home, dogs that were sometimes or regularly in the yard were similarly infected with *E. multilocularis* (OR and 95% CI: 1.0; 0.1-8.5) but dogs that were always kept in the yard were 5.0 times (95% OR: 0.5-37.8) more likely to be affected (*Figure 10*).

Spatial Factors

Almost half (6/13; 46.2%) of the infected dogs lived near Bowmont Park (BM) (51.1024° N, 114.2089° W) (*Figure 11*). When comparing the proportion of infected BM dogs to those living in all

other sampled areas, we found that more BM dogs were infected than all other dogs ($\chi^2 = 5.0$, df = 1, $p = 0.03$) (*Figure 9*) and BM dogs were 3.3 (95% CI: 1.1-10.0) times likely to be infected than dogs living near other city parks (*Figure 10*).

DISCUSSION

Previously, only two studies have been conducted on the prevalence of *E. multilocularis* in domestic dogs in Canada (103, 104), both of which failed to find evidence of infection. However, in this study, we found that the prevalence in dogs living around dog parks in Calgary, AB resembled reported estimates from Eastern Europe and Asia (133). Additionally, parasite eggs recovered from the fecal samples of infected dogs in this study were revealed to belong to the E haplotype of *E. multilocularis* (145) which more closely resembles the strains endemic to Europe (M1 specifically) and has been detected in both wildlife and humans in AB (18). The absence of the North American haplotype in dogs could indicate that it occurs less frequently in urban environments and/or that it is less infectious than the European-like haplotypes that are now found most often in dogs, wildlife, and humans across AB (18).

Compared to other dog breeds, the high numbers of infected scent hounds, which showed a significant relationship with likelihood of intestinal infection by *E. multilocularis*, and terriers in our study could be explained by their historically human-selected behavioural traits. Over 400 dog breeds currently exist and are distinguished by varying appearance and behaviour (146). This large number of distinguishable breeds historically developed due to selective breeding so that dogs could fulfill certain functions and achieving standards like those set by kennel clubs such as the CKC (147). Specifically, hound and terrier classes were bred for independent hunting and for flushing and catching rodents (146) and hunting behaviour remains an intrinsic trait of these breeds (148). It is therefore likely that this study's hounds and terriers captured and consumed more rodents and had a higher per capita rate of exposure to *E. multilocularis* through infected prey.

No *E. multilocularis* study has ever estimated the level of risk associated with specific dog breeds. The results of *E. multilocularis* studies are frequently in disagreement about whether purebred dogs are more likely to carry gastrointestinal parasites than mixed breeds (149) or *vice versa* (150), whether likelihood of infection is dependent on the dog breed and type of parasite (151), or whether the difference in infection levels between breeds is actually absent (152-155). It is therefore important to determine the relationship between dog breed, resulting intrinsic behaviours, and the likelihood of carrying dangerous zoonotic parasites such as *E. multilocularis*.

Unexpectedly, our study found that dogs were more likely to be infected with intestinal *E. multilocularis* when they were always kept on-leash at city dog parks. A previous study found that dogs kept more frequently off-leash were more likely to be infected with *Toxocara canis* – which employs a similar route of transmission to *E. multilocularis* (156). Similarly, Smith *et al* found that park-attending dogs that were frequently off-leash were more likely to be parasitized by *Giardia* spp. (137). The discrepancy with previous findings could perhaps be explained by the fact that the two rodent-hunting breeds that were most infected in this study (scent hounds and terriers) are also known to be less trainable to follow owners off-leash (148). It is however plausible that such dogs were in contact with rodents in other contexts than off-leash areas in parks (see below).

While several studies on gastrointestinal parasitism in dogs have found a positive correlation between park attendance and likelihood of infection (92, 137, 157, 158), our study seems to imply the opposite when *E. multilocularis* is the target parasite. Rather, infection was more highly associated with time spent in the yard at home. Roaming a yard for hours a day unsupervised may present opportunities for dogs to predate small mammals that may live on the property such as rabbits, mice, and voles (159). Dogs that prey upon rodents are 2.9 times more likely to be parasitized by endoparasites (160) and thus, the presence of rodents in the yard could provide a likely route of transmission of *E. multilocularis* from intermediate hosts to dogs.

The influence of the neighborhood's environment on *E. multilocularis* infections in dogs is one of the most important risk factors highlighted in this study. Dogs that were sampled around BM had a significantly higher prevalence of *E. multilocularis* than those living around other parks (*Figure 10*) even though demographics were constant across all groups. This could be due to the high prevalence of the parasite in wild intermediate and definitive hosts that were also sampled in and around BM at the same time (136). Our findings support the notion that wild canids and rodents present in urban areas can be sources of zoonotic infection in accidental hosts such as humans and dogs (160), with rodents acting a reservoirs for 46% of all global zoonoses (161).

In 2016, there were 350,070 pet dogs licensed in Calgary, AB (2016 Census Data). If our study is a true representation of the overall dog population in Calgary, it could suggest that up to 14,000 dogs in this city could be shedding infectious eggs through their feces which could be ingested by their owners (and possibly resulting in AE (61)) via multiple routes: directly, by petting or handling dog hair where eggs have attached (35, 55); indirectly, through defecation of eggs into vegetable gardens (112) or by transfer to the household (56, 57, 112). However, we have thus far only summarized the *E. multilocularis* situation of Calgary in 2012, and due to the recent increase in human AE numbers in Calgary and across AB (17, 18), intestinal infections in domestic dogs need to be more thoroughly studied and updated. More broadly, urbanization and the encroachment of residential areas upon wild landscapes provides ample opportunity for parasites like *E. multilocularis* to take advantage of new routes of transmission provided by the increase in urban-adapted wildlife hosts (31). Overlapping habitat between domestic dogs and urban-adapted wild hosts (56, 57) thereby enables the *E. multilocularis* cycle to be maintained by dogs (21), and for the infection to be transmitted to human owners (61) in metropolitan areas like Calgary, AB.

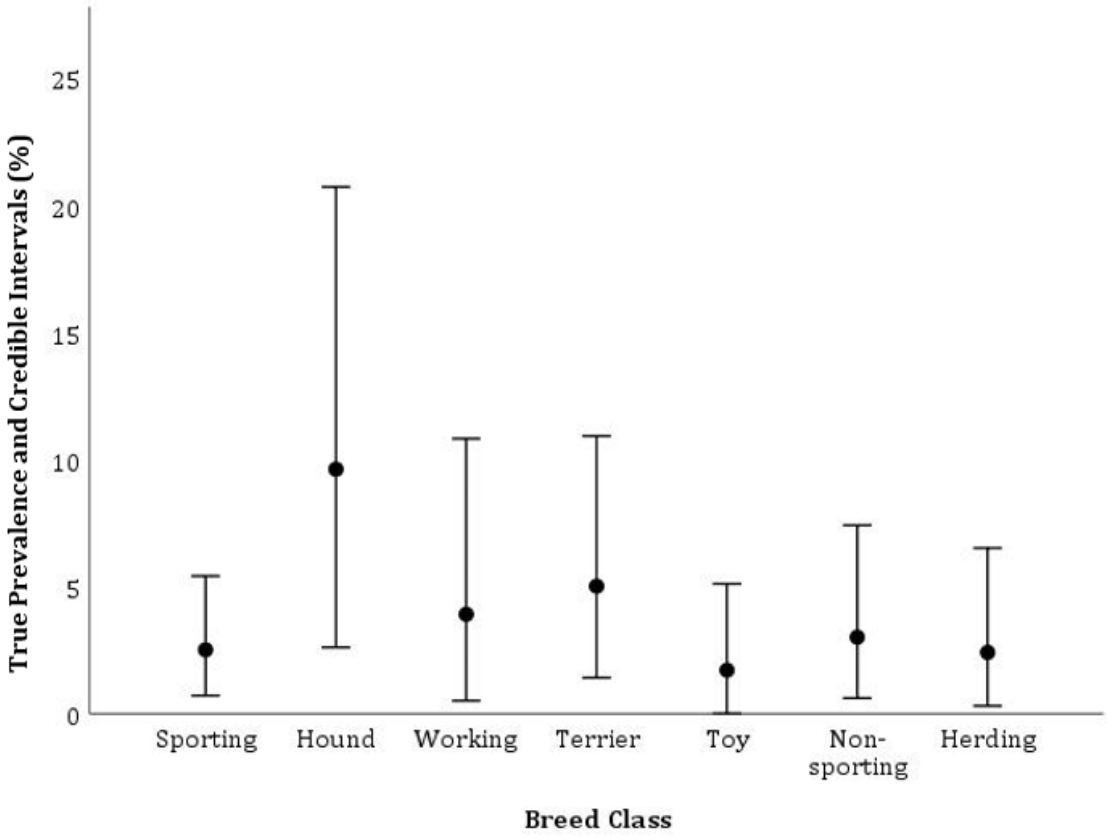


Figure 8: Bayesian true prevalence and associated 2.5 and 92.5% credible intervals of intestinal *Echinococcus multilocularis* infection in each class of dog breed, as designated by the Canadian Kennel Club. Dogs were sampled in communities bordering several parks in Calgary, Alberta, in 2012.

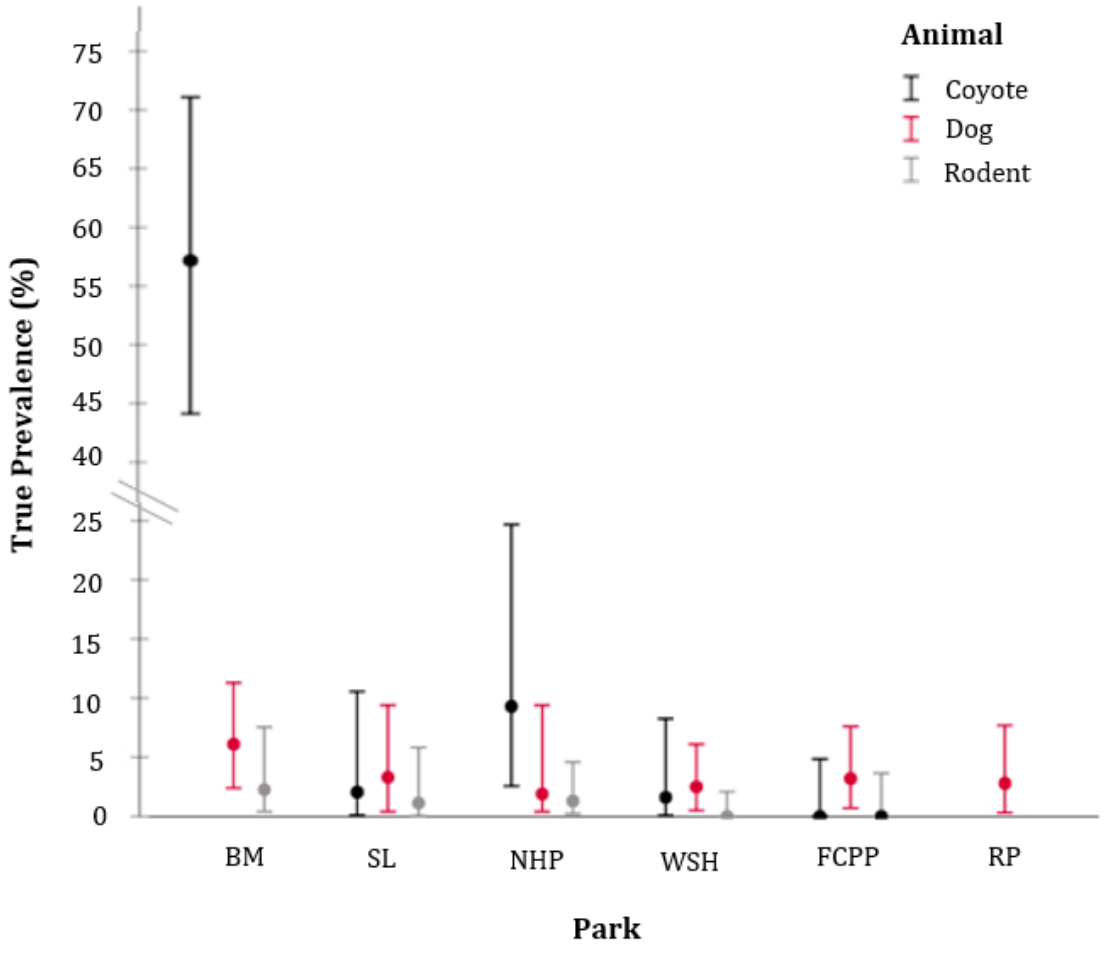


Figure 9: Bayesian true prevalence (and credible intervals) of *Echinococcus multilocularis* in dogs, coyotes, and rodents in Calgary, Alberta, Canada in 2012. Dogs were sampled in postal codes adjacent to six city parks: Bowmont (BM), Southland Lowlands (SL), Nosehill Park (NHP), Weaselhead Flats (WSH), Fish Creek Provincial Park (FCPP), and River Park (RP). Coyote feces (n=385) and rodents (n=645) were sampled in and around all parks except RP. Coyote and rodent Bayesian true prevalence values were estimated using data taken from Liccioli *et al*, 2014 (136).

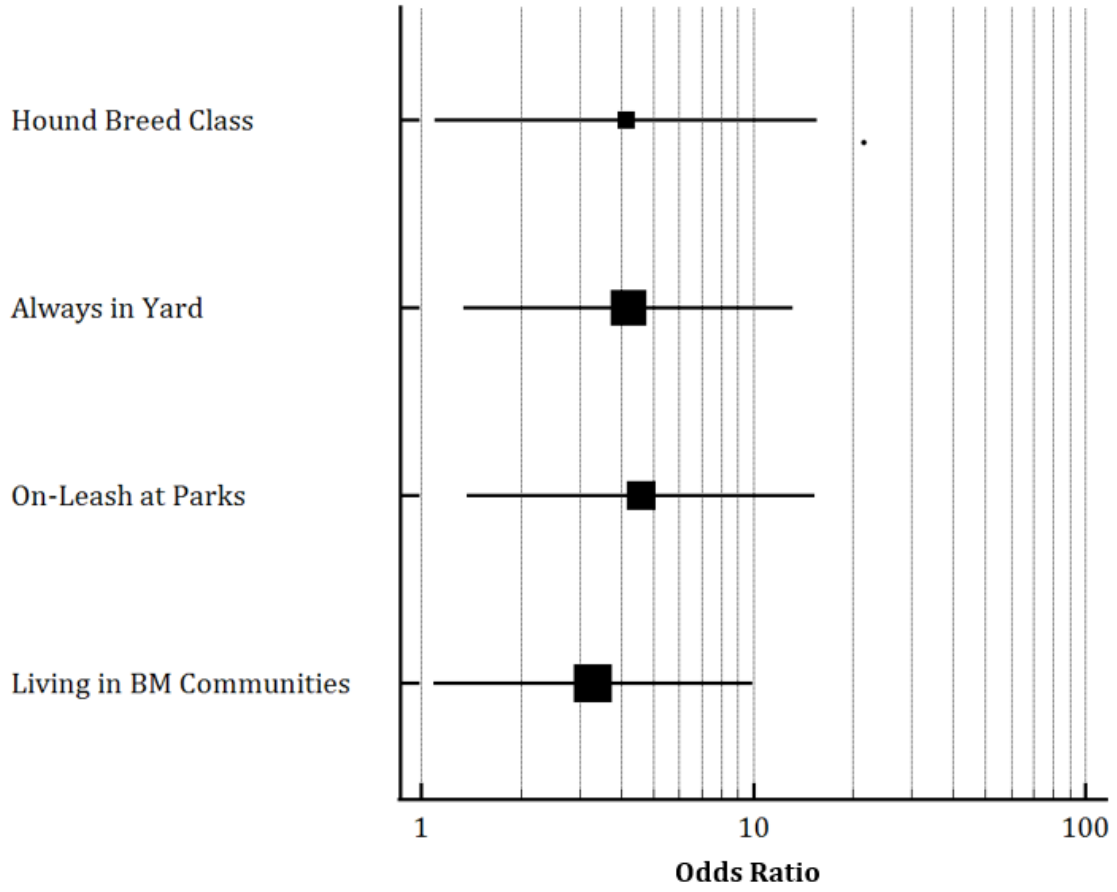


Figure 10: Odds ratios with associated confidence intervals for each risk factor that was found to have a significant relationship with presence of intestinal *E. multilocularis* infection in domestic dogs sampled in communities surrounding six Calgary dog parks (Bowmont (BM), Southland Lowlands (SL), Nosehill Park (NHP), Weaselhead Flats (WSH), Fish Creek Provincial Park (FCPP), and River Park (RP)) in 2012. Box size is scaled to the number of dogs with the associated risk factor.

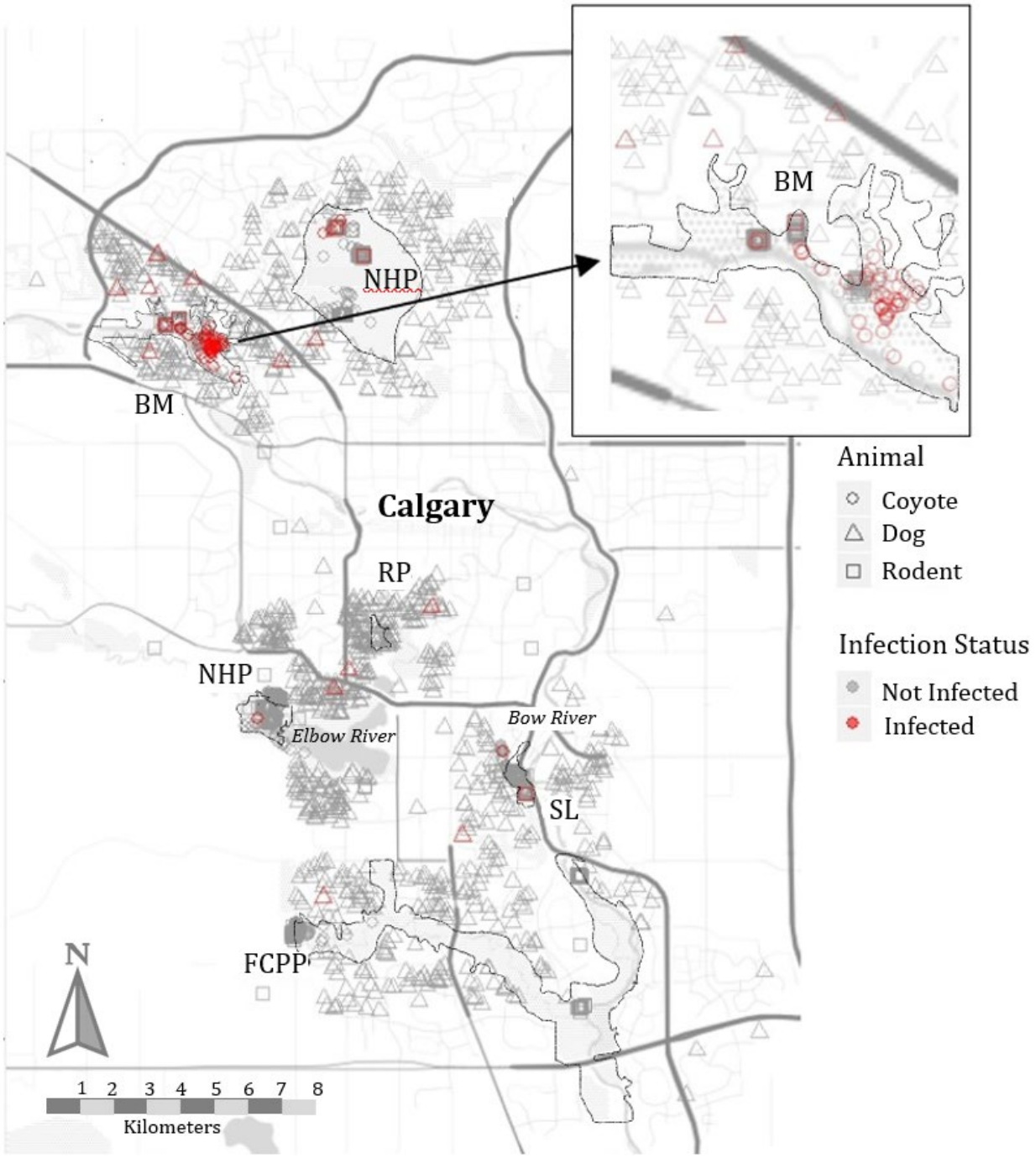


Figure 11: Location of dogs with and without intestinal *Echinococcus multilocularis* infection compared to the location of wildlife (coyotes and rodents) with and without *E. multilocularis* infections in Calgary, Alberta, Canada in 2012 (data from Liccioli *et al*, 2014 (136)). A total of 696 dogs were sampled from communities adjacent to six city parks: Bowmont (BM), Southland Lowlands (SL), Nosehill Park (NHP), Weaselhead Flats (WSH), Fish Creek Provincial Park (FCPP), and River Park (RP). Coyote feces (n=385) and rodents (n=645) were sampled in all parks and communities adjacent to all parks except RP.

Table 5: Intrinsic factors, including age, sex, neuter and spay status, and dog breed as described by the Canadian Kennel Club, characterizing 696 dogs sampled around six Calgary, Alberta dog park areas in 2012 which were screened for intestinal *Echinococcus multilocularis* infections in 2018-2020.

	Age (%)			Sex (%)		Neutered/ Spayed (%)		CKC [a] Breed Class (%)						
Park areas [b]	**Pup (<1y)**	**Adult (3-8y)**	**Senior (>8y)**	**Male**	**Female**	**Yes**	**No**	**Sporting**	**Hound**	**Working**	**Terrier**	**Toy**	**Non-Sporting**	**Herding**
WSH	1.3	57.0	41.6	51.4	48.7	96.6	3.4	33.1	6.1	8.1	14.9	9.5	14.9	13.5
SL	1.4	66.7	31.9	58.3	41.7	97.2	2.8	25.0	8.3	9.7	12.5	12.5	18.1	13.9
RP	0.9	57.4	41.7	55.7	44.4	95.7	4.4	31.3	12.2	10.4	13.0	10.4	10.4	12.2
NHP	2.4	72.2	25.4	39.7	60.3	96.8	3.2	25.4	7.1	9.5	17.5	8.7	14.3	17.5
FCPP	0.0	69.8	30.2	56.5	43.5	96.5	3.5	18.6	8.1	7.0	11.6	14.0	26.7	14.0
BM	2.7	66.7	30.6	48.0	52.1	94.6	5.4	30.8	2.7	7.5	12.3	8.2	22.6	15.8
All	1.6	64.5	34.0	50.6	49.4	96.1	3.9	28.3	7.1	8.7	13.9	10.1	17.5	14.6

[a] Canadian Kennel Club
[b] WSH = Weaselhead Park; SL = Southland Lowlands; RP = River Park; NHP = Nosehill Park; FCPP = Fish Creek Provincial Park; BM = Bowmont Park

Table 6: Proportion of time 696 dogs sampled around Calgary, Alberta dog park areas spent in the yard instead of the house, and the proportion of time these dogs were walked in other locations (parks, sidewalks and streets, mountains, acreages, or none of these).

	Time spent in yard (%)					Area most often frequented outside the yard (%)					
Park areas [a]	**Never/No Yard**	**Rarely**	**Sometimes**	**Regularly**	**Always**	**Park**	**Sidewalks/ Streets**	**School/ Sport fields**	**Mountains**	**None**	**Acreage**
WSH	6.8	4.7	6.1	68.2	14.2	55.8	37.4	0.0	0.0	6.1	0.7
SL	2.8	0.0	9.7	73.6	13.9	47.1	38.2	2.9	0.0	10.3	1.5
RP	10.5	1.8	9.6	69.3	8.8	56.1	34.6	0.9	2.8	5.6	0.0
NHP	6.4	3.2	8.0	65.6	16.8	49.2	37.7	1.6	3.3	6.6	1.6
FCPP	7.0	4.7	5.8	67.4	15.1	39.0	40.2	3.7	3.7	12.2	1.2
BM	12.2	2.7	8.8	63.9	12.2	53.6	37.9	2.1	1.4	4.3	0.7
All	8.1	3.0	7.9	67.5	13.4	51.2	37.5	1.7	1.8	6.9	0.9

[a] WSH = Weaselhead Park; SL = Southland Lowlands; RP = River Park; NHP = Nosehill Park; FCPP = Fish Creek Provincial Park; BM = Bowmont Park

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Table 7: Characteristics of 13 dogs living adjacent to Calgary, Alberta dog park areas that tested positive for intestinal *Echinococcus multilocularis* infection.

Park areas[a]	**Breed (CKC**[b] **breed class)**	**High prey drive breed**	**Sex**	**Age (y)**	**Area most-walked**	**Epg**[c]
BM	Bichon frise (6)	Yes	Female	6	Sidewalk/street	3.8
BM	German shepherd/boxer (3)	Yes	Female	2	nd[d]	12.5
BM	Labradoodle (1)	No	Female	3	Dog park	4.8
BM	Labrador/shepherd (1)	Yes	Female	3	Sidewalk/street	5.0
BM	Bichon frise (6)	Yes	Female	10	Dog park	2.4
BM	Golden retriever (1)	No	Female	2	Sidewalk/street	2.5
FCPP	German shepherd/Belgian Malinois (7)	Yes	Male	7	Dog park	10.0
NHP	Collie/terrier (4)	Yes	Female	8	Mountains	2.5
RP	Miniature dachshund (2)	Yes	Male	14	Dog park	16.0
RP	Terrier (4)	Yes	Male	9	Sidewalk/street	14.3
SL	Basset hound (2)	Yes	Female	5	Dog park	0.9
WSH	Redbone coonhound (2)	Yes	Male	11	Sidewalk/street	2.6
WSH	Kerry blue terrier (4)	Yes	Female	6	Sidewalk/street	19.1

[a] Park areas include: Bowmont Park (BM), Fish Creek Provincial Park (FCPP), Nosehill Park (NHP), RP (River Park), Southland Lowlands (SL), and Weaselhead Park (WSH)
[b] Canadian Kennel Club
Eggs per gram of fecal sample
[c] No data supplied by participant

CHAPTER 4: SURVEYING INTESTINAL AND HEPATIC INFECTIONS OF *ECHINOCOCCUS MULTILOCULARIS* IN CLIENT-OWNED DOGS: PROTOCOL AND RECOMMENDATIONS

ABSTRACT

Domestic dogs are unique in their ability to act as two different types of hosts for *E. multilocularis*, a parasitic helminth. First, as definitive hosts, they can contract intestinal echinococcosis, shedding infective eggs through their feces to be ingested by intermediate hosts. Also, when dogs accidentally ingest these infective eggs, they can develop alveolar echinococcosis as dead-end hosts and the resulting lesions on the liver and other organs can be lethal. Apparent prevalence of intestinal echinococcosis in dogs is documented in many countries across the northern hemisphere and several risk factors for infection have also been identified. However, studies have been relatively unstructured, often lacking true prevalence estimation, risk factor questionnaires and analyses, or both. Alveolar echinococcosis prevalence in dogs has never been estimated. Therefore, I conducted a pilot study in Calgary, Alberta (AB) to assess the feasibility of a future investigation into the prevalence of both intestinal and alveolar echinococcosis in dogs. I sampled apparently healthy dogs from veterinary clinics, collecting blood sera and feces for alveolar and intestinal echinococcosis determination respectively, While the presence of *E. multilocularis* could not be confirmed in this study, we anticipate that a future, larger investigation utilizing our study design and sample methods could accurately detect infections and determine the significance of various risk factors. Sampling dogs out of veterinary clinics both encourages collaboration with veterinarians and simplifies serum collection. Dog ownership is a significant risk factor for human alveolar echinococcosis, but this risk may be heightened in veterinarians who

work with numerous potentially infected dogs each week. Estimation of intestinal and alveolar echinococcosis in dogs is therefore important from both human and animal health perspectives.

INTRODUCTION

Echinococcus multilocularis, a zoonotic tapeworm, normally cycles through wild canids (definitive hosts) and rodents (intermediate hosts) (20, 24) but can also be maintained by domestic dogs (21). As definitive hosts, dogs can pass on the infection to humans (27, 61), other dogs, and themselves (29, 49). Dogs can also become infected with alveolar echinococcosis (AE) (48, 49), contracting the same – often lethal – infections as intermediate hosts and dead-end hosts (*e.g.* humans) (4, 24, 48). The close association between humans and their pets (36) enables *E. multilocularis* to be transmitted from dogs to humans directly (through petting dog fur where *E. multilocularis* eggs have transferred and attached) (35, 55) or indirectly (through fecal contamination of households). They can therefore be considered both sentinels of infection – indicating high levels of environmental contamination with *E. multilocularis* (39) – and also sources of infection to the human population (99, 100). In humans, AE has been widely studied, as *E. multilocularis* is globally considered a highly important parasite (12, 44), including in Canada, where human AE cases have been increasing noticeably in the last decade (17, 18).

Few studies have analyzed the prevalence of *E. multilocularis* infections in dogs living in urban areas (133) despite the known effect of urbanization on the transmission of *E. multilocularis* (55, 58). In rural environments, the main definitive hosts responsible for sustaining the *E. multilocularis* lifecycle are wild canids such as coyotes, foxes, wolves, and raccoon dogs (20, 21, 46). In urban and suburban areas, these species are scarcer (but still present) (54, 55) so domestic dogs gain increasing importance in maintaining the *E. multilocularis* lifecycle (58). Compared to wild canids, the worm burden carried by domestic dogs infected by *E. multilocularis* is much lower (21). However, on average, dogs shed more eggs per adult worm through their feces than their wild

counterparts do and have been proven capable of perpetuating the spread of *E. multilocularis* in urban areas (21, 59, 101).

If pet-owners face some risk of *E. multilocularis* infection transmitted from their dog, the potential risk to small animal veterinarians, who treat and examine several dogs per day, could be markedly higher (41, 42). It is therefore important to involve veterinarians in any studies evaluating prevalence of this parasite (40, 42). Veterinarians and animal care staff can play many important roles in the study of *E. multilocularis* in dogs. Not only can they spread awareness of zoonoses among their clients, they can also deworm and vaccinate animals against these pathogens. Lastly, veterinary clinics provide a source for efficient sampling in domestic dog studies because testing for zoonoses such as *E. multilocularis* can be performed easily during regularly-scheduled appointments (42). We therefore designed a pilot study to take place within veterinary clinics in Calgary, AB, Canada to guide future studies in estimating the true prevalence of *E. multilocularis* infections in urban domestic dogs as well as important risk factors for these infections.

The overall objective of this pilot study was to develop a feasible study on *E. multilocularis* infections (both hepatic and enteric) in owned domestic dogs recruited by veterinary clinics in a metropolitan area. Specifically, we aimed to: (1) generate a survey for dog owners that adequately assessed risk factors for dog infection by *E. multilocularis*; (2) create an efficient protocol for sampling blood and feces from dogs owned by existing clients of several veterinary clinics; and (3) analyze data on dog demographics and *E. multilocularis* infection status in a small sample of urban domestic dogs.

MATERIALS AND METHODS

STUDY AREA AND PARTICIPANT RECRUITMENT

The investigators received ethics approval from the Calgary Faculties Research Ethics Board, #REB18-1471.

A convenience sample of four private veterinary clinics in Calgary, AB (51.0447° N, 114.0719° W) were selected to participate in the pilot study. Recruitment materials were shared with veterinarians in a mailout from the Alberta Veterinary Medical Association (ABVMA) (*Appendix B1*). The same information was shared again at a local veterinary workshop on zoonotic diseases. Interested clinics approached the investigators about participating in the study. Clinics were included for the study if they were companion animal veterinary clinics in the city of Calgary, licensed by the ABVMA. We met with the lead veterinary practitioner at each selected veterinary practice between June and December of 2019 to inform staff members on the participant recruitment and sample collection processes, providing them with written background on the study (*Appendix B2*) , instructions for the participant recruitment (*Appendix B3*) and sampling supplies. At each clinic, staff were instructed to randomly select ten owned dogs from their appointment schedule based on the following criteria: (1) the dog was healthy (*i.e.* they were simply scheduled for a vaccination appointment or general wellness check-up); (2) the dog was not of a "toy breed" (as defined by the Canadian Kennel Club (CKC)) because we did not expect to find infections in toy breeds; (3) the dog owner ("client" hereafter) consented during their appointment to completing a risk factor questionnaire and having their dog's blood and feces collected and submitted to the study. Clinics were asked to not sample more than two dogs per week, so as not to overtly bias toward appointments occurring all in the same week.

SURVEY DESIGN AND ADMINISTRATION

During the client's scheduled appointment, this study was introduced by their regular veterinarian, who distributed recruitment information (*Appendix B4*) and obtained informed consent if the client was interested (*Appendix B5*). The dog behaviour questionnaire was also shared at this time (*Appendix B6*),

The dog behaviour questionnaire was comprised of 15 questions, which were separated into four sections which were titled: (A) "Screening Questions"; (B) "Questions about your Dog"; (C) "Walking your Dog"; and (D) "Your Dog's Eating Behaviour". A similar questionnaire was used by Smith *et al* (137) to determine risk factors for gastrointestinal parasites in urban dogs, which we adapted for our *E. multilocularis* study. Specifically, we shortened the survey to improve participant completion We added more detailed questions (*e.g.* on predation and scavenging behaviour) to focus the questionnaire more specifically on *E. multilocularis* risk factors, rather than gastrointestinal parasites more generally.

Sampling procedure and collection

Blood and fecal samples were collected from each dog upon completion of the dog behaviour questionnaire. Blood serum samples were collected by veterinary practitioners during the client's appointment. The client was then asked to collect a fecal sample from their backyard in a labeled plastic bag and return it to the clinic at their earliest convenience. Once each clinic had collected ten sets of completed questionnaires, serum samples, and fecal samples, all samples were collected (between August 2019 and January 2020). Materials from each individual client were labelled with a unique identifier; the veterinary clinic kept the key for all the unique identifiers of their clients.

Sample processing and DNA extraction

Whole blood samples were spun and serum was collected. Serum samples were immediately frozen and stored at -20°C for future processing. Fecal samples were first frozen at -80°C for 72 hours to inactivate *E. multilocularis* eggs (23)and then stored at -20°C until processing.

DNA extraction

DNA was extracted from 200mg of each fecal sample using the Omega Mag-Bind® Universal Pathogen DNA extraction kit (#M4029-01) as per the manufacturer's instructions, with one

amendment: we added five cycles of freezing with dry ice for 1 minute and heating on a 70°C heat block for 1 minute as outlined in Klein *et al*, 2014 (162) between the initial homogenization step and the addition of proteinase K to the sample in order to release the parasite oncosphere from its resilient outer shell (23). Automated DNA extraction was performed using a MagMAX™ Express 96 (Applied Biosystems).

qPCR FOR DETECTION OF E. MULTILOCULARIS

To detect presence of *E. multilocularis*, a duplex qPCR reaction of the mitochondrial gene *nad2* (139) and an internal amplification control (IAC) (140) was performed as described in Santa *et al*, 2018 (139) using a C1000™ Thermal Cycler Chassis with CFX96™ Optical Reaction Module (Bio-Rad) and visualized using the CFX Maestro™ Software. Nad234 primers were used to amplify *E. multilocularis* DNA while IAC was used to assess the presence of PCR inhibitors in the sample. The sensitivity and specificity of this test were 87.1% and 100% respectively (141). Samples negative for IAC amplification were diluted using a ten-fold dilution and re-run (139) again in duplicate using the same procedure to overcome PCR inhibition.

DATA ANALYSIS

Completed dog behaviour questionnaires were inputted into the social statistics computer software SPSS *v.26* (IBM®, Armonk, NY, US). For categorical data, we used chi-square goodness of fit to compare the proportions of the sample that fell into different demographic categories (*e.g.* dog breed, gender). For non-parametric Likert-scale responses, we used Kruskal Wallis one-way ANOVA to compare medians between different groups of dogs (*e.g.* by walking locations such as dog parks, sidewalks and streets, and school and sports fields, and by predation or scavenging behaviour). Response rates to individual questions were also summarized using cross-tabulation in *SPSS v.26*. Optimum sample size for estimating the true prevalence of *E. multilocularis* at 95% confidence using the qPCR technique described above was calculated in *R* (*version 3.5.1*) in

accordance with Humphry *et al* (163). All Likert-scale data was reported using median and interquartile ranges while means and standard errors were utilized for continuous data.

RESULTS

A total of 34 clients participated in the study. Completed questionnaires, serum and fecal samples were the available for 34 dogs. Each of the first three clinics recruited ten participants, while the last was only able to recruit four participants due to time and logistical constraints during.

QUESTIONNAIRE AND DATA ANALYSIS

Completion of individual questions was generally high, although several clients neglected to answer four of the questions, with completion rates ranging from 29/34 questions (85%) to 33/34 questions (97%) with the exception of one question, which was missing ten responses (71% completion rate). Among the 14 questionnaires with missing data, the average survey completion rate (how many questions were answered in each survey) was 93±2% while the average completion rate for individual questions was 97.0±1%.

On average, dogs in this sample were 5.2±0.6 years old and all were either spayed females (19/34; 55.9%) or neutered males (15/34; 44.1%). The breeds of the sampled dogs were distributed similarly to the population of dogs studied previously (Chapter 3) (χ^2=6.7, df = 5, p=0.2). Breeds were distributed in this pilot study as follows: 15 dogs of sporting breeds (44%), five herding dogs (15%), four hounds (12%), four terriers (12%), three working dogs (9%), two non-sporting dogs (6%), and one dog of unknown mixed-breed. No toy dogs were sampled.

All owners reported walking their dog outside their home at least two to three times per month and 56% (19/34) of dogs were walked at least weekly and equally in the following areas: city dog

parks, sidewalks and streets, school and sports fields, provincial and national parks, farms and ranches, and acreages, although they were walked in each category at a similar frequency (H=0.04, p=0.8, df=1). At least weekly, most dogs (33/34; 97%) were walked along streets and sidewalks (median: 5; IQR: 4-5) and many dogs (18/34; 53%) were also walked in dog parks weekly (median 4; IQR 1-4) (*Figure 12a*). On streets and sidewalks, dogs were always leashed (median: 0; IQR: 0-5), whereas dogs were mostly off-leash when in dog parks (median: 3; IQR: 0-5) (*Figure 12b*). Less frequently, dogs were walked in school and sports fields only approximately once per month (median: 2; IQR: 0-4) (*Figure 12a*) and were rarely off-leash here (median: 0; IQR: 0-2) (*Figure 12b*). Dogs were almost never walked in provincial and national parks (median: 0; IQR: 0-3), farms and ranches (median: 0; IQR: 0-1.75), and acreages (median: 0 IQR: 0-2.5) (*Figure 12a*) and were also never off-leash in these areas (median: 0, IQR: 0-2; median: 0, IQR: 0-2; median: 0.5, IQR: 0-3 respectively) (*Figure 12b*).

Predatory behaviour was reported in 58.8% (20/34) of dogs in this study, although success rates were low for all types of prey (*Figure 13a*). Rodents were most frequently caught (by six dogs; 17%), although still only rarely, at most (median: 0; IQR: 0-1) (*Figure 13a*). Other prey was also occasionally caught, including hares and rabbits (3/34; 8.8%), birds (3/34; 8.8%), and unknown prey (1/34) (*Figure 13a*). Scavenging behaviour was reported by almost all participants (85.3%; 29/34) (*Figure 13b*), although dogs showed no clear preference for one scavenging target over the others (H=1.3, p=0.7. df=3). Scavenged food sources included grass and plant matter (median: 2; IQR: 2-3), fecal matter (median: 1; IQR: 0-2), rodent and animal carcasses (median: 0; IQR: 0-1), and unknown matter such as anthropogenic food and garbage (median: 0; IQR: 0-0.5) (*Figure 13b*). Eighteen participants reported their dog either predating or scavenging upon rodents (52.9%). Finally, only one owner reported feeding moose, deer, or elk offal to their dog.

PARASITE PREVALENCE

The qPCR did not detect any *E. multilocularis* DNA in the 34 samples that were tested. Adjusting for the sensitivity and specificity of the qPCR, the true prevalence of *E. multilocularis* in this small sample of dogs was 0.03% (95% CI: 0.0-10.0%).

DISCUSSION

The survey completion rate was very high during this pilot study, likely due to the concise survey and straightforward administration. Participants completed the survey while at the recruiting veterinary clinic, either during their appointment or directly after, submitting the completed survey before leaving the clinic. As well, the survey (*Appendix B6*) was short (12 questions total, including two 6-part questions and three 4-part questions). Our findings agree with other researchers' conclusions that shorter surveys encourage higher response and completion rates (164).

The sample of dogs in this pilot was regularly active, especially in city dog parks and on streets and sidewalks, and their walking frequency was similar to that of the another Calgary Study (133). Additionally, in this pilot study, dogs were frequently off-leash in city dog parks, which enabled dogs to practice scavenging and predation behaviours more freely and might therefore expose them to zoonotic parasites. In Chapter 3, we found no corresponding increase in *E. multilocularis* intestinal infections and walking frequency or frequency off-leash while on walks, several other studies have found off-leash frequency to be an important risk factor for infection with *E. multilocularis* (56, 95, 165) and other gastrointestinal parasites (137, 156).

Most of the dogs sampled in this pilot were of sporting and other active breeds. Some dog breeds may practice predatory behaviour that could increase the risk of encountering *E. multilocularis* in rodent prey. We previously found that dogs in breed classes with high prey drive may have a higher probability of intestinal *E. multilocularis* infection in Calgary, AB (Chapter 3). No other study has analyzed the relationship between behaviours associated with specific dog breeds and likelihood of

E. multilocularis infection. However, the association between dog breed or breed class and infection by *E. multilocularis* is currently unclear and deserves further research. More broadly, one study found crossbred dogs to be at higher risk for infection by gastrointestinal parasites than purebred dogs (149) while another study found the opposite to be true (150). Still more research suggests there is no effect of pedigree on gastrointestinal parasitism in dogs (152, 153, 155, 166), and that all dog breeds have equal chance of contracting infection if exposed to infected materials by predation or scavenging (154).

We previously estimated through meta-analysis that dogs consuming small mammals were 4.61 times more likely to be infected with *E. multilocularis* (133). Over half of participants in this pilot reported that their dog actively predated or scavenged rodents, while only 15.5% of participants in a previous study (Chapter 3) reported these dog behaviours. This discrepancy could be due to the different breed distribution across the sampled dogs in each study and should be explored further.

In particular, scavenging behaviour of dogs and the subsequent potential risk for *E. multilocularis* infections has never been addressed before this study. Participants in this pilot study reported high frequencies of scavenging by their dogs while on walks, and that grass and other plant matter were consumed the most often. Not only can infective rodent material be consumed by dogs, resulting in intestinal *E. multilocularis*, but ingesting infective eggs in feces and water or attached to plant matter can result in canine AE (48). The mechanisms of AE contraction in dogs might be similar to those described for humans. As a food-borne disease, AE is regularly transmitted to humans through the consumption of unwashed fruits and vegetables (27, 61). While risk factors for canine AE have never been explored (133), it is likely this same route of transmission could be exploited by *E. multilocularis* eggs attached to plant matter after being expelled from other definitive hosts.

No dogs in this pilot study were positive for intestinal infection by *E. multilocularis.* However, in order to accurately estimate true prevalence in future studies at 0.95 confidence and 0.05 precision using the qPCR technique described above, the optimum sample size needed for a random sample is at least 35 (163).

STUDY LIMITATIONS

Sampling dogs out of veterinary clinics only could also be a limitation and bias this study. Dogs recruited within a veterinary clinic could be dewormed more consistently than the overall population. Vaccinating or deworming dogs can eliminate the presence of gastrointestinal infections (167, 168) and can thus negatively affect their reliability as sentinels of zoonotic diseases (37). According to veterinary records, all the dogs in our pilot had been dewormed in the last year, which could account for the lack of *E. multilocularis* cases found.

Limitations also exist in the practice of using owner questionnaires to quantify dog behaviour. Besides lower response rates in some of the survey questions, some intrinsic information, including deworming status, age, and breed, would be more reliable if gleaned from veterinary records rather than owner surveys (169). As well, participant answers to survey questions are not always accurate (169) and accuracy could further decrease when quantifying dog behaviour in off-leash areas, where the dog may be out of the owner's sight.

Lastly, *E. multilocularis* eggs may not be shed in pre- and post-patent infections (68, 69), hindering the ability to detect these infections through copro-PCR. Therefore, any study using copro-PCR as a diagnostic method will be limited by the normal lifecycle of this parasite.

RECOMMENDATIONS

There are several limitations facing this pilot study that would need to be considered before performing a full-scale study on *E. multilocularis* infections in domestic dogs. First, this pilot

excluded toy dog breeds from the sampling procedure, as budget and logistical considerations caused other breeds to be perceived as higher priority. However, the association between dog breed and possibility of infection by *E. multilocularis* has not yet been adequately quantified and we therefore should not exclude any breed from future studies. In theory, it is possible that all dogs have the same probability of infection by a pathogen if they encounter that pathogen equally (154). Encounter rates are an unknown parameter in this context, implying that our study is limited also by failing to consider the spatial aspects of infection. Specifically, we could not collect location data from participants and their dogs, and Chapter 2 shows that this is actually a very relevant factor influencing *E. multilocularis* infection in domestic dogs in Calgary, AB.

We conducted this pilot study to explore the integrity of the study protocol and randomization, test the data collection forms and questionnaire, and assess the temporal feasibility of this study (170, 171). To efficiently conduct a full-scale analysis of the prevalence of E. multilocularis infections (hepatic and enteric) in domestic dogs in AB, Canada, we recommend several adaptations to the current pilot study.

First, we recommend drastically increasing the sample size of dogs across AB to adequately estimate a true prevalence of *E. multilocularis* likely sampling an equal number of dogs in each major metropolitan area (Calgary and Edmonton). To address the spatial aspect of *E. multilocularis* infections, several clinics in each city quadrant should be randomly selected to the study. In addition to fecal samples, we also recommend taking serum samples from each animal to assess the prevalence of canine AE in each urban center.

We recommend that sampling should continue to occur in veterinary clinics so that veterinarians can continue to be involved in this study, as they are potentially at increased risk of infection by *E. multilocularis* due to the large number of dogs they interact with daily. As well, clients will be comfortable with their veterinarian, and therefore more likely to enroll in the study. Working with veterinary clinics also ensures that serum collection is performed by trained

personnel. Lastly, positive results can be communicated to veterinarians, who can guide the participant through any necessary veterinary care the affected dog may need. Owners and other contacts could also be alerted to take precautions to preserve their health.

Amendments to our current study design should be made, to properly assess the entire dog population. All dog breeds should be considered for the full-scale study so that any association between breed and *E. multilocularis* infection status can be determined. It also may be useful to obtain specific deworming information for each dog from the veterinary clinic, so researchers have insight on whether there is a relationship between lack of parasite and prophylaxis. Other information should also be gleaned from veterinary records, including dog age and breed (as susceptibility may vary), when possible.

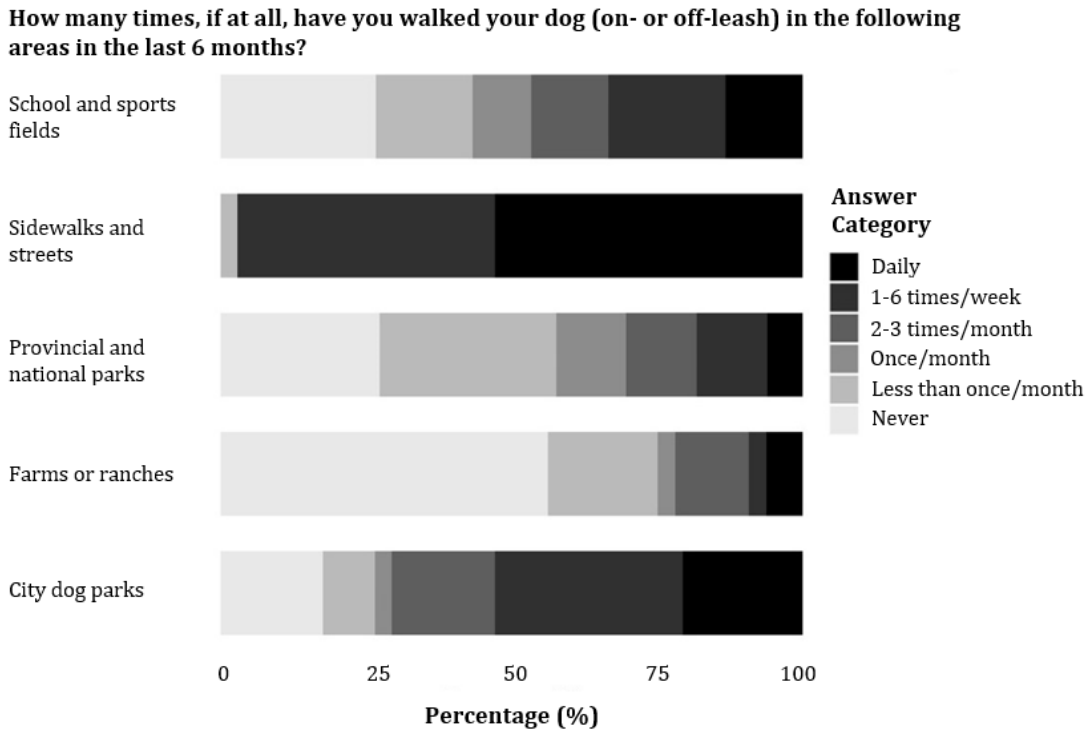
We also recommend changes be made to the existing dog behaviour questionnaire. Specifically, recording the location where the dog lives (*e.g.* postal-code, community name, or house address) should be considered, as well as the name or location of the dog park the dog is walked in most frequently. This will help to address the spatial component of *E. multilocularis* infection, as we have seen varying levels of infection in different areas within Calgary, AB (Chapter 2), but the causative factors are unknown. For the full-scale study, the questionnaire should also be administered online. Web-based surveys are more efficient in larger studies, can be designed to ensure participants answer every question before submitting, and are a cost-effective alternative to paper surveys (172).

Lastly, survey questions should also be added that address the possibility of infection in the owner's backyard, as time in yard contributed to increased risk of infection in dogs in Calgary, AB (Chapter 2). Specifically, participants should be asked about the amount of time their dog spends in the backyard during the week, and the presence of possible shelters for rodents – potential intermediate hosts for *E. multilocularis* which could be predated by dogs – such as woodpiles, sheds, decks and compost piles (159, 161).

In this pilot study, no dogs tested positive for intestinal *E. multilocularis* infection. However, it allowed us to assess several factors influencing the success of a full-scale study on the prevalence of hepatic and enteric *E. multilocularis* infections in domestic dogs. We were able to identify and propose solutions for issues in both the study design and survey. From this pilot, we then formulated a procedure for future studies sampling dogs out of veterinary clinics (*Figure 14*) that offers two methods of conducting surveys and sampling during client appointments, depending on the preference of the veterinary clinic. Future studies can choose to recruit participants either before their appointment – in the case of busy clinics with efficient communication methods in place – or during their appointments – for clinics with longer appointment times and less electronic communication with clients prior to appointments. Standardizing study design for zoonotic studies on domestic dogs can encourage more research in this area, which is important for increasing our knowledge on *E. multilocularis* and similarly transmitted pathogens affecting both dogs and humans.

Calgary, AB has a dog population of above 350,000 (2016 census data) and may be inhabited by numerous dogs that are shedding *E. multilocularis* eggs in their feces. Infective eggs can attach to the fur of their canine host (35), which can then be tracked into the living areas of their owners and potentially be ingested by humans resulting in AE, for which dog-ownership is a known potential risk factor (27, 61). Therefore, it is important to develop a full-scale investigation to provide an updated estimate of the prevalence of *E. multilocularis* in domestic dogs in this area using the sampling design and techniques recommended in this pilot study.

(a)



(b)

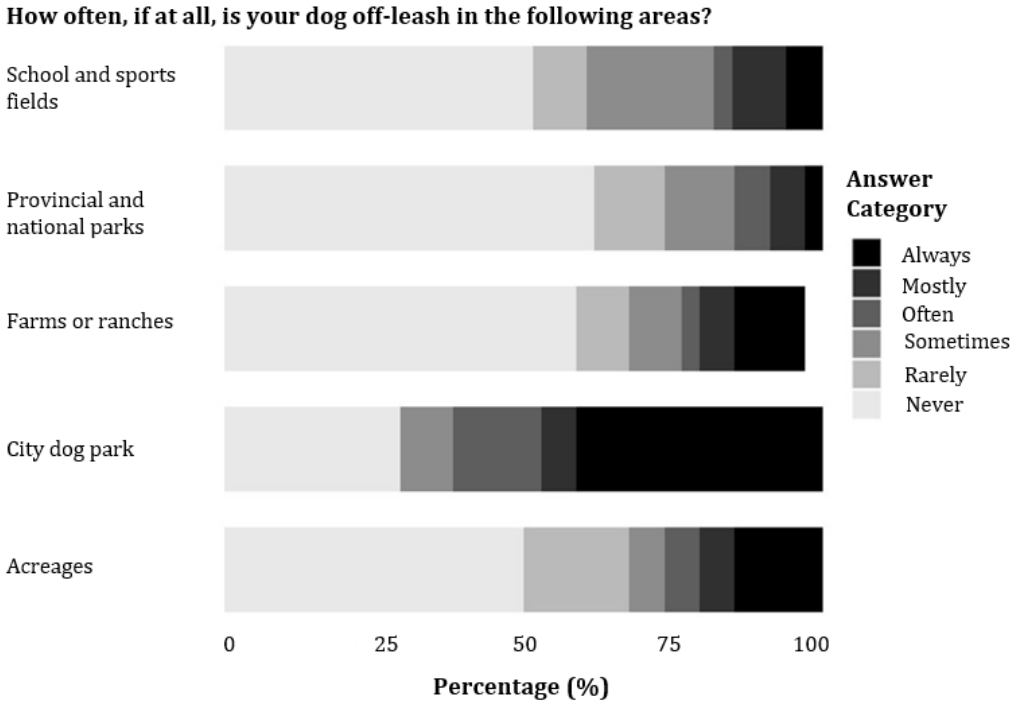
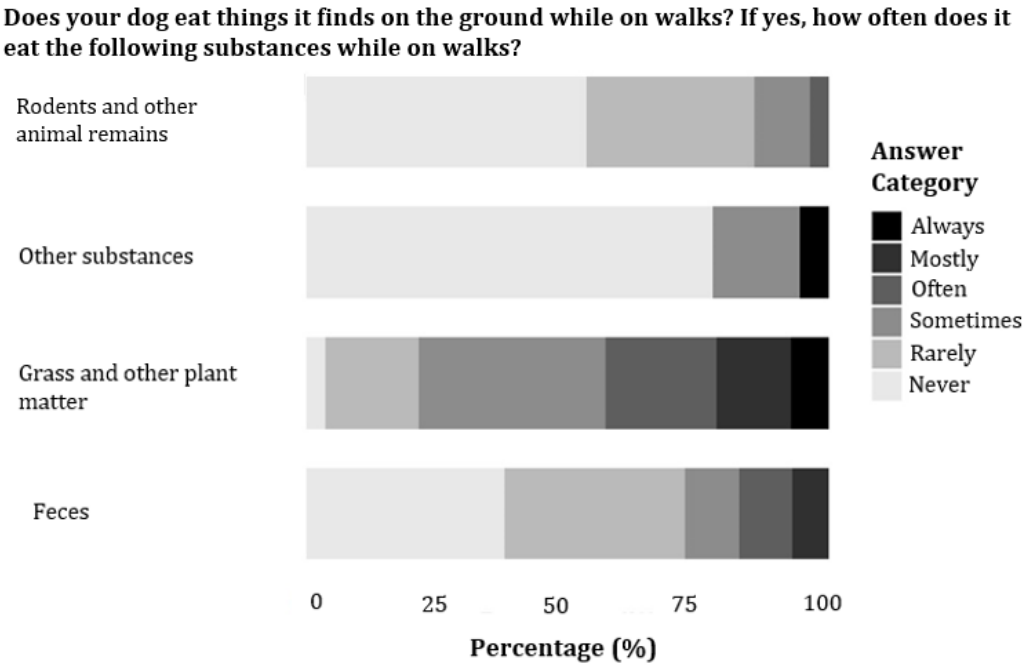


Figure 12: (a) Proportion of 34 dog-owning participants from a pilot study in Calgary, Alberta who walked their dogs in different types of areas and *(b)* the proportion of time dogs were walked off-leash in those types of areas.

(a)



(b)

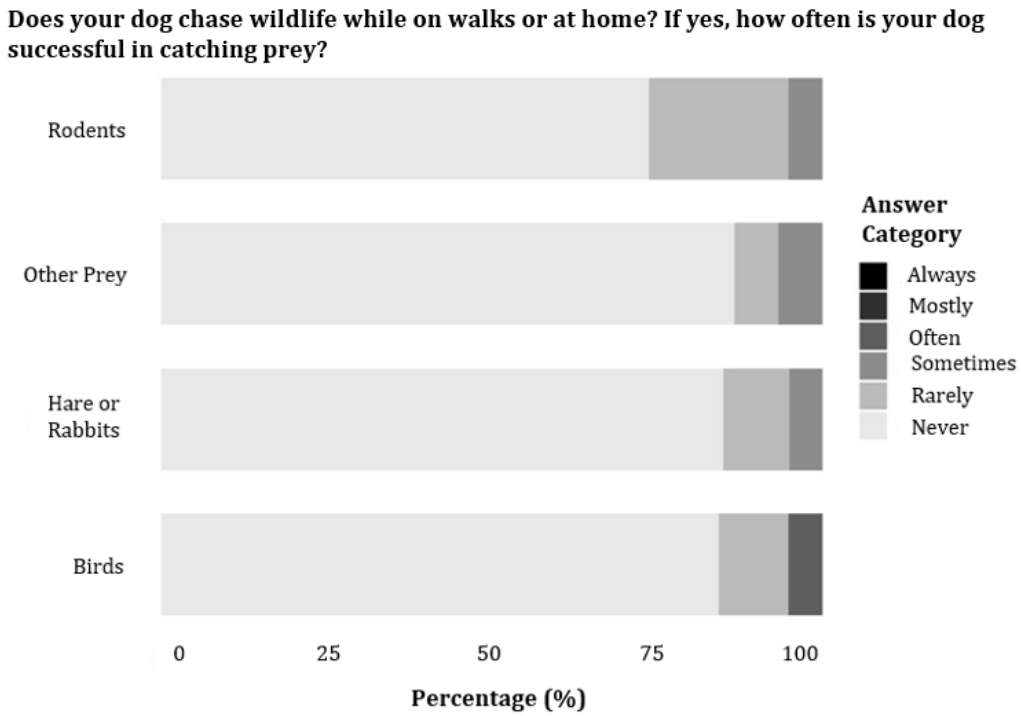


Figure 13: (a) The proportion time 34 dogs owned by participants in a pilot study in Calgary, Alberta that were successful in catching wildlife prey and *(b)* the proportion of time these dogs scavenged various food and materials while on walks with their owner.

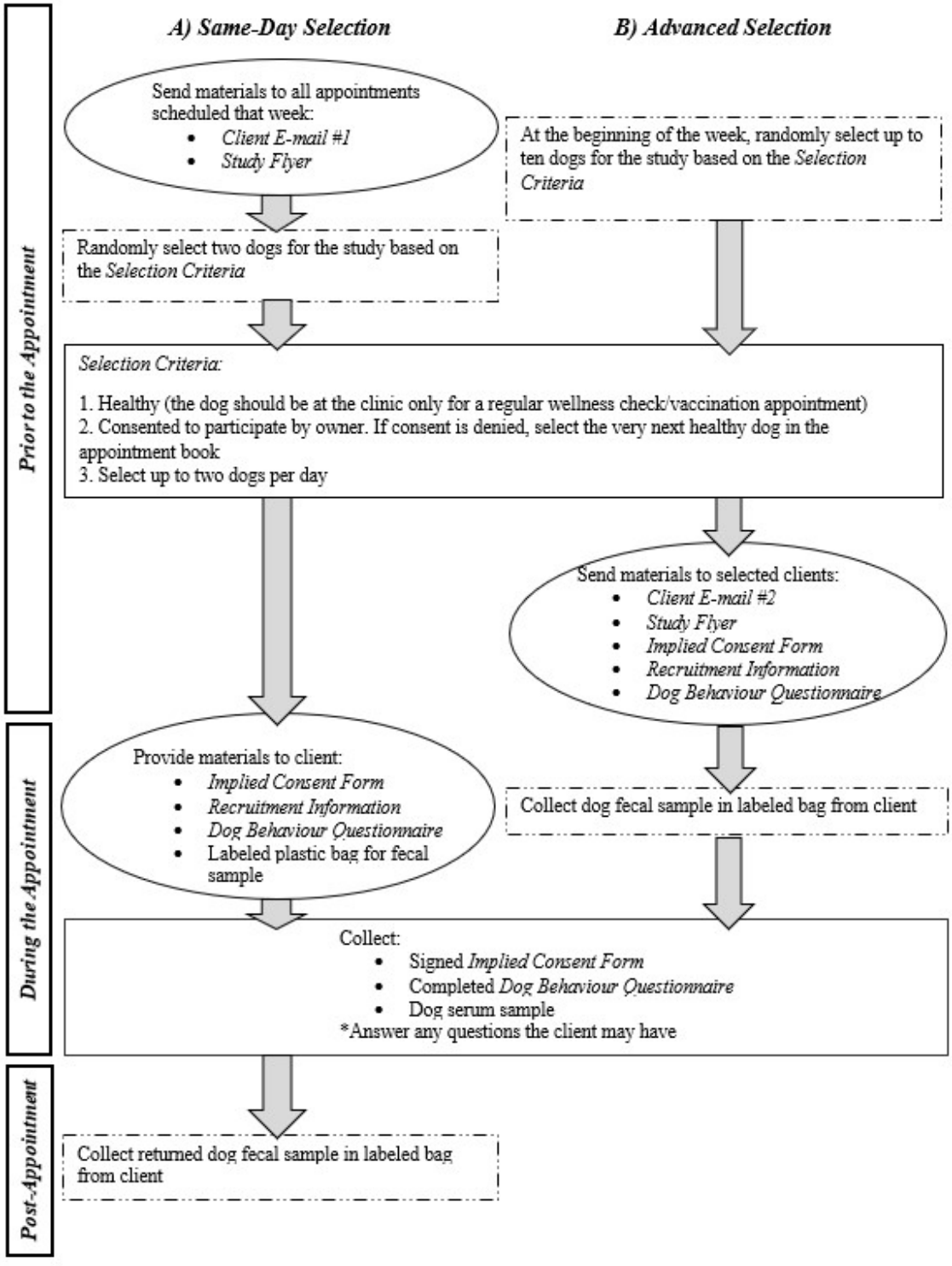


Figure 14: Recommended study design for sampling and testing domestic dogs in veterinary clinics to efficiently estimate the prevalence of hepatic and enteric *E. multilocularis* in client-owned dogs. Recommended protocol was assessed during a small pilot study on dogs in veterinary clinics in Calgary, AB in 2019 to 2020.

CHAPTER 5: SUMMARY AND STUDY LIMITATIONS

SUMMARY OF RESULTS

In my thesis I analyzed gaps in published literature investigating the prevalence of *Echinococcus multilocularis* in domestic dogs worldwide. I then attempted to fill some of these gaps by documenting intestinal *E. multilocularis* infections in dogs living in Calgary, Alberta (AB) and analyzing risk factors associated with infection. Finally, I conducted a pilot study which would account for limitations in my first study, outlining criteria for future population studies on *E. multilocularis* in domestic dogs. My results from these investigations (Chapters 2 to 4 of this thesis) are summarized below.

In **Chapter 2**, I describe and report the results from a systematic review and meta-analysis that I carried out on all the published studies on *E. multilocularis* infections in domestic dogs worldwide, estimating the true prevalence in each country. These infections were highly prevalent in Asia, especially China, which also carries the largest burden of human alveolar echinococcosis (AE) cases (4). Only three studies have been conducted in North America (84, 103, 104), and neither of the two Canadian investigations confirmed the presence of intestinal infection by *E. multilocularis* in dogs. In this review, I also estimated the pooled odds ratios for several important risk factors (living in a rural area, predation of rodents, being used for hunting, and free-roaming) affecting these infections in dogs

In **Chapter 3**, I report on my work in investigating *E. multilocularis* infections in fecal samples collected in 2012 by another graduate student (Dr. A. Smith) from domestic dogs living in communities adjacent to dog parks in Calgary, AB. I estimated the true prevalence and risk factors associated with intestinal *E. multilocularis* in domestic dogs, finding infections in 13 of 696 dogs (2.4%; 95% Credible interval: 1.3-4.0%) – a result that was higher than in previous studies. The

hound breed class showed higher frequency of infection than expected, likely due to a higher prey drive in these breeds (146, 148). Other risk factors seemed to be related to the frequency of dog park use (although, typically hounds and terriers may be left off leash less than other breeds), to time spent in the backyard each week, or living in communities surrounding Bowmont Park, an area which has already been shown to have high incidence of *E. multilocularis* infections in wildlife (142).

In **Chapter 4**, I detailed a pilot study than I ran to plan and provide recommendations on the proper estimation of true prevalence of *E. multilocularis* in a client-owned domestic dogs to estimate the frequency of exposure of veterinary professionals in veterinary clinics (practitioners, animal health technicians, and other clinic workers) in AB. In the pilot study, I tested a questionnaire previously developed for other gastrointestinal infections (137) and proposed a simplification of it to be used to better assess risk factors for both intestinal and hepatic infections with this parasite in dogs. We did not detect any intestinal *E. multilocularis* infections in the 34 dogs included in this study but did find that more dogs were observed to attempt or succeed at predating small animals including rodents, which can be intermediate hosts for *E. multilocularis*. This pilot study will provide the outline for a future study on *E. multilocularis* infections in client-owned dogs in the two metropolitan centers of AB: Calgary and Edmonton. Future findings will update the true prevalence estimated in Chapter 3 and expand upon our previous findings regarding risk factors for infection in veterinary clinics and for owners.

LIMITATIONS OF THE STUDY

As with all epidemiological studies, the determination of true prevalence of *E. multilocularis* in domestic dogs may be limited by both sample size and error due to imperfect tests. Many diagnostic techniques have been developed to detect *E. multilocularis* in purged material, scraped intestinal material, blood, and feces (173), although a gold standard for testing does not currently exist (1).

While many diagnostic techniques are commonly compared against intestinal scraping due to its relatively high sensitivity and specificity (1, 2), it is not useful when determining infection in live dogs (173). The lack of perfect testing makes evident the need to estimate true prevalence – which accounts for the sensitivity and specificity of diagnostic tests – rather than apparent prevalence – which is calculated using just the number of infected animals and the total sample size (90, 174). However, most studies analyzed in Chapter 2 did not account for diagnostic parameters, and therefore largely underestimated the actual prevalence of *E. multilocularis* in domestic dogs.

Chapter 2 was also limited by publication bias. Grey literature and other unpublished reports were not included in our systematic review. Therefore, government-run studies could have been overlooked and excluded from the analysis. This is particularly important for countries such as China, which has been running its National *Echinococcus* Control Programme since 2006 (175) but may not have published all results in scientific journals.

The original purpose of the survey and research design used in Chapter 3 was to estimate the prevalence of a broad range of gastrointestinal parasite species, rather than a narrow focus on *E. multilocularis*. Therefore, some of the risk factors addressed by the questionnaire (*e.g.* swimming, wading) correspond to other parasites (*e.g. Giardia*), while other factors which might also affect *E. multilocularis* infection (*e.g.* predation, scavenging) are not adequately addressed. As well, stratified random sampling was used to recruit participants that lived in postal codes surrounding several major city dog parks, as parks were considered potential hotspots. Thus, dogs living further away from these dog parks were not considered for the study and we cannot therefore consider the prevalence estimated by this study to be the true prevalence for the whole metropolitan area of Calgary, AB.

As well, a small sample size hinders both an accurate estimate of prevalence and the assessment of risk factors. The construction of a risk factor model for the dogs infected with intestinal *E. multilocularis* in Calgary was attempted, like in Budke *et al* 2005 (59) but it did not provide

informative results due to the stratified study design and low number of positive cases. The dogs sampled around each Calgary park were treated as separate populations – a random factor in our GLMM – and thus the number of positive dogs in each population was too small for the building of an accurate risk factor model. Additionally, survey data was not complete for all the samples we analyzed, so variables were selected to the model only if that data could be obtained for all thirteen positive samples. Factors such as walking and off-leash behaviours should be more adequately investigated to determine the precise relationship between *E. multilocularis* incidence in dogs and the behaviours and practices of them and their owners. However, the factors described here as significant, including breed, time spent in yard, and location of abode, were also subject to similar sample size limitations, and therefore should be considered strongly correlated to prevalence of *E. multilocularis*.

Our results in Chapter 3 could also have been affected by sample decay. Long-term storage of fecal samples is often accompanied by degradation of DNA (176-178). DNA degradation after storing fecal samples at -20°C (as in this study) has been found to occur after as early as seven weeks (178) and significant decline in PCR detection of host DNA has been recorded after freezing at -20°C for six months (177). Our samples had been preserved in this manner for six years prior to analysis, likely causing some DNA degradation. The high Ct values in our qPCR and the fact that sequences could not be obtained for most eggs were also consistent with degradation. Likely, this might have caused underestimation of the actual prevalence of *E. multilocularis* in this sample of dogs.

Overall, future studies should adopt the study design outlined at the end of Chapter 2, using the pilot study and recommendations in Chapter 3 as a guide for sampling domestic dogs for *E. multilocularis* testing. Sample sizes should be increased if factors influencing infections by *E. multilocularis* in dogs are to be accurately assessed. Also, as dual hosts for this parasite, dog populations should be investigated for hepatic infection prevalence as well, as this has never before

been estimated (133). While intestinal *E. multilocularis* infections in dogs can last a little longer than 90 days (21, 134), untreated hepatic echinococcosis can persist for months, and even years (48). Therefore, investigation of canine AE prevalence is the next logical step in the surveillance of *E. multilocularis* in urban environments if we are to adequately assess the risk of AE in both dog and human populations.

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APPENDICES

APPENDIX A

Appendix A1: Keyword and Boolean vector combinations used in five scientific databases to find peer-reviewed articles on *Echinococcus multilocularis* infection in domestic dogs. The number of hits from each search was recorded. The final keyword and vector combination was used to supply the literature for the current study on July 21, 2020.

Keywords	Alternate keywords (per database instruction)	Database	Hits
echinococc AND dog AND prevalence*	dog, prevalence, echinococcus, OR echinococcosis	WS	470
	(echinococcus OR echinococcosis), dog, prevalence	PM	932
		SC	617
		SD	74
echinococc AND dog AND population AND prevalence*	dog, prevalence, population, echinococcus, OR echinococcosis	WS	158
	(echinococcus OR echinococcosis), dog, prevalence, population	PM	246
		SC	229
		SD	31
echinococc AND dog AND population*	dog, population, echinococcus OR echinococcosis	WS	270
	(echinococcus OR echinococcosis), dog, population	PM	286
		SC	409
		SD	56
(multilocularis OR canadensis) AND echinococc AND dog*	(multilocularis OR canadensis) AND (echinococcus OR echinococcosis) AND dog	WS	431
	(echinococcus OR echinococcosis), (mutlilocularis OR canadensis), dog	PM	348
		SC	479
		SD	56
(multilocularis OR canadensis) AND echinococc AND dog AND prevalence*	dog, prevalence, (echinococcus OR echinococcosis), (multilocularis OR canadensis)	WS	193
	(echinococcus OR echinococcosis), (multilocularis OR canadensis), dog, prevalence	PM	226
		SC	173
		SD	24
(multilocularis OR candensis) AND echinococc AND dog AND prevalence AND population*	dog, prevalence, population, (echinococcus OR echinococcosis), (multilocularis OR canadensis)	WS	70
	(echinococcus OR echinococcosis), (multilocularis OR canadensis), dog, prevalence, population	PM	54
		SC	70
		SD	7
multilocularis AND echinococc AND dog AND prevalence*	dog, prevalence, multilocularis, echinococcus OR echinococcosis	WS	186
	(echinococcus OR echinococcosis), multilocularis, dog, prevalence	PM	207
		SC	166

		SD	23
multilocularis AND echinococc AND dog AND prevalence AND population*	dog, prevalence, population, multilocularis, echinococcus OR echinococcosis	WS	68
	(echinococcus OR echinococcosis), multilocularis, dog, prevalence, population	PM	50
		SC	70
		SD	7
(alveolar OR multilocularis) AND echinococc AND dog AND prevalence AND population*	dog, prevalence, population, (multilocularis OR alveolar), (echinococcus OR echinococcosis)	WS	77
	(echinococcus OR echinococcosis), (alveolar OR multilocularis), dog, prevalence, population	PM	57
		SC	74
		SD	8
(alveolar OR multilocularis) AND echinococc AND dog AND population*	dog, population, (multilocularis OR alveolar), (echinococcus OR echinococcosis)	WS	127
	(echinococcus OR echinococcosis), (alveolar OR multilocularis), dog, population	PM	66
		SC	121
		SD	15
(multilocularis OR canadensis) AND echinococc AND dog AND population*	dog, prevalence, (multilocularis OR alveolar), (echinococcus OR echinococcosis)	WS	119
	(echinococcus OR echinococcosis), (multilocularis OR canadensis), dog, population	PM	64
		SC	120
		SD	14
multilocularis AND echinococc AND dog AND population*	dog, population, multilocularis, echinococcus OR echinococcosis	WS	115
	(echinococcus Or echinococcosis), multilocularis, dog, population	PM	59
		SC	114
		SD	14
(alveolar OR multilocularis) AND echinococc AND (dog OR canine) AND (prevalence OR population)*	(dog OR canine), (population OR prevalence), (multilocularis OR alveolar), (echinococcus OR echinococcosis)	WS	257
	(echinococcus OR echinococcosis), (alveolar OR multilocularis), (dog OR canine), (prevalence OR population)	PM	252
		SC	228
		SD	34
(alveolar OR multilocularis) AND echinococc AND dog AND (prevalence OR population)*	dog, (population OR prevalence), (multilocularis OR alveolar), (echinococcus OR echinococcosis)	WS	277
	(echinococcus OR echinococcosis), (alveolar OR mutlilocularis), dog, (population OR prevalence)	PM	259
		SC	246
		SD	672

WS, Web of Science; PM, PubMed; SC, Scopus; GS, Google Scholar; SD, Science Direct.

Appendix A2: Exclusion criteria for the second round of screening and subsequent elimination of 122 papers from the study after the literature search was performed on July 21, 2020. Studies were removed if they did not supply an original measure of *Echinococcus multilocularis* prevalence in domestic dogs. Review papers were scanned for additional references, leading to the addition of 11 articles to the pool.

Reason for Exclusion	**Number Excluded**
Not original data	49
Echinococcus granulosus (sensu lato), not *E. multilocularis*	9
No prevalence determination (case study or experimental)	16
Human, not dog	13
wild canids, not dog	11
Other helminths, not *E. multilocularis*	17
Other animals, not dog	3
No species determination	4
Total	**122**
Number Added from Other Reviews	11

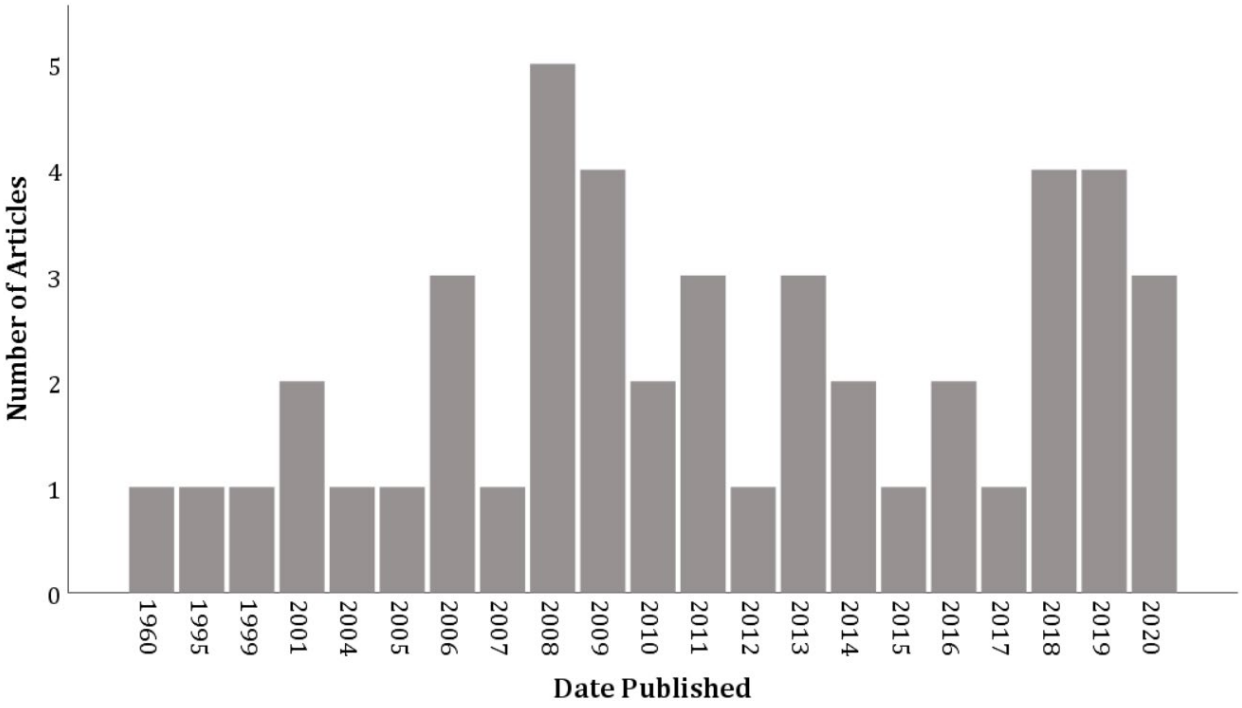
Appendix A3: A summary of the study data and diagnostic parameters used to estimate the apparent, true, and re-assessed true prevalence of *Echinococcus multilocularis* in 59 globally published studies as well as the modelled estimates for sensitivity and specificity together with their credible intervals. Bayesian methods were used to estimate apparent prevalence (AP) with 95% confidence intervals (CIs), and true (TP) and re-assessed true prevalence (ATP) with 2.5% and 97.5% credible intervals (CrI) or 0% and 95% CrI (for studies with zero positive cases). The priors used in these estimations are here reported and are based on the CIs of diagnostic sensitivity and specificity used in these studies. For studies which did not report their diagnostic parameters, the overall range of sensitivities and specificities calculated in recent re-assessments were used as prior.

Country	**Dates of study**	**Analysis Method**	**Source**	**Positive dogs**	**N**	**Apparent Prevalence**				**True Prevalence**				**Re-assessed True Prevalence**					
						prior SE(%)[a]	**prior SP(%)[b]**	**AP (%)[c]**	**AP CI[d]**	**prior SE (%)**	**prior SP(%)**	**TP (%)[e]**	**TP CrI (%)[f]**	**prior SE (%)**	**prior SP (%)**	**ATP (%)[g]**	**ATP CrI(%)**	**SE CrI(%)**	**SP CrI (%)**
Austria	2004-2005	Nested PCR	(102)	0	812	100.0	100.0	0.0	0-0.4	89.0	100.0	0.1	0-0.4	unif[h](78.9,96.3)	unif(88.2, 97.9)	0.1	0-0.4	87.3(78.9-95.4)	97.8(96.9-97.9)
Canada	2009-2010	Flotation-PCR	(103)	0	1086	100.0	100.0	0.0	0-0.3	94.0	100.0	0.1	0-0.3	unif(48.5, 61)	unif(87.3, 99.1)	0.2	0-0.5	54.5(48.5-60.3)	99(98.2-99.1)
Canada	2018	qPCR	(104)	0	44	100.0	100.0	0.0	0-8	76.0	98.0	2.9	0-8.7	76.0	98.0	2.9	0-8.5	76.0	98.0
China	2001-2007	Copro-PCR	(60)	no data	228	100.0	100.0	0.0	0-0			0.0	0-0			0.0	0-0		
China	2000	SCT	(131)	8	22	100.0	100.0	36.4	17.2-59.3	98.0	100.0	38.3	20.1-58.3	unif(82.7, 93.4)	100.0	42.7	22.3-65.4	87.9(82.9-93.1)	100.0
China	2000	SCT	(105)	8	23	100.0	100.0	34.8	16.4-57.3	98.0	100.0	36.9	19-56.5	unif(82.7, 93.4)	100.0	40.8	21.3-63.3	88.0(83-93.1	100.0
China	2002-2003	Arcoline purgation	(59)	45	371	100.0	100.0	12.1	9-16	67.0	92.0	7.4	2.1-13.2	unif(54.9, 94.2)	100.0	17.3	11.4-25	72.9(55.7-92.9)	100.0
China	2004-2005	Nested PCR (modified)	(106)	1	30	100.0	100.0	3.3	0.1-17.2	89.0	100.0	7.1	0.9-19.1	unif(78.9, 96.3)	unif(88.2, 97.9)	5.2	0.2-17	87.2(79.3-95.8)	94.5(88.9-97.8)
China	2006-2007	Nested PCR	(107)	32	142	100.0	100.0	22.5	16-30.2	85.0	100.0	27.0	19.3-35.5	unif(70,100)	100.0	27.6	18.7-38.4	84.0(70.6-99)	100.0
China	2006-2007	unknown	(108)	5	9	100.0	100.0	55.5	21.2-86.3	unif(67, 98)	unif(92, 100)	63.6	27.2-95.8	unif(40.8, 100)	unif(55.8, 100)	56.1	5.8-97.3	70.7(43-98.2)	75(56.6-98.4)
China	2004-2007	Arcoline purgation	(109)	4	74	100.0	100.0	5.4	1.5-13.3	67.0	92.0	3.9	0.1-12.5	unif(54.9, 94.2)	100.0	9.3	3-19.3	72.9(55.7-92.9)	100.0
China	2006-2007	Copro-PCR	(110)	31	276	100.0	100.0	11.2	7.8-15.6	69.0	100.0	16.7	11.6-22.4	unif(40.8, 68.9)	unif(65.3, 76.7)	1.9	0-7.6	54.2(41.2-67.8)	76.2(74.9-76.7)
China	2006-2007	Copro-PCR	(110)	4	311	100.0	100.0	1.3	0.3-3.3	69.0	100.0	2.3	0.8-4.7	unif(40.8, 68.9)	unif(65.3, 76.7)	0.9	0-3.7	52.6(41.2-67.8)	76.4(75.7-76.7)
China	2012	qPCR[i]	(82)	106	750	100.0	100.0	14.1	11.7-16.8	unif(67, 98)	100	17.6	13.3-22.8	unif(40.8, 100)	unif(55.8, 100)	12.0	0.6-29	65.5(41.7-97.5)	92.4(85.3-99.6)
China	2015	qPCR	(111)	0	256	100.0	100.0	0.0	0-1.4	86.0	93.0	0.5	0-1.4	86.0	93.0	0.5	0-1.3	86.0	93.0
China	2015-2017	Copro-PCR	(101)	25	105	100.0	100.0	23.8	16-33.1	69.0	100.0	35.3	24.3-48	69.0	100.0	35.2	24-47.7	69.0	100.0

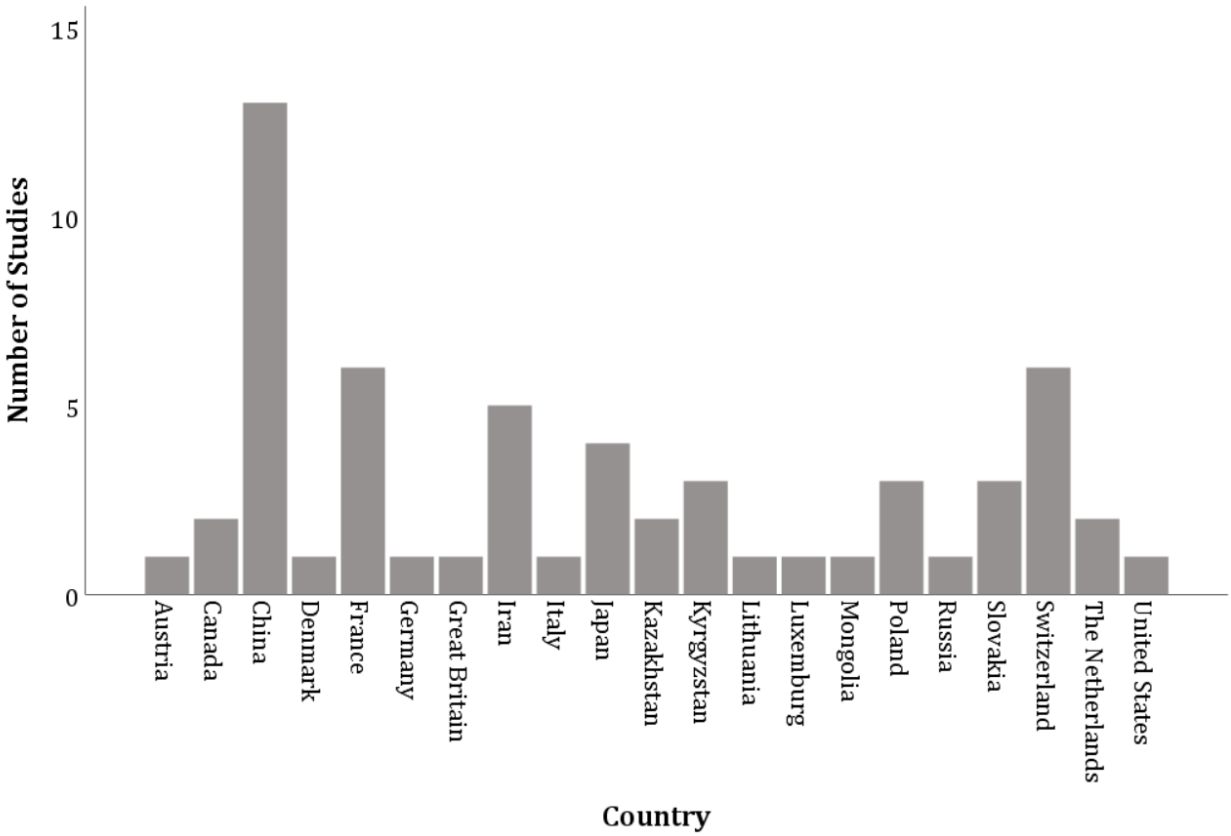
Denmark	2004-2005	Nested PCR	(102)	0	517	100.0	100.0	0.0	0-0.7	89.0	100.0	0.2	0-0.7	unif(78.9, 96.3)	unif(88.2, 97.9)	0.2	0-0.7	87.4(78.9-95.4)	97.7(96.3--97.9)
France	2008-2010	Flotation-PCR	(85)	0	367	100.0	100.0	0.0	0-1	94.0	100.0	0.3	0-0.8	unif(48.5, 61)	unif(87.3, 99.1)	0.5	0-1.5	54.5(48.5-60.3)	98.8(96.5-99.1)
France	2008-2010	Flotation-PCR	(85)	0	493	100.0	100.0	0.0	0-0.7	94.0	100.0	0.2	0-0.6	unif(48.5, 61)	unif(87.3, 99.1)	0.4	0-1.1	54.5(48.5-60.3)	98.9(97.5-99.1)
France	2006-2008	Flotation-PCR	(56)	4	817	100.0	100.0	0.5	0.1-1.2	94.0	100.0	0.7	0.2-1.3	unif(48.5, 61)	unif(87.3, 99.1)	0.4	0-1.2	54.5(48.8-60.7)	98.9(98.4-99.1)
France	2004-2005	Nested PCR	(102)	0	980	100.0	100.0	0.0	0-0.4	89.0	100.0	0.1	0-0.3	unif(78.9, 96.3)	unif(88.2, 97.9)	0.1	0-0.4	87.2(78.9-95.3)	97.8(96.8-97.9)
France	2011-2013	qPCR	(112)	2	18	100.0	100.0	11.1	1.4-34.7	89.0	93.0	11.8	0.6-32.6	86.0	93.0	12.2	0.6-34.1	86.0	93.0
France	2012-2015	qPCR	(83)	4	748	100.0	100.0	0.5	0.1-1.4	86.0	93.0	0.2	0-0.6	86.0	93.0	0.2	0-0.6	86.0	93.0
Germany	2004-2005	Nested PCR	(102)	43	17894	100.0	100.0	0.2	0.17-0.3	89.0	100.0	0.3	0.2-0.4	unif(78.9, 96.3)	unif(88.2, 97.9)	0.0	0-0	87.3(79.3-95.8)	97.9(97.9-97.9)
Great Britain	2004-2006	Nested PCR	(102)	0	121	100.0	100.0	0.0	0-3	89.0	100.0	0.9	0-2.7	unif(78.9, 96.3)	unif(88.2, 97.9)	0.9	0-2.8	87.3(78.9-95.3)	97.1(91.4-97.9)
Iran	Not specified	SCT[j]	(113)	0	29	100.0	100.0	0.0	0-12	98.0	100.0	3.4	0-10	unif(82.7, 93.4)	100.0	3.5	0-10.4	90.3(87.2-93.1)	100.0
Iran	2009-2010	Flotation-PCR	(132)	5	77	100.0	100.0	6.5	2.1-14.5	94.0	100.0	8.1	3-15	unif(48.5, 61)	unif(87.3, 99.1)	7.9	0.4-21.4	54.5(48.8-60.7)	95.1(89-98.9)
Iran	2013	Flotation-PCR	(115)	0	100	100.0	100.0	0.0	0-3.6	94.0	100.0	1.0	0-3.1	unif(48.5, 61)	unif(87.3, 99.1)	1.8	0-5.4	54.5(48.5-60.3)	98.1(88.8-99)
Iran	2013-2014	Flotation-PCR	(115)	0	167	100.0	100.0	0.0	0-2.2	94.0	100.0	0.6	0-1.9	unif(48.5, 61)	unif(87.3, 99.1)	1.1	0-3.3	54.5(48.5-60.3)	98.5(93.7-99.1)
Iran	Not specified	Copro-ELISA	(117)	0	59	100.0	100.0	0.0	0-6.1	80.0	95.0	2.1	0-6.1	80.0	95.0	2.1	0-6.3	80.0	95.0
Italy	2004-2005	Nested PCR	(102)	0	249	100.0	100.0	0.0	0-1.5	89.0	100.0	0.4	0-1.3	unif(78.9, 96.3)	unif(88.2, 97.9)	0.5	0-1.4	87.3(78.9-95.3)	97.5(94.4-97.9)
Japan	1997-2007	Copro-ELISA	(57)	18	4768	100.0	100.0	0.4	0.2-0.6	94.9	100.0	0.4	0.3-0.6	94.90	100.00	0.4	0.3-0.6	94.9	100.0
Japan	2003-2004	Nested PCR	(118)	1	183	100.0	100.0	0.5	0.01-3	89.0	100.0	1.2	0.2-3.4	unif(78.9, 96.3)	unif(88.2, 97.9)	0.7	0-2.5	87.3(79.3-95.8)	97.3(95.7-97.9)
Japan	2013-2017	Copro-PCR	(119)	3	156	100.0	100.0	2.0	0.4-5.5	unif(67, 98)	unif(92, 100)	2.0	0.1-5.8	unif(40.8, 100)	unif(55.8, 100)	2.6	0.1-8	65.6(41.7-97.6)	98.4(95.5-99.9)
Japan	2018-2019	Copro-PCR	(93)	7	98	100.0	100.0	7.1	3-14.2	unif(67, 98)	unif(92, 100)	6.1	0.4-14.9	unif(40.8, 100)	unif(55.8, 100)	7.6	0.3-21.1	65.7(41.8-97.7)	95.4(88.3-99.8)
Kazakhstan	2002	Nested PCR	(120)	6	131	100.0	100.0	4.6	1.7-9.7	89.0	100.0	5.9	2.4-10.9	unif(78.9, 96.3)	unif(88.2, 97.9)	2.6	0.1-7.8	87.3(79.3-95.8)	95.7(91.6-97.8)
Kazakhstan	2003-2005	Arcoline purgation	(61)	29	632	100.0	100.0	4.6	3.1-6.5	67.0	92.0	0.5	0-1.8	unif(54.9, 94.2)	100.0	6.6	4-10.1	73.2(55.7-93)	100.0
Kyrgyzstan	2012	Copro-PCR	(122)	4	204	100.0	100.0	2.0	0.5-4.9	69.0	100.0	3.6	1.2-7.2	unif (69,69)	100.0	3.5	1.2-7.1	69.0	100.0

Kyrgyzstan	2012	Arcoline purgation	(122)	1	20	100.0	100.0	5.0	0.1-24.9	67.0	92.0	9.8	0.3-32.2	unif(54.9, 94.2)	100.0	12.9	1.7-34.7	72.7(55.6-92.8)	100.0
Kyrgyzstan	2005	Arcoline purgation	(95)	50	466	100.0	100.0	10.8	8.1-13.9	21.0	100.0	51.8	39.1-65.7	unif(54.9, 94.2)	100.0	15.3	10-22	72.8(55.7-93)	100.0
Lithuania	2005-2006	Flotation-PCR	(94)	2	240	100.0	100.0	0.8	0.1-3	94.0	100.0	1.3	0.3-3.1	unif(48.5, 61)	unif(87.3, 99.1)	1.2	0-3.9	54.5(48.8-60.6)	98.5(97-99.1)
Luxemburg	2004-2005	Nested PCR	(102)	0	165	100.0	100.0	0.0	0-2.2	89.0	100.0	0.7	0-2	unif(78.9, 96.3)	unif(88.2, 97.9)	0.7	0-2.1	87.3(78.9-95.4)	97.3(92-97.9)
Mongolia	Not specified	Copro-ELISA	(123)	17	67	100.0	100.0	25.4	15.5-37.5	94.0	100.0	27.7	17.5-39.1	94.9	100.0	27.5	17.6-39.1	94.9	100.0
The Netherlands	2004-2005	Nested PCR	(102)	0	734	100.0	100.0	0.0	0-0.5	89.0	100.0	0.1	0-0.4	unif(78.9, 96.3)	unif(88.2, 97.9)	0.2	0-0.5	87.3(78.9-95.3)	97.8(96.6-97.9)
The Netherlands	2012-2013	qPCR	(81)	0	142	100.0	100.0	0.0	0-2.6	unif(67, 98)	unif(92, 100)	0.9	0-2.6	unif(40.8, 100)	unif(55.8, 100)	1.1	0-3.6	65.6(40.8-95.4)	99.3(94.1-100)
Poland	2015	Nested PCR	(124)	2	148	100.0	100.0	1.4	0.2-4.8	89.0	100.0	2.2	0.5-5.2	unif(78.9, 96.3)	unif(88.2, 97.9)	1.1	0-3.7	87.3(79.3-95.8)	97(94.7-97.9)
Poland	2017-2018	Nested PCR	(125)	2	145	100.0	100.0	1.4	0.2-4.9	89.0	100.0	2.3	0.5-5.5	unif(78.9, 96.3)	unif(88.2, 97.9)	1.1	0-3.9	87.2(79.3-95.8)	96.9(94.6-97.9)
Poland	2017-2018	Nested PCR	(125)	2	123	100.0	100.0	1.6	0.2-5.8	89.0	100.0	2.7	0.6-6.5	unif(78.9, 96.3)	unif(88.2, 97.9)	1.4	0-4.7	87.3(79.3-95.8)	96.7(93.8-97.9)
Russia	2017-2018	Unknown necropsy	(126)	1	28	100.0	100.0	3.6	0.1-18.3	unif(67, 98)	unif(92, 100)	6.5	0.2-20.6	unif(40.8, 100)	unif(55.8, 100)	8.5	0.3-28.1	65.4(41.6-97.7)	94.8(83.8-99.8)
Slovakia	2006	Nested PCR	(127)	1	752	100.0	100.0	0.1	0.003-0.7	89.0	100.0	0.3	0-0.8	unif(78.9, 96.3)	unif(88.2, 97.9)	0.2	0-0.6	87.4(79.3-95.8)	97.8 (97.4-97.9)
Slovakia	2002-2005	Copro-ELISA	(128)	8	289	100.0	100.0	2.8	1.2-5.4	80.0	95.0	0.8	0-2.8	80.0	95.0	0.8	0-2.9	80.0	95.0
Slovakia	2016-2019	Nested PCR	(129)	3	110	100.0	100.0	2.7	0.6-7.8	95.0	100.0	3.8	1-8.2	unif(78.9, 96.3)	unif(88.2, 97.9)	1.9	0.1-6.4	87.3(79.3-95.8)	96.3(92.8-97.9)
Switzerland	1996-1997	Flotation-PCR	(5)	6	86	100.0	100.0	7.0	2.6-14.6	89.0	100.0	8.9	3.7-16.3	unif(48.5, 61)	unif(87.3, 99.1)	8.1	0.4-21.2	54.5(48.8-60.6)	95(88.8-98.9)
Switzerland	2009-2010	Flotation-PCR	(35)	0	118	100.0	100.0	0.0	0-3.1	94.0	100.0	0.9	0-2.8	unif (48.5,61)	unif(87.3, 99.1)	1.5	0-4.7	54.5(48.5-60.3)	98.3(92.3-99.1)
Switzerland	2009-2010	Flotation-PCR	(35)	3	124	100.0	100.0	2.4	0.5-6.9	94.0	100.0	3.4	0.9-7.3	unif (48.5,61)	unif(87.3, 99.1)	3.2	0.1-10	54.5(48.8-60.7)	97.4(93.9-99)
Switzerland	2009-2010	Flotation-PCR	(35)	0	49	100.0	100.0	0.0	0-7.3	94.0	100.0	2.1	0-6.3	unif (48.5,61)	unif(87.3, 99.1)	3.5	0-10.4	54.5(48.5-60.3)	97.2(87.4-99)
Switzerland	not specified	pAb-copro ELISA[k]	(130)	2	505	100.0	100.0	0.4	0.05-1.4	84.0	99.5	0.4	0-1.3	unif (48,63.9)	unif(55.8, 75.6)	0.5	0-1.9	55.2(48.3-63.4)	75.5(75.1-75.6)
Switzerland	not specified	pAb-copro ELISA	(69)	2	660	100.0	100.0	0.3	0.04-1.1	96.0	99.5	0.2	0-0.8	unif (48,63.9)	unif(55.8, 75.6)	0.4	0-1.4	55.2(48.3-63.4)	75.5(75.2-75.6)
United States	1951	SCT	(84)	5	89	100.0	100.0	5.6	1.8-12.6	98.0	100.0	6.7	2.5-12.8	unif(82.7, 93.4)	100.0	7.5	2.8-14.1	87.9(83-93.1)	100.0

[a] Diagnostic sensitivity
[b] Diagnostic specificity
[c] Apparent prevalence
[d] 95% Confidence interval
[e] True prevalence
[f] Credible intervals. If the study had zero positives the credible intervals are given as 0, 95 otherwise they are 2.5, 97.5 credible intervals
[g] Re-assessed true prevalence
[h] Uninformed prior
[i] Quantitative PCR
[j] Sedimentation and counting technique
[k] Polyclonal antibody copro-ELISA



Appendix A4: Publication dates of the 46 dog *Echinococcus multilocularis* articles which were published prior to 21 July, 2020.



Appendix A5: Countries in which published data for *Echinococcus multilocularis* infections in domestic dogs has been investigated prior to 21 July, 2020.

APPENDIX B

APPENDIX B1

Mailout distributed electronically to Alberta (Calgary and Edmonton) veterinary clinics registered with the Alberta Veterinary Medical Association (ABVMA) for recruitment to the pilot study

Attention to all ABVMA veterinary clinics in Calgary and Edmonton:

As part of an ongoing study at the University of Calgary, we will be randomly recruiting veterinary clinics in Calgary and Edmonton to participate in a study estimating the prevalence of the *Echinococcus multilocularis* tapeworm in domestic dogs. You may be contacted by someone from our research group.

Please note: If any clinic has a dog with a suspected *E. multilocularis* infection that they wish to have tested, please contact:

Sylvia Checkley, DVM PhD
Associate Professor, Ecosystem and Public Health
University of Calgary, Faculty of Veterinary Medicine
Ph: 403-210-7409

Or

Dr. Alessandro Massolo
Adjunct Professor in Wildlife Health Ecology
Dept Ecosystem and Public Health
University of Calgary, Calgary, Alberta, Canada
Mail: amassolo@ucalgary.ca

APPENDIX B2

Study information sheet distributed to veterinary clinics that participated in the pilot study

Name of Researcher, Faculty, Department, Telephone & Email:

Emilie Toews
Faculty of Science, Department of Biological Sciences at University of Calgary
(403)-820-4812
emilie.toews1@ucalgary.ca

Supervisor:

Dr. Marco Musiani
Department of Biological Sciences, Faculties of Science and Veterinary Medicine (Joint Appointment) at University of Calgary
(403)-220-2604
mmusiani@ucalgary.ca

Dr. Alessandro Massolo
Adjunct Professor in Wildlife Health Ecology, Department of Ecosystem and Public Health at University of Calgary
amassolo@ucalgary.ca

Co-PI:
Dr. Sylvia Checkley, Department of Ecosystem and Public Health/Faculty of Veterinary Medicine
(403) 210-7409,
slcheckl@ucalgary.ca

Title of Project:

Echinococcus multilocularis in domestic dogs: intestinal and hepatic infections and risk factors

Sponsor:

Bayer Animal Health, MITACS Accelerate

The University of Calgary Conjoint Faculties Research Ethics Board has approved this research study.

Participation is completely voluntary, and confidential.

Purpose of the Study

The objectives of this study are the following:

1. To estimate the prevalence of *Echinococcus multilocularis* in dogs in Calgary and Edmonton
2. To identify risk factors for *Echinococcus multilocularis* infection in domestic dogs
3. To interpret potential implications for human and dog health
4. To determine potential implications for parkland management of pets, humans, and wildlife

What will Clinics/Veterinarians be Asked to Do?

Clinics/veterinarians will be asked to select randomly 10 dogs from their client list who are scheduled for a regular wellness check-up and/or vaccine/booster appointment. Toy dog breeds are not being sampled at this time so veterinarians should try to recruit larger, more active breeds to the study.
There are two options for selecting dogs for the study:

a) **Pre-Appointment:** Contact randomly selected clients before their appointment with information about the study, and, if they agree to participate, ask them to bring in a fecal sample to their appointment. During the appointment, collect the following: dog fecal sample, signed consent form, completed survey, and a dog blood sample to be spun down to serum.
b) **During Appointment:** Discuss the study with the client, have them sign the consent form and fill out the survey during the appointment. Collect the dog blood sample to be spun down to serum at that time and ask the participant to bring their dog's fecal sample to the clinic as soon as possible.

If a client declines to participate in the study, recruit the next client on the appointment list.

Once 10 sets of consent forms, surveys, serum samples, and fecal samples have been completely collected, contact the researchers at the bottom of this form and they will pick up the sample.

What will dog-owners be Asked to Do?

Participating dog-owners will be asked to complete a survey about their dog-walking behaviour and the medical history, age, breed, and gender of your dog. They will also be asked to provide a fecal sample from their dog which can be collected from their backyard or while walking their dog. Lastly, the participant will be asked for their permission for a veterinarian to take a blood sample from their dog. Their participation is voluntary and can be withdrawn at any point if they are uncomfortable with providing these three things. If the participant's consent is withdrawn after the collection and analysis of the samples, the samples and survey will be removed from the study. However, consent cannot be withdrawn after the first paper on this study is submitted for publication.

What Type of Personal Information Will Be Collected?

Should a dog-owner agree to participate, they will only be asked to provide the name of their dog and access to the deworming history of their dog. Other personal information will be retained by their regular veterinary clinic as only anonymized data will be used in this study. In case of a positive result, we will contact the veterinarian with the dog name and sample number and they will reach out to the participant rather than supplying us with their personal information.

Are there Risks or Benefits to the Participant?

We do not expect any risks to participants of this study. Their dog may be uncomfortable in the blood sample collection, but this will be performed by trained veterinary staff and is a safe and common procedure. If a dog has a positive result, veterinary intervention to ensure the health and safety of the participant's dog and/or family may be necessary. These treatment options will not be financially covered by this study and it will be up to the participant and their veterinarian to discuss these options. Similarly, if researchers think the participant or their family may be at risk due to a positive result from their dog, it may be recommended to the participant to visit a physician for a check-up.

If the client agrees to participate in this study, their dog's blood and feces will be tested for infection with the *Echinococcus multilocularis* tapeworm. These tests will be entirely free to them, and, with their permission, will be communicated to their veterinarian in case of a positive result so that they can make informed decisions on their pet's health. Further analyses as ultrasounds or biopsies, should they be recommended by the veterinarians, will not be covered by the project.

Through participating in this study, participants will also gain valuable knowledge into how to keep their dog safe from parasites that may be circulating in its environment. They will also be helping us raise awareness of a potentially harmful disease which will benefit both this study and the larger community of dog owners.

What Happens to the Information that is Provided?

The participant's full name will be removed from the dataset by the veterinary staff and replaced by a random sample number before their data is given to the research team mentioned at the top of this form. In case of a positive test result, the sample number and name of the participant's dog will be communicated to their veterinarian who will discuss the information with them. Survey data and corresponding sample numbers will be destroyed 5 years after publication of the data. Any future use of this research data is required to undergo review by a Research Ethics Board.

Electronic versions of the sample results and survey data will be kept on a password-protected computer and distributed only to the research team. Participants are free to withdraw until 2 years after data collection or until the first paper on the results is submitted for publication, whichever comes first. If they choose to withdraw their consent within this timeline, their survey data, along with the corresponding blood and fecal sample will be destroyed and all the associated data removed from the study.

Questions/Concerns

If you have any further questions or want clarification regarding this research and/or your participation, please contact:

Ms. Emilie Toews
Department of Biological Sciences/Faculty of Science
(403)-820-4812, emilie.toews1@ucalgary.ca
or
Dr. Sylvia Checkley
Department of Ecosystem and Public Health/Faculty of Veterinary Medicine
(403) 210-7409, slcheckl@ucalgary.ca

If you have any concerns about the way participants are being treated, please contact the Research Ethics Analyst, Research Services Office, University of Calgary at (403) 220-6289/220-4283; email cfreb@ucalgary.ca. A copy of this consent form will be given to participants to keep for their records and reference. The investigator will also keep a copy of the signed consent form.

APPENDIX B3

Sampling instruction sheet distributed to veterinarians in clinics participating in the pilot study

Veterinarian Instruction Sheet

Selection Criteria

For a dog to be considered for the study, they must be:

i. **Healthy**
   - The dog must not have any pre-existing conditions
   - The dog should be at the clinic only for a regular wellness check/vaccination appointment at this time

ii. **Of an "at-risk" breed**
   - We are not accepting "toy" dogs currently. Otherwise, sample regardless of breed
   - Toy dogs that are of mixed breed with a higher risk breed (ie. terrier) **are** acceptable

iii. **Consented to participate by owner**
   - Owner of the dog must agree to participate in this study by signing the provided "Informed Consent" form before samples can be taken

Selection Procedure (Pick one "Option")

1. **Same-Day Selection**
   i. Send client email #1 and study flyer to all appointments scheduled for the week
   ii. Each day, randomly select up to 2 dogs that fit the selection criteria by using a random number generator, flipping a coin, or other suitable method
   iii. If a dog fits the first two criteria but the owner does not wish to participate, select the very next dog that fits all three criteria
2. **Advanced Selection**
   i. At the beginning of the week, select 2 dogs per day that fit the selection criteria by using a random number generator, flipping a coin, or other suitable method
   ii. Send information package (client email #2, flyer, implied consent form, recruitment information, and dog behaviour questionnaire) to owners of the selected dogs
   iii. If the owner agrees to the study, collect the necessary forms and samples during the appointment
   iv. If the owner does not agree to the study, select the very next dog fitting all three criteria on the appointment list, and send the email package to them

Sample/Form Collection

During the appointment with the dog and owner:

1. If using **Same-Day Selection**, Provide owner with *Informed Consent Form* (2 copies), *Dog Behaviour Questionnaire* (1 copy), *Recruitment Information Letter* (1 copy), and the labeled

plastic bag. If using **Advanced Selection**, these materials should already have been sent to the participant
2. Answer any questions the owner may have about the study
3. Ensure owner **signs/has signed** both copies of the ***Informed Consent Form.*** Collect **1 signed copy**
4. **Collect blood sample** from dog
5. **Collect completed *Dog Behaviour Questionnaire*** from owner at the end of the appointment. Alternatively, if using **Same-Day Selection,** the owner can return the completed questionnaire when they submit their dog's fecal sample
6. If using **Same-Day Selection,** instruct owner on the **collection of fecal sample.** They may collect existing feces from their backyard, or the next time their dog defecates during a walk. The sample must be returned in the provided bag with sample label. If using **Advanced Selection**, the participant should have brought in the fecal sample with them. Collect it at this time.

* If the sample is not returned within a week, sample a new dog or follow up with the owner

Once 10 sets of fecal samples, blood samples, questionnaires, and consent forms have been submitted (see sample checklist), contact Emilie Toews for pick-up via email **and/or** phone at:

Email: emilie.toews1@ucalgary.ca

Phone: (403)-820-4812

APPENDIX B4

Recruitment information distributed to dog owners who consented to participating in the pilot study

Dear Dog Owner,

Introduction:

Echinococcus multilocularis is a tapeworm parasite infecting wolves, coyotes, and foxes in Alberta and across the world. It is spread through their feces to rodents which also become infected. When wolves, coyotes, or foxes catch and eat infected rodent prey, the cycle of the tapeworm is complete; however, sometimes dogs can be infected accidentally with this parasite. When this happens, the infection can be very dangerous to the dog and can, in rare cases, be passed on to their human owners.

This study aims to work closely with your veterinarian to investigate the rates of *E. multilocularis* infection in dogs and to determine what factors (behavioural and demographic) can cause some dogs to be more likely to be infected than others. To do so, you and approximately 400 other dog-owners under the care of 40 veterinary clinics in Calgary and Edmonton, will be asked to answer a quick survey about your dog and provide both a fecal sample collected from your yard and a blood sample which will be collected by your veterinarian.

Your Role:

You will be asked to complete a 10-minute survey either online, following the web-link provided by your veterinarian, or by filling out a paper copy of the same survey which can also be provided by your veterinarian.

In addition to completion of the survey, there are two types of samples we wish to collect from your dog:

1. Feces:

We ask that you collect a sample of your dog's feces (poop) either from your backyard or collected while on a walk in a bag that will be supplied to you and bring this sample back to your veterinary clinic. We will test these feces for **free** and notify your veterinarian (with your consent) if *E. multilocularis* eggs are present which could be passed on to your or your family. If eggs are present, your veterinarian may recommend a de-worming routine for your dog to keep your pets and family safe!

2. Blood:

We also ask that you allow your veterinarian to take a sample of blood from your dog. This is very routine procedure which will cause no harm to your animal besides the regular stress of a visit to your veterinary clinic. We will test this blood sample for **free** to check for the presence of adult *E. multilocularis* which could be a health concern to your dog, but of no risk of being spread to you or your family. If this parasite is present in the sample, we will contact your veterinarian (with your consent) who will help you figure the best course of action for further diagnosis of medical issues and a treatment plan for your dog.

Benefits:
Through participation in this important research, not only will you be gaining two laboratory tests for free for your dog, but you will also receive information on how to prevent your dog from being infected with these parasites, increasing the health and safety of your pets and family.

For information on the larger project please visit: https://www.mitacs.ca/en/projects/one-health-approach-echinococcosis-echinococcus-multilocularis-client-owned-dogs-alberta

If you have any further questions or comments, please contact: slcheckl@ucalgary.ca, amassolo@ucalgary.ca, or emilie.toews1@ucalgary.ca
Thank you very much for taking the time to help us with this project.

Emilie Toews, B.Sc.
M.Sc. Candidate at University of Calgary
emilie.toews1@ucalgary.ca

APPENDIX B5

Informed consent form distributed to dog owners to consented to participate in the pilot study

Name of Researcher, Faculty, Department, Telephone & Email:

Emilie Toews
Faculty of Science, Department of Biological Sciences at University of Calgary
(403)-820-4812
emilie.toews1@ucalgary.ca

Supervisor:

Dr. Marco Musiani
Department of Biological Sciences, Faculties of Science and Veterinary Medicine (Joint Appointment) at University of Calgary
(403)-220-2604
mmusiani@ucalgary.ca

Dr. Alessandro Massolo
Adjunct Professor in Wildlife Health Ecology, Department of Ecosystem and Public Health at University of Calgary
amassolo@ucalgary.ca

Title of Project:

Echinococcus multilocularis in domestic dogs: intestinal and hepatic infections and risk factors

Sponsor:

Bayer Animal Health, MITACS Accelerate

This consent form, a copy of which has been given to you, is only part of the process of informed consent. If you want more details about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

The University of Calgary Conjoint Faculties Research Ethics Board has approved this research study.

Participation is completely voluntary, and confidential.

Purpose of the Study

The objectives of this study are the following:
1. To estimate the prevalence of *Em* in dogs in Calgary and Edmonton
2. To identify risk factors for *Em* infection in domestic dogs

3. To interpret potential implications for human and dog health
4. To determine potential implications for parkland management of pets, humans, and wildlife

What Will I Be Asked to Do?

You will be asked to complete a survey about your dog-walking behaviour and the medical history, age, breed, and gender of your dog. You will also be asked to provide a fecal sample from your dog which can be collected from your backyard or while walking your dog. Lastly, you will be asked for your permission for your veterinarian to take a blood sample from your dog, a common and safe procedure for your pet. Your participation is voluntary and can be withdrawn at any point if you are uncomfortable with providing these three things. If your consent is withdrawn after the collection and analysis of the samples, the samples and survey will be removed from the study. However, consent cannot be withdrawn after the first paper on this study is submitted for publication.

What Type of Personal Information Will Be Collected?

Should you agree to participate, you will only be asked to provide the name of your dog and access to the deworming history of your dog. Other personal information will be retained by your regular veterinary clinic as only anonymized data will be used in this study. In case of a positive result, we will contact your veterinarian with the dog name and sample number and they will reach out to you rather than supplying us with your personal information.

"I grant permission for you to obtain my dog's de-worming treatment history from my veterinarian:
Yes: ___ No: ___."

"I grant permission for you to contact my veterinarian in case of a positive result on my dog's fecal and/or blood test:
Yes: ___ No: ___. "

"I grant permission to be contacted by my veterinarian in case of a positive result on my dog's fecal and/or blood test:
Yes: ___ No: ___."

Are there Risks or Benefits if I Participate?

I do not expect any risks to you in participating in this study. Your dog may be uncomfortable in the blood sample collection, but this will be performed by trained veterinary staff and is a safe and common procedure. If your dog has a positive result, veterinary intervention to ensure the health and safety of your dog and/or family may be necessary. These treatment options will not be financially covered by this study and it will be up to you and your veterinarian to discuss these options. Similarly, if researchers think you or your family may be at risk due to a positive result from your dog, it may be recommended to you to visit a physician for a check-up.

If you agree to participate in this study, your dog's blood and feces will be tested for infection with the *Em* tapeworm. These tests will be entirely free to you, and, with your permission, will be communicated to your veterinarian in case of a positive result so that you can make informed decisions on your pet's health.

Through participating in this study, you will also gain valuable knowledge into how to keep your dog safe from parasites that may be circulating in its environment. You will also be helping us raise awareness of a potentially harmful disease which will benefit both this study and the larger community of dog owners.

What Happens to the Information I Provide?

Your full name will be removed from the dataset by the veterinary staff and replaced by a random sample number before your data is given to the research team mentioned at the top of this form. In case of a positive test result,

the sample number and name of your dog will be communicated to your veterinarian who will discuss the information with you. Survey data and corresponding sample numbers will be destroyed 5 years after publication of the data. Any future use of this research data is required to undergo review by a Research Ethics Board.

Electronic versions of the sample results and survey data will be kept on a password-protected computer and distributed only to the research team. Participants are free to withdraw until 2 years after data collection or until the first paper on the results is submitted for publication, whichever comes first. If you choose to withdraw your consent within this timeline, your survey data will be destroyed, and the corresponding blood and fecal sample removed from the study.

"I grant permission for my answers to the provided survey to be used in this research study:
Yes: ___ No: ___."

"I grant permission for my dog's fecal and blood samples to be used in this research study:
Yes: ___ No: ___."

Signatures

Your signature on this form indicates that 1) you understand to your satisfaction the information provided to you about your participation in this research project, and 2) you agree to participate in the research project.

In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from this research project at any time. You should feel free to ask for clarification or new information throughout your participation.

Participant's Name: (please print) _____

Participant's Signature: _____ Date: _____

Researcher's Name: (please print) _____

Researcher's Signature: _____ Date: _____

Questions/Concerns

If you have any further questions or want clarification regarding this research and/or your participation, please contact:

Ms. Emilie Toews
Department of Biological Sciences/Faculty of Science
(403)-820-4812, emilie.toews1@ucalgary.ca
or
Dr. Sylvia Checkley
Department of Ecosystem and Public Health/Faculty of Veterinary Medicine
(403) 210-7409, slcheckl@ucalgary.ca

If you have any concerns about the way you've been treated as a participant, please contact the Research Ethics Analyst, Research Services Office, University of Calgary at (403) 220-6289/220-4283; email cfreb@ucalgary.ca. A copy of this consent form has been given to you to keep for your records and reference. The investigator has kept a copy of the consent form.

We ask that you:

1. Walk your dog as usual
2. When your dog poops, please pick up the poop using either the coded Ziploc bag that we gave you, or if you prefer, your own bag
3. If you use your own bag, please place it into the Ziploc bag that we gave you
4. When you return to the staging area/parking lot, please place the Ziploc bag containing your dog's poop into the provided cooler

We also ask for your written consent to participate in this project, indicating that you understand to your satisfaction the information provided in this letter and in the attached survey. You are free to withdraw your consent from the study at any time. If you do withdraw your consent, the fecal sample and survey will continue to be used but will be anonymized and record of your involvement in this project will be destroyed.

APPENDIX B6

Dog behaviour questionnaire distributed to dog owners who consented to participate in the pilot study

Sample #:
Date:
Time:
Investigator:

UNIVERSITY OF CALGARY

Part A. Screening Questions				
1. Are you over the age of 18?	*Yes*	○	*No*	○
2. Is this your dog?	*Yes*	○	*No*	○
3. What is the reason for your dog's veterinary appointment today?	*Regular check-up*	○	*Other*	○
			(Please specify): _____	

Part B. Questions about your dog

1. What is the name of your dog? _____

2. What is the age of your dog? _____

3. What is the breed of your dog? *Please specify:* _____

Unknown ○

4. What is the sex of your dog? *Male* ○ *Female* ○

5. Is your dog neutered or spayed? *Yes* ○ *No* ○

6. Has your dog been dewormed in the last year (including heartworm)? *Yes* ○ *No* ○

Part C. Walking your dog

1. How many times, if at all, have you walked your dog (on or off-leash) in the following areas in **the last 6 months**?

	Never	*Less than once/month*	*Once/ month*	*2-3 times/month*	*1-6 times/week*	*Daily*
a. City dog parks:	○	○	○	○	○	○
b. Sidewalk/Street:	○	○	○	○	○	○
c. Sport/School field:	○	○	○	○	○	○
d. Provincial/National Parks:	○	○	○	○	○	○
e. Farm/Ranch:	○	○	○	○	○	○
f. Acreage:	○	○	○	○	○	○

*For **Part C question 2** and **Part D questions 1 and 2** below, use the following as a scale:

Never:	0% of the time
Rarely:	1% - 24% of the time
Sometimes:	25% - 49% of the time
Often:	50% - 74% of the time
Mostly:	75% - 99% of the time
Always:	100% of the time

2. How often, if at all, is your dog **off-leash** in the following areas:

	Never	*Rarely*	*Sometimes*	*Often*	*Mostly*	*Always*
a. City dog parks:	○	○	○	○	○	○
b. Sidewalk/Street:	○	○	○	○	○	○
c. Sport/School field:	○	○	○	○	○	○
d. Provincial/National Parks:	○	○	○	○	○	○
e. Farm/Ranch:	○	○	○	○	○	○
f. Acreage:	○	○	○	○	○	○

Part D. Your dog's eating behaviour

1. Does your dog chase wildlife on walks or at home? Yes ○ No ○

If yes, how often is your dog successful in catching prey?

	Never	Rarely	Sometimes	Often	Mostly	Always
a. Rodents	○	○	○	○	○	○
b. Hare/rabbit	○	○	○	○	○	○
c. Birds	○	○	○	○	○	○
d. Others, specify: _____	○	○	○	○	○	○

2. Does your dog eat things it finds on the ground while on walks? Yes ○ No ○

If yes, how often does your dog eat the following substances on walks?

	Never	Rarely	Sometimes	Often	Mostly	Always
a. Feces	○	○	○	○	○	○
b. Grass/plant matter	○	○	○	○	○	○
c. Rodent/other animal remains	○	○	○	○	○	○
d. Others. Specify: _____	○	○	○	○	○	○

3. At home, what do you feed your dog?

	Dry Kibble	Wet/Canned Food	Homemade (vegetable-based)	Homemade (meat-based)
a. Primary Choice (choose **one**):	○	○	○	○
b. Secondary Choice (choose **all that apply**)	○	○	○	○

4. Has your dog ever been fed entrails and internal organs from: Yes ○ No ○

a. Hunted deer/elk/moose?
b. Sheep
c. Cattle

a. **If yes**, how many times in the last year? _____