International Journal for Parasitology xxx (xxxx) xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

A global assessment of *Echinococcus multilocularis* infections in domestic dogs: proposing a framework to overcome past methodological heterogeneity

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ARTICLE INFO

Article history:
Received 20 August 2020
Received in revised form 13 October 2020
Accepted 18 October 2020
Available online xxxx

Keywords:
Echinococcosis
Prevalence
Risk assessment
Canine alveolar echinococcosis
Echinococcus multilocularis
Diagnostic tests
Parasite
Sensitivity and specificity

ABSTRACT

Echinococcus multilocularis, the aetiological agent of human Alveolar Echinococcosis, is transmitted between small mammals and wild or domestic canids. Dogs infected with *E. multilocularis* 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 Alveolar Echinococcosis. 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 urbanisation plays in the transmission of zoonotic diseases, especially those utilising pets as definitive hosts. Lastly, population studies on canine Alveolar Echinococcosis 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.

1. Introduction

Alveolar Echinococcosis (AE) is a hepatic infection caused by *Echinococcus multilocularis*, a parasitic taeniid helminth. It causes cyst-like lesions in organs of intermediate (small mammals) and dead-end (dogs and humans) (Pawlowski et al., 2001) hosts. Whereas most human AE cases (~18,000 per year) occur in China (Torgerson et al., 2010) due to the Asian strain (Nakao et al., 2009), *E. multilocularis* (including the Asian, European and North American strains) is present in most of the cold and temperate regions of the northern hemisphere (Eckert et al., 2001c; Romig et al., 2017). As this parasite was ranked by the World Health Organization (WHO) as the third most important food-borne zoonotic parasite worldwide (FAO/WHO, 2014), and of highest importance in Europe (Bouwknegt et al., 2018), 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) (Massolo et al., 2019).

To complete its lifecycle, *E. multilocularis* requires a complex two-host predator–prey system. Definitive hosts (DHs; mostly wild canids such as foxes, coyotes, wolves, and raccoon dogs, but also domestic dogs) (Eckert et al., 2001b; Kapel et al., 2006;

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https://doi.org/10.1016/j.ijpara.2020.10.008
0020-7519/

Moro and Schantz, 2009; Otero-Abad and Torgerson, 2013; Romig et al., 2017) present intestinal *E. multilocularis* infection (also known as enteric infection) with adult worms producing eggs that, once fecundated, are shed with faeces into the environment. These embryonated eggs can endure harsh conditions (Thompson, 2017) until accidental ingestion by intermediate hosts (IHs; small mammals) (Eckert et al., 2001b; Eckert and Deplazes, 2004; Giraudoux et al., 2006; Romig et al., 2017) 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) (Torgerson et al., 2010; Thompson, 2017). Here, metacestode larvae mature and proliferate asexually (Torgerson et al., 2010) causing tumour-like lesions and developing protoscoleces, thereby becoming infective (Thompson, 2017). When protoscoleces are ingested by DHs following predation upon infectious IHs, they attach to the DH intestinal wall and develop into adults (Romig et al., 2017).

Domestic dogs can host two stages of the *E. multilocularis* lifecycle – adult worms, when acting as DHs preying on infected small mammals, and larval stages, when acting occasionally as dead-end IHs, where they do not contribute to the cycle, (Romig et al., 2017) developing liver lesions (i.e., canine AE), often with fatal consequences (Corsini et al., 2015; Peregrine, 2015). It is still unknown whether canine AE occurs when dogs consume eggs present in the environment (Staebler et al., 2006; Peregrine, 2015) or by self-infection following intestinal *E. multilocularis* infection (Peregrine, 2015), or both.

Urbanisation is an emerging phenomenon known to impact wildlife movements (Villaseñor et al., 2014), behaviours (Riley et al., 2003), density (Chernousova, 2001), and distribution (Bonnington et al., 2014). Wild and domestic canids are regularly found living among people in urban and suburban areas (Deplazes et al., 2004; Gehrt et al., 2010). The spatial overlap between domestic dogs and wild hosts in these areas (Nonaka et al., 2009; Umhang et al., 2014) allows the *E. multilocularis* sylvatic lifecycle, once established by wild hosts, to be maintained by domestic dogs due to their high population density compared with wild DHs (Liccioli et al., 2015). In a similar manner, free-roaming domestic dogs in rural environments may become the primary DHs for *E. multilocularis* (Budke et al., 2005; Wang et al., 2010).

As DHs, dogs can contribute to *E. multilocularis* transmission to humans directly (e.g., petting or handling) (Deplazes et al., 2004; Nagy et al., 2011), or indirectly (through faecal contamination of households) (Nonaka et al., 2009; Umhang et al., 2014). 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 (FAO/WHO, 2014), with dog ownership possibly being a greater risk factor for human AE than consumption of unwashed, contaminated food (Kern et al., 2004; Torgerson et al., 2020).

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: ELISA to detect antibodies in blood (Deplazes and Gottstein, 1991; Staebler et al., 2006; Frey et al., 2017), and PCR to detect parasite DNA in biopsied lesions (Gottstein et al., 2001; Stieger et al., 2002). 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 (Deplazes and Eckert, 1996). However, some ELISAs (Allan et al., 1992; Deplazes et al., 1992; Morishima et al., 1999) have been developed to detect coproantigens in faecal 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 (Sakai et al., 1998; Deplazes et al., 1999). Often, genus-specific copro-ELISAs are used to detect *Echinococcus* spp. 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 faeces of live DHs and can be performed directly on faecal samples (Dinkel et al., 1998; Isaksson et al., 2014; Knapp et al., 2014) or on concentrated egg solutions obtained through zinc chloride sedimentation analysis (Mathis et al., 1996; Trachsel et al., 2007). However, the sensitivity of copro-PCR depends largely on the worm burden of the infected host (Mathis et al., 1996). In addition, arcoline purgation can be used to obtain purged worms from the DH small intestine (Budke et al., 2005) 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 (Eckert et al., 2001a; Gesy et al., 2013) has traditionally been used to microscopically identify *E. multilocularis* in carcasses of DHs including stray domestic dogs.

Despite the wide distribution of *E. multilocularis* (Eckert et al., 2001c; Romig et al., 2017) and the potential role of dogs in its maintenance and transmission to humans, 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 those 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 AE in domestic dogs to gain more comparable data on *E. multilocularis* infections in dogs, potentially high-risk carriers of this severe zoonosis.

2. Materials and methods

2.1. Literature review

The literature search followed PRISMA guidelines for reporting systematic reviews and meta-analyses (Moher et al., 2009) 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 (Supplementary Table S1).

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 (Supplementary Table S2). Studies reporting *Echinococcus* spp. 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* spp. 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 fitted the criteria. For non-English articles,

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

Diagnostic test	Source sensitivity	LCA[a] sensitivity (95% CI)	Source specificity	LCA [a] specificity (95% CI)	LCA source
Arcoline purgation (Budke et al., 2005)	na	0.758 (0.549–0.942)	1.00	1.00	(Hartnack et al., 2013)
Flotation-PCR (Mathis et al., 1996; Trachsel et al., 2007)	0.94	0.548 (0.485–0.610)	1.00	0.934 (0.873–0.991)	(Otero-Abad et al., 2017a)
Nested PCR (Dinkel et al., 1998)	0.89	0.892 (0.789–0.963)	1.00	0.928(0.882–0.979)	(Hartnack et al., 2013)
pAb-copro-ELISA[b] (Deplazes et al., 1999)	0.836	0.56 (0.480–0.639)	0.995	0.659 (0.558–0.756)	(Otero-Abad et al., 2017a)
mAb-copro-ELISA[c] (Deplazes et al., 1992)	0.94	0.632 (55.3–70.8)	1.00	0.700 (0.601–0.794)	(Otero-Abad et al., 2017a)
Copro-ELISA (Allan et al., 1992; Craig et al., 1995)	0.83	0.55 (0.408–0.689)	0.96	0.706 (0.653–0.767)	(Hartnack et al., 2013)
SCT/IST[d] (Eckert et al., 2001a)	0.98	0.885 (0.827–0.934)	1.00	1.00	(Otero-Abad et al., 2017a)

[a] LCA was used to determine sensitivity and specificity.
[b] Polyclonal antibody-copro-ELISA.
[c] Monoclonal antibody-copro-ELISA.
[d] Sedimentation and counting technique/intestinal scraping technique.

data were gleaned from abstracts and tables while methods and results were translated via Google translate.

2.2. Evaluation of study designs

Included studies were characterised 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 characterised 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.

2.3. 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 a common diagnostic test. We report the apparent prevalence, with the exact binomial confidence intervals (CIs) (Brown et al., 2001), 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 (Speybroeck et al., 2013; Flor et al., 2020) implemented using the *R* package '*prevalence*' (version 0.4.0) to estimate true prevalence and its credibility 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 (Supplementary Table S3).

Subsequently, we calculated an updated true prevalence based on two recent re-assessments of common *Echinococcus* spp. diagnostic techniques (Table 1) (Hartnack et al., 2013; Otero-Abad et al., 2017a). This provided new sensitivity and specificity estimates for several *E. multilocularis* tests and enabled true prevalence calculations for studies in which these parameters were not reported (Table 1). The ranges 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 10,000 "burn-in" samples and 10,000 samples that were retained; a multivariate Brooks-Gelman-Rubin statistic was assessed 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 zero positive cases we report the 0% and 95% credible intervals. The modelled estimates for sensitivity and specificity, together with their credibility intervals, are given in Supplementary Table S3.

Re-assessed true prevalence and 95% CIs for each country were then weighted by sample size, bootstrapped and mapped using R (Manly, 2006).

A risk factor meta-analysis was conducted using odds ratios of known extrinsic risk factors for *E. multilocularis* infection in dogs (Budke et al., 2005) including: being used for hunting, living in a rural area, roaming untethered, and predation on rodents. Individual and pooled weighted odds ratios (ORs) were calculated using MedCalc Statistical Software version 18.11.6 (MedCalc Software Ltd., Ostend, Belgium).

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 generalised 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 a random effect in both conditional and zero-inflated components (Brooks et al., 2017). The model was formulated using the R package 'glmmTMB' version 1.0.2.1, and assumptions were verified using 'DHARMa', both run on R Software version 4.0.2 (2020-06-22).

An ANOVA with Tukey's post hoc tests 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% CIs, whereas other proportions and means are reported with their S.E.M., unless otherwise stated.

3. Results

3.1. 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, Supplementary Table S1) on 21 July, 2020. From the resulting 695 articles (after removal of duplicates), 527 were excluded after 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 the absence of original data (e.g., critical and systematic reviews), the analysis of other helminth or *Echinococcus* spp. instead of *E. multilocularis*, and the absence of prevalence determination (e.g., case studies and experimental infection studies) (Supplementary Table S2).

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. The last search was completed on 21 July, 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

Thus, 46 articles on enteric *E. multilocularis* were included in the review (Fig. 1).

3.2. Canine alveolar echinococcosis

No articles provided an estimate of canine AE prevalence. However, eight articles (three from Canada, two from Switzerland, and one 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 frise, 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.

3.3. Intestinal Echinococcus multilocularis infection

3.3.1. Analysis of study designs

Forty-six articles from the search ("articles" hereafter) 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" hereafter).

The 46 articles were published between 1960 and 2020 (Supplementary Fig. S1); surveillance of *E. multilocularis* prevalence

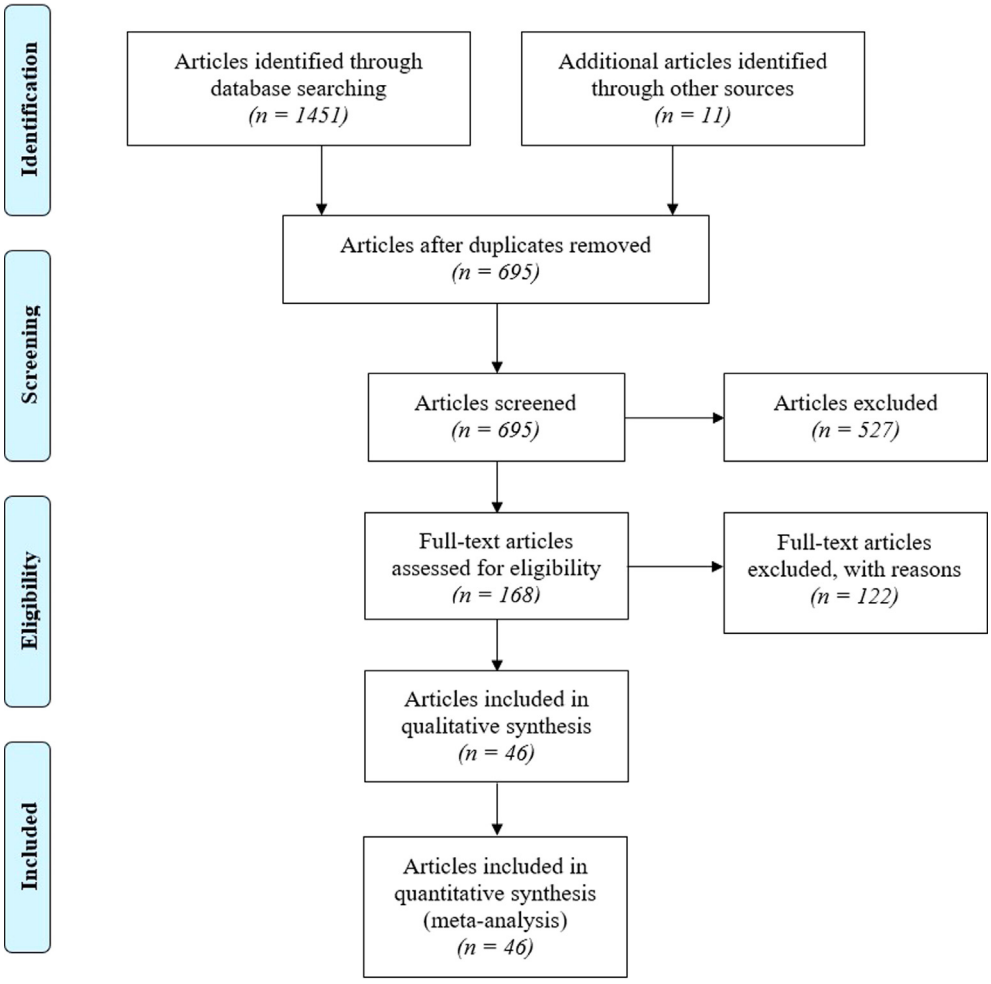


Fig. 1. Process flowchart describing the outcome of the literature search and review of papers on *Echinococcus multilocularis* in dogs (completed on July 21, 2020) outlined using the PRISMA protocol (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). Modified from Moher et al. (2009) with permission under Creative Commons Attribution Licence © Copywright 2015 PRISMA.

Table 3
Sample design information on studies (literature search was completed on 21 July, 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	Veterinary clinic	Sampling method	Ownership, locality	Risk factors	Strain confirmed	Source
Austria	2004–2005	All	Yes	Not specified	Owned, Mixed	No	No	(Dyachenko et al., 2008)
Canada	2009–2010	Not specified	No	Stratified	Stray, Mixed	Yes	Yes	(Villeneuve et al., 2015)
	2018	Spring, Summer	No	Convenience	Owned, Urban	No	No	(Tse et al., 2019)
China	2001–2007	Spring, Autumn	No	Stratified	Mixed, Rural	No	No	(Wang et al., 2010)
	2000	Not specified	No	Convenience	Stray, Rural	No	No	(Yang et al., 2009)
	2000	Not specified	No	Not specified	Stray, Rural	No	No	(Huang et al., 2008)
	2002–2003	Spring, Autumn	No	Not specified	Owned, Rural	Yes	No	(Budke et al., 2005)
	2004–2005	Autumn, Winter, Spring	No	Convenience	Stray, Rural	No	Yes	(Zhang et al., 2006)
	2006–2007	Spring	No	Stratified	Owned, Rural	No	No	(Vaniscotte et al., 2011)
	2006–2007	Not specified	No	Convenience	Stray, Rural	No	No	(Han et al., 2009)
	2004–2007	All	No	Not specified	Mixed, Rural	No	No	(Zhao et al., 2009)
	2006–2007	Spring, Summer, Autumn	No	Not specified	Owned, Rural	No	No	(Moss et al., 2013)
	2006–2007	Spring, Summer, Autumn	No	Not specified	Owned, Rural	No	No	(Moss et al., 2013)
	2012	Not specified	No	Cluster	Owned, Rural	Yes	No	(Liu et al., 2018)
	2015	Spring	No	Not specified	Owned, Rural	Yes	No	(Giraudoux et al., 2019)
	2015–2017	Summer	No	Systematic	Owned, Rural	Yes	Yes	(Weng et al., 2020)
Denmark	2004–2005	All	Yes	Not specified	Owned, Mixed	No	No	(Dyachenko et al., 2008)
France	2008–2010	Spring, Summer	Yes	Not specified	Owned, Mixed	Yes	No	(Umhang et al., 2012)
	2008–2010	Spring, Summer	Yes	Not specified	Owned, Mixed	Yes	No	(Umhang et al., 2012)
	2006–2008	Not specified	Yes	Not specified	Owned, Mixed	Yes	Yes	(Umhang et al., 2014)
	2004–2005	All	Yes	Not specified	Owned, Mixed	No	No	(Dyachenko et al., 2008)
	2011–2013	All	No	Not specified	Unknown, Rural	No	No	(Poulle et al., 2017)
	2012–2015	Winter, Spring	No	Convenience	Unknown, Mixed	No	Yes	(Knapp et al., 2018)
Germany	2004–2005	All	Yes	Not specified	Owned, Mixed	No	No	(Dyachenko et al., 2008)
Great Britain	2004–2006	All	Yes	Not specified	Owned, Mixed	No	No	(Dyachenko et al., 2008)
Iran	Not specified	Not specified	No	Convenience	Stray, Unknown	No	No	(Fallah et al., 1995)
	2009–2010	Winter	No	Not specified	Mixed, Rural	No	Yes	(Beiromvand et al., 2011)
	2013	All	No	Not specified	Owned, Rural	No	No	(Rahimi et al., 2016)
	2013–2014	All	Yes	Random	Unknown, Rural	No	No	(Beiromvand et al., 2018)
	Not specified	Not specified	No	Not specified	Unknown, Rural	No	No	(Mobedi et al., 2013)
Italy	2004–2005	All	Yes	Not specified	Owned, Mixed	No	No	(Dyachenko et al., 2008)
Japan	1997–2007	All	Yes	Not specified	Owned, Mixed	Yes	Yes	(Nonaka et al., 2009)
	2003–2004	Not specified	No	Not specified	Owned, Mixed	No	Yes	(Morishima et al., 2006)
	2013–2017	All	No	Not specified	Stray, Urban	No	No	(Irie et al., 2018)
	2018–2019	All	Yes	Not specified	Owned, Rural	Yes	Yes	(Irie et al., 2019)
Kazakhstan	2002	Autumn	No	not specified	Mixed, Rural	No	Yes	(Štefanić et al., 2004)
	2003–2005	Summer, Autumn	No	Convenience	Mixed, Rural	Yes	No	(Torgerson et al., 2009)
Kyrgyzstan	2012	Spring	No	Stratified	Mixed, Rural	Yes	No	(Van Kesteren et al., 2013)
	2012	Spring	No	Stratified	Mixed, Rural	No	Yes	(Van Kesteren et al., 2013)
	2005	Summer, Autumn	No	Cluster	Mixed, Rural	Yes	Yes	(Ziadinov et al., 2008)
Lithuania	2005–2006	Autumn, Winter	No	Convenience	Mixed, Rural	Yes	Yes	(Bružinskaitė et al., 2008)
Luxemburg	2004–2005	All	Yes	Not specified	Owned, Mixed	No	No	(Dyachenko et al., 2008)
Mongolia	Not specified	Not specified	No	Convenience	Stray, Rural	No	No	(Zoljargal et al., 2001)
The Netherlands	2004–2005	All	Yes	Not specified	Owned, Mixed	No	No	(Dyachenko et al., 2008)
	2012–2013	All	Yes	Convenience	Owned, Urban	Yes	No	(Maas et al., 2014)
Poland	2015	Spring	Yes	Convenience	Owned, Rural	Yes	Yes	(Karamon et al., 2016)
	2017–2018	All	Yes	Not specified	Owned, Rural	Yes	Yes	(Karamon et al., 2019)
	2017–2018	All	Yes	Convenience	Stray, Unknown	Yes	Yes	(Karamon et al., 2019)
Russia	2017–2018	All	No	Convenience	Unknown, Rural	No	Yes	(Andreyanov, 2020)

(*continued on next page*)

Table 3 (*continued*)

Country	Dates of study	Seasonality	Veterinary clinic	Sampling method	Ownership, locality	Risk factors	Strain confirmed	Source
Slovakia	2006	Spring, Summer, Autumn	No	Convenience	Owned, Rural	Yes	Yes	(Szabová et al., 2007)
	2002–2005	All	No	Not specified	Mixed, Mixed	Yes	No	(Antolova et al., 2009)
	2016–2019	All	No	Not specified	Mixed, Mixed	Yes	Yes	(Jarošová et al., 2020)
Switzerland	1996–1997	All	Yes	Not specified	Owned, Unknown	No	No	(Gottstein et al., 2001)
	2009–2010	Autumn, Winter	Yes	Not specified	Owned, Unknown	No	No	(Nagy et al., 2011)
	2009–2010	Autumn, Winter	No	Not specified	Stray, Urban	No	No	(Nagy et al., 2011)
	2009–2010	Autumn, Winter	Yes	Not specified	Owned, Urban	No	No	(Nagy et al., 2011)
	2006 (published)	Not specified	Yes	Random	Owned, Unknown	Yes	No	(Sager et al., 2006)
	not specified	Not specified	No	Not specified	Mixed, Mixed	No	No	(Deplazes et al., 1999)
United States	1951	Not specified	No	Not specified	Owned, Rural	No	No	(Rausch et al., 1990)

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%) (Supplementary Fig. S2), 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 (Torgerson et al., 2010) (Spearman's rho: $r_s = 0.605$, degrees of freedom (df) = 37, $P < 0.01$). Sample sizes in the studies ranged from nine to 17,894 (650.97 ± 308.94; median = 156, interquartile range (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, Fig. 2).

Diagnostic techniques for intestinal *E. multilocularis* were also inconsistent across the articles. Nested PCR directly on faecal samples (Dinkel et al., 1998) was the most used diagnostic technique (15/59; 25.42%), followed by zinc chloride flotation/sedimentation analysis (Mathis et al., 1996), and then PCR (Trachsel et al., 2007) (12/59; 20.34%). Other techniques included various copro-ELISA tests (6/59; 10.17%) (Allan et al., 1992; Craig et al., 1995; Deplazes et al., 1999; Morishima et al., 1999), quantitative PCR (qPCR) (5/59; 8.47%) (Maas et al., 2014; Knapp et al., 2018; Liu et al., 2018), arcoline purgation (5/59; 8.47%) (Budke et al., 2005), a post-mortem SCT (4/59; 6.78%) (Rausch et al., 1990; Eckert et al., 2001a), a magnetic bead capture PCR (1/59; 1.69%) (Isaksson et al., 2014), and two unknown diagnostic techniques following necropsy (3.39%).

3.3.2. 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 (Torgerson et al., 2010) (Fig. 3B, 4B). 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 biassed. 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 (Hartnack et al., 2013; Otero-Abad et al., 2017a), 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, Fig. 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 (Wang et al., 2010) as not enough data were reported in literature (Table 4).

3.3.3. Risk factor analysis

Risk factors such as 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%) ($\chi^2 = 16.44$, $df = 2$, $P < 0.001$) 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 those on mixed (260.92 ± 62.22) (ANOVA, $F_{2,51} = 6.207$, $P = 0.004$; Tukey test, stray versus 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%) ($\chi^2 = 18.78$, $df = 1$, $P < 0.001$). Eighteen studies (30.51%) did not distinguish between rural and urban dogs, and five (8.47%) did not specify this information.

Table 4
Sample size, diagnostic parameters, and results in studies on *Echinococcus multilocularis* in dogs from the literature search completed on July 21, 2020. Apparent prevalence (AP) recorded by each study (as a percentage) 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	True prevalence reported	Methods of analysis	Se (%); Sp (%)	Sample size	Dogs infected	AP (%); CI[a]	TP (%); CrI[b]	LCA[c] Se (%); Sp (%)	ATP (%); CrI[b]	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	(Dyachenko et al., 2008)
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	(Villeneuve et al., 2015)
	No	Mag-PCR[d]	88; 99.9	44	0	0; 0–8	2.9; 0–8.7		2.9; 0–8.5	(Tse et al., 2019)
China	No	Copro-PCR	no data; no data	228	no data	14.8; 10.38–19.62	NA			(Wang et al., 2010)
	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	(Yang et al., 2009)
	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	(Huang et al., 2008)
	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	(Budke et al., 2005)
	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	(Zhang et al., 2006)
	No	Nested PCR	85; 100	142	32	22.5; 16–30.2	27; 19.3–35.5		27.6; 18.7–38.4	(Vaniscotte et al., 2011)
	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	(Han et al., 2009)
	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	(Zhao et al., 2009)
	No	Copro-PCR	69; 100	276	31	11.2; 7.8–15.6	16.7; 11.6–22.4		1.9; 0–7.6	(Moss et al., 2013)
	No	Copro-PCR	69; 100	311	4	1.3; 0.3–3.3	2.3; 0.8–4.7		0.9; 0–3.7	(Moss et al., 2013)
	No	qPCR[e]	no data; 100	750	106	14.1; 11.7–16.8	17.6; 13.3–22.8		12; 0.6–29	(Liu et al., 2018)
	No	qPCR	86; 93	256	0	0; 0–1.4	0.5; 0–1.4		0.5; 0–1.3	(Giraudoux et al., 2019)
	No	Copro-PCR	69; 100	105	25	23.8; 16–33.1	35.3; 24.3–48		35.2; 24–47.7	(Weng et al., 2020)
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	(Dyachenko et al., 2008)
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	(Umhang et al., 2012)
	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	(Umhang et al., 2012)
	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	(Umhang et al., 2014)
	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	(Dyachenko et al., 2008)
	No	qPCR	86; 93	18	2	11.1; 1.4–34.7	11.8; 0.6–32.6		12.2; 0.6–34.1	(Poulle et al., 2017)
	No	qPCR	86; 93	748	4	0.5; 0.1–1.4	0.2; 0–0.6		0.2; 0–0.6	(Knapp et al., 2018)
Germany	No	Nested PCR	89; 100	17,894	43	0.2; 0.2–0.3	0.3; 0.2–0.4	89.2; 92.8	0; 0–0	(Dyachenko et al., 2008)
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	(Dyachenko et al., 2008)
Iran	No	SCT	98; 100	29	0	0; 0–3	0.9; 0–2.7	88.5; 100	3.5; 0–10.4	(Fallah et al., 1995)
	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	(Beiromvand et al., 2011)
	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	(Rahimi et al., 2016)
	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	(Beiromvand et al., 2018)
	No	Copro-ELISA[f]	80; 95	59	0	0; 0–6.1	2.1; 0–6.1		2.1; 0–6.3	(Mobedi et al., 2013)
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	(Dyachenko et al., 2008)
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	(Nonaka et al., 2009)
	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	(Morishima et al., 2006)
	No	Copro-PCR	no data, no data	156	3	2; 0.4–5.5	2; 0.1–5.8		2.6; 0.1–8	(Irie et al., 2018)
	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	(Irie et al., 2019)
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	(Štefanić et al., 2004)
	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	(Torgerson et al., 2009)
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	(Van Kesteren et al., 2013)
	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	(Van Kesteren et al., 2013)
	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	(Ziadinov et al., 2008)
Lithuania	No	Flotation-PCR	94; 100	240	2	0.8; 0.1–3	1.3; 0.3–3.1		1.2; 0–3.9	(Bružinskaitė et al., 2008)
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	(Dyachenko et al., 2008)
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	(Zoljargal et al., 2001)
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	(Dyachenko et al., 2008)
	No	qPCR	no data, no data	142	0	0; 0–2.6	0.9; 0–2.6		1.1; 0–3.6	(Maas et al., 2014)
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	(Karamon et al., 2016)
	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	(Karamon et al., 2019)
	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	(Karamon et al., 2019)
Russia	No	Necropsy (technique 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	(Andreyanov, 2020)
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	(Szabová et al., 2007)
	No	Copro-ELISA[f]	80, 95	289	8	2.8; 1.2–5.4	0.8; 0–2.8		0.8; 0–2.9	(Antolova et al., 2009)

(*continued on next page*)

Table 4 (*continued*)

Country	True prevalence reported	Methods of analysis	Se (%); Sp (%)	Sample size	Dogs infected	AP (%); CI[a]	TP (%); CrI[b]	LCA[c] Se (%); Sp (%)	ATP (%); CrI[b]	Source
	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	(Jarošová et al., 2020)
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	(Gottstein et al., 2001)
	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	(Nagy et al., 2011)
	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	(Nagy et al., 2011)
	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	(Nagy et al., 2011)
	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	(Sager et al., 2006)
	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	(Deplazes et al., 1999)
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	(Rausch et al., 1990)

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

ORs revealed that hunting dogs were significantly more likely to become infected (pooled OR = 4.02, 95% CI = 2.31–7.02, z = 4.91, P < 0.001) (Fig. 5A). 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) (Fig. 5B). 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) (Fig. 5C). Unexpectedly, the analysis of pooled ORs 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* (Fig. 5D), although, given the P value was close to the 0.05 threshold, we could not exclude that this could be due to a low sample size (n = 2).

4. Discussion

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 assessments for dog owners. Lastly, no attempt to estimate the prevalence of canine AE has ever been reported (Massolo et al., 2019).

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 (Torgerson et al., 2010) (Fig. 3B). 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 about *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 (Budke et al., 2005) and the role of dogs in perpetuating the lifecycle of *E. multilocularis* and potentially affecting humans (Umhang et al., 2012).

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 (Rausch et al., 1990; Budke et al., 2005; Torgerson et al., 2010; Nagy et al., 2011). This lack of urban dog studies is of concern, due to the increased density of infective faeces in urban green spaces compared with rural areas (Knapp et al., 2018) even though rural dogs may have a higher intestinal *E. multilocularis* prevalence (Bradley and Altizer, 2007) (Fig. 5C). 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

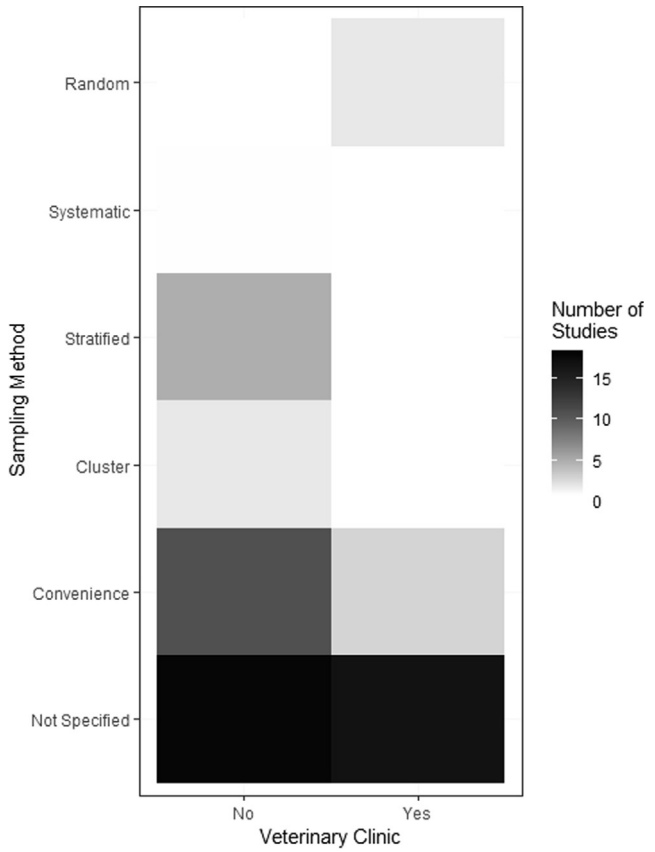


Fig. 2. Characteristics of the sampling design (stratified, cluster, and random) and whether dogs were recruited by veterinary clinics in 59 studies on the prevalence of intestinal *Echinococcus multilocularis* in domestic dogs selected through a formal literature review completed on 21 July, 2020.

(Torgerson et al., 2010). However, we found few studies that fulfilled this attribute. The heavy occurrence of sampling during spring (Table 3) coincides with higher rates of predation on small mammals such as rodents and lagomorphs (Giraudoux et al., 2006; Liccioli et al., 2015), 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 (Lewis et al., 2014; Liccioli et al., 2015; Wang et al., 2016; Otero-Abad et al., 2017b).

The primary methodological issue detected by this review was the lack of true prevalence estimates, which normally account for inadequate diagnostic techniques (Rogan and Gladen, 1978). By basing true prevalence calculations on diagnostic parameters, biases inherent to apparent prevalence estimates are reduced (Blaker, 2000). However, uncertainty in true prevalence estimates increases when sample size, number of positives, or diagnostic parameters are too small (Rogan and Gladen, 1978). 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 the absence of these measurements in the calculation of the prevalence value (Speybroeck et al., 2013; Flor et al., 2020). 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 (Fig. 4). This was likely due to both the low sensitivity of the diagnostic tests that were used and the uncertainty that accompanies estimating diseases of low prevalence in the population (Rogan and Gladen, 1978; Speybroeck et al., 2013). 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 practise of reporting only apparent prevalence estimates in *E. mul-*

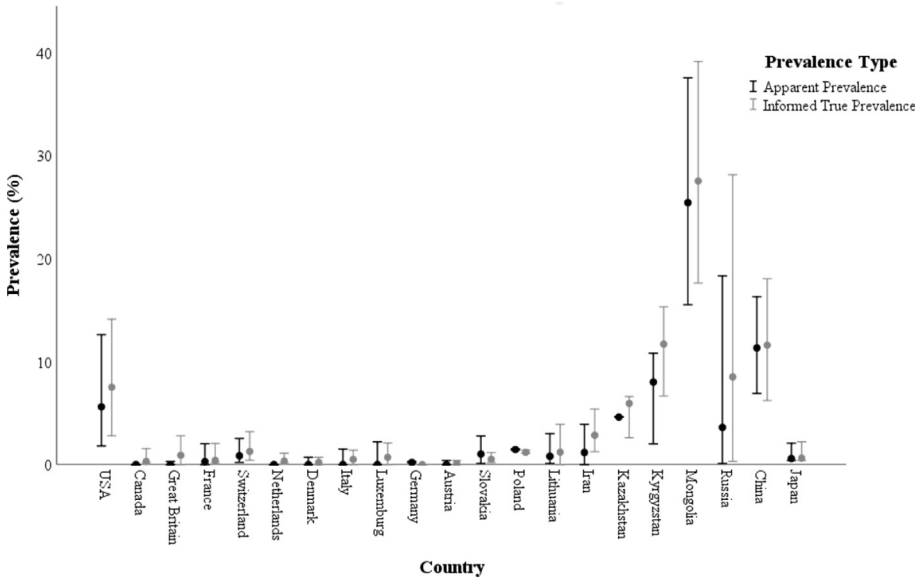


Fig. 3. The true prevalence of *Echinococcus multilocularis* in domestic dogs distributed in countries across the northern hemisphere. (A) Mean apparent prevalence ± 95% confidence intervals and re-assessed true prevalence ± credible intervals calculated via Bayesian methods, accounting for diagnostic sensitivity and specificity (Hartnack et al., 2013; Otero-Abad et al., 2017a) (and weighted by sample size for country means) of intestinal infection by *E. multilocularis* in domestic dogs in each country as reported in a selection of studies obtained through a formal literature review completed on 21 July, 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 with black and white stripes even though they are known to be endemic for *E. multilocularis* (Torgerson et al., 2010).

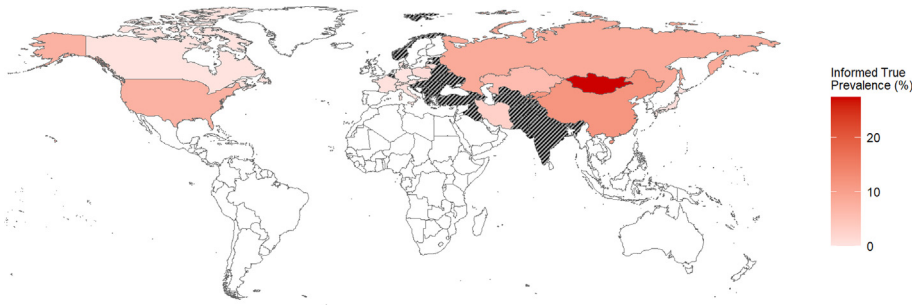


Fig. 3 (*continued*)

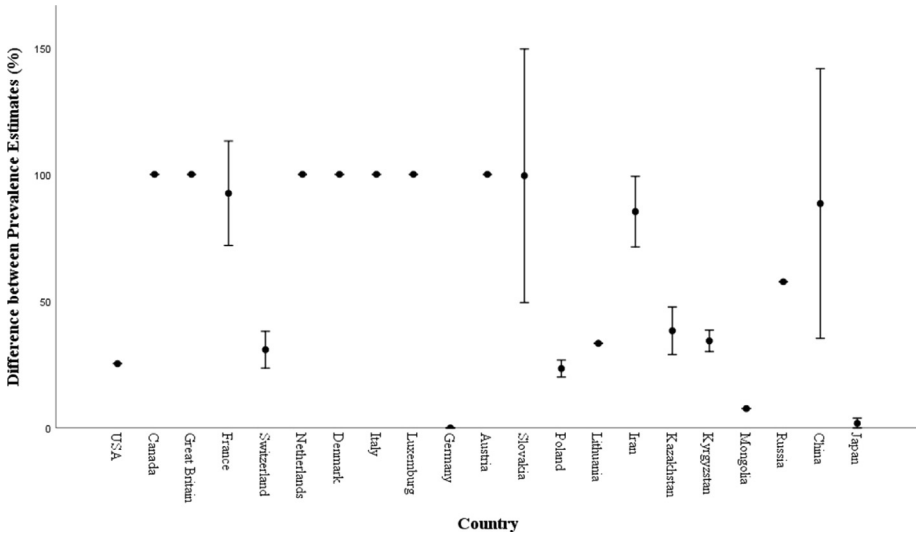


Fig. 4. The difference between apparent and re-assessed true prevalence estimates was calculated for each country in which *Echinococcus multilocularis* has been studied in domestic dogs. (A) Measurement differences (expressed as the percentage of change in prevalence estimates when adjusted for diagnostic precision) ± S.E.M. between re-assessed true prevalence and apparent prevalence of intestinal infection by *E. multilocularis* in domestic dogs globally as reported in a selection of studies obtained through a formal literature review completed on 21 July, 2020. (B) A visual description of this trend shows the difference between re-assessed true prevalence and apparent prevalence (%) in a green scale. Several countries (black and white striped) are known to be endemic for *E. multilocularis* (Torgerson et al., 2010) but do not have published data for *E. multilocularis* in domestic dogs.

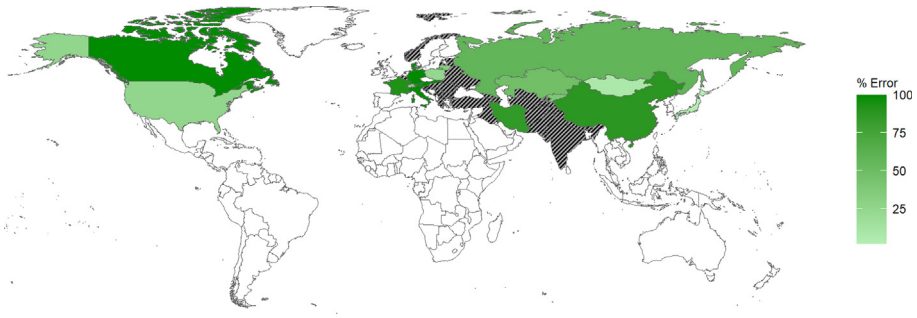


Fig. 4 (*continued*)

tilocularis studies has considerably underestimated the actual presence of the parasite. It is therefore necessary for researchers 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 (e.g. copro-PCR on faecal samples from live dogs, SCT on necropsied dogs), especially in domestic dogs where worm burden may be lower than in other DHs (Kapel et al., 2006), 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 (Flor et al., 2020). 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 para-

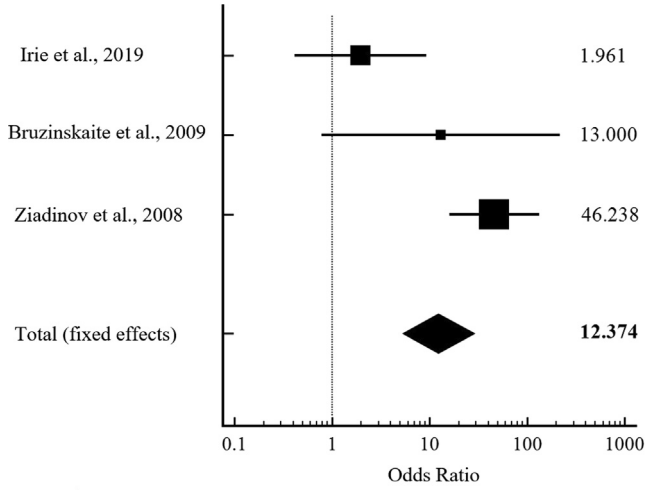
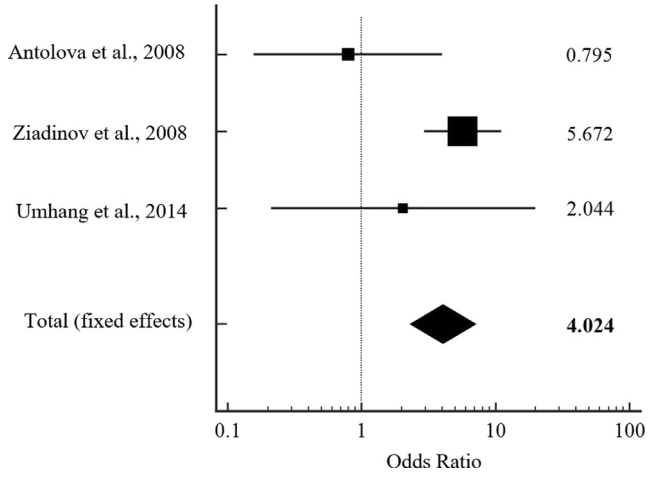


Fig. 5. Pooled weighted log-odds ratios (and 95% confidence intervals) for intestinal infection by *Echinococcus multilocularis* in domestic dogs which (A) were not tethered to their owner's property, (B) were used for hunting, (C) lived in rural environments, and (D) preyed upon rodents, as reported in a selection of studies obtained through a formal literature review completed on 21 July, 2020. Box size is scaled with sample size and odds ratios are reported on the right-hand margin.



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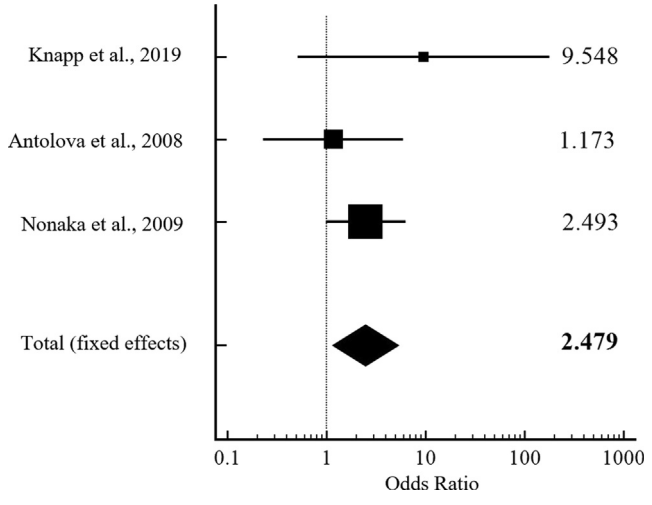

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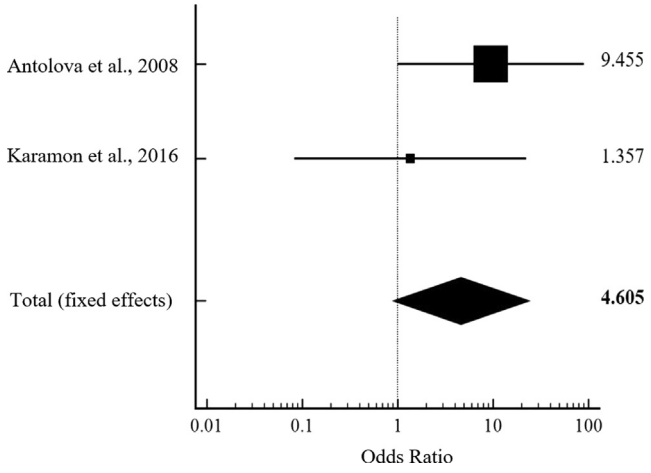

Fig. 5 (*continued*)

sites (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 enrol domestic dogs into research (Lipton et al., 2008) 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 (Fig. 5A) and if they live in rural areas (Fig. 5C). Off leash frequency has previously been indicated as a significant risk factor for gastrointestinal parasitic infection in park-attending dogs in Canada (Smith et al., 2014). Only three studies in this review (Bružinskaitė et al., 2008; Ziadinov et al., 2008; Irie et al., 2019) investigated the relationship between tethering and intestinal *E. multilocularis* infection, and there is therefore a need to address this further.

Hunting dogs also had a higher risk of intestinal *E. multilocularis* infection (Fig. 5B). However, the hunted game was not specified. It is possible that hunting dogs have a 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 DHs contracting intestinal *E. multilocularis*, and dead-end IHs, developing liver infection and AE (Peregrine, 2015; Romig et al., 2017). 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 (Jenkins et al., 2015).

In newly endemic areas, human AE cases may not be present due to lower levels of *E. multilocularis* in the environment (Romig, 2003), or may not be detected because AE cases go unnoticed as the disease is not on the differential diagnosis list (Massolo et al., 2014). However, our meta-analysis confirmed that the pre-

dominant 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 (Jenkins et al., 2015). Given the ability of dogs to both act as sentinels and contribute to the environmental contamination by *E. multilocularis* (Salb et al., 2008; Aguirre, 2009; Schmidt, 2009), 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 (Lindenmayer et al., 1991; Schurer et al., 2014).

The impact of urbanisation on *E. multilocularis* infections in dogs must also be analysed, as few population studies were conducted on dogs in urban areas, despite the known effect of urbanisation on the transmission of *E. multilocularis* (Deplazes et al., 2004; Liccioli et al., 2015). While dogs tend to have a lower worm burden than wild DHs, they shed, on average, greater numbers of eggs per adult worm through their faeces than wild DHs and have thus been proven to be capable of perpetuating the spread of *E. multilocularis* (Budke et al., 2005; Kapel et al., 2006; Weng et al., 2020). Similarly, pet dogs acting as DHs may provide opportunities for the sylvatic *E. multilocularis* cycle to spill over to domestic hosts (Bradley and Altizer, 2007). Therefore, increased *E. multilocularis* surveillance in domestic dogs – for both intestinal *E. multilocularis* and canine AE – is key for the management of human AE.

Finally, to make future globally collected data comparable, we recommend a methodological framework and subsequent workflow (Fig. 6) based on key recommendations: (i) clearly define the study objectives (surveillance, prevalence/risk assessment, trend); (ii) identify the study population based on the objectives, geographical area, type of dogs, etc.; (iii) carry out surveys in different seasons and years, if feasible, to account for seasonal and year-to-year fluctuations; (iv) provide an assessment of diagnostic sensitivity and specificity, and integrate these parameters into prevalence estimates, and (v) develop targeted questionnaires to collect ancillary data, allowing for subsequent assessment of risk factors to be tested at broader scales. It also would be desirable to apply such a framework to areas where human AE is not reported yet, but the presence of *E. multilocularis* 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 risks for humans (Massolo et al., 2019).

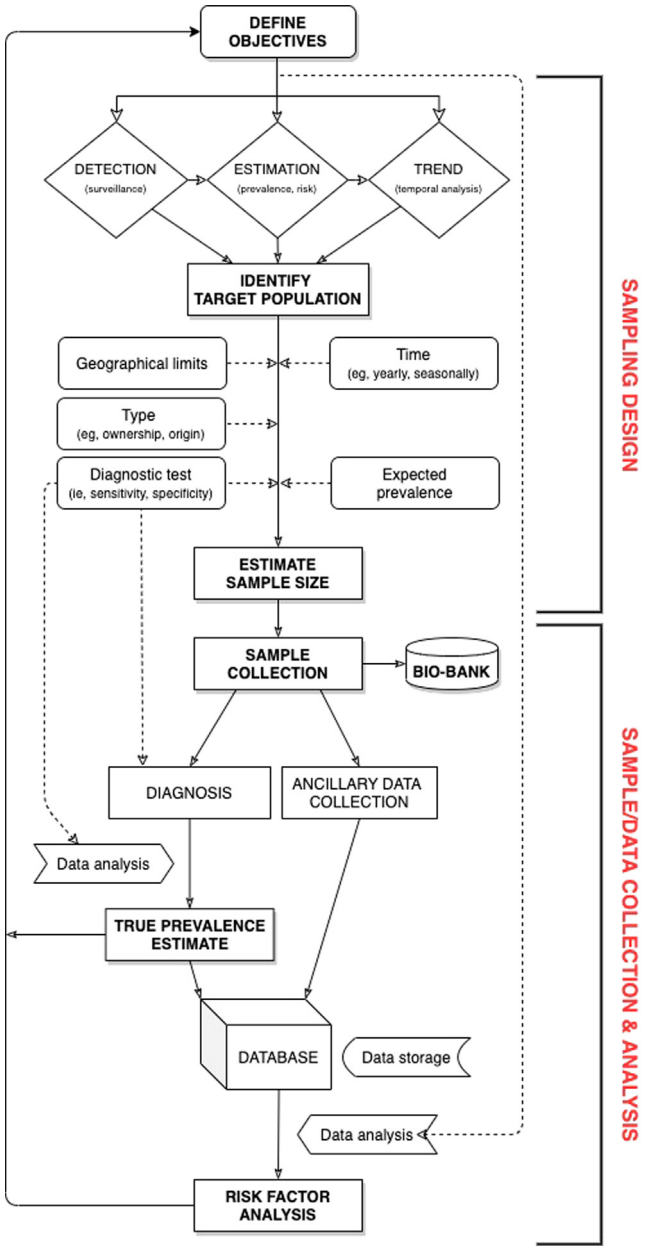

Fig. 6. Recommended framework for future investigations into *Echinococcus multilocularis* infection in domestic dogs.

Acknowledgements

This work was supported by Elanco Canada Limited (Grant number 10017067), Mathematics of Information Technology and Complex Systems (MITACS) Accelerate, Canada (Grant number 10018836), and Alberta Conservation Association, Canada (Grant number 030-00-90-269). Our special thanks to Dr. Dimitri Giunchi for his support in the development of the Beta-regression model in R.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2020.10.008.

References

Aguirre, A.A., 2009. Wild canids as sentinels of ecological health: a conservation medicine perspective. Parasit Vectors 2 (Suppl. 1), S7.

Allan, J.C., Craig, P.S., Noval, J.G., Mencos, F., 1992. Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitology 104, 347–455.

Andreyanov, O.N., 2020. Examining *Echinococcus multilocularis* infection in some Midland Russia predatory animal species. Infektsiia Immun. 10, 193–196.

Antolova, D., Reiterova, K., Miterpakova, M., Dinkel, A., Dubinsky, P., 2009. The first finding of *Echinococcus multilocularis* in dogs in Slovakia: An emerging risk for spreading of infection. Zoonoses Public Health 56, 53–58.

Beiromvand, M., Akhlaghi, L., Massom, S.H.F., Mobedi, I., Meamar, A.R., Oormazdi, H., Motevalian, A., Razmjou, E., 2011. Detection of *Echinococcus multilocularis* in carnivores in Razavi Khorasan Province, Iran using mitochondrial DNA. PLoS Negl.Trop. Dis. 5, e1379.

Beiromvand, M., Rafiei, A., Razmjou, E., Maraghi, S., 2018. Multiple zoonotic helminth infections in domestic dogs in a rural area of Khuzestan Province in Iran. BMC Vet. Res. 14.

Blaker, H., 2000. Confidence curves and improved exact confidence intervals for discrete distributions. Can. J. Stat. 28, 783–798.

Bonnington, C., Gaston, K.J., Evans, K.L., 2014. Squirrels in suburbia: influence of urbanisation on the occurrence and distribution of a common exotic mammal. Urban Ecosyst. 17, 533–546.

Bouwknegt, M., Devleesschauwer, B., Graham, H., Robertson, L.J., van, J.W.B., der, Geissen, Euro-FBP workshop, participants, 2018. Prioritisation of food-borne parasites in Europe, 2016. Euro Surv. 23, 17–61.

Bradley, C.A., Altizer, S., 2007. Urbanization and the ecology of wildlife diseases. Trends Ecol. Evol. 22, 95–102.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Machler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9, 378–400.

Brown, L.D., Cai, T.T., DasGupta, A., 2001. Interval estimation for a binomial proportion. Stat. Sci., 101–117

Bružinskaitė, R., Šarkūnas, M., Torgerson, P.R., Mathis, A., Deplazes, P., 2008. Echinococcosis in pigs and intestinal infection with *Echinococcus* spp. in dogs in southwestern Lithuania. Vet. Parasitol. 160, 237–241.

Budke, C.M., Campos-Ponce, M., Qian, W., Torgerson, P.R., 2005. A canine purgation study and risk factor analysis for echinococcosis in a high endemic region of the Tibetan plateau. Vet. Parasitol. 127, 43–49.

Chernousova, N.F., 2001. Specific features of the dynamics of murine rodent communities under the effects of urbanization: 1. Dynamics of species composition and abundance. Russ. J. Ecol. 32, 122–125.

Corsini, M., Geissbühler, U., Howard, J., Gottstein, B., Spreng, D., Frey, C.F., 2015. Clinical presentation, diagnosis, therapy and outcome of alveolar echinococcosis in dogs. Vet. Rec. 177, 569.

Craig, P.S., Gasser, R.B., Parada, L., Cabrera, P., Parietti, S., Borgues, C., Acuttis, A., Agulla, J., Snowden, K., Paolillo, E., 1995. Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. Vet. Parasitol. 56, 293–301.

Deplazes, P., Alther, P., Tanner, I., Thompson, A.R.C., Eckert, J., 1999. *Echinococcus multilocularis* coproantigen detection by enzyme-linked immunosorbent assay in fox, dog, and cat populations. J. Parasitol. 85, 115–121.

Deplazes, P., Eckert, J., 1996. Diagnosis of the *Echinococcus multilocularis* infection in final hosts. Appl. Parasitol. 37, 245–252.

Deplazes, P., Gottstein, B., 1991. A monoclonal antibody against *Echinococcus multilocularis* Em2 antigen. Parasitology 103, 41–49.

Deplazes, P., Gottstein, B., Eckert, J., Jenkins, D.J., Ewald, D., Jimenez-Palacios, S., 1992. Detection of *Echinococcus* coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes and foxes. Parasitol. Res. 78, 303–308.

Deplazes, P., Hegglin, D., Gloor, S., Romig, T., 2004. Wilderness in the city: the urbanization of *Echinococcus multilocularis*. Trends Parasitol. 20, 77–84.

Dinkel, A., von Nickisch-Rosenegk, M., Bilger, B., Merli, M., Lucius, R., Romig, T., 1998. Detection of *Echinococcus multilocularis* in the definitive host: coprodiagnosis by PCR as an alternative to necropsy. J. Clin. Microbiol. 36, 1871–1876.

Dyachenko, V., Pantchev, N., Gawlowska, S., Vrhovec, M., Bauer, C., 2008. *Echinococcus multilocularis* infections in domestic dogs and cats from Germany and other European countries. Vet. Parasitol. 157, 244–253.

Eckert, J., Deplazes, P., 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev. 17, 107–135.

Eckert, J., Deplazes, P., Craig, P.S., Gemmell, M.A., Gottstein, B., Heath, D., Jenkins, D. J., Kamiya, M., Lightowlers, M., 2001a. Echinococcosis in animals: clinical aspects, diagnosis, and treatment. In: Eckert, J., Gemmell, M.A., Meslin, F.-X., Pawłowski, Z.S. (Eds.), WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Organisation for Animal Health, Paris, pp. 72–99.

Eckert, J., Rausch, R.L., Gemmell, M.A., Giraudoux, P., Kamiya, H., Liu, F.-J., Schantz, P. M., Romig, T., 2001b. Epidemiology of *Echinococcus multilocularis*, *Echinococcus vogeli*, and *Echinococcus oligarthrus*. In: Eckert, J., Gemmell, M.A., Meslin, F.-X., Pawłowski, Z.S. (Eds.), WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Organisation for Animal Health and World Health Organization, Paris, pp. 164–194.

Eckert, J.S., Schantz, P.M., Gasser, R.B., Torgerson, P.R., Bessonov, A.S., Movsessian, S. O., Thakur, A., Grimm, F., Nikogossian, M.A., 2001c. Geographic distribution and prevalence. In: Eckert, J., Gemmell, M.A., Meslin, F.-X., Pawlowski, Z.S. (Eds.), WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Organisation for Animal Health and World Health Organization, Paris, pp. 100–142.

Fallah, M., Taherkhani, H., Sadjjadi, M., 1995. Echinococcosis in the stray dogs in Hamadan, west of Iran. Iran. J. Med. Sci. 20, 170–172.

FAO/WHO [Food and Agriculture Organization of the United Nations and World Health Organization], 2014. Multicriteria-Based Ranking for Risk Management of Food-Borne Parasites. World Health Organization, Rome.

Flor, M., Weiβ, M., Selhorst, T., Müller-Graf, C., Greiner, M., 2020. Comparison of Bayesian and frequentist methods for prevalence estimation under misclassification. BMC Public Health 20, 1335.

Frey, C.F., Marreros, N., Renneker, S., Schmidt, L., Sager, H., Hentrich, B., Milesi, S., Gottstein, B., 2017. Dogs as victims of their own worms: Serodiagnosis of canine alveolar echinococcosis. Parasites Vectors 10, 422.

Gehrt, S.D., Riley, S.P.D., Cypher, B.L., 2010. Urban Carnivores: Ecology, Conflict, and Conservation. Johns Hopkins University Press, Baltimore.

Gesy, K., Pawlik, M., Kapronczai, L., Wagner, B., Elkin, B., Schwantje, H., Jenkins, E., 2013. An improved method for the extraction and quantification of adult *Echinococcus* from wildlife definitive hosts. Parasitol. Res. 112, 2075–2078.

Giraudoux, P., Pleydell, D., Raoul, F., Quéré, J.-P., Wang, Q., Yang, Y., Vuitton, D.A., Qiu, J., Yang, W., Craig, P.S., 2006. Transmission ecology of *Echinococcus multilocularis*: What are the ranges of parasite stability among various host communities in China? Parasitol. Int. 55, S237–S246.

Giraudoux, P., Zhao, Y., Afonso, E., Yan, H., Knapp, J., Rogan, M.T., Shi, D., Jia, W., Craig, P.S., 2019. Long-term retrospective assessment of a transmission hotspot for human alveolar echinococcosis in mid-west China. PLOS Negl. Trop. Dis. 13, e0007701.

Gottstein, B., Saucy, F., Deplazes, P., Reichen, J., Demierre, G., Busato, A., Zuercher, C., Pugin, P., 2001. Is high prevalence of *Echinococcus multilocularis* in wild and domestic animals associated with disease incidence in humans? Emerg. Infect. Dis. 7, 408–412.

Han, X.-M., Wang, H., Cai, H.-X., Ma, X., Liu, Y.-F., Wei, B.-H., Ito, A., Craig, P.S., 2009. Epidemiological survey on echinococcosis in Darlag County of Qinghai Province. Chinese J. Parasitol. Parasit. Dis. 27, 22–26.

Hartnack, S., Budke, C.M., Craig, P.S., Jiamin, Q., Boufana, B., Campos-Ponce, M., Torgerson, P.R., 2013. Latent-class methods to evaluate diagnostics tests for *Echinococcus* infections in dogs. PLoS Negl. Trop Dis., 7

Huang, Y., Heath, D.D., Yang, W., Qiu, J.-M., Chen, X.-W., Yang, Y., Wang, Q., Li, T.-Y., Xiao, Y.-F., Qiu, D.-C., Xiao, N., Chen, F.-X., Ge, S., Se, D., 2008. Epidemiology and risk factor analysis for canine echinococcosis in a Tibetan pastoral area of Sichuan. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 26, 245–252.

Irie, T., Mukai, T., Yagi, K., 2018. *Echinococcus multilocularis* surveillance using copro-DNA and egg examination of shelter dogs from an endemic area in Hokkaido, Japan. Vector Borne Zoonotic Dis. 18, 390–392.

Irie, T., Yamada, K., Morishima, Y., Yagi, K., 2019. High probability of pet dogs encountering the sylvatic cycle of *Echinococcus multilocularis* in a rural area in Hokkaido, Japan. J. Vet. Med. Sci. 81, 1606–1608.

Isaksson, M., Hagström, Å., Armua-Fernandez, M.T., Wahlström, H., Ågren, E.O., Miller, A., Holmberg, A., Lukacs, M., Casulli, A., Deplazes, P., Juremalm, M., 2014. A semi-automated magnetic capture probe based DNA extraction and real-time PCR method applied in the Swedish surveillance of *Echinococcus multilocularis* in red fox (*Vulpes vulpes*) faecal samples. Parasites Vectors 7, 583.

Jarošová, J., Antolová, D., Šnábel, V., Guimarães, N., Štofík, J., Urban, P., Cavallero, S., Miterpáková, M., 2020. The fox tapeworm, *Echinococcus multilocularis*, in grey wolves and dogs in Slovakia: epidemiology and genetic analysis. J. Helminthol. 94, e168.

Jenkins, E.J., Simon, A., Bachand, N., Stephen, C., 2015. Wildlife parasites in a One Health world. Trends Parasitol. 31, 174–180.

Kapel, C.M., Torgerson, P.R., Thompson, R.C., Deplazes, P., 2006. Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats. Int. J. Parasitol. 36, 79–86.

Karamon, J., Samorek-Pieróg, M., Kochanowski, M., Dąbrowska, J., Sroka, J., Gołąb, E., Umhang, G., Cencek, T., 2016. First detection of *Echinococcus multilocularis* in dogs in a highly endemic area of Poland. Folia Parasitol. 63, 1.

Karamon, J., Sroka, J., Dąbrowska, J., Bilska-Zając, E., Zdybel, J., Kochanowski, M., Różycki, M., Cencek, T., 2019. First report of *Echinococcus multilocularis* in cats in Poland: a monitoring study in cats and dogs from a rural area and animal shelter in a highly endemic region. Parasites Vectors 12, 313.

Kern, P., Ammon, A., Kron, M., Sinn, G., Sander, S., Petersen, L.R., Gaus, W., Kern, P., 2004. Risk factors for alveolar echinococcosis in humans. Emerg. Infect. Dis. 10, 2088–2093.

Knapp, J., Giraudoux, P., Combes, B., Umhang, G., Boué, F., Said-Ali, Z., Aknouche, S., Garcia, C., Vacheyrou, M., Laboissière, A., Raton, V., Comte, S., Favier, S., Demerson, J.-M., Caillot, C., Millon, L., Raoul, F., 2018. Rural and urban distribution of wild and domestic carnivore stools in the context of *Echinococcus multilocularis* environmental exposure. Int. J. Parasitol. 48, 937–946.

Knapp, J., Millon, L., Mouzon, L., Umhang, G., Raoul, F., Ali, Z.S., Combes, B., Comte, S., Gbaguidi-Haore, H., Grenouillet, F., Giraudoux, P., 2014. Real time PCR to detect the environmental faecal contamination by *Echinococcus multilocularis* from red fox stools. Vet. Parasitol. 201, 40–47.

Lewis, F.I., Otero-Abad, B., Hegglin, D., Deplazes, P., Torgerson, P.R., 2014. Dynamics of the force of infection: insights from *Echinococcus multilocularis* infection in foxes. PLoS Negl. Trop. Dis. 8, e2731.

Liccioli, S., Giraudoux, P., Deplazes, P., Massolo, A., 2015. Wilderness in the "city" revisited: different *urbes* shape transmission of *Echinococcus multilocularis* by altering predator and prey communities. Trends Parasitol. 31, 297–305.

Liccioli, S.B., Carly, Ruckstuhl,, Kathreen, E.,, Massolo,, Alessandro,, 2015. Feeding ecology informs parasite epidemiology: Prey selection modulates encounter rate with *Echinococcus multilocularis* in urban coyotes. PLoS ONE 10, e0121646.

Lindenmayer, J.M., Marshall, D., Onderdonk, A.B., 1991. Dogs as sentinels for Lyme disease in Massachusetts. Am. J. Public Health 81, 1448–1455.

Lipton, B.A., Hopkins, S.G., Koehler, J.E., Digiacomo, R.F., 2008. A survey of veterinarian involvement in zoonotic disease prevention practices. J. Am. Vet. Med. Assoc. 233, 1242–1249.

Liu, C.-N., Xu, Y.-Y., Cadavid-Restrepo, A.M., Lou, Z.-Z., Yan, H.-B., Li, L., Fu, B.-Q., Gray, D.J., Clements, A.A., Barnes, T.S., Williams, G.M., Jia, W.-Z., McManus, D.P., Yang, Y.-R., 2018. Estimating the prevalence of *Echinococcus* in domestic dogs in highly endemic for echinococcosis. Infect. Dis. Poverty 7, 77.

Maas, M., Dam-Deisz, W.D.C., Van Roon, A.M., Takumi, K., Van Der Giessen, J.W.B., 2014. Significant increase of *Echinococcus multilocularis* prevalence in foxes, but no increased predicted risk for humans. Vet. Parasitol. 206, 167–172.

Manly, B.F., 2006. The Bootstrap, Randomization, Bootstrap and Monte Carlo Methods in Biology. CRC Press, Boca Raton, FL.

Massolo, A., Klein, C., Kowalewska-Grochowska, K., Belga, S., Macdonald, C., Vaughan, S., Girgis, S., Giunchi, D., Bramer, S.A., Santa, M.A., Grant, D.M., Mori, K., Duignan, P., Slater, O., Gottstein, B., Müller, N., Houston, S., 2019. European *Echinococcus multilocularis* identified in patients in Canada. N. Engl. J. Med. 381, 384–385.

Massolo, A., Liccioli, S., Budke, C.M., Klein, C., 2014. *Echinococcus multilocularis* in North America: the great unknown. Parasite 21, 23.

Mathis, A., Deplazes, P., Eckert, J., 1996. An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. J. Helminthol. 70, 219–222.

Mobedi, I., Zare-Bidaki, M., Siavashi, M.R., Naddaf, S.R., Kia, E.B., Mahmoudi, M., 2013. Differential detection of *Echinococcus* spp. copro-DNA by nested-PCR in domestic and wild definitive hosts in Moghan Plain, Iran. Iran. J. Parasitol. 8.

Moher, D., Liberati, A., Altman, D.G., The, P.G., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 6, e1000097.

Morishima, Y., Sugiyama, H., Arakawa, K., Kawanaka, M., 2006. *Echinococcus multilocularis* in dogs, Japan. Emerg. Infect. Dis. 12, 1292–1294.

Morishima, Y., Tsukada, H., Nonaka, N., Oku, Y., Kamiya, M., 1999. Evaluation of coproantigen diagnosis for natural *Echinococcus multilocularis* infection in red foxes. Jpn. J. Vet. Res. 46, 185–189.

Moro, P., Schantz, P.M., 2009. Echinococcosis: a review. Int. J. Infect. Dis. 13, 125–133.

Moss, J.E., Chen, X., Li, T., Qiu, J., Wang, Q., Giraudoux, P., Ito, A., Torgerson, P.R., Craig, P.S., 2013. Reinfection studies of canine echinococcosis and role of dogs in transmission of *Echinococcus multilocularis* in Tibetan communities, Sichuan, China. Parasitology 140, 1685–1692.

Nagy, A., Ziadinov, I., Schweiger, A., Schnyder, M., Deplazes, P., 2011. Hair coat contamination with zoonotic helminth eggs of farm and pet dogs and foxes. Berl. Munch. Tierarztl. Wochenschr. 124, 503–511.

Nakao, M., Xiao, N., Okamoto, M., Yangida, T., Sako, Y., Ito, A., 2009. Geographic pattern of genetic variation in the fox tapeworm *Echinococcus multilocularis*. Parasitol. Int. 58, 384–389.

Nonaka, N., Kamiya, M., Kobayashi, F., Ganzorig, S., Ando, S., Yagi, K., Iwaki, T., Inoue, T., Oku, Y., 2009. *Echinococcus multilocularis* infection in pet dogs in Japan. Vector Borne Zoonotic Dis. 9, 201–205.

Otero-Abad, B., Armua-Fernandez, M., Deplazes, P., Torgerson, P.R., Hartnack, S., 2017a. Latent class models for *Echinococcus multilocularis* diagnosis in foxes in Switzerland in the absence of a gold standard. Parasites Vectors 10, 612.

Otero-Abad, B., Rüegg, S.R., Hegglin, D., Deplazes, P., Torgerson, P.R., 2017b. Mathematical modelling of *Echinococcus multilocularis* abundance in foxes in Zurich, Switzerland. Parasites Vectors 10, 21.

Otero-Abad, B., Torgerson, P.R., 2013. A Systematic Review of the Epidemiology of Echinococcosis in Domestic and Wild Animals. PLoS Negl. Trop Dis., 7

Pawlowski, Z.S.E., Eckert, J., Vuitton, R.W., Kern, P., Craig, P.S., Dar, K.F., De Rosa, F., Filice, C., Gottstein, B., Grimm, F., Macpherson, C.N.L., Sato, N., Todorov, T., Uchino, W., von Sinner, W., Wen, H., 2001. Echinococcosis in humans: clinical aspects, diagnosis and treatment. In: Eckert, J., Gemmell, M.A., Meslin, F.X., Pawlowski, Z.S. (Eds.), WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Organisation for Animal Health and World Health Organization, Paris, pp. 20–71.

Peregrine, A.S., 2015. Alveolar echinococcosis in dogs: an emerging issue?. Vet. Rec. 177. 567 567.

Poulle, M.-L., Bastien, M., Richard, Y., Josse-Dupuis, É., Aubert, D., Villena, I., Knapp, J., 2017. Detection of *Echinococcus multilocularis* and other foodborne parasites in fox, cat and dog faeces collected in kitchen gardens in a highly endemic area for alveolar echinococcosis. Parasite 24, 29.

Rahimi, M.T., Sarvi, S., Daryani, A., Sharif, M., Ahmadpour, E., Shokri, A., Mizani, A., 2016. Application of multiplex PCR for the simultaneous detection of Taenia spp. from domestic dogs in the north of Iran. Helminthologia 53, 285–289.

Rausch, R.L., Fay, F.H., Williamson, F.S.L., 1990. The ecology of *Echinococcus multilocularis* (Cestoda : Taeniidae) on St. Lawrence Island, Alaska. – II. — Helminth populations in the definitive host. Ann. Parasitol. Hum. Comp. 65, 131–140.

Riley, S.P.D., Sauvajot, R.M., Fuller, T.K., York, E.C., Kamradt, D.A., Bromley, C., Wayne, R.K., 2003. Effects of urbanization and habitat fragmentation on bobcats and coyotes in southern California. Conserv. Biol. 17, 566–576.

Rogan, W., Gladen, B., 1978. Estimating prevalence from the results of a screening test. Am. J. Epidemiol. 107, 71–76.

Romig, T., 2003. Epidemiology of echinococcosis. Langenbeck's. Arch. Surg. 388, 209–217.

Romig, T., Deplazes, P., Jenkins, D., Giraudoux, P., Massolo, A., Craig, P.S., Wassermann, M., Takahashi, K., de la Rue, M., 2017. Ecology and life cycle patterns of *Echinococcus* species. In: Thompson, R.C.A., Deplazes, P., Lymbery, A. J. (Eds.), *Echinococcus* and Echinococcosis, Part A. Academic Press, London, pp. 213–314.

Sager, H., Moret, C., Grimm, F., Deplazes, P., Doherr, M.G., Gottstein, B., 2006. Coprological study on intestinal helminths in Swiss dogs: temporal aspects of anthelminthic treatment. Parasitol. Res. 98, 333–338.

Sakai, H., Nonaka, N., Yagi, K., Oku, Y., Kamiya, M., 1998. Coproantigen detection in a routine fox survey of *Echinococcus multilocularis* infection in Hokkaido, Japan. Parasitol. Int. 47, 47–51.

Salb, A.L., Barkema, H.W., Elkin, B.T., Thompson, R.C.A., Whiteside, D.P., Black, S.R., Dubey, J.P., Kutz, J.S., 2008. Dogs as sources and sentinels of parasites in humans and wildlife, Northern Canada. Emerg. Infect. Dis. 14, 60–63.

Schmidt, P.L., 2009. Companion animals as sentinels for public health. Vet. Clin. N. Am-Small 39, 241–250.

Schurer, J.M., Ndao, M., Quewezance, H., Elmore, S.A., Jenkins, E.J., 2014. People, pets, and parasites: one health surveillance in southeastern Saskatchewan. Am. J. Trop. Med. Hyg. 90, 1184–1190.

Smith, A.F., Semeniuk, C.A.D., Kutz, S.J., Massolo, A., 2014. Dog-walking behaviours affect gastrointestinal parasitism in park-attending dogs. Parasites Vectors 7, 429.

Speybroeck, N., Devleesschauwer, B., Joseph, L., Berkvens, D., 2013. Misclassification errors in prevalence estimation: Bayesian handling with care. Int. J. Public Health 58, 791–795.

Staebler, S., Grimm, F., Glaus, T., Kapel, C.M., Haller, M., Hasler, A., Hanosset, R., Deplazes, P., 2006. Serological diagnosis of canine alveolar echinococcosis. Vet. Parasitol. 141, 243–250.

Štefanić, S., Shaikenov, B.S., Deplazes, P., Dinkel, A., Torgerson, P.R., Mathis, A., 2004. Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ("sheep strain") in naturally infected dogs. Parasitol. Res. 92, 347–351.

Stieger, C., Hegglin, D., Schwarzenbach, G., Mathis, A., Deplazes, P., 2002. Spatial and temporal aspects of urban transmission of *Echinococcus multilocularis*. Parasitology 124.

Szabová, E., Juriš, P., Miterpáková, M., Antolová, D., Papajová, I., Šefčíková, H., 2007. Prevalence of important zoonotic parasites in dog populations from the Slovak Republic. Helminthologia, 170–176.

Thompson, R.C., 2017. Biology and systematics of *Echinococcus*. In: Thompson, R.C. A., Deplazes, P., Lymbery, A.J. (Eds.), *Echinococcus* and Echinococcosis, Part A. Academic Press, London, pp. 65–109.

Torgerson, P.R., Keller, K., Magnotta, M., Ragland, N., 2010. The global burden of alveolar echinococcosis. PLoS Negl.Trop. Dis. 4, 722.

Torgerson, P.R., Robertson, L.J., Enemark, H.L., Foehr, J., Van Der Giessen, J.W.B., Kapel, C.M.O., Klun, I., Trevisan, C., 2020. Source attribution of human echinococcosis: A systematic review and meta-analysis. PLOS Negl. Trop. Dis. 14, e0008382.

Torgerson, P.R., Rosenheim, K., Tanner, I., Ziadinov, I., Grimm, F., Brunner, M., Shaiken, S., Shaikenov, N., Rysmukhambetova, A., Deplazes, P., 2009. Echinococcosis, toxocarosis and toxoplasmosis screening in a rural community in eastern Kazakhstan. Trop. Med. Int. Health 14, 341–348.

Trachsel, D., Deplazes, P., Mathis, A., 2007. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology 134, 911–920.

Tse, C., Bullard, J., Rusk, R., Douma, D., Plourde, P., 2019. Surveillance of *Echinococcus* tapeworm in coyotes and domestic dogs in Winnipeg, Manitoba. Can. Commun. Dis. Rep. 45, 171–176.

Umhang, G., Raton, V., Comte, S., Hormaz, V., Boucher, J.-M., Combes, B., Boué, F., 2012. *Echinococcus multilocularis* in dogs from two French endemic areas: No evidence of infection but hazardous deworming practices. Vet. Parasitol. 188, 1–5.

Umhang, G., Raton, V., Comte, S., Hormaz, V., Boucher, J.-M., Combes, B., Boué, F., 2014. *Echinococcus multilocularis* infections in dogs from urban and peri-urban areas in France. Parasitol. Res. 113, 2219–2222.

Van Kesteren, F., Mastin, A., Mytynova, B., Ziadinov, I., Boufana, B., Torgerson, P.R., Rogan, M.T., Craig, P.S., 2013. Dog ownership, dog behaviour and transmission of *Echinococcus* spp. in the Alay Valley, southern Kyrgyzstan. Parasitology 140.

Vaniscotte, A., Raoul, F., Poulle, M.L., Romig, T., Dinkel, A., Takahashi, K., Guislain, M. H., Moss, J., Tiaoying, L., Wang, Q., Qiu, J., Craig, P.S., Giraudoux, P., 2011. Role of dog behaviour and environmental fecal contamination in transmission of *Echinococcus multilocularis* in Tibetan communities. Parasitology 138.

Villaseñor, N.R., Driscoll, D.A., Escobar, M.A.H., Gibbons, P., Lindenmayer, D.B., 2014. Urbanization impacts on mammals across urban-forest edges and a predictive model of edge effects. PLoS ONE 9.

Villeneuve, A., Polley, L., Jenkins, E., Schurer, J., Gilleard, J., Kutz, S., Conboy, G., Benoit, D., Seewald, W., Gagné, F., 2015. Parasite prevalence in fecal samples from shelter dogs and cats across the Canadian provinces. Parasites Vectors 8.

Wang, Q., Raoul, F., Budke, C., Craig, P.S., Yong-fu, X., Vuitton, D.A., Compos-Ponce, M., Dong-chuan, Q., Pleydell, D., Giraudoux, P., 2010. Grass height and transmission ecology of *Echinococcus multilocularis* in Tibetan communities. China. Chin. Med. J. 123, 61–67.

Wang, Q., Yu, W.-J., Zhong, B., Shang, J.-Y., Huang, L., Mastin, A., Renqingpengcuo, Huang, Y., Zhang, G.-J., He, W., Giraudoux, P., Wu, W.-P., Craig, P.S., 2016. Seasonal pattern of *Echinococcus* re-infection in owned dogs in Tibetan communities of Sichuan, China and its implications for control. Infect. Dis. Poverty 5, 60.

Weng, X., Mu, Z., Wei, X., Wang, X., Zuo, Q., Ma, S., Ding, Y., Wang, X., Wu, W., Craig, P.S., Wang, Z., 2020. The effects of dog management on *Echinococcus* spp. prevalence in villages on the eastern Tibetan Plateau, China. Parasites Vectors 13 207.

Yang, Y.R., McManus, D.P., Huang, Y., Heath, D.D., 2009. *Echinococcus granulosus* infection and options for control of Cystic Echinococcosis in Tibetan communities of Western Sichuan Province, China. PLOS Negl. Trop. Dis. 3, e426.

Zhang, Y., Bart, J.-M., Giraudoux, P., Craig, P., Vuitton, D., Wen, H., 2006. Morphological and molecular characteristics of *Echinococcus multilocularis* and *Echinococcus granulosus* mixed infection in a dog from Xinjiang, China. Vet. Parasitol. 139, 44–48.

Zhao, Y.M., Tong, S.X., Jing, T., Chong, S.G., Cai, X.P., Jing, Z.Z., Han, J., 2009. Investigation on echinococcosis in animals in Gannan Tibetan Autonomous Prefecture. Chinese J. Parasitol. Parasit. Dis. 27, 27–30.

Ziadinov, I., Mathis, A., Traschel, D., Rysmukhambetova, A., Abdyjaparov, T.A., Kuttubaev, O.T., Deplazes, P., Torgerson, P.R., 2008. Canine echinococcosis in Kyrgyzstan: using prevalence data adjusted for measurement error to develop transmission dynamics models. Int. J. Parasitol. 38, 79–90.

Zoljargal, P., Ganzorig, S., Nonaka, N., Oku, Y., Kamiya, M., 2001. A survey of canine echinococcosis in Gobi Altai Province of Mongolia by coproantigen detection. Jpn. J. Vet. Res. 49, 125–129.