A One Health systematic review of diagnostic tools for *Echinococcus multilocularis* surveillance: Towards equity in global detection

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**A B S T R A C T**

*Echinococcus multilocularis* is a zoonotic cestode of canid definitive hosts that is emerging as a parasite of medical and veterinary concern in regions of North America, Europe and Asia. Infection with the metacestode stage (alveolar echinococcosis – AE) is life-threatening, especially for patients who reside in low resource countries and lack access to modern diagnostic tests and treatments. The overall objectives of this One Health review were to systematically describe the diagnostic tests currently employed in endemic countries to detect *E. multilocularis* in people, canids and the environment, and to report the test characteristics of new diagnostic techniques for population surveillance. In this systematic review of English and Chinese language databases, we identified 92 primary records of *E. multilocularis* surveillance in canids (N = 75), humans (N = 20) and/or the environment (food, soil; N = 3) and 12 grey literature records that reported *E. multilocularis* surveillance or health systems protocols between 2008 and 2018. Surveillance for *E. multilocularis* was conducted using a broad range of combined morphological, molecular, immunological and imaging techniques. Nine studies reporting diagnostic evaluations for cestode or metacestode detection were identified, including studies on copro-antigen ELISA, copro-PCR, intestinal examination, Western Blot, magnetic capture RT-PCR and immunochromatography. Our dataset includes prevalence estimates for *E. multilocularis* in canids, people, or environment in 27 of the 43 endemic countries and reports data gaps in surveillance, laboratory methods, and diagnostic sensitivity. International consensus on gold standard diagnostic techniques and harmonization of human, canid and environmental surveillance data across political boundaries are needed to comprehensively assess the global burden and distribution of this parasite.

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1. Introduction

Alveolar echinococcosis (AE) is a debilitating medical condition that affects people and animals infected with the metacestode stage of *Echinococcus multilocularis* (Eckert et al., 2001). Such hosts are infected when they accidentally ingest cestode eggs that
are shed into the environment in canid or felid fecal matter, contaminating soil, plants, and water. Definitive canid hosts are most commonly associated with human infections, namely, foxes (Vulpes vulpes, V. lagopus, V. ferrilata), wolves (Canis lupus), coyotes (Canis latrans), raccoon dogs (Nyctereutes procyonoides) and domestic dogs (Canis familiaris) (Deplazes et al., 2017). In the sylvatic lifecycle, rodents such as voles (e.g. Arvicola and Microtus spp.) are the primary intermediate hosts; however, AE is also reported in aberrant hosts such as domestic dogs, nonhuman primates, and swine (Deplazes et al., 2017).

Each year, E. multilocularis infects 11,400 to 29,600 new people, causes approximately 17,000 deaths, and incurs a global burden of 409,000 to 1.1 million Disability Adjusted Life Years (DALYs; Torgerson et al., 2015). For people and animals infected with AE, infections are characterized by multi-chambered cysts growing in liver tissue, with cysts occasionally expanding to other organs (Kern et al., 2017). Patients are often asymptomatic for years following infection, and eventually experience signs and symptoms related to the impaired function and eventual failure of the liver and affected tissues (Kern et al., 2017). The clinical outcomes for AE patients depend on cyst characteristics and immune status of the host, but especially on prompt diagnosis and access to modern treatment. If untreated, 90–100% of reported human patients die within 15 years of infection (Ammann and Eckert, 1996), and for that reason, AE continues to be a life-threatening condition for patients in low income endemic regions where medical access is limited.

Echinococcus multilocularis is geographically restricted to the northern hemisphere, but is widely distributed across countries in North America, Europe and Asia. The vast majority of human cases are reported from rural areas of western China (91%), followed by Russia (6%) (Torgerson et al., 2010). In focal regions of each of the three endemic continents, E. multilocularis is considered an emerging public health concern due to high prevalence in wild canids, detection of infected canids in new geographic areas, increased reports of AE in aberrant hosts (e.g. dogs, nonhuman primates), or increased incidence in human populations (Altintas, 2008; Romig et al., 2006; Schurer et al., 2015; Davidson et al., 2016). However, it is not always clear whether E. multilocularis is truly emerging or whether increased reports are the result of enhanced surveillance efforts and/or improved diagnostic techniques. In some regions, it is impossible to accurately characterize the burden of E. multilocularis, due to the unknown level of under-diagnosis, mis-diagnosis and under-reporting. These gaps can be partly attributed to the long interval between infection and disease onset, shortages of trained healthcare professionals, poor access to health services, lack of diagnostic tests, gaps in human and canid surveillance and lack of reporting infrastructure (EFSA and ECDC, 2016). Many endemic countries do not classify AE as nationally notifiable for people or animals (EFSA Panel on Animal Health and Welfare, 2015). Furthermore, human echinococcosis cases are often not identified to species level (Schurer et al., 2015; Pisceddu et al., 2017), causing challenges in regions that are co-endemic for cystic and alveolar echinococcosis (Kern et al., 2017).

A wide array of morphological, molecular, immunological, and imaging tests exist for detecting E. multilocularis in people, canids, and the environment. These tests vary in diagnostic accuracy, cost, and resource requirements, such as skilled technicians, laboratory or diagnostic equipment, and reagent access (Conraths and Deplazes, 2015; Siles-Lucas et al., 2017). There is a recognized need to harmonize diagnostic strategies within the veterinary and medical communities to improve epidemiological data and to characterize regions of potential emergence (Conraths and Deplazes, 2015). There is also a need to improve equitable access of AE patients to state-of-the-art diagnostics and treatments currently unavailable in many endemic regions. Previous systematic reviews on this topic have been limited by geographic region or host species. Therefore, the objectives of this One Health study were (i) to systematically describe the current methods reported for population level detection of E. multilocularis in people, canids, and the environment in endemic countries, and (ii) to report diagnostic test characteristics and resource requirements of novel techniques evaluated against a gold standard at the population level.

2. Methods

This systematic review was conducted and reported according to the established guidelines “Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement” (Moher et al., 2009). The protocol for the review is available on Prospero (registration #: CRD42018108935). Before starting, all reviewers received training in database searches, study selection, data extraction, and quality assessment to ensure uniformity within the research team. Ethics approval was not needed as this is a secondary literature-based study.

2.1. Search strategy

One author (JS) searched three English language databases (Embase, PubMed, Google Scholar) from January 1, 2008 up to and including Sept. 3rd, 2018. The following search strings were used:

PubMed: (“Echinococcus multilocularis” OR “E. multilocularis” OR “Alveolar echinococcosis”) AND (“diagnosis” OR “diagnostic” OR “diagnos” OR “test” OR “screen” OR “detect”) OR (“monitor” OR “surveillance” OR “epidemiolog” OR “prevalence” OR “burden”).

Embase: (“alveolar echinococcosis” OR “Echinococcus multilocularis” OR “E. multilocularis”).ab, OR (“alveolar echinococcosis” OR “Echinococcus multilocularis” OR “E. multilocularis”).ti, AND (:(diagnosis or diagnostic or diagnos* or test or screen* or detect* or monitor or surveillance or epidemiolog) or prevalence or burden).ab, or (diagnosis or diagnostic or diagnos* or test or screen* or detect* or monitor or surveillance or epidemiolog) or prevalence or burden).ti.

Google Scholar: With all of the words: “Echinococcus multilocularis” OR “E. multilocularis” OR “Alveolar echinococcosis”; with at least one of the words: “diagnosis” OR “diagnostic” OR “test” OR “screen” OR “detect” OR “monitor” OR “surveillance” OR “epidemiology” OR “prevalence” OR “burden”.

Searches of Google Scholar excluded patents but included citations. One author (JS) reviewed the first 10 pages of results, and each page thereafter containing at least one relevant citation.

To capture Chinese-language publications, two co-authors (YH, HL) searched the China National Knowledge Infrastructure (CNKI) database on November 1st, 2018 using the following terms:

“多房棘球绦虫”和诊断，“多房棘球绦虫”和检测以及“多房棘球绦虫”和监测

English translation: (“Echinococcus multilocularis” AND diagnosis) OR (“Echinococcus multilocularis” AND test) OR (“Echinococcus multilocularis” AND surveillance).

One author (JS) searched multiple databases (GreyLit.org, OpenGrey, Science.gov, European Centres for Disease Control (ECDC), European Food and Safety Authority (EFSA), Government of Canada) for grey literature published from January 1st, 2008 up to October 14th, 2018 using the terms “Echinococcus multilocularis” OR “E. multilocularis” OR “Alveolar echinococcosis”. Using the same terms, a second author (LM) searched The University of Toronto’s custom Canadian government document search engine (available at: https://cse.google.com/cse?q=&cx=00784386528685006037:3ajwn2j1weq) for relevant Canadian federal, provincial and municipal documents published from January 1st, 2008 up to October 14th, 2018. We were unable to access the search databases of relevant Chinese grey sources (National Institute of Parasitic Diseases, China Ministry of Health, National Health Commission).

Peer-reviewed and grey literature searches were not restricted by language. All collected studies were collated in Zotero reference manager (https://www.zotero.org/) and duplicates were removed according to title and author.

2.2. Study selection

Primary studies were included if they described diagnostic methods used for *E. multilocularis* surveillance and/or diagnostic method evaluation. Institutional laboratory or surveillance protocols and institutional surveillance reports outlining prevalence of *E. multilocularis* were also included. Reference lists of relevant grey literature were searched for additional sources. In addition, as part of our One Health approach, inclusion criteria selected studies that reported outcomes from humans, canids (i.e. wolves, foxes, coyotes, raccoon dogs and domestic dogs), and/or the environment (e.g. soil, plants, food, water). We excluded data related to non-canid definitive and aberrant hosts as population level surveillance is uncommon for these animals, and we excluded data related to intermediate hosts as post-mortem examination is a widely used and accepted method of diagnosis. We also excluded studies where diagnostic method efficacy was conducted using an early stage index test in Phase I or Phase II of evaluation (Boelaert et al., 2018). This review focused on articles published between 2008 and 2018 because we wanted to capture and report the diagnostic methods currently being used for surveillance, and because the accuracy of older diagnostic tests has been reported elsewhere. Further, studies must have reported on detection of *E. multilocularis* within an endemic country. Two global reviews of AE prevalence and distribution were completed in the last 10 years, and we included countries listed as endemic by these publications to create a list of countries to be included in this review (Torgerson et al., 2010; Deplazes et al., 2017). When discrepancies occurred between the two reports, we considered whether a country shared a border with an endemic country, whether autochthonous animal or human cases had been recently reported, and whether the authors had found reliable secondary sources on which to base their decision. The 43 countries that we considered endemic for AE are listed in Table 1.

| Country            | Country          | Country       |
|--------------------|------------------|---------------|
| Asia (N = 13)      | Kyrgyzstan       | Tadjikistan   |
| Azerbaijan         | Japan            | Turkey        |
| China              | Mongolia         | Uzbekistan    |
| Georgia            | Russia           | Turkmenistan  |
| Iran               | Kazakhstan       |               |
| Kazakhstan         |                  |               |
| Europe (N = 28)    |                  |               |
| Austria            | Germany          | Romania       |
| Belgium            | Hungary          | Russia        |
| Belarus            | Italy            | Serbia        |
| Bulgaria           | Latvia           | Slovakia      |
| Croatia            | Lithuania        | Slovenia      |
| Czech Republic     | Luxembourg       | Sweden        |
| Denmark            | Moldova          | Switzerland   |
| Estonia            | Norway (Svalbard Island only) | The Netherlands |
| France             | Poland           | Ukraine       |
| Fürstentum Lichtenstein |            |               |
| North America (N = 2) | Canada            | United States of America |

*Note: Russia listed on 2 continents.*
inclusion/exclusion criteria; (2) articles that were deemed to be relevant, or for which more information was required, were read in full; (3) any studies that met relevance criteria following review of the full article proceeded to data extraction. The eligibility of each study was assessed independently by two reviewers (JS, AN; LM, ET; DH, JPM; YH, HL). Disagreements regarding eligibility were resolved by consensus.

2.3. Data extraction

Data from each relevant article was extracted independently by two reviewers (JS, AN; LM, ET; DH, JPM; YH, HL), using pre-tested forms. Extracted information from primary studies included: first author, publication year, title, language, study objective, host/source, location of data collection, location of lab analysis, study design, sampling method, inclusion/exclusion criteria, sample size, reported prevalence, detection method(s), tests in series/parallel, criteria for assigning case status, test characteristics, method(s) for determining test characteristics, and if *E. multilocularis* samples were sequenced and/or submitted to GenBank® (Clark et al., 2016), an open access nucleotide sequence database. When a study reported the criteria to assign case status, this was extracted and recorded. If no explicit criteria were stated, we assumed that the results of a single test were used to assign case status but classified the case definition as Not Reported when multiple tests were used. Data elements extracted from grey literature included title, first author, publication year, report type, host/source, case definition(s), reportable/notifiable directive, diagnostic method(s) used and reported prevalence.

2.4. Quality assessment

Only studies that evaluated diagnostic test accuracy for detection of the parasite were assessed for quality. Each study that passed through quality assessment was independently evaluated by two separate reviewers (LM, JS) using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS 2) tool (Whiting et al., 2011). QUADAS 2 measures risk of bias and applicability to the review question within four domains: patient selection, index test, reference standard, and flow/timing. Disagreement was settled by discussion and consensus.

![Fig. 1. Systematic search strategy of English and Chinese language peer-reviewed literature databases (CNKI – Chinese National Knowledge Infrastructure).](image-url)
Table 2
Summary of data extracted from English and Chinese language primary literature reporting surveillance for *Echinococcus multilocularis* in wild and/or domestic canids (2008–2018).

| Authors, year (language) | Host | Location | Study design | Sampling method | Sample size | Prevalence % (95% CI) | Detection method(s) | Case definition | Seq/submissiona |
|--------------------------|------|----------|--------------|-----------------|-------------|-----------------------|---------------------|-----------------|----------------|
| Hanosset et al. 2008 (English) | Red foxes | Wallonoia, Southern Belgium | Cross-sectional | Convenience | 990 | 24.55 (22.38–27.87) | (i) Intestines: mucosal scraping (ii) Cestode morphology | Morphology +ve | NA |
| Dyachenko et al. 2008 (English) | Dogs | Austria Denmark Germany France Italy Luxembourg Netherlands | Cross-sectional | Convenience | Austria: 812 Denmark: 517 Germany: 17894 France: 980 Italy: 249 Luxembourg: 165 Netherlands: 734 | Germany: 0.24 (0.17–0.32) | Other countries: 0 | Microscopy and PCR +ve | Not sequenced |
| Bagrade et al. 2008 (English) | Red foxes | Latvia | Cross-sectional | Convenience | 45 | 35.60 | (i) Intestinal examination (ii) Cestode morphology (iii) PCR (CO1, ND1, rrnS, ATP6, actII) (iv) Sequencing | NR | Sequenced |
| Guislain et al. 2008 (English) | Foxes | French Ardennes region, France | Cross-sectional | Convenience | 149 | 53 (45.4–60.6) | (i) Intestines: SCT (ii) Cestode morphology (iii) Fragment size analyses (EmsB microsatellite target) | NR | Not sequenced |
| Ziadinov et al. 2008 (English) | Dogs (no puppies, pregnant bitches) | At-Bashy, Naryn province, Kyrgyzstan | Cross-sectional | Cluster | 466 | 11 | (i) Arecoline purgation (ii) Cestode morphology (iii) Fecal: ZnCl₂ sieving/flotation (v) Multiplex PCR | Morphology or PCR +ve | Not sequenced |
| Burecka et al. 2008 (English) | Red foxes | Malopolskie voivodship, Poland | Cross-sectional | Convenience | 214 | 20.1 (14.72–25.46) | (i) Intestines: IST² (ii) Cestode morphology | Morphology +ve | NA |
| Antolova et al. 2009 (English) | Dogs (not dewormed in 4 mos) | Presov and Kosice regions, Slovakia | Cross-sectional | NR | 289 | 2.8 | (i) Feces-Sheather's sucrose flotation (ii) Copro-antigen ELISA³ (iii) Nested copro-PCR (12S) | PCR +ve | Not sequenced |
| Takumi et al. 2008 (English) | Foxes | Groningen & Limburg Provinces, The Netherlands | Cross-sectional | Convenience | 1996–1997: 39 2003: 195 | 1996–1997: 7.7 2003: 11.7 | (i) Intestines: IST (ii) PCR | NA | Not sequenced |
| Malczewski et al. 2008 (English) | Red foxes | Northeast & Southeast Poland | NR | NR | 1514 | 23.8 (21.63–25.92) | (i) Intestines: IST (ii) Cestode morphology | Morphology +ve | NA |
| Robardet et al. 2008 (English) | Red foxes | Nancy, France | Cross-sectional | Convenience | 127 | 30 | (i) Intestines: SCT (ii) Cestode morphology | Morphology +ve | NA |
| Nonaka et al. 2009 (English) | Dogs | Hokkaido, Honshu, Kyushu, Japan | Cross-sectional | Convenience | Hokkaido: 4768 Honshu/Kyushu: 348 | Hokkaido: 0.86 Honshu/Kyushu: 0.86 | (i) Feces: Sieving/flotation (ii) Copro-antigen ELISA (iii) PCR (CO1, 12S, U1 snRNA) (v) Multiplex PCR | NR | Sequenced |
| Nonaka et al. 2009 (English) | Dogs (in AE +ve villages) | Lithuania | Cross-sectional | Convenience | 240 | 0.8% (0.1–3) | (i) Feces: McMaster method (ii) ZnCl₂ sieving/flotation (iii) Multiplex PCR | NR | Not sequenced |
| Nonaka et al. 2009 (English) | Silver foxes, red foxes, raccoon dogs, | Hokkaido, Japan | Cross-sectional | NR | Foxes: 209 Raccoon dogs: 3 | Foxes: 12.9 Raccoon dogs: 0 | (i) Feces: Sucrose flotation (ii) Copro-antigen ELISA | PCR +ve | Not sequenced |

(continued on next page)
| Authors, year (language) | Host | Location | Study design | Sampling method | Sample size | Prevalence % (95% CI) | Detection method(s) | Case definition | Seq/submissiona |
|--------------------------|------|----------|--------------|-----------------|-------------|------------------------|---------------------|----------------|----------------|
| (English) Hurnikova et al. 2009 (English) | Dogs, Red foxes, Raccoon dogs | Tatra National Park, Slovakia | Cross-sectional | Convenience | Dogs: 3 Red foxes: 328 Raccoon dogs: 2 | Dogs: 0 Red foxes: 42.7 Raccoon dogs: 50 | (iii) PCR (CO1) (i) Intestines: SCT (ii) Cestode morphology | Morphology +ve | NA |
| (English) Bagrade et al. 2009 (English) | Wolves | Latvia | NR | NR | 34 | 5.9 | (i) Intestinal examination (ii) Cestode morphology | Morphology +ve | NA |
| (English) Barabasi et al. 2010 (English) | Red foxes | Romania | Cross-sectional | Convenience | 561 | 4.8 (3.2–6.9) | (i) Intestines: Sedimentation & mucosal scraping (ii) Cestode morphology (iii) Multiplex PCR (iv) Sequencing | NR | Sequenced |
| (English) Casulli et al. 2010 | Red foxes | Hungary | Cross-sectional | NR | 840 | 10.7 (9.7–11.7) | (i) Intestines: SCT (ii) Cestode morphology (iii) Fluorescent PCR & fragment size analyses (EmsB microsatellite target) | NR | Not sequenced |
| (English) Stien et al. 2010 (English) | Arctic foxes | Svalbard Islands, Norway | Cross-sectional | Convenience | 353 | 8.5 (6–11.9) | (i) Intestines: IST (ii) Cestode morphology | Morphology +ve | NA |
| (English) Wang et al. 2010 (English) | Dogs | Shiqu County, Ganzi Tibetan Autonomous Prefecture, China | Cross-sectional | Clustered, non-random | 228 | 14.80 (i) Arecoline purgation (ii) Cestode morphology (iii) Copro-PCR (125) | Morphology +ve | NA |
| (English) Ziadinov et al. 2010 (English) | Red foxes | Naryn Oblast, Kyrgyzstan | Cross-sectional | Convenience | 151 | 63.6 (55.4–71.3) | (i) Intestines – SCT (ii) Cestode morphology | Morphology +ve | NA |
| (English) Siko et al. 2011 (English) | Red foxes | Romania | Cross-sectional | Convenience | 561 | 4.8 (3.2–6.9) | (i) Intestines: SCT and/or mucosal scraping (ii) Cestode morphology (iii) Multiplex PCR (iv) Sequencing | NR | Sequenced |
| (English) Beiromvand et al. 2011 (English) | Dogs, foxes, wolves | Chenaran, Razavi Khorasan Province, Iran | Cross-sectional | Convenience | Dogs: 77 Foxes: 3 Wolf: 1 | Dogs: 6.5 (2.8–14.3) Foxes & wolf: 100 (78.5–100) | (i) Fecal ZnCl₂ sieving/flotation (ii) Multiplex PCR Foxes & wolf: (i) Intestines: IST or SCT (ii) Cestode morphology Intestines: SCT | SR | Sequenced and submitted |
| (English) Karamon et al. 2011 (English) | Red foxes | Świętokrzyskie & Lubelskie Provinces, Poland | Cross-sectional | NR | 353 | 13.6 | Reference: SCT Index: SSCT | SCT +ve | NA |
| (English) Umhang et al. 2011 (English) | Foxes | France | Cross-sectional | NR | 358 | 32.7 | Reference: SCT Index: SSCT* | SCT +ve | NA |
| (English) Miterpakova et al. 2011 (English) | Red foxes | Slovakia | Cross-sectional | Stratified cluster | 4761 | 30.3 | Intestines: SCT | SCT +ve | NA |
| (English) Umhang et al. 2012 (English) | Dogs | Meuse and Haute-Saone, France | Cross-sectional | Convenience | Meuse: 493 Haute-saone: 367 | Meuse: <1 Haute-saone: <0.75 | (i) Fecal ZnCl₂ sieving/flotation (ii) PCR (ND1) | PCR +ve | Not sequenced |
| Study                        | Species                     | Location                        | Study Design | Sample Size | Prevalence | Methods                                                                 |
|------------------------------|-----------------------------|---------------------------------|--------------|-------------|------------|------------------------------------------------------------------------|
| Catalano et al. 2012 (English) | Coyotes                     | Alberta, Canada                 | Cross-sectional Convenience | 91          | 25.30      | (i) Intestinal sieving (ii) Cestode morphology (iii) Multiplex PCR Reference: RFLP-PCR Index: (i) Nested multiplex copro-PCR (CO1) (ii) Sequencing Intestines: SSCT Morphology+ve Not sequenced |
| Jiang et al. 2012a (English)  | Tibetan foxes, Red foxes    | Shiqu County, China             | Cross-sectional Convenience | 184         | 35         | (i) Nested multiplex copro-PCR (CO1) Morphology+ve Not sequenced       |
| Compte et al. 2012 (English)  | Foxes                       | France                          | Cross-sectional Stratified  | 3307        | 17 (16–19)| (i) Intestines: mucosal scraping (ii) Cestode morphology PCR+ve Sequenced and submitted |
| Jiang et al. 2012b (Chinese)  | Tibetan foxes               | Yunbo Gou, Shiqu county, Sichuan Province, China | Cross-sectional Cluster | 120         | 19         | Nested duplex copro-PCR Morphology+ve NA                               |
| Takahashi et al. 2013 (English) | Foxes                       | Nemuro peninsula, Hokkaido, Japan | Cross-sectional Simple random | Bait zone: 411 Control zone: 163 | Pre-bait² (1994–1999): Bait: 49.4 (43.7–55) Control: 70.5 (60.2–79.2) Post-bait: (2003–2006): Bait: 15.8 (7.9–28.4) Control: 65 (40.9–83.7) | (i) Intestines: mucosal scraping (ii) Cestode morphology Morphology+ve NA |
| Tolnai et al. 2013 (English)  | Red foxes                    | Hungary                         | Cross-sectional Random     | 2008: 840   | 2012: 772 | 2008: 10.7 (9.7–11.7) 2012: 7.9 (6.9–8.9) (i) Intestines: SCT (ii) Cestode morphology (iii) Microsatellite analysis (iv) PCR (12S) Morphology+ve Not sequenced |
| Gesy et al. 2013 (English)    | Red foxes, coyotes           | Quesnel, Canada                 | Cross-sectional Convenience | Red foxes: 6 Coyotes: 27 | Coyotes: 71 (i) Intestines: SFCt (ii) Cestode morphology (iii) PCR (NDY, NDZ, COB, CO1) (iv) Sequencing Morphology+ve Sequenced |
| Mobedi et al. 2013 (English)  | Dogs, red foxes              | Moghan Plain, Iran              | Cross-sectional NR         | Dogs: 59    Red foxes: 84 | Dogs: 0 (i) Copro-antigen ELISA (ii) Nested copro-PCR (12S) NR Not sequenced |
| Moss et al. 2013 (English)    | Dogs                         | Shiqu and Yajiang counties, China | Cohort NR             | 592         | 11.20      | (i) Copro-antigen ELISA (ii) Copro-PCR (ND1) NR Not sequenced          |
| Li et al. 2013 (English)      | Tibetan sand foxes and red foxes | Qinghai, China                 | Cross-sectional Convenience | Intestines: 36 Feces: 70 | Intestines: 3 (i) Intestinal examination (ii) Feces-Sucrose flotation (iii) PCR (CO1) (iv) Sequencing Copro-antigen ELISA SEQUENCED |
| Comte et al. 2013 (English)   | Foxes                        | Annemasse & Pontarlier, France  | Cross-sectional Purposive  | Annemasse: 700 Pontarlier: 700 | Pre-bait² (2006) Annemasse: 13.3 Pontarlier: 10.9 Post-bait (2007–2009) Annemasse: 2.2 Pontarlier: 7.1 | (i) Intestines: SFCt (ii) Cestode morphology ELISA+ve NA |
| Schurer et al. 2014 (English) | Wolves                       | Saskatchewan (SK), Manitoba (MB), | Cross-sectional Convenience | SK: 17 MB: 3 | SK: 23.5 MB: 67 | (i) Intestines: SFCt (ii) Cestode morphology PCR+ve Sequenced and submitted |

(continued on next page)
| Authors, year (language) | Host | Location | Study design | Sampling method | Sample size | Prevalence % (95% CI) | Detection method(s) | Case definition | Seq/submission* |
|--------------------------|------|----------|--------------|-----------------|-------------|------------------------|---------------------|-----------------|----------------|
| Umhang et al. 2014 (English) | Dogs | Northwest Territories (NT) | Cross-sectional | NR | NT: 73 | NT: 8.2 (0.1–1.3) | PCR (CO1, ND1) (iv) Sequencing | | PCR +ve | Sequenced |
| Isaksson et al. 2014 (English) | Red foxes | Eastern Switzerland and Sweden | Cross-sectional | NR | Switzerland: 177 Sweden: 2158 | NA | Reference: Intestines: SCT Index: Fecal Magnetic Capture RT-PCR | | SCT +ve | Not sequenced |
| Denzin et al. 2014 (English) | Red foxes | Saxony-Anhalt, Germany | Cross-sectional | NR | 1998–2005: 1882 2006–2010: 2307 | 1998–2005: 13.6 (11.6–15.6) 2006–2010: 23.4 | (i) Intestinal Smear Technique (ii) Cestode morphology | 'Smear' +ve | NA |
| Maas et al. 2014 (English) | Red foxes, Dogs (>6 mos, not dewormed in 1 mo) | South Limburg, Maastricht, The Netherlands | Cross-sectional | Convenience | Red foxes: 37 Dogs: 142 | Red foxes: 59 (43–74) Dogs: 0 | PCR +ve | | Not sequenced |
| Liccioli et al. 2014 (English) | Coyotes | Calgary, Canada | Cross-sectional | Convenience | 385 | 21.42 (i) Fecal ZnCl\textsubscript{2} sieving/flotation (ii) PCR | | | |
| Karamon et al. 2014 (English) | Red foxes | Poland | Cross-sectional | Convenience | 1546 | 16.5 (14.7–18.4) | Intestines: SCT | | SCN +ve | NA |
| Gesy et al. 2014 (English) | Arctic foxes, Red foxes, Coyotes | Canada | Cross-sectional | Convenience | Arctic foxes: 404 Red foxes: 6 Coyotes: 48 | Arctic foxes: 0.74 Red foxes: 17 Coyotes: 58 | (i) Fecal Sucrose Flotation (ii) Intestines: SCT (iii) Simplex/multiplex PCR (iv) Sequencing | | | |
| Ma et al. 2014 (Chinese) | Red foxes | Zhaosu basin, China | Cross-sectional | Stratified cluster | 6 | 50 | (i) Intestinal examination (ii) Cestode morphology | Morphology +ve | Not sequenced |
| Karamon et al. 2015 (English) | Red foxes | Śląskie, Opalskie, Lubelskie, Podkarpackie, Poland | Cross-sectional | Convenience | 500 | Śląskie: 11.7 (6.7–19.4) Opalskie: 3.9 (1.6–8.4) Podkarpackie: 54.6 (46.7–62.3) Lubelskie: 18.9 (12.0–28.3) Overall: 23.6 | Intestines: SCT | | SCN +ve | NA |
| Melotti et al. 2015 (English) | Coyotes, red foxes, grey foxes | Michigan, USA | Cross-sectional | Convenience | Coyotes: 223 Grey foxes: 45 Red foxes: 34 | Coyotes: 0.4 Grey foxes: 0 Red foxes: 0 | All canids: (i) Intestines: SCT (ii) Multiplex PCR (iii) Sequencing | | SCN +ve | Sequenced |
| Villeneuve et al. 2015 (English) | Shelter dogs (not dewormed in 5 mos), Raccoon dogs | Canada | Cross-sectional | Quota | 1086 | 1.6 (0.4–4.1) | (i) Intestinal morphology (ii) Cestode morphology | | SCN +ve | Sequenced and submitted |
| Study                  | Species               | Location                  | Design                  | Samples | Prevalence | Test Methods                                                                 | Morphology | Sequencing |
|------------------------|-----------------------|---------------------------|-------------------------|---------|------------|-----------------------------------------------------------------------------|------------|------------|
| Laurimaa et al. 2016   | Red foxes             | Estonia                   | Cross-sectional         | 111     | 31.5       | (iv) Sequencing (i) Intestines: SCT (ii) Cestode morphology                | NA         | NR         |
| Bagrade et al. 2016    | Red foxes, raccoon dogs | Latvia                    | Cross-sectional         | NR      | NR         | NA                                                                          | NR         | Sequenced  |
| Miller et al. 2016     | Red fox               | Katrineholm, Uddevalla, Gnesta, Nyköping, Vetlanda, Växjö, Sweden | Cross-sectional         | 714     | 5.7        | (ii) Multiplex PCR (iii) Sequencing                                         | PCR and sequence +ve | Sequenced and submitted |
| Umhang et al. 2016     | Wild: foxes Captive: red foxes, Alaskan tundra wolves, Eurasian wolves | Moselle, France          | Purposive               | NR      | NR         | Intestines: SSCT (ii) EmsB microsatellite analysis                          | NR         | Sequenced  |
| Comte et al. 2017      | Foxes                 | Nancy, France             | Cross-sectional         | 445     | 57%        | Intestines: SSCT                                                            | SSCT +ve   | NA         |
| Frey et al. 2017       | Dogs (clinical AE cases, presumed uninfected, negative controls) | Switzerland              | Diagnostic investigation Presumed uninfected - random; Clinical AE and negative controls - NR | 75      | NA         | Ultrasound Index (i) ELISA (EmVF, Em2-antigen, rEm18, rEm95) (ii) In-house Western Blot (iii) Anti-Echinococcus EUROLINE-Western Blot (IgG, rEm18, rEm95, rEgAgB) | Ultrasound +ve | NA         |
| Schuster & Shimalov 2017 | Raccoon dogs, red foxes | Uckermark district, Brandenburg state, Germany | Cross-sectional         | 101     | 0.99       | (i) Intestines: Sedimentation Technique (ii) Cestode morphology             | Morphology +ve | NA         |
| Maksimov et al. 2017   | Foxes                 | NR                        | NR                      | NR      | 120        | Reference: intestines: IST Index 1: (i) ZR Faecal DNA MiniPrep™ (ii) FastDNA® SPIN Kit for Soil (iii) QIAamp® Fast DNA Stool MiniKit (iv) NucleoSpin® Soil Index 2: (i) PCR (rrn5), (ii) IQS-qPCR (rrnL) (iii) QT-qPCR (rrnL) | NR         | Not sequenced |
| Poule et al. 2017      | Dogs, red foxes       | Ardennes, France          | Cross-sectional         | 18      | 11.1       | qPCR +ve                                                                    | Not sequenced |

(continued on next page)
| Authors, year (language) | Host | Location | Study design | Sampling method | Sample size | Prevalence % (95% CI) | Detection method(s) | Case definition | Seq/submission* |
|--------------------------|------|----------|--------------|-----------------|-------------|----------------------|---------------------|-----------------|----------------|
| Kohansal et al. 2017 (English) | Stray dogs | Zanjan Province, Iran | Cross-sectional | NR | 450 | 0 (23.5–47.6) | (i) Fecal Formalin ethyl acetate concentration test (ii) ZnCl₂ sieving/flotation (iii) Multiplex PCR | PCR +ve | Not sequenced |
| Hermosilla et al. 2017 (English) | Wolves | Gorski Kotar region, Croatia | Cross-sectional | NR | 400 | 0 (23.5–47.6) | (i) Fecal Sodium acetate formalin test (ii) Nested PCR (12S) (iii) Sequencing | NR | Sequenced and submitted |
| Otero-Abad et al. 2017 (English) | Red foxes | Switzerland | Cross-sectional | Convenience | 300 | 59.5 (43.1–66.4) | Reference: Intestines – SCT Index (i): Polyclonal copro-antigen ELISA Index (ii): Monoclonal copro-antigen ELISA Index (iii): Multiplex PCR | SCT +ve | Not sequenced |
| Schurer et al. 2018 (English) | Wolves, coyotes, red foxes, Arctic foxes | Quebec Canada (QC), Maine USA (ME) | Cross-sectional | Convenience | QC: 284 | QC: 0 (23.5–47.6) | Red foxes: 1.8 (0.7) Raccoon dogs: 1.2 | (i) SCT (ii) Cestode morphology Feces: Magnetic Capture RT-PCR | NR | Sequenced and submitted |
| Petersen et al. 2018 (English) | Red foxes, Raccoon dogs | Denmark | Cross-sectional | Convenience | Red foxes: 1073 | 1.5 (0.7) | Red foxes: 1.8 Raccoon dogs: 0.7 | (i) SCT (ii) Simplex/multiplex PCR (CO1, ND1) (iii) Sequencing | All canids: intestines - SCT | PCR +ve | Sequenced |
| Massolo et al. 2018 (English) | Wolves, dogs | Parco Regionale delle Alpi Liguri, Italy | Cross-sectional | Convenience | Dogs: 32 | Dogs: 12.5 | 1.2 | (i) Feces - ZnCl₂ sieving/flotation (ii) PCR (ND1, COB) (iii) Sequencing | (i) Fecal flotation (ii) Multiplex PCR | PCR +ve | Sequenced |
| Gurler et al. 2018 (English) | Red foxes | Central Anatolia and Thrace, Turkey | Cross-sectional | Random | 405 | 1.90 | 0 (1.8) | (i) Fecal flotation (ii) Multiplex PCR | PCR +ve | Not sequenced |
| Beirovand et al. 2018 (English) | Domestic dogs | Ahvaz County, Khuzestan Province, Iran | Cross-sectional | Simple random | 167 | 0 (1.8) | (i) ZnCl₂ sieving/flotation (ii) Multiplex PCR | PCR +ve | Not sequenced |
| Knapp et al. 2018 (English) | Red foxes, Dogs | Franche-Comté and Ile-de-France, France | Cross-sectional | Systematic | Red foxes: 590 | Red foxes: 27.9 (23.8–32.4) | 1.2 | (i) Copro-qPCR (rnrL) (ii) Sequencing | Sequencing | Sequenced |
| Liu et al. 2018 (English) | Dog | Xiji County, Ningxia Hui Autonomous Region, China | Cross-sectional | Convenience | 750 | 14.1 | 1.2 | (i) Multiplex copro-PCR (ND5) | Copro-PCR +ve | Not sequenced |

NR - not reported; NA - not applicable.
* E. multilocularis PCR products sequenced and submitted to GenBank.
1) SCT - Sedimentation and Counting Technique.
2) IST - Intestinal Scraping Technique.
3) Enzyme-linked Immunosorbent Assay.
4) SSCT - Segmental Sedimentation and Counting Technique.
5) Praziquantel-based baits.
6) SFCT - Sedimentation, Filtration, and Counting Technique.
| Authors, year (language) | Host | Location | Study design | Sampling method | Sample size | Prevalence % (95%CI) | Detection method(s) | Case definition | Seq/submission* |
|--------------------------|------|----------|--------------|-----------------|-------------|-----------------------|---------------------|-----------------|----------------|
| Yang et al. 2008 (English) | Children (7–18 yrs) | Xiji County, China | Cross-sectional | Purposive | 861 | US: 0/0 Serology: 18 | (i) US (ii) Serology - EmP-ELISA | ELISA +/– | NA |
| Zhao et al. 2008 (Chinese) | Humans | Gannan Tibetan Autonomous Prefecture, Gansu Province, China | Cross-sectional | Stratified | 1040 | 0.29 | (i) US (ii) Serology - ELISA | US and ELISA +/– | NA |
| Han et al. 2009 (Chinese) | Humans | Darlag County of Qinghai Province, China | Cross-sectional | Stratified | 1723 | 8.20 | (i) US (ii) Serology - IHA | US or IHA +/– | NA |
| Wang et al. 2009 (Chinese) | Humans | Hobuksar Mongolian Autonomous County, Xining, China | Cross-sectional | Cluster | 712 | 0.30 | (i) US (ii) Serology | US or ELISA +/– | NA |
| Feng et al. 2010 (English) | Humans | Xiji County in Ningxia Hui Autonomous Region, Gansu Province, China | Cross-sectional | Stratified cluster | 3191 | 3.38 | (i) Reference: US (ii) Index: Em2-DIGFA (Dot immunogold filtration assay) | US +/– | NA |
| Li et al. 2010 (English) | Humans | Peking, China | Cross-sectional | Convenience | 10,186 | 3.05 | (i) US (ii) Serology - Em18-ELISA | US +/– | NA |
| Poeppel et al., 2013 (English) | Humans (18–60 yrs) | Austria | Cross-sectional | Convenience | 1046 | 0 | (i) Serology - Em-ELISA (ii) Serology - Western Blot | ELISA and Western Blot +/– | NA |
| Liu et al. 2014 (Chinese) | Children (4–18 yrs) | Xiji county, Ningxia Hui Autonomous Region, China | Cross-sectional | Stratified cluster | 1772 | 6.72 | Serology - ELISA | US +/– | NA |
| Cisak et al. 2015 (English) | Humans (rural east Poland) | Bialystok, Lublin and Rzeszow, Poland | Cross-sectional | Purposive | 172 | 1.7 | (i) Serology - Em29s ELISA (ii) Serology - Western Blot | ELISA and Western Blot +/– | NA |
| Cai et al. 2017 (English) | Students (6–16 yrs) | Golog Tibetan Autonomous Prefecture, China | Cross-sectional | NR | 11,260 | 1.29 | (i) US (ii) Serology (IgG) | US and serology +/– | NA |
| Han et al. 2017 (Chinese) | Children (6–12 yrs) | Yushu and Guoluo prefectures, Qinghai Province, China | Cross-sectional | Cluster | 19629 | 1.13 | Serology: 9888 | US or serology +/– | NA |
| Cadavid Restrepo et al. 2018 (English) | Children (6–18 yrs) | Xiji County, Ningxia Hui Autonomous Region, China | Cross-sectional | Cluster | 5110 | 12.20 | (i) US (ii) Serology - EmP-ELISA | ELISA +/– | NA |

(continued on next page)
2.5. Data synthesis

We reported the geographic location of population level *E. multilocularis* surveillance, the detection tools used by such studies and the diagnostic test characteristics of detection tools evaluated as part of Phase III or Phase III field studies. Maps display one study location per citation and were created using ArcGIS v10.6. Elements of the QUADAS 2 assessment were rated as high risk of bias, low risk of bias or unclear according to the method’s recommendations for analysis (Whiting et al., 2011).

3. Results

3.1. Primary literature search

Our searches of English and Chinese language primary literature identified 1393 articles that matched our search terms for the years 2008–2018 (Fig. 1). Of these, 1295 records were excluded because they were duplicates, unavailable, did not meet inclusion criteria, or were written in a language other than English or Chinese. The primary literature search retrieved eight reports deemed to be grey literature, which were then included in grey literature relevance screening. In total, the search team extracted data from 92 peer-reviewed articles reporting *E. multilocularis* prevalence or diagnostic test evaluation in canids (N = 69; Table 2), humans (N = 14; Table 3), multiple hosts (N = 6; Table 4), or the environment (N = 3; Table 5). Prevalence estimates were most frequently reported for China (N = 21), France (N = 11), Poland (N = 10) and Canada (N = 7), with an overall dataset that spanned 27 of the 43 AE endemic countries in North America, Europe and Asia (Fig. 2a,b). The countries that reported some form of surveillance for this parasite were most frequently categorized as high (74%, 20 of 27) or upper middle (22%, 6 of 27) income by the World Bank (The World Bank, 2018). Countries not reporting data were evenly categorized as high, upper-middle, and low-middle (31%, 5 of 16, each) with one country categorized as low income. Only 34 of the 92 studies reported the location of sample analysis; of these, most research teams analyzed their samples in the same country where they were collected (85%, 29 of 34). Only two studies estimated *E. multilocularis* prevalence in a low income country (Kyrgyzstan) and these occurred through collaboration between Kyrgyz and Swiss researchers, with molecular analyses performed in Switzerland (Ziadinov et al., 2008; Ziadinov et al., 2010).

3.2. Surveillance for *E. multilocularis* in canids

Our search collected 75 primary research studies that carried out case detection and population surveillance of *E. multilocularis* cestodes in wild and domestic canids (Table 2). Of these, six studies reported simultaneous surveillance in canids and humans (Table 4). Canid surveillance was most frequently reported from France and China (N = 11 each), followed by Canada (N = 7), Poland (N = 6), and Iran (N = 5) (Fig. 2a). Many studies reported prevalence estimates for multiple canid species, and out of all canid or canid/human studies the majority focused on red foxes (49%), followed by dogs (32.5%) and foxes (unspecified, 12.5%). Just under 10% reported on each of coyotes, raccoon dogs and wolves, and our search also found studies that assessed prevalence in silver foxes, arctic foxes, grey foxes, Tibetan foxes, and a single study that reported on captive canids. Most studies used a variety of diagnostic techniques to identify and confirm *E. multilocularis* infection, including necropsy or arecoline hydrobromide purgation followed by morphological identification of cestodes, copro-PCR, fecal sieving/ flotation followed by PCR, and coproantigen ELISA (Enzyme-Linked Immunosorbent Assay). Post-mortem examination of canid intestinal tracts was employed using various techniques (Sedimentation and Counting Technique - SCT, Intestinal Scraping Technique - IST, Segmental Sedimentation and Counting Technique - SSCT, Sedimentation, Filtration and Counting Technique - SFTCT) to collect *E. multilocularis* adult cestodes for species level identification (N = 44). Molecular identification of taeniid eggs or *Echinococcus* cestodes, either as...
Table 4
Summary of data extracted from English and Chinese primary literature reporting surveillance for *Echinococcus multilocularis* in canids and humans (2008–2018).

| Authors, year (language) | Host | Study location | Study design | Sampling method | Sample size | Prevalence % (95% CI) | Detection method(s) | Case definition | Seq/submissiona |
|--------------------------|------|----------------|--------------|-----------------|-------------|----------------------|---------------------|-----------------|----------------|
| Torgerson et al. 2009 (English) | Humans, Dogs | Jalanash, Kazakhstan | Cross-sectional | Convenience | Humans: 3126 Dogs: 632 | Humans: 0 Dogs: 5 | Humans: (i) Abdominal ultrasound (ii) Serology: Em2G11-ELISA<sup>b</sup> Dogs: Arecoline purgation | NR | NA |
| Han et al. 2015 (English) | Humans, Dogs | Minle County, China | Cross-sectional | Convenience | Humans: 362 Dogs: 356 | Humans: 0.29 Dogs: 0 | Humans: (i) Abdominal ultrasound (ii) Serology: Colloidal gold rapid diagnostic kit Dog feces: Kato-Katz technique | NR | NA |
| Ma et al. 2015 (English) | Humans (echinococcosis surgical cases), Dogs, Tibetan foxes | Qinghai, China | Cross-sectional | NR | Humans: 163 Dogs: 21 Foxes: 2 | Humans: 10.4 Dogs: 76 Foxes: 0 | Humans and canids: (i) Simplex PCR (CO1) (ii) Sequencing Humans: Serology: Em-ELISA Dogs: Copro-PCR | NR | Sequenced and submitted |
| Xu et al. 2015 (Chinese) | Children (4–18 years), Dogs | Haiyuan Counties and Guyuan District, Ningxia Hui Autonomous Region, China | Cross-sectional | Stratified random | Humans: 5706 Dogs: 2175 | Humans: Xiji: 8.38 Haiyun: 7.03 Guyuan: 20.48 Dogs: Xiji: 6.40 Haiyun: 1.52 Guyuan: 3.37 | Humans: Serology: IgG-ELISA Dogs: Copro-PCR | Not sequenced |
| Karamon et al. 2016 (English) | Humans (*E. multilocularis* +ve dog owners), Dogs, red foxes | Podkarpackie Province, Poland | Cross-sectional | Purposive | Humans: 8 Dogs: 148 Foxes: 59 | Humans: 0 Dogs: 1.4 (0.4–4.8) Foxes: 46 | Humans: Serum - IgG-ELISA Dogs: Copro-PCR (12S) Foxes: Intestines – SCT<sup>c</sup> | NR | Sequenced |
| Sen-Hai et al. 2008 (English) | Humans (≥5 years), Dogs | Jiuzhi County, China | Cross-sectional | Convenience | Humans: Ultrasound: 1549 Serology: 1113 Stray dogs: 8 Dog feces: 149 | Humans: 2.5 Stray dogs: 8 Dog feces: 0 | Humans: (i) Abdominal ultrasound (ii) Serology –IHA<sup>d</sup> (iii) Immunoblot Dogs: (i) Necropsy (ii) Copro-antigen ELISA (iii) Wisconsin flotation (iv) Copro-PCR (12S) (v) RFLP<sup>e</sup> (AseI, SspI) | NR | Not sequenced |

NR - not reported; NA - not applicable.

<sup>a</sup> *E. multilocularis* PCR products sequenced and submitted to GenBank.
<sup>b</sup> Enzyme-linked Immunosorbent Assay.
<sup>c</sup> SCT - Sedimentation and Counting Technique.
<sup>d</sup> IHA - Indirect Hemagglutination Assay.
<sup>e</sup> Restriction Fragment Length Polymorphism.
Table 5
Summary of data extracted from primary literature surveillance for *Echinococcus multilocularis* on environmental samples (2008–2018).

| Authors, year | Host/source | Study location | Study design | Sampling method | Sample size | Prevalence % | Detection method(s) | Case definition | Seq/submission* |
|---------------|-------------|----------------|--------------|----------------|-------------|--------------|---------------------|----------------|----------------|
| Szostakowska et al. 2014 (English) | Soil from kitchen gardens, farmlands, arable fields, forests, and areas near fox dens/lairs | Varmia-Masuria Province, Poland | Cross-sectional Purposive | 62 | 11.3 | (i) ZnCl₂ flotation (ii) Nested PCR (125) (iii) Sequencing | PCR +ve | Sequenced and submitted |
| Lass et al. 2015 (English) | Fruits, vegetables, mushrooms from forests, kitchen gardens and plantations | Varmia-Masuria Province, Poland | Cross-sectional Convenience | 103 | 23.3 | (i) ZnCl₂ flotation/sieving (ii) Nested PCR (125) (iii) Sequencing | PCR +ve | Sequenced and submitted |
| Lass et al. 2017 (English) | Fruits, vegetables, mushrooms from forests, kitchen gardens and plantations | Pomerania Province, Poland | Cross-sectional Convenience | 104 | 6.73 | (i) ZnCl₂ flotation/sieving (ii) Nested PCR (125) (iii) Sequencing | PCR +ve | Sequenced and submitted |

NR - not reported.

* E. multilocularis PCR products sequenced and submitted to GenBank.

A stand-alone assay or to confirm morphological results, was conducted by 55 research teams. These methods included a variety of PCR techniques - conventional simplex, nested, multiplex, copro-, fluorescent, qPCR, RFLP-PCR, magnetic capture RT-PCR, as well as microsatellite analysis. Various loci were targeted, including cytochrome *c* oxidase subunit 1 (*cox1*), NADH dehydrogenase subunits 1 and 5 (*nd1, nd5*), ATPase subunit 6 (*atp6*), cytochrome *b* (*cob*), and the small and large subunit rRNA genes (*rrnS, rrnL*). Multiplex PCR was the single most popular molecular technique (*N* = 20), with the majority carried out using Cest1/Cest2 primers (*nd1*) to differentiate *E. multilocularis* from *E. granulosus* and *Taenia* spp. (Trachsel et al., 2007). Only two studies used alternative multiplex primers (Jiang et al., 2012; Liu et al., 2018). Of the studies using PCR to detect *E. multilocularis* in canids, approximately half (57%, 25 of 44) sequenced PCR products and one-third (34%, 15 of 44) submitted these nucleotide sequences into GenBank®. The highest number of records that sequenced and/or submitted *E. multilocularis* sequences originated from Canada (Gesy et al., 2013, 2014; Schurer et al., 2014; Schurer et al., 2018), China (Jiang et al., 2012; Ma et al., 2015; Li et al., 2013; 蒋伟炽, 2012), and Poland (Karamon et al., 2016; Lass et al., 2015; Lass et al., 2017; Szostakowska et al., 2014) (*N* = 4 each), followed by France (Umhang et al., 2016; Knapp et al., 2018; Umhang et al., 2014) (*N* = 3). In addition to these morphological and molecular techniques, our review identified eight studies from six countries where immunological tests (copro-antigen ELISA) were used to screen fecal samples collected from wild foxes (*N* = 4) and/or domestic dogs (*N* = 6) (Sen-Hai et al., 2008; Otero-Abad et al., 2017; Nonaka et al., 2009a, 2009b; Mobedi et al., 2013; Comte et al., 2013; Antolova et al., 2009; Moss et al., 2013) for *E. multilocularis*. Although canid studies often did not explicitly state their sample collection strategy, most appeared to use convenience sampling and a cross-sectional approach. Furthermore, 36% (29/80) of studies reporting surveillance of *E. multilocularis* in canids did not describe the case definition (Tables 2, 3).

### 3.3. Surveillance for *E. multilocularis* in humans

In total, 20 studies from four countries (China, Kazakhstan, Poland, Austria) conducted population surveillance for AE in people (Tables 3–4). Prevalence based on abdominal ultrasound and serology was estimated to be 0–13.45% in China (Yang et al., 2008; Gao et al., 2018), and 0% in Kazakhstan (Torgerson et al., 2009). Seroprevalence was estimated to be 0% in Austria (Poeppl et al., 2013) and 0–1.7% in Poland (Karamon et al., 2016; Cisak et al., 2015). Most case definitions (70%, 14/20) were based on abdominal ultrasound and confirmatory serology (ELISA and/or Western Blot), while the remainder were based on serology only. Case definitions for AE were not reported for 30% of studies where humans were surveyed for infection (Ma et al., 2015; Karamon et al., 2016; Sen-Hai et al., 2008; Torgerson et al., 2009; Han et al., 2015; 许曜阳 et al., 2015). Case definitions were reported or implied for all studies of human patients (Table 3) but were only provided for one study that addressed human/canid infection (Table 4). A variety of sampling methods were reported including convenience, purposive, randomized, and cluster. Only one study sequenced AE cyst tissue removed from human patients and submitted the DNA sequences to GenBank® (Ma et al., 2015).

### 3.4. Surveillance for *E. multilocularis* in the environment

Two studies conducted surveillance for *E. multilocularis* eggs in food (Lass et al., 2015; Lass et al., 2017), and one conducted surveillance in soil between 2008 and 2018 (Szostakowska et al., 2014; Table 5). All three studies collected taeniid eggs using
ZnCl₂ flotation, identified the eggs using nested PCR and sequencing, and submitted amplified PCR product sequences into GenBank®. The reported contamination levels ranged from 6.73% to 23.3% in Poland where the studies took place; however, these levels have been called into question.

Fig. 2. a. Economic status of countries where *Echinococcus multilocularis* surveillance of canids, humans, or the environment was reported in English or Chinese language primary literature (2008–2018). b. Economic status of countries where *Echinococcus multilocularis* surveillance included DNA sequencing and/or submission of DNA sequences into GenBank® (an open access nucleotide sequence database).
### Table 6
Diagnostic test characteristics and resource requirements of protocols identified in this systematic review for surveillance of *Echinococcus multilocularis* in canids, humans, and/or the environment (2008–2018).

| Diagnostic test                                           | Through-put\(^a\) | Equipment requirements                                                                 | Sn% (95%CI)\(^b\) | Sp% (95%CI)\(^c\) | Notes                                                                                                                                                                                                 | Citation                   |
|-----------------------------------------------------------|--------------------|----------------------------------------------------------------------------------------|--------------------|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| Canids - post-mortem intestinal examination               | Low                | Biosafety space and/or 80 °C freezer, microscope                                       | 88.5 (82.7–93.4)   | 100                | -Requires skilled microscopist  
- Sample quality impacts cestode identification  
- Quantifying worm burden can be time intensive  
- See SCT notes  
- Selective examination of *E. multilocularis* predilection sites to increase throughput  
- Lower intensity estimate accuracy than SCT                                                                                                                                 | Otero-Abad et al., 2017 |
| Sedimentation & Counting Technique (SCT)                  |                    |                                                                                        |                    |                    |                                                                                                                                                                                                     |                            |
| Segmental Sedimentation & Counting Technique              | Low                | Biosafety space and/or 80 °C freezer, microscope                                       | 56.4–98.3 (depending on segment) | <100               | -See SCT notes  
- Selective examination of *E. multilocularis* predilection sites to increase throughput  
- Lower intensity estimate accuracy than SCT                                                                                                                                                        | Umhang et al., 2011       |
| Canids – fecal examination                                |                    |                                                                                        |                    |                    |                                                                                                                                                                                                     |                            |
| Arecoline hydrobromide purgation                          | Low                | Microscope                                                                             | 21 (11–34)         | 100                | -Not all canids purge after arecoline dose  
- Canids must be restrained  
- Adverse effects possible; contraindicated for pregnant bitches, puppies, and older canids                                                                                                                                 | Ziadinov et al., 2008     |
| Taenid egg recovery/multiplex PCR\(^d\)                   | Low                | 80 °C freezer, centrifuge, PCR thermocycler, gel electrophoresis system, UV visualization | (1) 50 (29–72)     | (1) 100 (97–100)   | -Requires skilled technician  
- Detection depends on egg recovery assay, DNA extraction technique, and PCR protocol  
- DNA extraction kits limited to 0.15–0.5 g/reaction  
- Copro-inhibitors impact extraction  
- Detects patent and pre-patent infections  
- Sensitivity depends on worm burden  
- Requires skilled technician  
- Throughput can be optimized using an automated nucleic acid extraction robot                                                                                                                                 | (1) Ziadinov et al., 2008; (2) Otero-Abad et al., 2017 |
| Copro-DNA/PCR (various DNA extraction kits/PCR system combinations) | Low | 80 °C freezer, microcentrifuge, PCR/qPCR system                                        | QIAamp/QT-qPCR: 81 | QIAamp/PCR: 100    | -Requires skilled technician  
- DNA extraction kits limited to 0.15–0.5 g/reaction  
- Copro-inhibitors impact extraction  
- Requires skilled technician  
- Detects patent and pre-patent infections  
- Sensitivity depends on worm burden  
- Requires skilled technician  
- Throughput can be optimized using an automated nucleic acid extraction robot                                                                                                                                 | Maksimov et al., 2017     |
| Copro-antigen ELISA\(^e\)                                 | High               | Incubator, ELISA plate washer, ELISA reader                                           | Monoclonal: 63.2 (55.3–70.8)  
Polyclonal: 56 (48.0–63.9) | Monoclonal: 70.0 (60.1–79.4)  
Polyclonal: 65.9 (55.8–75.6) | -Requires skilled technician  
- Throughput can be optimized using an automated nucleic acid extraction robot                                                                                                                                 | Otero-Abad et al., 2017   |
| Magnetic Capture RT-PCR                                   | Moderate           | 80 °C freezer, Tissue homogenizer, magnet, rotator, heat block, RT-PCR system          | 88.2 (79.8–93.9)   | 99.9 (99.7–100)   | -Requires skilled technician  
- Throughput can be optimized using an automated nucleic acid extraction robot                                                                                                                                 | Isaksson et al., 2014      |
| Canids – serology EUROLINE®-WB (IgG, rEm18, rEm95, rEgAgB) | Moderate           | SDS-PAGE electrophoresis machine, incubator, UV visualization                          | 100 (74–100)       | 98 (91–100)       | -Requires skilled technician  
- Commercially available; recombinant antigens widely available  
- Throughput can be optimized using an automated nucleic acid extraction robot                                                                                                                                 | Frey et al., 2017          |
| Western Blot (EmVF)                                       | Moderate           | SDS-PAGE electrophoresis machine, incubator, UV visualization                          | 100 (77–100)       | 100 (94–100)      | -Requires skilled technician  
- Throughput can be optimized using an automated nucleic acid extraction robot                                                                                                                                 | Frey et al., 2017          |
| ELISA (EmVF, Em2, rEm95, rEm18)                           | High               | SDS-PAGE electrophoresis machine, incubator, UV visualization                          | EmVF: 100 (78–100)  
Em2: 79 (52–92)  
rEm18: 79 (52–92)  
rEm95: 100 | EmVF: 85 (74–92)  
Em2: 97 (89–99)  
rEm18: 85 (74–92)  
rEm95: 100 | -Requires skilled technician  
- Throughput can be optimized using an automated nucleic acid extraction robot                                                                                                                                 | Frey et al., 2017          |
Table 6 (continued)

| Diagnostic test                          | Through-puta | Equipment requirements                                      | Sn% (95%CI)b | Sp% (95%CI)c | Notes                                                                 | Citation         |
|------------------------------------------|--------------|-------------------------------------------------------------|--------------|--------------|----------------------------------------------------------------------|------------------|
| Humans – serology                        | High         | DIGFA test kit                                              | 77.8         | 97.1         | -Commercially available -Rapid diagnostic test suitable for field conditions | Feng et al., 2010 |
| Em2-DIGFA†                               | Low          | −80 °C freezer, centrifuge, RT-PCR system                  | 33–100       | NR           | -Requires skilled technician -Detection depends on egg recovery assay, DNA extraction technique and PCR primers | Umhang et al., 2017 |
| Antibody Gold Immunochromatographic assay| High         | Immunochromatographic test kit                             | 97.5±       | 95.8         | -Commercially available -Rapid diagnostic test suitable for field conditions | Gao et al., 2018  |
| Environment – soil                       |              |                                                             |              |              |                                                                      |                  |
| Taenid egg recovery/RT-PCR               |              |                                                             |              |              |                                                                      |                  |

*a Low - <20 samples/technician/day; Moderate - 21–50 samples/technician/day; High - >50 samples/technician/day.

*b Sensitivity.

*c Specificity.

*d Multiplex Polymerase Chain Reaction using Trachsel et al., 2007 primers

*e Enzyme-linked Immunosorbent Assay.

*f Dot Immunogold Filtration Assay.

*g Other test characteristics: PLR: 23.0 (17.3–30.7); NLR: 0.03 (0.01–0.07); PPV: 78.2 (72.8–82.7); NPV: 99.6 (99.0–99.9); Accuracy: 96 (94.8–97.0).

3.5. *E. multilocularis* diagnostic test evaluations

Our review identified nine studies that conducted Phase III or Phase III field surveillance to evaluate the diagnostic accuracy of techniques to detect *E. multilocularis* in people, canids or the environment (Table 6). Seven of these protocols screened canids for cestode or metacestode infection, and were categorized as assays for (i) intestinal examination (N = 1), (ii) fecal analysis (N = 1), or (iii) serology (N = 1):

(i) The SSCT is a modification of the gold standard SCT protocol that reduces processing time by examining cestode predilection sites in the intestinal tract (Umhang et al., 2011). The SSCT has a high sensitivity (>98.3%) and specificity compared to SCT (both depend on the intestinal segment), but lower accuracy in estimating infection intensity.

(ii) Fecal analysis is a non-invasive method of sampling canids; however, the eggs shed by *E. multilocularis* cestodes are morphologically indistinguishable for those of other taeniid species and molecular analyses can lack sensitivity due to inconsistent shedding of eggs and the difficulty of extracting DNA from taeniid eggs. We identified one study that aimed to optimize molecular detection by testing different combinations of commercially available kits for DNA extraction with PCR and qPCR protocols (Maksimov et al., 2017). Using IST as the reference standard, the authors identified QIAamp® Fast DNA Stool Mini Kit with qPCR (using QuantiTect® Multiplex-Master Mix) as having the highest sensitivity (97%) of the combinations tested. An alternative technique, DNA fishing using magnetic probes to extract DNA in combination with RT-PCR, is less sensitive (88.2%) relative to the SCT but is appealing for mass surveillance as it can be partially automated for high throughput (Isaksson et al., 2014). This systematic review identified other techniques, such as arecoline purgation, copro-antigen ELISAs and PCR, which had even lower sensitivity but that generally had acceptable specificity (close to 100%; Maksimov et al., 2017; Ziadinov et al., 2008; Otero-Abad et al., 2017).

(iii) Lastly, an evaluation of native and recombinant antigens highlighted EmVF-Western Blot and rEm95-ELISA as two highly sensitive (100%) and specific (100% and 98%, respectively) options for serological diagnosis of AE in canids (Frey et al., 2017).

Of the two human serological rapid diagnostic tests evaluated against abdominal ultrasound as a reference standard, the antibody gold immunochromatographic assay demonstrated higher sensitivity (97.8%) and specificity (95.8%) than the Em2-DIGFA assay. Both tests are commercially available, field stable, and can be used to rapidly screen large numbers of people (Table 6).

Six of the nine studies reported the analytical approach used to calculate sensitivity and specificity, and these were as follows: standard 2 × 2 calculation and modelling methods (Receiver Operating Curve/Area Under the Curve, Hui-Walter maximum likelihood estimation, and Bayesian Latent Class model; Iskenderali Ziadinov et al., 2008; Jiang et al., 2012; Otero-Abad et al., 2017; Gao et al., 2018; Isaksson et al., 2014; Frey et al., 2017). Ziadinov et al., 2008 used maximum likelihood estimation to determine test characteristics in dog populations from different villages in Kyrgyzstan, while Otero-Abad et al., 2017 employed Bayesian latent class analysis to jointly estimate test characteristics of four tests, prevalence and covariates related to prevalence in foxes.
Both methods allow for determination of sensitivity and specificity in the absence of a gold standard. No studies were identified that evaluated new methods of detecting *E. multilocularis* in environmental samples at the Phase III field level.

### 3.6. QUADAS 2 quality appraisal

Only one of the nine studies that evaluated diagnostic assays for *E. multilocularis* surveillance in people, canids or the environment was considered low risk of bias and concern across all QUADAS2 criteria (Table 7; Gao et al., 2018). High risk of bias related to index and reference tests was generally a result of poor transparency on interpreting test thresholds and/or lack of clarity on whether diagnosticians were blinded between index and reference test results. The high risk of bias associated with participant selection was due to the frequent use of case-control studies and unclear reporting on exclusion criteria. Similarly, lack of information regarding time intervals between reference and index tests contributed to the assessment of high bias risk for flow and timing for approximately half of studies. In general, few applicability concerns were identified, indicating that participant selection and the use and interpretation of index and reference tests matched the questions posed by the systematic review.

### 3.7. Grey literature search

Searches performed on grey literature search engines and government databases collected 276 reports, of which 12 were health system protocols or surveillance reports from endemic countries and deemed relevant (Table 8). Nine of these were European Union (EU) reports on surveillance methodology and outcomes in canids and humans, of which two provided information on diagnostic methods (Madslien et al., 2013; Antolova et al., 2014). Annual surveillance for *E. multilocularis* in red foxes in Norway is conducted to maintain official “free-from” status, for which the 2011–2012 assessment utilized magnetic capture real-time PCR to detect positive cases (Madslien et al., 2013). Antolova et al., 2014 carried out multi-year surveillance in Slovakia, using nested PCR for dogs, SCT for red foxes as well as ELISA, western blot and imaging techniques for humans (Table 8). While diagnostic techniques were not reported in EFSA surveillance reports, the 2015 citation states that the human case definition of echinococcosis does not differentiate between the two forms of the disease (EFSA, 2015). The EU defines human echinococcosis as at least one of the following: (1) histopathology or parasitology compatible with *E. multilocularis* OR *E. granulosus* (direct visualization of the protoscolex in cyst fluid); (2) detection of *E. granulosus* pathognomonic macroscopic morphology of cyst(s) in surgical specimens; (3) typical organ lesions detected by imaging techniques (CT, sonography or MRI) AND confirmed by a serological test; (4) *Echinococcus* spp. specific serum antibodies by high-sensitivity serological test AND confirmed by a high specificity serological test; (5) detection of *E. multilocularis* or *E. granulosus* nucleic acid in a clinical specimen (ECDC, 2018b).

Three Canadian health system protocols were found, including two 2018 Ontario Public Health Standards outlining guidance on conditions and appropriate diagnostic methods for case detection and surveillance, for measurement error to develop transmission dynamics models (Maksimov et al., 2008). Appendix B provided provincial case definitions and appropriate diagnostic methods for case detection and surveillance.

### Table 7

Quality appraisal of studies that reported diagnostic test characteristics of novel methods for *Echinococcus multilocularis* surveillance in people, canids, or the environment at the population level (2008–2018).

| Title | Author, year | Risk of bias | Applicability concerns |
|-------|--------------|--------------|------------------------|
| Dot immunogold filtration assay (DIGFA) with multiple native antigens for rapid serodiagnosis of human cystic and alveolar echinococcosis | Feng et al. 2010 | ●●●○○ | ○○○○ ○○○○ |●●●●○○○○ |
| Field evaluation of an immunochromatographic test for diagnosis of cystic and alveolar Echinococcus | Gao et al. 2018 | ○○○○○ | ○○○○○ |○○○○○○○○ |
| Dogs as victims of their own worms: serodiagnosis of canine alveolar echinococcosis | Frey et al. 2017 | ● ● ○ ○ | ● ● ● ○ |○○○○○○○○ |
| 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) fecal samples | Isaksson et al. 2014 | ●●●●○ | ○○○○○ |○○○○○○○○ |
| Specific detection of Echinococcus spp. from the Tibetan fox (Vulpes ferrilata) and the red fox (V. vulpes) using copro-DNA PCR as reference | Jiang et al. 2012 | ○●●●● | ○●●●○ |○○○○○○○○ |
| Latent class models for Echinococcus multilocularis diagnosis in foxes in Switzerland in the absence of a gold standard | Otero-Abad et al. 2017 | ● ● ●●● | ○ ○ ○ ○ |○○○○○○○○ |
| Segmental Sedimentation and Counting Technique (SSCT): An adaptable method for qualitative diagnosis of Echinococcus multilocularis in fox intestines | Umhang et al. 2011 | ●●●●○ | ○●●●○ |○○○○○○○○ |
| Canine echinococcosis in Kyrgyzstan: Using prevalence data adjusted for measurement error to develop transmission dynamics models | Zaidinov et al. 2008 | ●●●●○ | ○●●●○ |○○○○○○○○ |
| Comparison of different commercial DNA extraction kits and PCR protocols for the detection of Echinococcus multilocularis eggs in fecal samples from foxes | Maksimov et al. 2017 | ●●●●○ | ○●●●○ |○○○○○○○○ |

Not consistent with criteria, high risk of bias; ○ Consistent with criteria, low risk of bias; Δ Unknown risk of bias.
Table 8
English language grey literature reports of country level surveillance or regulations pertaining to *Echinococcus multilocularis* in canids, humans, or environment (2008–2018).

| Title                                                                 | Author, year | Report type         | Host/source | Case definition(s)                                                                 | Reportable/notifiable | Diagnostic method (s) | Prevalence % (95%CI) |
|-----------------------------------------------------------------------|--------------|---------------------|-------------|------------------------------------------------------------------------------------|-----------------------|-----------------------|----------------------|
| Scientific and technical assistance on *Echinococcus multilocularis* infection in animals | EFSA, 2012   | Health system protocol | Canids      | Any definitive host animal confirmed positive for *E. multilocularis* based on the results of the diagnostic tests described in Annex II of Regulation (EU) No 1152/2011 and having epidemiological information consistent with infection in the country | EU Regulation No 1152/2011 | NA                    | NA                   |
| The surveillance and control programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway. Hunting season 2011–2012. | Madslien et al., 2013 | Annual surveillance report | Red foxes   | Dogs, foxes: NR Humans: At least 2 of following 4 criteria: (i) presence of typical organ lesions, (ii) presence of antibodies to *E. multilocularis*, (iii) histological findings compatible with *E. multilocularis* metacestode, or (iv) presence of *E. multilocularis* DNA | NR                    | Magnetic Capture RT-PCR | 0 (0–0.51) |
| Alveolar echinococcosis in a highly endemic area of northern Slovakia between 2000 and 2013 | Antolova et al., 2014 | Multi-year surveillance report | Dogs, foxes | Dogs: Nested PCR Red foxes: SCT Humans: ELISA, Western blot, Imaging (Ultrasound, MRI, CT) | NR                    | Dogs: 2.9 Red fox: 26–50 Humans: 26 cases |
| The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013 | EFSA and ECDC, 2015 | Annual surveillance report | Foxes (Human data reported in ECDC annual reports) | Zoonoses Directive 2003/99/EC, | NR                    | Germany = 21.87 Slovakia = 22.3 Lux = 5.41 Sweden = 3.17 |
| The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014 | EFSA and ECDC, 2015 | Annual surveillance report | Foxes (Human data reported in ECDC annual reports) | Zoonoses Directive 2003/99/EC, | NR                    | Sweden 0.1 Denmark 2.0 Germany 23.4 Slovakia 15.8 Hungary 9.9 |
| Annual epidemiological report 2014 - Echinococcosis | ECDC, 2016 | Annual surveillance report | Humans | 2008 or 2012* Case definition acceptable. | NR                    | NR                    | 82 cases from 7 EU/EEA countries |
| The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015 | EFSA and ECDC, 2016 | Annual Surveillance Report | Foxes (Human data reported in ECDC annual reports) | Zoonoses Directive 2003/99/EC, | NR                    | Luxembourg 26.9 Switzerland 28.6 Germany 23.6 France 21.5 Slovakia 21.5 Denmark 8.06 Hungary 5.5 Sweden 0.1 |
| Echinococcosis - Annual Epidemiological Report for 2015 | ECDC, 2017 | Annual Surveillance Report | Humans | 2008 or 2012* Case definition acceptable. | NR                    | NR                    | 135 cases and 1 death from 6 countries (Table 2 lists countries for 2014 and 2015) |
| Echinococcosis - | ECDC, | Annual | Humans | 2008 or 2012* Case | NR | NR | 104 cases |

(continued on next page)
4. Discussion

Despite recent improvements to diagnostic technologies, AE remains a life-threatening infection for humans and animals; in part because patients across endemic countries do not have equal access to modern diagnostics and treatments. This systematic

| Title                                                                 | Author, year | Report type          | Host/source       | Case definition(s)                                         | Reportable/notifiable | Diagnostic method                                                                 | Prevalence % (95%CI) |
|----------------------------------------------------------------------|--------------|----------------------|-------------------|------------------------------------------------------------|-----------------------|-------------------------------------------------------------------------------------|---------------------|
| Annual Epidemiological Report for 2016                              | MOHLTC, 2018a| Surveillance Report   | Humans            | definition acceptable.                                     | NR                    | Health Protection and Promotion Act, R.R.O. 1990, Reg. 569, Reports, (2018), and as per Requirement #3 of the “Reporting of Infectious Diseases” section of the Infectious Disease Protocol, 2018. Serology performed at the Institute of Parasitology, University of Berne, Switzerland: Em2-ELISA, II/3-10-ELISA, Em2Plus-ELISA. | NA                  |
| Ministry of Health and Long-term Care Infectious Diseases Protocol, Appendix A, Chapter: Echinococcus multilocularis infection | MOHLTC, 2018b| Health system protocol | Humans            | Ontario provincial case definition for human infection with *E. multilocularis* (in the presence of clinically compatible signs and symptoms): • Demonstration of antibodies to *E. multilocularis* in blood or serum sample OR • Demonstration of larval stages of *E. multilocularis* in histopathology samples from tissue biopsies | Conﬁrmatory and probable cases of disease are provincially reportable. Serology performed at the Institute of Parasitology, University of Berne, Switzerland: Em2-ELISA, II/3-10-ELISA, Em2Plus-ELISA. Confirmatory assays: PCR (tissue biopsies), direct immunofluorescence | NA                  |
| Management of *Echinococcus multilocularis* Infections in Animals Guideline, 2018 | MOHLTC, 2018c| Health system protocol | NR                | Communicable Diseases Regulation (R.R.O. 1990, Reg. 557). A veterinarian or laboratory director who knows or suspects that one or more animals is infected with *E. multilocularis* shall notify the Medical Officer of Health within one business day. The board of health shall report all cases of *E. multilocularis* in animals to the ministry after receiving the report. | NR                    | NA                                                                                 | NA                  |

EFSA = European Food Safety Authority; ECDC = European Centers for Disease Control; OMHLTC = Ontario Ministry of Health and Long-term Care; NA = Not applicable; NR = Not reported, RT-PCR = Real Time Polymerase Chain Reaction.

a 2012 case definition for echinococcosis is at least one of the following five: (1) histopathology or parasitology compatible with *E. multilocularis* OR *E. granulosus* (direct visualization of the protoscolex in cyst fluid); (2) detection of *E. granulosus* pathognomonic macroscopic morphology of cyst(s) in surgical specimens; (3) typical organ lesions detected by imaging techniques (computerized tomography, sonography or MRI) AND confirmed by a serological test; (4) *Echinococcus* spp. specific serum antibodies by high-sensitivity serological test AND confirmed by a high specificity serological test; (5) detection of *E. multilocularis* or *E. granulosus* nucleic acid in a clinical specimen.
review identified examples of case finding in 27 of 43 countries where _E. multilocularis_ is known to be present, confirming that there continue to be knowledge gaps in the global distribution and burden of this parasite (Deplazes et al., 2017; Torgerson et al., 2010; EFSA and ECDC, 2016). Prevalence estimates were most notably missing from Russia, which has the second highest reported annual incidence after China (Torgerson et al., 2010), and were also missing from other Central Asian countries (Azerbaijan, Georgia, Mongolia, Tadjikistan, Uzbekistan, Turkmenistan). Time period and language limitations of our search strategy might partially explain these gaps; however, it is also likely that limitations in reporting infrastructure, access and availability of diagnostic tests, poor physician and veterinarian awareness, long asymptomatic period, and presence of other closely related cestodes contribute to the general issues of under-diagnosis and under-reporting universal to characterizing this parasite (Eckert et al., 2001; EFSA and ECDC, 2016). These barriers to understanding the true burden of AE are cause for concern because _E. multilocularis_ appears to be emerging in areas of North America, Europe and Asia.

This review identified nine protocols that were evaluated through Phase III and Phase III field trials for accuracy in diagnosing AE. There is currently no international consensus on specific gold standard protocols to detect this parasite in humans, animals, or the environment. Recommendations by the World Health Organization-Informal Working Group on Echinococcosis state that diagnostic criteria for AE in humans require parasite detection using at least one of the following: (i) imaging, (ii) serology, (iii) histopathology, (iv) nucleic acid detection (Brunetti et al., 2010). Many laboratories consider SCT to be the gold standard for case detection in wild canids (Eckert et al., 2001; Conraths and Deplazes, 2015), and as a result, new diagnostic techniques are often evaluated against this standard. However, two recent studies, one comparing SCT and three other diagnostic techniques in latent class models (Otero-Abad et al., 2017) and the second comparing SCT directly to magnetic capture RT-PCR (Isaksson et al., 2014), provide evidence that the sensitivity of SCT is lower than originally thought (Eckert et al., 2001). Therefore, it would be sensible to reconsider the value of SCT in surveillance and as a reference standard for diagnostic test evaluations (Conraths and Deplazes, 2015). As importantly, intestinal examination requires death of the animal, which has ecological effects when conducted as part of mass surveillance, and which is also not suitable for diagnosing infection in domestic or captive canids. We noted some studies conducting intestinal examination as a stand-alone determinant of _E. multilocularis_ infection did not report morphologically identifying cestodes, which would delay early detection of other emerging _Echinococcus_ species. Our study suggests that the latest ante-mortem developments in commercial and in-house technology lack diagnostic sensitivity and/or specificity (Table 5), although older techniques excluded by the timeline of this study do exist (EFSA and ECDC, 2016). Magnetic capture RT-PCR performed on fecal matter had the highest reported diagnostic accuracy of these assays and can be semi-automated for mass surveillance but requires costly equipment and reagents (Isaksson et al., 2014). In contrast, several promising serological tests were identified for detecting metacestode infection in human and canid hosts, each with sensitivity and specificity exceeding 95%. These included a commercially available but modified EUROLINES®-Western Blot (based on IgG, rEm18, rEm95, rEgAgB), an in-house Western Blot (EmVF) and an in-house ELISA (rEm95) for use in dogs, and a commercially available antibody gold immunochromatographic rapid diagnostic test for use in people (Gao et al., 2018; Frey et al., 2017). Diagnostic tests have differing capacity for detecting pre-patent, early metacestode and low intensity infections (Conraths and Deplazes, 2015). Furthermore Phase III field evaluations of test accuracy are often performed on one host in one locale, ignoring potential differences in accuracy across host species and prevalence (Conraths and Deplazes, 2015). Therefore, mass screening campaigns should consider the epidemiological situation of a region and the detection limits of diagnostic tests when creating a surveillance strategy.

Laboratory capacity is an integral component of health system infrastructure, and such services play a key role in detection, assessment, response, notification, and monitoring of public health events. According to the World Bank, seven AE endemic countries were classified as low income (Kyrzgyzstan, Tadjikistan) or low-middle income (Georgia, Mongolia, Uzbekistan, Moldova, Ukraine) at the mid-point of this review of these, our search only captured prevalence estimates for Kyrzgyzstan (The World Bank, 2018; Ziadinov et al., 2008; Ziadinov et al., 2010). Some _E. multilocularis_ detection techniques, such as SCT, arecoline hydrobromide purgation and Em2-DIGFA, require minimal equipment and are feasible for a range of settings. However, most molecular and immunological detection techniques require significant investments to laboratory infrastructure and technician training as well as access to expensive reagents, making them inaccessible to diagnosticians in low resource regions. It is also important to consider the safety aspects of various diagnostic tests. For example, dogs treated with arecoline hydrobromide have died of bone splinters puncturing the intestinal tract. Technicians collecting and analyzing freshly purged fecal matter must be properly equipped with personal protective equipment, and environments housing purged animals must be thoroughly decontaminated from infectious _E. multilocularis_ eggs (Eckert et al., 2001). High income countries currently engaged in _E. multilocularis_ surveillance can play a greater role in building capacity for surveillance, diagnostics, research and treatment among lower resource countries, which would ultimately deliver mutual benefits given the ability of infected wild canid to move freely between endemic and non-endemic regions. Moreover, it is not only low income countries experiencing capacity shortages. The outsourcing of Ontario medical diagnostics to Swiss laboratories for confirmatory testing suggests the need for improved laboratory capacity in Canada (MOH, 2018c). As well, _E. multilocularis_ specimens collected from humans do not appear to be sequenced routinely (our study found only one example (Ma et al., 2015)). This represents a lost opportunity to explore parasite origin, to confirm the emergence of specific haplotypes into new areas, or to investigate the biological and clinical significance of parasite diversity.

While our findings show Europe as a leading region in AE surveillance and reporting, diagnostic methods were not explicitly stated within ECDC reports, likely due to the lack of standardization across Member State (MS) laboratories (EFSA Panel on Animal Health and Welfare, 2015). Moreover, there exists a high degree of discrepancy of diagnostic test characteristics reported between MS, and as reported by pooled meta-analysis evaluations (Conraths and Deplazes, 2015; EFSA Panel on Animal Health and Welfare, 2015; Casulli et al., 2015). One third of diagnostic accuracy evaluations in our study did not report how sensitivity
and specificity were calculated, while others utilized advanced modelling methods to compare measures across prevalence parameters. These findings indicate the need for standardized internal AE protocols for laboratory diagnostics within endemic and newly emerging regions. Our team did not carry out meta-analysis for test characteristics given the small number of studies collected and the variation of test types, host populations and population prevalence. We chose not to report pooled estimates of population prevalence, as combining studies by higher-level geographical designations would misrepresent prevalence variations by regions.

These diagnostic considerations bear strongly on mechanisms of disease reporting, and particularly on case definitions for surveillance. Definitions utilized by the EU and Ontario (Canada) denote a case positive by the outcome of any one of a number of tests. Moreover, some components of the case definition algorithms require a positive result from multiple diagnostic tests run in series to yield a final positive classification. In our study, case definitions in primary surveillance studies were often not described, and it was difficult to interpret the relationships between multiple tests in a diagnostic procedure. The reliance of case definitions on multiple test outcomes, combined with substantial variation in test characteristics, predictive values and performance of gold standards, is a barrier to accurately assessing the confidence of reported prevalence estimates. This is further compounded by a lack of consistency in application of case definitions within health jurisdictions. For example, while the EU has an established annual reporting system for *E. multilocularis*, only 23 countries in 2016 reported echinococcosis cases using the 2008 or 2012 case definitions, neither of which are species specific (ECDC, 2018b). Species specific reporting is especially important for co-endemic regions, and is also a problem in Canada, where patient records obtained from the Canadian Institute for Health Information show that doctors often do not differentiate between *Echinococcus* species (Schurer et al., 2015). Development of case definitions should consider the test characteristics of diagnostic procedures wherever possible, be standardized for classifying/counting cases consistently across reporting jurisdictions, and should, at minimum, differentiate between *Echinococcus* species.

Our literature search did not identify a source that summarized mandatory versus voluntary *E. multilocularis* reporting all AE endemic countries. In the EU, notification of human echinococcosis is mandatory for 22 MS, although other countries do voluntarily report cases (EFSA and ECDC, 2016). Echinococcosis in animals is notifiable in 17 MS, and contamination of food is notifiable in 10 MS (EFSA and ECDC, 2016). Surveillance for *E. multilocularis* in Europe is usually carried out on red foxes, and is predominantly diagnosed using morphological (SCT) or molecular (PCR) methods. Four EU countries (Finland, Ireland, Malta and the United Kingdom) are considered free from *E. multilocularis* and must conduct annual surveillance to retain this status (as per Regulation (EU) No 1152/201117). Human AE is not nationally notifiable in Canada, but it became provincially reportable in 2018 in response to heightened prevalence in wild canids and the novel detection of AE in domestic dogs within Ontario (Government of Ontario, 2018). Detection of *E. multilocularis* in animals and food is not currently notifiable and we did not find any documents to suggest that the government was involved in active surveillance in people, animals, or food. Human AE is reportable in China (Ding and Li, 2016), Turkey (Altintas, 2008) and Kyrgyzstan (Usubalieva et al., 2013). Considering the increased concern for this parasite in regions of Canada, Europe and Asia, there exists a need for mandatory reporting frameworks based on consistent case definitions. Moreover, developers of surveillance and reporting frameworks should consider applying a One Health approach to create enhanced systems that work synergistically in monitoring human, animal and environmental sources, especially as this parasite continues to build in importance.

4.1. Conclusions

Individuals infected with AE require early, affordable, and accurate diagnosis as well as access to modern treatment to ensure a favourable prognosis. Our study identified barriers to this goal that included scarce surveillance in low and low-middle income AE endemic countries, lack of consensus on diagnostic gold standards, reliance on convenience sampling for human and canid studies, poor reporting of case definitions, genus versus species level diagnosis, and infrequent submission of nucleotide sequences public databases. Improving reporting infrastructure systems is an important next step to comprehensively defining the global health burden and geographic distribution of *E. multilocularis*, as well as to monitoring emergence of this parasite into new areas and host types. Mandatory reporting of human cases in endemic countries and animal cases in non-endemic countries, data sharing between government agencies engaged in human, animal and environmental surveillance, and strengthened partnerships between low and high resource countries are all strategies to optimize health equity for AE patients. Furthermore, national *E. multilocularis* control programs should consider diagnostic test limitations with respect to host species, estimated local prevalence, and presence of other *Echinococcus* species when designing surveillance strategies. The formation of a joint WHO/OIE world reference laboratory with a mandate to develop diagnostic protocols, identify *Echinococcus* species, and design integrated human-animal surveillance systems could go a long way in achieving these goals.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fawpar.2019.e00048.
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