IJP: Parasites and Wildlife 7 (2018) 111–115

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

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

Detecting co-infections of *Echinococcus multilocularis* and *Echinococcus canadensis* in coyotes and red foxes in Alberta, Canada using real-time PCR

Maria A. Santa[a], Sonya A. Pastran[a], Claudia Klein[b], Padraig Duignan[c], Kathreen Ruckstuhl[a], Thomas Romig[d], Alessandro Massolo[c,e,f,*]

[a] Department of Biology, University of Calgary, Alberta, T2N 1N4, Canada
[b] Department of Veterinary Clinical Diagnostic Sciences, Faculty of Veterinary Medicine, University of Calgary, Alberta, T2N 4Z6, Canada
[c] Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, T2N 4Z6, Canada
[d] Institute of Zoology/Parasitology, University of Hohenheim, 70593, Stuttgart, Germany
[e] Ethology Unit, Department of Biology, University of Pisa, 56126, Pisa, Italy
[f] UMR CNRS 6249 Chrono-environnement, Université Bourgogne Franche-Comté, 25030, Besançon, France

ARTICLE INFO

Keywords:
Echinococcus canadensis
Echinococcus multilocularis
Coyote
Red fox
Canada
Co-infection
Diagnostics

ABSTRACT

The continued monitoring of *Echinococcus* species in intermediate and definitive hosts is essential to understand the eco-epidemiology of these parasites, as well to assess their potential impact on public health. In Canada, co-infections of *Echinococcus canadensis* and *Echinococcus multilocularis* based on genetic characterization have been recently reported in wolves, but not yet in other possible hosts such as coyotes and foxes. In this study, we aimed to develop a quantitative real-time PCR assay to detect *E. multilocularis* and *E. canadensis* and estimate the occurrence of co-infections while inferring about the relative abundance of the two parasites within hosts. We tested DNA extracted from aliquots of *Echinococcus* spp. specimens collected from intestinal tracts of 24 coyote and 16 fox carcasses from Alberta, Canada. We found evidence of co-infections of *E. multilocularis* and *E. canadensis* in 11 out of 40 (27%) samples, with 8 out of 24 (33%) in coyote samples and 3 out of 16 (19%) in red fox samples. DNA concentrations were estimated in three samples with Cq values within the range of the standard curve for both parasites; two of them presented higher DNA concentrations of *E. multilocularis* than *E. canadensis*. The use of qPCR aided detection of co-infections when morphological discrimination was difficult and quantification of DNA for samples within the standard curve. This is the first molecularly confirmed record of *E. canadensis* in coyotes and the first evidence of co-infections of *E. multilocularis* and *E. canadensis* in coyotes and red foxes.

1. Introduction

The genus *Echinococcus* currently includes at least nine species of parasites (reviewed in Romig et al., 2017) some of which have a cosmopolitan distribution and represent an important concern for animal and public health. As a typical taeniid cestode, *Echinococcus* spp. cycle between two mammalian hosts which may include both domestic and wild animals. Carnivores act as definitive hosts, harbouring the adult worms in the intestine and shedding the parasite's embryonated eggs into the environment, while a wide variety of intermediate hosts develop larval stages in their viscera upon ingestion of eggs. Humans can be affected as aberrant hosts and develop echinococcosis disease by accidental ingestion of embryonated eggs (Thompson, 2017).

In Canada, only two *Echinococcus* species have been confirmed so far: *Echinococcus multilocularis* and *Echinococcus canadensis* (G8 and G10 genotypes) (Davidson et al., 2016) which are causative agents of human alveolar echinococcosis (AE) and cystic echinococcosis (CE), respectively. Previously, *E. canadensis* was classified as a subspecies of *Echinococcus granulosus* (Sweatman and Williams, 1963) but was later recognized as a cryptic species within the *E. granulosus* (sensu lato) complex (Romig et al., 2015). *E. canadensis* is widely distributed across Canada with mainly wolves (*Canis lupus*), but also coyotes (*Canis latrans*) and dogs as definitive hosts, whereas intermediate hosts include cervids such as moose (*Alces alces*), elk (*Cervus canadensis*), caribou (*Rangifer tarandus*) and deer (*Odocoilecus* spp.) (Sweatman, 1952; Rausch, 2003; Romig et al., 2015).

The historical distribution of *E. multilocularis* in Canada encompasses two disjunct geographic areas: Northern Tundra Zone and North Central Region, both involving different predator-host communities (Eckert et al., 2001). However, recent findings of the parasite in

* Corresponding author. Ethology Unit, Department of Biology, University of Pisa, 56126, Pisa, Italy.
E-mail address: alessandro.massolo@unipi.it (A. Massolo).

https://doi.org/10.1016/j.ijppaw.2018.03.001
Received 10 January 2018; Received in revised form 27 February 2018; Accepted 1 March 2018

Table 1
Primers and hydrolysis probes designed for a partial sequence of the *nad2* gene of *Echinococcus multilocularis* and for the *cox1* gene of *Echinococcus canadensis* used to detect infections in coyotes and foxes from Alberta, Canada.

Target species	Primer/Probe[a]	Oligonucleotide sequence (5′–3′)	Amplicon size
Echinococcus multilocularis	Nad234_F	TTGTTGAGCTATGTAATAATGTGTGGAT	
	Nad234_R	CATAAATGGAAACAAACCAAACTTCA	126bp
	Nad234_P	FAM-CTGTGCTATTAGTACTC-MGB-NFQ	
Echinococcus canadensis	Cox143_F	ATGAGGTGTTGGGTTCGTATAGG	143bp
	Cox143_R	ACAATCATCAACCCAACGCA	
	Cox143_P	FAM-TTGGTTTGGTGGATTATT-MGB-NFQ	

[a] F: forward; R: reverse; P: probe.

areas previously considered as nonendemic suggest an expansion of its geographic range (Schurer et al., 2013b, 2016; Gesy et al., 2014). This parasite circulates primarily through smaller canids as definitive hosts: foxes (*Vulpes* spp.) and coyotes; whereas intermediate hosts include several species of small mammals, mostly small rodents such as *Peromyscus*, *Myodes* and *Microtus* species (Holmes et al., 1971; Eckert et al., 2001; Liccioli et al., 2013). Domestic species such as dogs can be involved in semi-synanthropic cycles; however, the lifecycle of the parasite is considered primarily sylvatic (Eckert et al., 2001).

Morphological identification of *Echinococcus* spp. worms can be labour-intensive and difficult. At present, molecular tools are commonly used to identify species and genotypic variations and can be applied to fecal samples collected in the environment. However, microscopic screening of intestinal content to identify and count *Echinococcus* worms is still the most accurate way to estimate the intensity of infection with a high sensitivity and specificity (Gesy et al., 2013; Conraths and Deplazes, 2015). Nevertheless, in areas co-endemic for *Echinococcus* species, difficulties in morphological identification in early stages of infection could lead to misdiagnosis (Conraths and Deplazes, 2015).

The recent findings of the first *E. multilocularis* infections in wolves in Canada, including mixed infections with *E. canadensis* (Schurer et al., 2013b), led us to the hypothesis that co-infections with these parasites could also be present in other definitive hosts. Therefore, we aimed to develop a real-time PCR assay to be used for detection of mixed infections of *E. multilocularis* and *E. canadensis,* to estimate the occurrence of co-infections in coyotes and red foxes in Alberta, Canada. We also aimed to assess the feasibility of using quantitative real-time PCR (qPCR) results to infer about the relative abundance of the two species within hosts.

2. Materials and methods

2.1. Collection of host and parasite samples

We used aliquots of *Echinococcus* spp. specimens collected from intestinal tracts of 24 coyote and 16 fox carcasses of road-killed and trapped animals collected between 2012 and 2016, from rural and urban areas in Alberta, Canada. Intestinal tracts were collected upon post-mortem examination, frozen at −80 °C for 72 h to inactivate eggs and then stored at −20 °C upon further processing. Carcasses and intestines were processed during the same year of collection. The intestines were examined for the presence of *Echinococcus* spp. using the scraping, filtration and counting technique (SFCT) described by Gesy et al. (2013) with the following modifications: intestinal content was filtered through sieves of decreasing mesh size (1 mm, 250 µm and 75 µm), and 25% of each aliquot was analyzed to determine the worm burden. The total filtrate from the 250 µm and 75 µm sieves was collected in a final volume of 50 ml of 70% ethanol for further extraction of DNA from the worm population per host (mixed worm samples).

2.2. DNA extraction

Total DNA extraction was performed from mixed worm samples per host using 5 ml of the 50 ml aliquot that represents approximately 10% of the worm population per host. The E.Z.N.A stool DNA kit (Omega bio-tek, US) was used to prepare bulk DNA lysates to remove PCR inhibitors commonly found in intestinal content samples. After careful mixing, five milliliters of the sample, which included worms and the intestinal content that passed through the sieves, were centrifuged to remove the ethanol (5 min, 13,000 × *g*). Tubes were left open for an additional 20 min for evaporation to occur and the remaining pellet was used for DNA extraction (up to 200 mg per extraction) following the manufacturer's instructions. If needed, more than one extraction was performed per sample according to the amount of pellet and then combined in only one DNA extract. The final elution volume was 100 µL and following extraction, DNA was stored at −20 °C until use.

2.3. Real-time PCR on worm populations

2.3.1. qPCR conditions

To evaluate the occurrence of *E. multilocularis* and *E. canadensis* co-infections and quantify the DNA from each, we designed species-specific primers and hydrolysis probes (supplied by Applied Biosystems, US) from highly polymorphic regions of the mitochondrial *nad2* gene for *E. multilocularis* (GenBank accession number AB018440.2) and *cox1* gene for *E. canadensis* (GenBank accession number AB745463.1) using Primer3 software (http://primer3.ut.ee/) (Table 1). Simplex qPCR assay was performed for each target in a final volume of 10 µL containing 5 µl TaqMan Fast Advanced master mix (Applied Biosystems, US), 900 nM forward and reverse primer respectively, 300 nM of hydrolysis probe and 1 µL of DNA extract. We performed reactions in duplicates using a StepOnePlus Real-Time PCR System (Applied Biosystems, US) with the following conditions: 95 °C for 2 min, 40 cycles of 95 °C for 1 s, and 60 °C for 20 s. Additionally, we performed a duplex qPCR with an internal amplification control (IAC) to assess the presence of PCR inhibitors as described by Deer et al. (2010) with modifications of plasmid preparation as described by Klein et al. (2014). For this duplex qPCR, each final 10 µL reaction mixture contained 450 nM of each primer (forward and reverse Nad234/IAC), 125 nM of each hydrolysis probe, 100 copies of the IAC plasmid and 1 µL of total extracted DNA. The threshold for detection of PCR inhibitors was calculated as 34 cycles based on the average Cq value for 100 copies/µL of IAC plasmid run in 7 replicates without DNA extract. PCR inhibitors were considered present when the IAC Cq value of the sample was over 1.5 cycles greater than the IAC Cq average (Klein et al., 2014). In these cases, Cq values were normalized with the IAC Cq average as described by Knapp et al. (2014) to account for the lower efficiency of PCR. If the IAC was not detected, the samples were diluted in ten-fold dilutions to overcome inhibition.

The 143bp and 126bp amplicons obtained from samples positive for both *E. canadensis* and *E. multilocularis* were sequenced with the respective forward primer to validate the results. For samples with low concentration of qPCR product, we performed classical PCR in order to

obtain a higher volume and concentration. The sequences obtained were aligned and compared with nucleotide databases using NCBI Nucleotide BLAST tool (https://blast.ncbi.nlm.nih.gov). The samples that could not be sequenced were tested in a SYBR™ green assay evaluating the melt curve and by agarose gel electrophoresis confirming the presence of single PCR product of expected size. Reactions were carried out in a final volume of 10 µL using Power SYBR™ Green PCR Master Mix (Applied Biosystems, US) 5 µl, 500 nM forward and reverse primers, 3 µL of H2O and 1 µL of DNA. We performed reactions using a StepOnePlus Real-Time PCR System (Applied Biosystems, US) using the default thermocycler program for the standard quantification curve and dissociation curve using SYBR™ green reagents. PCR products were resolved on 1.5% agarose gels containing 0.5 µg/ml ethidium bromide. Additionally, we reanalyzed the aliquots of *Echinococcus* spp. that were positive for co-infection in qPCR, to try to identify and compare both species morphologically (Jones and Pybus, 2008) and validate the molecular results.

2.3.2. Analytic specificity and sensitivity of qPCR

Primers and probes were tested for specificity *in silico* using the NCBI Primer BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Additionally, the specificity was assessed with DNA extracted from single adult worms of *E. multilocularis* (North American and European genotypes), *E. canadensis* (G10 genotype), *Taenia crassiceps* and *Taenia pisiformis* collected from wild canids in Alberta. The identity of the species used for this test was confirmed through sequencing.

The method detection limits (MDLs) and the efficiency of PCR assay were evaluated based on a standard curve of 8 ten-fold serial dilutions of pure DNA from one adult worm each from *E. multilocularis* and *E. canadensis*, ranging from 2 ng/µL to 2×10^{-7} ng/µL. The initial DNA concentration was measured with Nanodrop™ 2000 spectrophotometer (Thermo Fisher Scientific, US). Seven replicates were performed from each ten-fold dilution. The last point of the DNA range representing 7 out of 7 positive trials was considered the limit of *E. multilocularis* and *E. canadensis* DNA detection and accurate quantification.

3. Results

3.1. Analytic specificity and sensitivity of qPCR

The specificity of the primer pair for *E. multilocularis* (Nad234) was 100% according to *in silico* analysis and when tested with DNA from *E. canadensis* and *Taenia* spp. The primers for *E. canadensis* (Cox143) also revealed no amplification of DNA from *E. multilocularis* and *Taenia* spp. For *in silico* analysis, Cox143 forward and reverse primers had also a perfect match with *E. ortleppi*, but presented two mismatches within the probe which may prevent unspecific fluorescent signal.

Based on the standard curve of ten-fold serial dilutions the limit of detection of *E. multilocularis* with Nad234 primers was 35 cycles corresponding to a DNA concentration of 2×10^{-5} ng/µL. Beyond this limit, DNA was considered to be detectable but not quantifiable. The efficiency of PCR assay (E = 10^(-1/slope)) was 95%. The limit of detection for *E. canadensis* with Cox143 primers was 34 cycles corresponding to 2×10^{-4} ng/µL. Based on the standard curve the efficiency was 99% (Fig. 1).

3.2. qPCR on worm populations

All the 40 mixed worm samples were positive for *E. multilocularis* with Cq values ranging from 11.28 to 37.15. DNA from both parasites *E. canadensis* and *E. multilocularis* was detected in 11 out of 40 (27%) samples, with 8 out of 24 (33%) in coyote samples and 3 out of 16 (19%) in red fox samples. Cq values for *E. canadensis* ranged from 20.53 to 38.54 (Table 2). There was no disagreement between replicates from each host and parasite.

Only three samples presented some grade of inhibition and the Cq values were normalized accordingly. In one sample showing total inhibition, the IAC was detected after a 1:10 dilution but the sample was positive only to *E. multilocularis*. For the other two samples showing partial inhibition, only one was positive for co-infection.

The sequences obtained were ~90 long, and the percentages identities were 100% for *E. canadensis* G10 and *E. multilocularis* (European-like strains). Within these sequences, there were at least 10 SNPs to differentiate between *E. multilocularis* and *E. canadensis*, and 3 SNPs to identify *E. canadensis* G10 and G8 strains. For the samples that could not be sequenced, the melt curve and the band on agarose gel corresponded to the targeted amplicon. After reanalyzing aliquots of positive samples for co-infections in qPCR, we identified morphologically *E. multilocularis* adult worms from ~1800 µm to ~3100 µm long. In only a single sample from a coyote were we able to identify *E. canadensis* adult worms, which were ~2300 µm long.

DNA concentrations of *E. canadensis* and *E. multilocularis* in positive samples for co-infection were estimated using the standard curve calculated for each target and the Cq values. Considering the limits of detection for each target, only three samples had Cq values under these limits for both parasites, two of them presenting higher DNA concentrations of *E. multilocularis* than *E. canadensis* (Table 2).

4. Discussion

This study represents the first evidence of co-infections of *E. multilocularis* and *E. canadensis* in red foxes and coyotes and the first molecularly confirmed record of *E. canadensis* infection in coyotes after morphological identification of adult worms. Red foxes have been shown to occasionally act as definitive hosts for *E. granulosus* s.s. in different locations such as Great Britain and Australia (Thompson and Smyth, 1975; Jenkins and Morris, 2003). In North America, Rausch and Schiller (1951) reported *Echinococcus granulosus* s.l. in red and arctic foxes in northern Alaska. This is most likely due to scavenging on domestic and wild host carcasses, although they do not seem to play a significant role in transmission (Carmena and Cardona, 2014; Thompson, 2017). There is no reason to doubt that the presence of *E. canadensis* in red foxes in Alberta is due to scavenging behavior. Although red foxes mainly prey on small animals such as rodents, which are the intermediate host to *E. multilocularis*, they are generalists and opportunistic predators that could feed on cervid carcasses infected with *E. canadensis* cysts. Availability of ungulate carcasses is likely influenced by prey abundance, seasonality, hunting pressure and presence of large carnivores (Theberge and Wedeles, 1989; Needham et al., 2014), and so could be the encounters with *E. canadensis*. For example, post-mortem surveys of cervid hosts and molecular characterization of hydatid cysts indicated a higher prevalence of *E. canadensis* in moose than in caribou, elk, or white-tailed deer (Schurer et al., 2013a). At the same time, it has been suggested that higher parasite intensity in moose may increase the level of predation by wolves and human hunters, due to reduced lung function, making these infected carcasses more accessible to scavengers (Joly and Messier, 2004).

Echinococcus granulosus s.l. has been reported in coyotes in Canada (Alberta, Manitoba, and Ontario); it can be assumed that these parasites have indeed been *E. canadensis*, but in the absence of genotype identification retrospective proof is not possible (Freeman et al., 1961; Holmes and Podesta, 1968; Samuel et al., 1978). Recent studies failed to detect any species of the *E. granulosus* s.l complex in coyotes (Davidson et al., 2016). Although mixed infections of *E. canadensis* and *E. multilocularis* were found in wolves in Western Canada (Schurer et al., 2013b), the present study is the first evidence of co-infections by these parasites in coyotes and foxes. In this study, all the cases positive for *E. canadensis* were identified as genotype G10, which could be related to an apparently higher abundance of G10 over G8 in Canada, according to molecular characterization in both intermediate and definitive hosts (Davidson et al., 2016). Indeed, Schurer et al. (2016), found a prevalence of 6% G10-*E. multilocularis* co-infections in wolves, and an

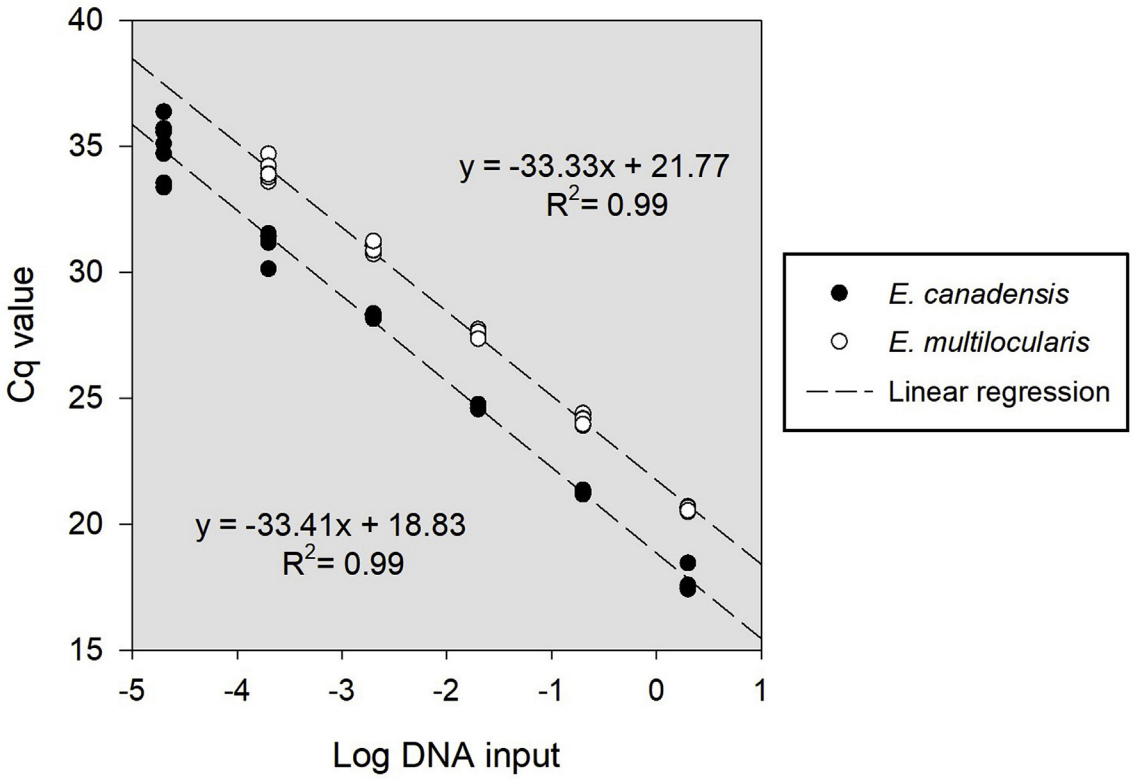

Fig. 1. Standard curve for qPCR assays to detect *E. canadensis* and *E. multilocularis* using Cox143 and Nad234 primers/probes, respectively.

overall prevalence of G8 and G10 genotypes of 6% and 24%, respectively. Interestingly, G8-*E. multilocularis* co-infections were not found.

The detection of mixed infections could be limited by the techniques of intestinal screening and the number of adult worms processed per host (Schurer et al., 2013b). Additionally, morphological identification of the parasites could be affected by the level of autolysis and the stage of development of worms (Conraths and Deplazes, 2015). The use of qPCR allowed for the rapid and sensitive detection of mixed infections using DNA extracted from worm subpopulations per host instead of only using a few worms. Using this approach, *E. canadensis* DNA could be detected in samples where morphological discrimination was not possible or the low burden of worms did not allow for detection.

Interestingly, no *E. canadensis* co-infection was ever found in coyotes and foxes using the intestinal scraping investigation, despite a considerable number of animals that have been examined in recent years (Liccioli et al., 2012; Gesy et al., 2014). This could be due to a reduced development of the species that may make it more difficult to distinguish from *E. multilocularis*. Indeed, Holmes (1961) compared *E. granulosus* adult worms from coyotes and wolves and found that worms in coyotes were smaller and only 5% contained normally developed ova. From all the positive cases to co-infection, we could only identify *E. canadensis* worms in one coyote where the differences in size were more than 500 µm compared to *E. multilocularis* worms and the uterus shape was clearly distinguishable. Additionally, the size of these *E. canadensis* worms only reached ~2300 µm, while it could reach up to 11 mm, as described for *E. granulosus* s.l. species (Jones and Pybus, 2008). On the other hand, the detection of DNA in foxes could possibly result from the consumption of cysts without the establishment of a true infection, as they are generally considered less suitable hosts for *E. granulosus* s.l. The presence of immature worms that established (but would never shed eggs) in a marginally suitable host would also lead to positive PCR results and can therefore not be ruled out. Additional studies (e.g. on the presence of *E. canadensis* eggs in fox faeces) will be needed to draw definite conclusions on the host role of foxes for *E.*

Table 2
Positive samples for co-infection of *Echinococcus canadensis* (*Ec*) and *Echinococcus multilocularis* (*Em*) from coyotes and foxes from Alberta, Canada.

Host	Sample ID	*Em* Cq	*Ec* Cq	DNA *Em* (ng/µL)	DNA *Ec* (ng/µL)	Worm burden
Vulpes vulpes	F7-2016	11.28	38.39	ne	ne	12,200
	F9-2016	26.82	35.26	4.55×10^{-3}	ne	40
	F13-2016	29.74	36.26	6.35×10^{-4}	ne	40
Canis latrans	5–2016	11.22[a]	36.61[a]	ne	ne	91,144
	13–2016[b]	24.06	33.50	2.93×10^{-2}	3.05×10^{-4}	6276
	14–2016[b]	35.08	20.53	1.73×10^{-5}	2.35	260
	17–2016	36.63	31.34	ne	1.35×10^{-3}	404
	27–2016	37.15	23.99	ne	2.16×10^{-1}	608
	35–2016	14.52	38.45	ne	ne	6628
	48–2016	21.68	36.81	1.46×10^{-1}	ne	500
	51–2016[b]	21.84	28.72	1.31×10^{-1}	8.25×10^{-3}	728

ne: not estimated (values out of the range of the standard curve).
[a] Cq values normalized.
[b] Samples with Cq values under limit of detection for both targets.

canadensis.

The use of qPCR allowed a quantitative estimation of the DNA of each parasite, but only for samples with Cq values within the range of the standard curve. A significant negative correlation between the Cq value and the worm burden of *E. multilocularis* was found in previous studies designed to detect DNA of *E. multilocularis* in fecal samples (Isaksson et al., 2014; Knapp et al., 2014). Nevertheless, the Cq value was suggested as an indicator, but not as a precise quantification of the worm burden (Isaksson et al., 2014). In our case, DNA concentrations might be used an indicator of the parasites relative abundance in samples were differences are notably high. However, we could only calculate DNA concentrations for both parasites in three samples, and validation of results through morphological identification and counting of *E. canadensis* worms was not possible. Therefore, these results are not conclusive, and many limitations exist for the comparison of the abundance of these parasites based solely on qPCR, including differences in size, stage of infection and presence of gravid worms. As an alternative, qPCR could be used as a complementary test to detect mixed infections in co-endemic areas, helping to avoid a possible overestimation of worm burden of one or another *Echinococcus* species via morphological discrimination. Additionally, this assay could be used on fecal samples to monitor environmental contamination by *E. multilocularis* and *E. canadensis* eggs. According to the standard curve, up to 2×10^{-5} and 2×10^{-6} ng/μL of DNA could be detected for each parasite respectively. Therefore, the high sensitivity of the test would allow for the detection of DNA from only one egg (5×10^{-3} ng) (Knapp et al., 2014), while overcoming the presence of inhibitors.

The monitoring of *Echinococcus* species in intermediate and definite hosts is essential to study the distribution and prevalence of these parasites, as well to assess the potential impact on public health, especially in areas with insufficient surveillance data (Massolo et al., 2014; Davidson et al., 2016). The finding of mixed infections of *Echinococcus* in coyotes and foxes may not represent a threat to the health of these animals, as the parasites do not cause significant egregious effects on them. However, the presence of *E. canadensis* in a higher-than-expected prevalence in coyotes and foxes may impact the distribution of this parasite and the risk of transmission to humans. The significance of the role played by foxes and coyotes in the transmission of *E. canadensis* is still unclear and more accurate estimates of prevalence and intensity of infection in these hosts are needed. The use of molecular techniques has proven to be a valuable tool to investigate the eco-epidemiology of these parasites.

Acknowledgments

We gratefully acknowledge the ATA (Alberta Trappers' Association), the trappers who provided animal carcasses, Emily Jenkins (University of Saskatchewan) who provided advice and genetic material and all the undergraduate students that helped in our study. This research was supported by the ACA (Alberta Conservation Association) (N030-00-90-247).

Declarations of interest

None.

References

Carmena, D., Cardona, G.A., 2014. Echinococcosis in wild carnivorous species: epidemiology, genotypic diversity, and implications for veterinary public health. Vet. Parasitol. 202, 69–94.

Conraths, F.J., Deplazes, P., 2015. Echinococcus multilocularis: epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. Vet. Parasitol. 213, 149–161.

Davidson, R.K., Lavikainen, A., Konyaev, S., Schurer, J., Miller, A.L., Oksanen, A., Skírnisson, K., Jenkins, E., 2016. *Echinococcus* across the north: current knowledge, future challenges. Food Waterborne Parasitol. 4, 39–53.

Deer, D.M., Lampel, K.A., González-Escalona, N., 2010. A versatile internal control for use as DNA in real-time PCR and as RNA in real-time reverse transcription PCR assays. Lett. Appl. Microbiol. 50, 366–372.

Eckert, J., Gemmell, M.A., Meslin, F.X., Pawlowski, Z.S., 2001. WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Organization for Animal Health, Paris, France.

Freeman, R.S., Adorjan, A., Pimlott, D.H., 1961. Cestodes of wolves, coyotes, and coyote-dog hybrids in Ontario. Can. J. Zool. 39, 527–532.

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

Gesy, K.M., Schurer, J.M., Massolo, A., Liccioli, S., Elkin, B.T., Alisauskas, R., Jenkins, E.J., 2014. Unexpected diversity of the cestode *Echinococcus multilocularis* in wildlife in Canada. Int. J. Parasitol. Parasites. Wildl. 3, 81–87.

Holmes, J.C., 1961. The importance of coyotes (*Canis latrans*) in the maintenance of sylvatic echinococcosis: preliminary observations. J. Parasitol. 47, 55.

Holmes, J.C., Mahrt, J.L., Samuel, W.M., 1971. The occurrence of *Echinococcus multilocularis* Leuckart, 1863 in Alberta. Can. J. Zool. 49, 575–576.

Holmes, J.C., Podesta, R., 1968. The helminths of wolves and coyotes from the forested regions of Alberta. Can. J. Zool. 46, 1193–1204.

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

Jenkins, D.J., Morris, B., 2003. *Echinococcus granulosus* in wildlife in and around the Kosciuszko National Park, south-eastern Australia. Aust. Vet. J. 81, 81–85.

Joly, D.O., Messier, F., 2004. The distribution of *Echinococcus granulosus* in moose: evidence for parasite-induced vulnerability to predation by wolves? Oecologia 140, 586–590.

Jones, A., Pybus, M.J., 2008. Taeniasis and echinococcosis. In: Samuel, W.M., Pybus, M.J., Kocan, A.A. (Eds.), Parasitic Diseases of Wild Mammals. Iowa State University Press, Ames, Iowa, USA, pp. 150–192.

Klein, C., Liccioli, S., Massolo, A., 2014. Egg intensity and freeze-thawing of fecal samples affect sensitivity of *Echinococcus multilocularis* detection by PCR. Parasitol. Res. 113, 3867–3873.

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

Liccioli, S., Catalano, S., Kutz, S.J., Lejeune, M., Verocai, G.G., Duignan, P.J., Fuentealba, C., Hart, M., Ruckstuhl, K.E., Massolo, A., 2012. Gastrointestinal parasites of coyotes (*Canis latrans*) in the metropolitan area of Calgary, Alberta, Canada. Can. J. Zool. 90, 1023–1030.

Liccioli, S., Duignan, P.J., Lejeune, M., Deunk, J., Majid, S., Massolo, A., 2013. A new intermediate host for *Echinococcus multilocularis*: the southern red-backed vole (*Myodes gapperi*) in urban landscape in Calgary, Canada. Parasitol. Int. 62, 355–357.

Massolo, A., Liccioli, S., Budke, C., Klein, C., 2014. *Echinococcus multilocularis* in North America: the great unknown. Parasite 21, 73.

Needham, R., Odden, M., Lundstadsveen, S.K., Wegge, P., 2014. Seasonal diets of red foxes in a boreal forest with a dense population of moose: the importance of winter scavenging. Acta Theriol. 59, 391–398.

Rausch, R., Schiller, E.L., 1951. Hydatid disease (echinococcosis) in Alaska and the importance of rodent intermediate hosts. Science 113, 57–58.

Rausch, R.L., 2003. Cystic echinococcosis in the arctic and sub-arctic. Parasitology 127, S73–S85.

Romig, T., Deplazes, P., Jenkins, D., Giraudoux, P., Massolo, A., Craig, P.S., Wassermann, M., Takahashi, K., de la Rue, M., 2017. Ecology and life cycle patterns of *Echinococcus* species. Adv. Parasitol. 95, 213–314.

Romig, T., Ebi, D., Wassermann, M., 2015. Taxonomy and molecular epidemiology of *Echinococcus granulosus* sensu lato. Vet. Parasitol. 213, 76–84.

Samuel, W.M., Ramalingam, S., Carbyn, L.N., 1978. Helminths in coyotes (*Canis latrans* Say), wolves (*Canis lupus* L.), and red foxes (*Vulpes vulpes* L.) of southwestern Manitoba. Can. J. Zool. 56, 2614–2617.

Schurer, J., Shury, T., Leighton, F., Jenkins, E., 2013a. Surveillance for *Echinococcus canadensis* genotypes in Canadian ungulates. Int. J. Parasitol. Parasites. Wildl. 2, 97–101.

Schurer, J.M., Gesy, K.M., Elkin, B.T., Jenkins, E.J., 2013b. *Echinococcus multilocularis* and *Echinococcus canadensis* in wolves from western Canada. Parasitology 141, 159–163.

Schurer, J.M., Pawlik, M., Huber, A., Elkin, B., Cluff, H.D., Pongracz, J.D., Gesy, K., Wagner, B., Dixon, B., Merks, H., Bal, M.S., Jenkins, E.J., 2016. Intestinal parasites of gray wolves (*Canis lupus*) in northern and western Canada. Can. J. Zool. 94, 643–650.

Sweatman, G.K., 1952. Distribution and incidence of *Echinococcus granulosus* in man and other animals with special reference to Canada. Can. J. Public Health 43, 480–486.

Sweatman, G.K., Williams, R.J., 1963. Comparative studies on the biology and morphology of *Echinococcus granulosus* from domestic livestock, moose and reindeer. Parasitology 53, 339–390.

Theberge, J.B., Wedeles, C.H.R., 1989. Prey selection and habitat partitioning in sympatric coyote and red fox populations, southwest Yukon. Can. J. Zool. 67, 1285–1290.

Thompson, R.C.A., 2017. Biology and systematics of *Echinococcus*. Adv. Parasitol. 95, 65–109.

Thompson, R.C.A., Smyth, J.D., 1975. Equine hydatidosis: a review of the current status in Great Britain and the results of an epidemiological survey. Vet. Parasitol. 1, 107–127.