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Unexpected diversity of the cestode *Echinococcus multilocularis* in wildlife in Canada

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ABSTRACT

Echinococcus multilocularis is a zoonotic cestode with a distribution encompassing the northern hemisphere that causes alveolar hydatid disease in people and other aberrant hosts. *E. multilocularis* is not genetically uniform across its distribution, which may have implications for zoonotic transmission and pathogenicity. Recent findings of a European-type haplotype of *E. multilocularis* in wildlife in one location in western Canada motivated a broader survey of the diversity of this parasite in wildlife from northern and western Canada. We obtained intact adult cestodes of *E. multilocularis* from the intestines of 41 wild canids (wolf – *Canis lupus*, coyote – *Canis latrans*, and red fox – *Vulpes vulpes*), taeniid eggs from 28 fecal samples from Arctic fox (*Vulpes lagopus*), and alveolar hydatid cysts from 39 potential rodent intermediate hosts. Upon sequencing a 370-nucelotide region of the NADH dehydrogenase subunit 1 (*nad1*) mitochondrial locus, 17 new haplotypes were identified. This constitutes a much higher diversity than expected, as only two genotypes (European and an Asian/North American) had previously been identified using this locus. The European-type strain, recently introduced, may be widespread in wildlife within western Canada, possibly related to the large home ranges and wide dispersal range of wild canids. This study increased understanding of the biogeographic distribution, prevalence and genetic differences of a globally important pathogenic cestode in northern and western Canada.

1. Introduction

Echinococcus multilocularis is a zoonotic tapeworm with an indirect life cycle involving definitive carnivore and intermediate small mammal hosts. The oncosphere, or first larval stage, migrates to the target organ (primarily the liver) of intermediate hosts, where it develops into an alveolar hydatid cyst containing protoscolices (Thompson and McManus, 2002). Protoscolices are infectious to the carnivore definitive host upon ingestion but there are no clinical signs associated with definitive host infection. A wide range of mammal species, including humans and domestic dogs can serve as aberrant intermediate hosts, in which there can be variable development of protoscolices. Rapid growth of the cysts and proliferation of the germinal membrane can lead to extensive destruction of host tissue and metastasis to other organs (Eckert et al., 2001). If not detected early and treated aggressively, debilitating disease and even host death can occur (Webster and Cameron, 1967; Wilson and Rausch, 1980; Rausch and Wilson, 1985).

The genetic differences found within *E. multilocularis* are not as abundant as in other *Echinococcus* species; however, fine-scale genetic differences may have relevance for variation in levels of infectivity for humans (Nakao et al., 2009). Globally, there is an estimated annual incidence of over 18, 000 cases, the vast majority occurring in Asia followed by Europe (Torgerson et al., 2010). This may be due to higher levels of human exposure to the infectious eggs and/or higher pathogenicity associated with Eurasian strains (Bartel et al., 1992; Nakao et al., 2009). In contrast, central North America, although considered an endemic area, has seen only two autochthonous cases of human infection (James and Boyd, 1937; Gamble et al., 1979). It is possible that the occurrence of

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human alveolar echinococcosis in Canada has been underestimated, as historically, cases have been based on histological description, with potential for misdiagnosis as hepatic carcinoma or cystic echinococcosis (caused by *Echinococcus granulosus* which is present in most of Canada) (Hildreth et al., 2000; Somily et al., 2005).

Early investigation into the genetic diversity of *E. multilocularis* began with the analysis of short mitochondrial (*mt*) gene sequences; these included 366 nucleotide (*nt*) sites from cytochrome *c* oxidase subunit 1 (*cox1*) and 471 *nt* sites from NADH dehydrogenase subunit 1 (*nad1*) (Bowles et al., 1992; Okamoto et al., 1995). From this, a total of four single polymorphisms, or mutations, differentiated isolates into one of two geographic genotypes, M1 (Europe) or M2 (Japan, China, Alaska and North America) (Bowles et al., 1992; Okamoto et al., 1995). The classification of strains into two genotypes was subsequently reinforced by the amplification and analysis of the nuclear DNA genes 18S rRNA and homeobox (Haag et al., 1997; Rinder et al., 1997). These findings, associated with amplification of the two partial mtDNA genes (*cox1* and *nad1*) and the nuclear genes (18S rRNA and homeobox), implied that *E. multilocularis* was primarily uniform across its Holarctic distribution. This apparent uniformity led to the use of the *nad1* gene only for species level identification. However, more recent classification schemes using multiple mitochondrial loci and microsatellites suggested greater diversity in *E. multilocularis* than previously thought, possibly linked to geographic variation of host community structure and/or biological differences of the parasite itself (Nakao et al., 2009; Jenkins et al. 2012; Knapp et al., 2012). Genetic and geographic diversity has become more apparent with an increased scope of sampling.

The discovery of a European-type strain of *E. multilocularis* in a domestic dog and wild canids in Quesnel, British Columbia (BC) in western Canada, prompted study in other regions of Canada. The apparently low occurrence of this strain and unusual clinical manifestation (the dog had the alveolar stage and was serving as intermediate host) is consistent with a relatively recent introduction of European-type strain into North America (Jenkins et al., 2012; Gesy et al., 2013a). Increasing reports of infected coyotes (*Canis latrans*) in urban centers in western Canada, and the recent identification of free-ranging wolves (*Canis lupus*) as significant definitive hosts in northern Canada, also highlighted the need to better understand the distribution and diversity of this cestode (Catalano et al., 2012; Schurer et al., 2013). This study explored parasite assemblages in definitive and intermediate hosts, the geographic distribution of *E. multilocularis* in northern and western Canada, and the similarities or differences of sequences of Canadian isolates to from across the circumpolar north.

2. Materials and methods

2.1. Definitive hosts – adult cestodes

Adult *Echinococcus* spp. cestodes were isolated from the small intestines of coyotes, red fox (*Vulpes vulpes*) and wolves collected from BC, Alberta (AB), Saskatchewan (SK), Manitoba (MB) and the Northwest Territories (NT) at the Zoonotic Parasite Research Unit (ZPRU) in Saskatoon, SK (Table 1; Fig. 1). Standard safety precautions as recommended by the World Health Organization and World Organization for Animal Health included freezing the intestines at −80 °C for a minimum of 3 days to inactivate eggs infectious to people (Eckert et al., 2001). Animals sampled for *Echinococcus* were trapped for other purposes, as part of a fur harvest or pest control, or were natural mortalities submitted to the Canadian Cooperative Wildlife Health Centre (Saskatoon, SK) for

Table 1
Sources, prevalence, and haplotypes of the NADH dehydrogenase subunit 1 gene of *Echinococcus multilocularis* in northern and western Canada in the current study.

Species	Location	Year	No. test	No. positive (%[a])	No. of sequences	Haplotype (No. of sequences)[f]
Definitive hosts – intestinal tracts (positive denotes adult Echinococcus)						
Coyote (*Canis latrans*)	Quesnel, BC	2011–12	27	10 (37%)	21[b]	D (1), E (7), L (1), M (4)
	Edmonton, AB	2009	5	5	5[c]	E (3), F (2)
	Unknown, AB	2012	6	4	12[b]	
	Unknown, SK	2010	4	3	3[b]	
	Saskatoon, SK	2012	6	6	18[b]	A (1), B (1), C (1), E (1)
Red fox (*Vulpes vulpes*)	Quesnel, BC	2011–12	6	1 (17%)	0	
Wolf (*Canis lupus*)	Sahtu, North Slave, NT	2011	73	6 (8%)	5[b]	A (3), G (1), H (1)
	Northern SK	2009	17	4 (24%)	5[b]	A (4), I (1)
	Riding Mtn, MB	2009	3	2 (67%)	4[b]	A (9), E (2)
Sub-total			147	41	73[b]	9 (36)
Definitive hosts – feces (positive denotes taeniid-type eggs detected)						
Arctic fox (*Vulpes lagopus*)	Karrak Lake, NU	2000–03	278	3 (1%)	6 [d]	A (1), E (1), N (1), O (1), P (1), Q (1)
	Karrak Lake, NU	2011	76	14 (18%)	0	
	Bylot Island, NU	2010	50	11 (22%)	0	
Sub-total			404	28	6	6 (6)
Intermediate hosts – (positive denotes detection of protoscolices)						
Brown lemming (*Lemmus trimucronatus*)	Karrak Lake, NU	2011	37	0	0	
Collared lemming (*Dicrostonyx groenlandicus*)	Karrak Lake, NU	2011	72	0	0	
Deer Mice (*Peromyscus maniculatus*)	100 Mile House, BC	2010	72	0	0	
	Southwest, SK	2009–10	783	40 (5%)[e]	39 [e]	A (8), I (4), J (1), K (1)
Meadow voles (*Microtus pennsylvanicus*)	100 Mile House, BC	2010	59	0	0	
North American jumping mice (*Zapus hudsonius*)	100 Mile House, BC	2010	16	0	0	
Shrews (*Sorex* spp.)	100 Mile House, BC	2010	7	0	0	
Northern red-backed voles (*Myodes rutilus*)	Karrak Lake, NU	2011	8	0	0	
Sub-total			1054	40	39	4 (14)
Total			1604	108	121	17 (56)

[a] Prevalence (percent of hosts or samples positive) is reported only for those collections for which infection status was not know a priori.
[b] Amplification was attempted on 3 intact cestodes from each canid host.
[c] Only 5 individual cestodes were sent from coyotes in Alberta (AB).
[d] Two clonal colonies from each fecal sample positive for *E. multilocularis* were used in analysis.
[e] One larval cyst proved to be a cysticercus of *Taenia polyacantha* on molecular characterization.
[f] Only sequences, which were of high quality and verified on protein translation were used in the haplotype network analysis.

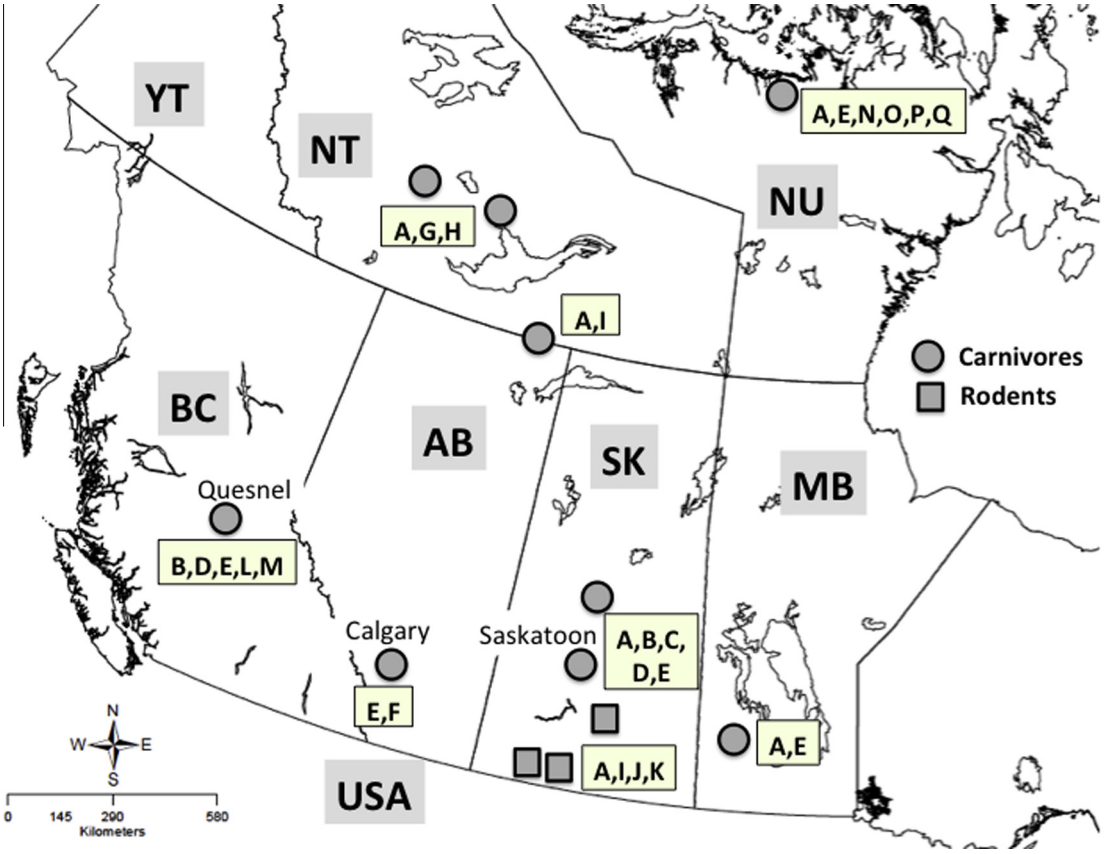

Fig. 1. Map of Canada showing sites with samples positive for *Echinococcus multilocularis* in the current study. Carnivore icons (circles) in the Northwest Territories and rodent icons (squares) in southern Saskatchewan represent centrum data, not exact sampling locations. Boxed lettering represents haplotypes from this study. Provincial and territory abbreviations are as follows: Yukon territory (YT), Northwest territories (NT), Nunavut (NU), British Columbia (BC), Alberta (AB), Saskatchewan (SK), and Manitoba (MB).

routine diagnostic pathology. Cestodes from five coyotes were obtained from the University of Calgary in Alberta (AB), collected as described in Catalano et al. (2012). Adult cestodes were recovered using the sedimentation, filtration and counting technique (Schurer et al., 2013; Gesy et al., 2013a,b) and kept in 70% ethanol. If possible, DNA was extracted from up to 3 intact adult worms from each animal. Individual cestodes were soaked in *Echinococcus* lysis buffer to rehydrate and remove excess ethanol (Catalano et al., 2012). After 10 min, each cestode was placed in an individual 0.2 ml PCR tube with 50 µl lysis buffer and 5 U Proteinase K (Qiagen, Toronto, ON, Canada), and placed in a thermocycler for 98 min at 60 °C to lyse the cestodes, then 94 °C for 15 min to denature the Proteinase K (Catalano et al., 2012). DNA was amplified with a simplex PCR using *E. multilocularis* primers for a 395 bp region of the *nad1* mitochondrial gene (Trachsel et al., 2007).

2.2. Definitive hosts – eggs

Arctic fox fecal samples ($n = 404$) were examined for the presence of taeniid eggs using a modified centrifugation/sucrose flotation technique on 1 g samples following freezing at −80 °C for minimum of 3 days to inactivate infectious eggs (Eckert et al., 2001; Salb et al., 2008). Of these samples, 278 were collected in 2000–2003 as part of dietary survey at Karrak Lake, Nunavut (NU), by Environment Canada and frozen at −20 °C since that time (Table 1; Fig. 1). The remaining samples from Karrak Lake and Bylot Island, NU, were collected in 2010 and 2011 and frozen at −20 °C prior to shipment to ZPRU. Taeniid eggs detected during microscopic examination of slides were collected, then washed with distilled H_2O (dH_2O) to remove any residual sucrose solution. From each sample, approximately 50 eggs were placed in 1.5 mL microcentrifuge tubes using a Pasteur pipette and washed 3 times in dH_2O. Tubes were subsequently frozen at −20 °C until DNA extraction using the FastDNA™ kit (MP Biomedicals, Solon, Ohio, USA) modified by substituting binding matrix A for binding matrix F (Da Silva et al., 1999; Kirk, 2012). As mixed infections with eggs of other taeniid species were possible, a multiplex PCR was performed using primers that differentiate *E. multilocularis*, *E. granulosus/Echinococcus canadensis,* and *Taenia* spp. (Trachsel et al., 2007). Samples with the expected 395 *nt* amplicon size for *E. multilocularis* were cloned using pGEM®-T Easy vector (Promega Corporation, Madison, WI, USA) to increase DNA yields and purify the PCR product. The two largest clonal colonies from each sample were chosen for analysis.

2.3. Intermediate hosts – metacestodes

A total of 1056 rodents and shrews (*Sorex* spp.), representing of 7 species from BC, SK and NU (Table 1; Fig. 1) were examined at necropsy for the presence of alveolar hydatid cysts of *E. multilocularis* under level 2 bio-safety conditions to minimize risk of human exposure to hantaviruses. Deer mice (*Peromyscus maniculatus*) were captured in southern Saskatchewan in 2009 and 2010, as part of a small mammal diversity study by the Royal Saskatchewan Museum and University of Regina, SK (University of Regina Animal Utilization Protocol 08-11). Rodents and shrews from BC and NU were captured in summers of 2010 and 2011, under BC wildlife permit WL 10-65638, NU wildlife research permit 2011-017, and

University of Saskatchewan Animal Use Protocol 2011-0005. Fluid, drawn from suspicious lesions, was examined under the microscope for the presence of protoscolices. The remaining cyst tissue (macerated in 70% EtOH) and fluid were placed in microcentrifuge tubes and frozen at −20 °C until DNA extraction. DNA was extracted from cyst fluid/tissue using the DNeasy Blood and Tissue Kit® (Qiagen, Toronto, ON, Canada), as per manufacturer's instructions, with an additional AW2 buffer wash, which improved PCR amplification results. Simplex PCR was used to amplify a 395 bp region of the *nad1* gene (Trachsel et al., 2007).

2.4. Analysis

PCR products were resolved by electrophoresis (110 V, 40 min) on a 1.5% agarose gel stained by RedSafe™ nucleic acid staining solution (ChemBio Ltd., Hertfordshire, UK), and viewed under UV light. PCR products from eggs and adult and larval cestodes with the amplicon size of 395 bp were purified using QIAquick® PCR Purification Kit (Qiagen Inc., Valencia, CA), and sequenced by Macrogen Inc. (Seoul, Korea). Sequencing of purified PCR products was performed using PCR primers and aligned using the Staden Software Package 2-1.0.0b9. All *nad1* sequences underwent protein translation for accuracy verification using the flatworm mitochondrial translation tool from eBIOx prior to further analysis.

All sequences from the current study were clipped to 370 base pairs for uniformity, and compared to each other, to previously published sequences, and to the European-type strain recently detected in a dog and coyotes from BC (Peregrine et al., 2010; Jenkins et al., 2012; Gesy et al., 2013a). The following programs were used for haplotype network creation: ClustalX 2.1; Alignment Transformation Environment (http://sing.ei.uvigo.es/ALTER/); and TCS 1.2.1, based on statistical parsimony. Representative sequences (Table 2) were entered into GenBank™ (http://www.ncbi.nlm.nih.gov/genbank/).

3. Results

This survey identified 17 previously undetected haplotypes in Canada, based on the analysis of the nad1 mitochondrial gene. Adult cestodes from 41 wild canids were positively identified as *E. multilocularis*. Prevalence, where relevant, is indicated in Table 1. We obtained sequences from 73 intact adult cestodes from 37 of 41 *E. multilocularis*-positive animals. Only 36 sequences from these 73 cestodes were deemed unambiguous by protein translation and used in the haplotype network analysis. These 36 sequences represent 9 of the 17 haplotypes; NT/MB wolves (5 haplotypes), NU fox samples (6 haplotypes), BC coyotes (4 haplotypes), AB coyotes (2 haplotypes) and SK coyotes (4 haplotypes) (Table 1, Figs. 1 and 2).

Taeniid eggs were detected in 28 (7%) of 404 fecal samples from Arctic fox from Karrak Lake and Bylot Island, Nunavut (NU). However, only 3 of the 28 samples (10.7%) were positively identified as *E. multilocularis*, all from Karrak Lake (Table 1). Sequences from the two clonal colonies, from each of three fecal samples, represent six different haplotypes (Table 1, Fig. 2).

Of the potential intermediate hosts surveyed, the only rodent species that had visible lesions compatible with alveolar hydatid cysts of *E. multilocularis* were deer mice from southern SK (Table 1; Fig. 1). Of the 783 deer mice, 40 displayed lesions characteristic of *E. multilocularis* infection. Infections identified visually were confirmed as *E. multilocularis* in 39 of 40 positive samples. The remaining sample was characterized as 99% identical to *Taenia polyacantha* across 395 nucleotides of the *nad1* gene (EU544647). Only 14 sequences from the 39 deer mouse isolates were unambiguous and used in analysis, belonging to 4 haplotypes (A, I–K) (Table 1, Fig. 2).

In total, there were 56 sequences suitable for haplotype network analysis, which represented 17 haplotypes (A – Q) (Table 1; Fig. 1, Fig. 2). None of the Canadian sequences exactly matched the previously described M1 and M2 genotypes, with one and three nucleotide mutations between the M1 (AJ237639) and M2 (AJ237640) genotypes, respectively, and their closest Canadian relative (haplotype E) (Fig. 2; Table 2). Haplotypes A and E dominated, with 19 and 14 sequences, respectively (Fig. 2). Haplotype A included isolates from SK, MB, NT and NU, while haplotype E included isolates from most Canadian locations (SK, MB, NU, BC and AB) (Fig. 2). Haplotype E also matched previously published sequences from China (EU704122), Poland (JX266825 and JX266826) and the dog from British Columbia (JN371771). Twelve haplotypes (B, C, D, G, H, J, K, L, N, O, P, Q) were represented by only one sequence. Haplotypes M (BC coyotes) and I (SK deer mice and NT wolf) contained four and five sequences, respectively. The AB coyotes grouped with haplotypes E ($n = 3$) and F ($n = 2$). Sequences from SK deer mice formed a cluster (A, I, J, and K), as did 5 of the 6 NU fox (E, N–Q).

Table 2
Single nucleotide mutations found in 17 NADH dehydrogenase subunit 1 (*nad1*) based haplotypes of Canadian *Echinococcus multilocularis*. A complete gene sequence, accession number NC_000928, was used for comparison. Positions of the mutations are numbered according to the nad1 gene taken from the complete gene sequence. The first position of the nad1 gene corresponds to position number 7491 of the complete genome.

Accession	Haplotype	No. Seq.	167	189	192	193	231	249	250	352	365	397	457	462	468	490	523	528	529
NC_000928	Complete*		A	G	T	T	C	T	T	T	C	T	G	T	T	A	A	G	T
KF962555	A	19					T				T					G			
KF962556	B	1					T				T		T			G			
KF962557	C	1					T						T			G			
KF962558	D	1					T						T						
KF962559	E	14					T												
KF962560	F	2					T							G					
KF962561	G	1					T									G		A	
KF962562	H	1					T				T					G			A
KF962563	I	5	T				T				T					G			
KF962564	J	1			A	A	T				T					G			
KF962565	K	1		T	A	A	T				T					G			
KF962566	L	1	T				T											A	
KF962567	M	4	T				T												
KF962568	N	1					T										G		
KF962569	O	1					T	A	A								G		
KF962570	P	1					T								C		G		
KF962571	Q	1					T	A	C	C							G		
TOTAL	17	56																	

* NAD1 sequence used for comparison of new haplotypes.

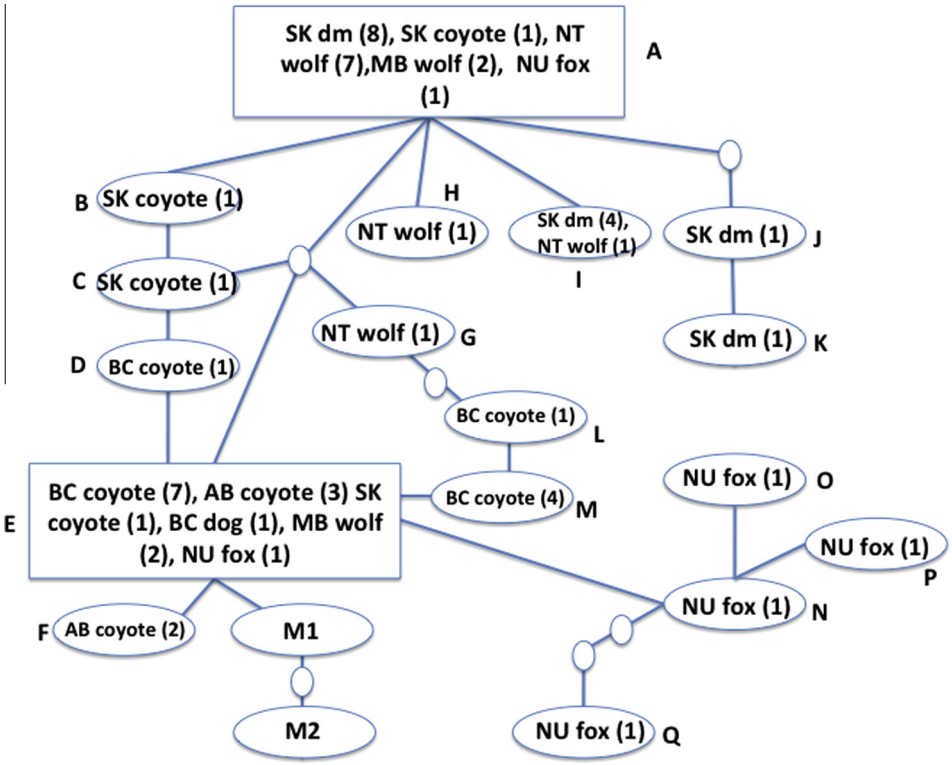

Fig. 2. Haplotype network of *Echinococcus multilocularis*. Network shows the relationships of haplotypes A–Q from a 370 base pair region of the *nad1* mitochondrial gene, described in the current study from wild carnivores (Arctic fox, wolf, and coyote) and deer mice (dm) in western Canada, and previously published sequences (M1, M2 and a European-type strain recently isolated from a dog in BC). Network is based on statistical parsimony. Small, unlabelled circles indicate hypothetical haplotypes separated by a single nucleotide change from adjacent sequences. Labelled ovals and rectangles represent distinct haplotypes. Abbreviations for Canadian provinces and territories as for Fig. 1.

4. Discussion

The identification of 17 new haplotypes of this cestode within Canada, resulting from analysis of *nad1*, indicates more diversity within this gene than previously assumed to be present within *E. multilocularis* across its global distribution. The original designation of only two genotypes, M1 (Asian and North American) and M2 (European), was likely the result of an underestimate of genetic diversity due to a smaller number of isolates from a limited geographic range than in our survey. The M1 genotype came from three infected human liver samples and the M2 genotype came from a single infected rodent from Germany (Bowles et al., 1992).

Haplotype E was present in all locations sampled in Canada except for the Northwest Territories. It was most closely related to the previously recognized M1 genotype. Haplotype E was identical to additional sequences from locations in China and Poland, suggesting that this haplotype may be more appropriately designated as a Holarctic genotype. The occurrence of haplotype E in most Canadian regions suggests that the European-type strain found in a dog and in coyotes in central BC may be widespread within western Canada. Such broad distribution is consistent with a high likelihood of transport and transmission by carnivore definitive hosts; for example, wolves can cover more than 62,000 km^2 (Walton et al., 2001; Schurer et al., 2013). In addition, the anthropogenic translocation of foxes for hunting purposes and the reintroduction of wolves into the western United States may also have spread the parasite within North America (Rausch, 1985; Foreyt et al., 2009).

There was some clustering of closely related isolates collected in close proximity (i.e. A, I, J and K from deer mice collected from south-western SK and N, O, P, and Q from Arctic fox at Karrak Lake), supporting the idea that there are highly endemic and well-established foci of *E. multilocularis* in both Arctic and central North America. This further supports the hypothesis that the central North American population of this parasite was not a recent introduction from the Arctic (Nakao et al., 2009). The deer mouse isolates (mostly haplotype A) are likely to be closely related to the central North American strain of *E. multilocularis* previously identified in South Dakota (Nakao et al., 2009). Identification of six haplotypes from three Arctic fox samples may suggest that definitive host infections with multiple haplotypes are common. Mixed infections are likely the result of multiple haplotypes being found in intermediate hosts in a given region. It is possible that the Arctic fox fecal samples came from the same individual, rather than three different fox, as these samples were ground collected from Karrak Lake, NU. Alternatively, this observation could indicate mitochondrial heteroplasmy. *E. granulosus* has been shown to exhibit heteroplasmy within some mitochondrial genes (Bowles et al. 1995). Whether or not this heteroplasmic condition also exists within *E. multilocularis*, to our knowledge, has not been investigated. Further analysis, such as cloning and sequencing amplicons from single worms, is needed to explore this possibility.

It is important to note that the differences among *E. multilocularis* haplotypes are often only single nucleotide mutations, as compared to much greater diversity observed within the closely related *E. granulosus* species complex (Nakao et al., 2009). Caution must be taken when interpreting data based on a single mitochondrial locus; however, the biogeographical significance of this genetic variation may become clearer with the increased availability of *nad1* sequences and comparison of samples from a broader geographic range. It is possible that single mutations were the result of errors during the amplification process; however, analysis in its entirety (from DNA extraction if possible) was repeated, for all haplotypes with a single sequence.

In Arctic Canada, as in Europe, it was assumed that the Arctic fox was the primary definitive host (Choquette et al., 1962; Eaton and Secord, 1979; Wilson et al., 1995). However, a recent study

suggested that wolves play a larger role in transmission than previously believed (Schurer et al., 2013). Our study detected a minimum prevalence in wolves of 13%, as compared to 2–9% found in mainland Arctic fox (Jenkins et al., 2013; Schurer et al., 2013). Likewise, the red fox was assumed to be the primary definitive host in southern Canada; however, recent studies suggest that coyotes may play a significant role (Catalano et al., 2012; Gesy et al., 2013a). The identification of *E. multilocularis* in 5% of deer mice, and absence from a wide range of other rodent hosts, is consistent with findings from previous studies in central North America, although more intensive sampling over a broader geographic range is recommended (Leiby et al., 1970). Analysis of both intermediate and definitive hosts from the same location would likely reveal haplotype similarities and identify definite ranges for each of the haplotypes detected.

Molecular characterization at multiple mitochondrial and nuclear loci of *E. multilocularis* from across the broad range occupied by this species in North America would provide greater genetic resolution at a broader spatial scale, significantly improving our ability to explore hypotheses about origins and relationships of *E. multilocularis* in Canada (Rausch, 1985; Wilson et al., 1995; Nakao et al., 2009). *E. multilocularis* was thought to have been introduced to Arctic North America through dispersal of Arctic fox across the Bering Strait (Rausch, 1994; Nakao et al., 2009). The origins of the central North American population however, remains unclear (Rausch, 1985; Wilson et al., 1995; Nakao et al., 2009). Prevailing theories include the anthropogenic introduction of European strains from the importation of European fox and/or genetic exchange between strains of *E. multilocularis* established in Arctic and prairie regions of North America through southward incursions of naturally infected Arctic fox (Rausch, 1985; Wilson et al., 1995; Nakao et al., 2009). Additional research will characterize isolates in the current study at multiple mitochondrial loci to more fully understand the origins and relationships of *E. multilocularis* in North America and those found elsewhere across the circumpolar North, where this parasite continues to emerge and re-emerge as a public and animal health concern.

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References

Bartel, M.H., Seesee, F.M., Worley, D.E., 1992. Comparison of Montana and Alaska isolates of *Echinococcus multilocularis* in gerbils with observations on the cyst growth, hook characteristics, and host response. J. Parasitol. 78, 529–532.

Bowles, J., Blair, D., McManus, D.P., 1992. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol. 54, 165–174.

Bowles, J., Blair, D., McManus, D.P., 1995. A molecular phylogeny of the genus *Echinococcus*. Parasitology 110, 317–328.

Catalano, S., Lejeune, M., Liccioli, S., Verocai, G.G., Gesy, K.M., Jenkins, E.J., Kutz, S.J., Fuentealba, C., Duignan, P.J., Massolo, A., 2012. Echinococcus multilocularis in urban coyotes, Alberta, Canada. Emerg. Infect. Dis. 18, 1625–1628.

Catalano, S., Lejeune, M., Liccioli, S., Verocai, G.G., Gesy, K.M., Jenkins, E.J., Kutz, S.J., Fuentealba, C., Duignan, P.J., Massolo, A., 1962. Note on the occurrence of *Echinococcus multilocularis* Leuckart, 1863 in the Arctic fox in Canada. Can. J. Zool. 40, 1167.

Da Silva, A.J., Bornay-Llinares, F.J., Moura, I.N.S., Slemenda, S.B., Tuttle, J.L., Pieniazek, N.J., 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Mol. Diagn. 4, 57–64.

Eaton, R.D.P., Secord, D.C., 1979. Some intestinal parasites of Arctic Fox, Banks Island, N.W.T. Can. J. Comparat. Med. 43, 229–230.

Eckert, J., Deplazes, P., Craig, P.S., Gemmel, M.A., Gottstein, B., Heath, D., Lightowlers, M., 2001. Echinococcus in animals: clinical aspects, diagnosis and treatment. In: Eckert, J., Gemmel, M.A., Meslin, F.-X., Pawloski, Z.S. (Eds.), WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern. World Organization for Animal Health (OIE), Institute of Parasitology, Zurich, Switzerland, pp. 72–100.

Foreyt, W.J., Drew, M.L., Atkinson, M., McCauley, D., 2009. *Echinococcus granulosus* in gray wolves and ungulates in Idaho and Montana, USA. J. Wildl. Dis. 45, 1208–1212.

Gamble, W.G., Segal, M.S., Schantz, P.M., Rausch, R., 1979. Alveolar hydatid disease in Minnesota. First human case acquired in the contiguous United States. JAMA 241, 904–907.

Gesy, K.M., Hill, J.E., Liccioli, S., Jenkins, E.J., 2013a. Establishment of a European-type strain of *Echinococcus multilocularis* in Canadian wildlife. Parasitology 40, 1133–1137.

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

Haag, K.L., Zaha, A., Araujo, A.M., Gottstein, B., 1997. Reduced genetic variability within coding and non-coding regions of the *Echinococcus multilocularis* genome. Parasitology 115, 521–529.

Hildreth, M.B., Sriram, S., Gottstein, B., Schantz, P.M., 2000. Failure to identify alveolar echinococcosis in trappers from South Dakota in spite of high prevalence of *Echinococcus multilocularis* in wild canids. J. Parasitol. 86, 75–77.

James, E., Boyd, W., 1937. *Echinococcus alveolaris*. Can. Med. Assoc. J. 36, 354–356.

Jenkins, E.J., Hill, J.E., Somers, C., Gesy, K.M., Barnes, B., Gottstein, B., Polley, L., 2012. Detection of European Strain of *Echinococcus multilocularis* in North America [letter]. Emerg. Infect. Dis. 18, 1010–1012.

Jenkins, E.J., Castrodale, L.J., de Rosemond, S., Dixon, B., Elmore, S.A., Gesy, K.M., Thompson, R.C.A., 2013. Tradition and transition: parasitic zoonoses in people and animals in northern North America and Greenland. Adv. Parasitol. 82, 33–204.

Kirk, C., 2012. Molecular Ecology of Echinococcus and Taeniinae in Arctic Fox (*Vulpes lagopus*) of Northern Alaska: Characterization of the nad1 Gene Fragment (Thesis). University of Colorado, Colorado, USA.

Knapp, J., Staebler, S., Bart, J.M., Stien, A., Yoccoz, N.G., Drogemuller, C., Gottstein, B., Deplazes, P., 2012. *Echinococcus multilocularis* in Svalbard, Norway: microsatellite genotyping to investigate the origin of a highly focal contamination. Infect. Genet. Evol. 12, 1270–1274.

Leiby, P.D., Carney, W.P., Woods, C.E., 1970. Studies on sylvatic echinococcosis. III. Host occurrence and geographic distribution of *Echinococcus multilocularis* in the north central United States. J. Parasitol. 56, 1141–1150.

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

Okamoto, M., Bessho, Y., Kamiya, M., Kurosawa, T., Horii, T., 1995. Phylogenetic relationships within *Taenia taeniaeformis* variants and other taeniid cestodes inferred from the nucleotide sequence of the cytochrome c oxidase subunit 1 gene. Parasitol. Res. 81, 451–458.

Peregrine, A.S., Jenkins, E.J., Barnes, B. 2010. Hepatic alveolar echinococcosis in a dog in Britsh Columbia, Canada. In: Proceedings of the 55th Annual Meeting of the American Association of Veterinary Parasitologists, Atlanta, Georgia, USA, pp. 72–73.

Rausch, R.L., 1985. Parasitology: retrospect and prospect. J. Parasitol. 71, 139–151.

Rausch, R.L., Wilson, J.F., 1985. The current status of alveolar hydatid disease in Northern regions. Circumpolar Health 84, 245–247.

Rausch, R.L., 1994. Transberingian dispersal of cestodes in mammals. Int. J. Parasitol. 24, 1203–1212.

Rinder, H., Rausch, R.L., Takahashi, K., Kopp, H., Thomschke, A., Loscher, T., 1997. Limited range of genetic variation in *Echinococcus multilocularis*. J. Parasitol. 83, 1045–1050.

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

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

Somily, A., Robinson, J.L., Miedzinski, L.J., Bhargava, R., Marrie, T.J., 2005. Echinococcal disease in Alberta, Canada: more than a calcified opacity. BMC Infect. Dis. 5, 34.

Thompson, R.C.A., McManus, D.P., 2002. Towards a taxonomic revison of the genus *Echinococcus*. Trends Parasitol. 18, 452–457.

Torgerson, P.R., Keller, K., Magnotta, M., Ragland, N., 2010. The global burden of alveolar echinococcosis. PLoS Negl. Trop. Dis. 4, e722.
Trachsel, D., Deplazes, P., Mathis, A., 2007. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology 134, 911–920.
Walton, L.R., Cluff, H.D., Paquet, P.C., Ramsay, M.A., 2001. Movement patterns of barren-ground wolves in the central Canadian Arctic. J. Mammal. 82, 867–876.
Webster, G.A., Cameron, T.W.M., 1967. Epidemiology and diagnosis of *Echinococcus* in Canada. Can. Med. Assoc. J. 96, 600–607.
Wilson, J.F., Rausch, R.L., 1980. Alveolar hydatid disease: a review of clinical features of 33 indigenous cases of *Echinococcus multilocularis*: infection in Alaskan Eskimos. Am. J. Trop. Med. Hyg. 29, 1340–1355.
Wilson, J.F., Rausch, R.L., Wilson, F.R., 1995. Alveolar hydatid disease: review of the surgical experience in 42 cases of active disease among Alaskan Eskimos. Ann. Surg. 221, 315–323.