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Introduced and Native Haplotypes of *Echinococcus multilocularis* in Wildlife in Saskatchewan, Canada

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ABSTRACT: Recent detection of a European-type haplotype of the cestode *Echinococcus multilocularis* in a newly enzootic region in British Columbia prompted efforts to determine if this haplotype was present elsewhere in wildlife in western Canada. In coyote (*Canis latrans*) definitive hosts in an urban region in central Saskatchewan (SK), we found a single haplotype of *E. multilocularis* that was most similar to a haplotype currently established in the core of this parasite's distribution in Europe and to the European-type haplotype found in coyotes and a dog (*Canis lupus familiaris*) in British Columbia. We found six haplotypes of *E. multilocularis* from deer mouse (*Peromyscus maniculatus*) intermediate hosts in southwestern SK that were closely related to, and one haplotype indistinguishable from, a haplotype previously reported in the adjacent north-central US. This is a higher level of diversity than has previously been recognized for this parasite, which suggests that the population native to central North America is well established, rather than a recent introduction from the Arctic. These findings, in combination with recent cases of alveolar hydatid cysts in dogs in Canada, raise concerns that European haplotypes of *E. multilocularis* may be increasing in distribution within wildlife in Canada. European haplotypes may pose greater risks to veterinary and human health than native haplotypes long established in central North America.

Key words: Alveolar hydatid, emerging, European, mitochondrial DNA.

The cestode *Echinococcus multilocularis* is emerging around the circumpolar North and poses a challenge for animal and public health (König et al. 2005; Davidson et al. 2012). The parasite usually cycles between carnivore definitive hosts and rodent intermediate hosts, occasionally spilling over into a wide range of aberrant intermediate hosts, including humans. The larval stage in intermediate hosts is an alveolar hydatid cyst.

Mitochondrial DNA (mtDNA) haplotyping holds promise for better understanding the biogeography of *E. multilocularis*, although the number of sequences available and sampling effort are limited, especially in North America. An Arctic North American haplotype (N1) was reported from 11 voles (*Microtus* spp.) from St. Lawrence Island, Alaska, and a central North American haplotype (N2) from one red fox (*Vulpes vulpes*) in Indiana and one laboratory strain in South Dakota (Nakao et al. 2009). There is little information about the genetic diversity of *E. multilocularis* in Canada, which falls between the N1 and N2 parasite populations. Previously, we reported the results of a single locus approach, which provided a limited amount of genetic information (Gesy et al. 2014); here we use a multiple locus approach using a previously published haplotyping scheme that increases the ability to detect diversity and describe relationships within a relatively conserved parasite species (Nakao et al. 2009).

Detection of a European-type haplotype of *E. multilocularis* in a dog (*Canis lupus familiaris*) in central British Columbia, Canada, and subsequently in wild coyotes (*Canis latrans*) in the surrounding area raised the question of whether European-type strains are established elsewhere in western Canada (Jenkins et al. 2012; Gesy et al. 2013). Additional cases of dogs with alveolar hydatid cysts have been reported in eastern Canada (Oscos-Snowball et al. 2014; Skelding et al. 2014). This suggests that this parasite may be emerging in North America as a veterinary, and potentially public health, issue. To better understand the origin, distribution, and diversity of this important wildlife parasite, we sought more sequences of *E. multilocularis* from naturally infected wildlife hosts in north-central North America.

TABLE 1. Sources of *Echinococcus multilocularis* from Saskatchewan (SK), Canada, and haplotyping results based on aggregate analysis of three mitochondrial genes.

Samples	Location	Year	No. animals positive	No. individual cestodes and alveolar hydatids[a]	No. sequences available	Haplotypes (no. sequences)
Coyotes (*Canis latrans*)	Saskatoon, SK	2012	6	18	18	SK1 (18)
Deer mice (*Peromyscus maniculatus*)	Southwest SK	2009–10	39	39	23	SK2 (5) SK3 (2) SK4 (3) SK5 (3) SK6 (4) SK7 (3) SK8 (3)
Total			45	57	41	8 (41)

[a] From which we extracted DNA (three cestodes/coyote, one alveolar hydatid cyst/deer mouse).

We extracted DNA from adult cestodes from coyotes and alveolar hydatid cysts from deer mice (*Peromyscus maniculatus*) acquired opportunistically in Saskatchewan (Table 1 and Fig. 1) using previously described methods (Catalano et al. 2012; Gesy et al. 2014). Segments from three mtDNA genes were amplified using published primers and protocols for DNA extracted from adult cestodes (Nakao et al. 2009). Published primers did not produce the expected amplicons for NADH dehydrogenase subunit 2 (*nad2*), cytochrome b (*cob*), and cytochrome c oxidase subunit 1 (*cox1)* for larval cysts from deer mice, so tailored primers for a smaller subset within these genes were developed for lengths of 623 (*nad2* nt 104–727), 693

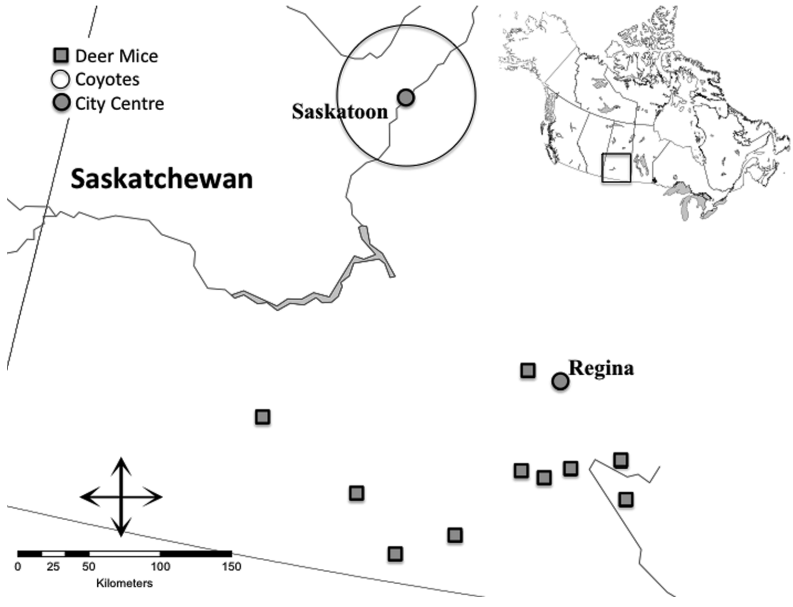

FIGURE 1. Locations of newly identified haplotypes of *Echinococcus multilocularis* in south and central Saskatchewan (SK). Deer mice (*Peromyscus maniculatus*) were collected from 10 locations (squares) in southern SK, and coyotes (*Canis latrans*) from within 100 km of Saskatoon, SK (delimited by the large circle).

TABLE 2. In-house developed primers and amplification parameters for mitochondrial DNA genes NADH dehydrogenase subunit 2 (*nad2*), cytochrome b (*cob*), and cytochrome c oxidase subunit 1 (*cox1*) of *Echinococcus multilocularis* from Saskatchewan deer mice (*Peromyscus maniculatus*).

Gene	Primer set (5′-3′)[a]	Amplification parameters	Amplicon	
			bp	nt
NADH dehydrogenase subunit 2 (*nad2*)	F: GGGTTTTTTTGGAGTTGTG R: AAGGCATAGAYACAGGAGTCA	95 C for 3 min; (94 C for 30 s, 54 C for 30 s, 72 C for 45 s)×40; 72 C for 5 min	623	104–727
Cytochrome B (*cob*)	F: TGCGTTATTGGCATATGGTAG R: GTGCCACCCTCAGTTGGTACT	As above	693	209–902
Cytochrome C oxidase subunit 1 (*cox1*)	F: TGGGTGCTGGGTGTTGGTTGG R: TACACACACGACGMGGAAC	94 C for 3 min; (94 C for 30 s, 55 C for 30 s, 72 C for 1 min)×40; 72 C for 5 min	966	362–1327

[a] F = forward; R = reverse.

(*cob* nt 209–902), and 899 (*cox1* nt 362–1327) nucleotides (Table 2). The resulting PCR products were purified using QIAquick® PCR Purification Kit (Qiagen Inc., Valencia, California, USA) and sequenced by Macrogen Inc. (Seoul, Korea) using PCR primers. Sequences were compared with previously published sequences (Nakao et al. 2009).

Sequence alignment, protein translation, and haplotype network creation were performed as per Gesy et al. (2014). Haplotype analysis was performed once with truncated sequences of *cob*, *nad2,* and *cox1* using the tailored primers and once using the longer sequences from published primers (Nakao et al. 2009). Aggregate analysis of the *cob*, *nad2,* and *cox1* genes allowed resolution of the different haplotypes better than if each gene was analyzed separately. Amplification and sequencing were repeated to verify haplotypes represented by a single isolate.

All sequences (three cestodes from each of six animals) from coyotes grouped as a single haplotype (SK1) and were most closely related to the E4 haplotype (France and Belgium), rather than North American haplotypes. SK1 differed from E4 by six mutations, from the European-type haplotype previously described from British Columbia (GenBank accession numbers KC550003–5) by nine mutations, and from the N1 and N2 native North American haplotypes (Nakao et al. 2009) by 26 and 32 mutations, respectively (Fig. 2).

Sequences at all 3 mt-DNA loci from 23 alveolar hydatid cysts were obtained using in-house developed primers. Five sequences from the Saskatchewan (SK) deer mice (SK2) were identical to the N2 haplotype described from the north-central US (Fig. 2) (Nakao et al. 2009). The remaining 18 sequences classified as six new haplotypes, SK3 through SK8 (Table 1), which were closely related to the N2 haplotype (Fig. 2). Representative sequences were entered into GenBank (http://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers NAD2: KC549993, KC550008, and KC582628–33; COX1: 550007 and KC582621–26; and COB KC549999, KC550006, and KC582614–19.

European-type strains of *E. multilocularis* described within wildlife in western Canada may have much greater significance for veterinary and human health than strains long established in North America. Saskatoon is the third urban center in western Canada in which infected coyotes have been detected (Catalano et al. 2012). In urban areas, both dogs and people may be exposed to eggs of *E. multilocularis* in dog parks and other green spaces where coyotes are present. Normally dogs serve as subclinical definitive hosts for adult cestodes of *E. multilocularis*, not aberrant

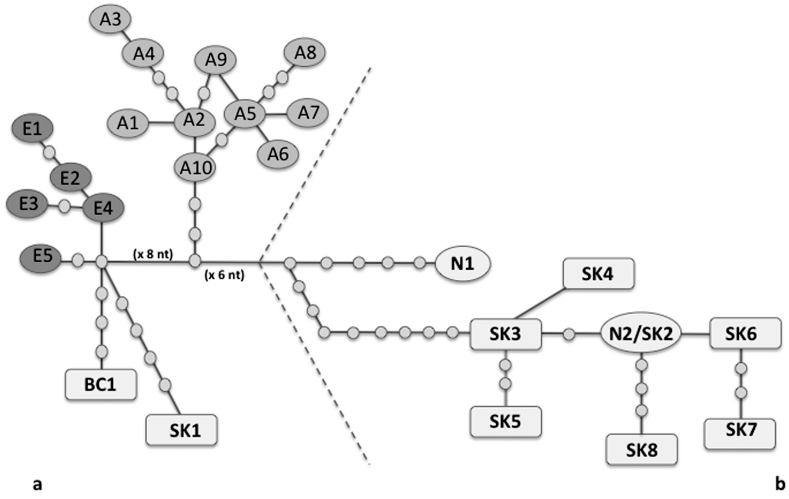

FIGURE 2. Haplotype network from the mitochondrial genes *nad2*, *cob*, and *cox1* of *Echinococcus multilocularis* based on statistical parsimony, showing sequences from coyotes (*Canis latrans*; SK1) in the European (E) cluster and those from deer mice (*Peromyscus maniculatus*; SK2–8) in the central North American cluster (N2). Isolates were amplified using published primer sets ("a" side of dashed line) and in-house primer sets ("b" side of dashed line). Gray ovals indicate Asian (A), European, and North American (N) haplotypes (Nakao et al. 2009). Small, unlabeled circles indicate hypothetical haplotypes and represent a single nucleotide mutation from adjacent sequences. Rectangles indicate newly identified haplotypes in Canada. BC1 is a European-type haplotype recently detected in western Canada (Jenkins et al. 2012; Gesy et al. 2013).

intermediate hosts infected with the larval stage of the parasite. Reports of alveolar hydatid cysts in dogs have been largely limited to the central core region of this parasite in Europe (Eckert and Deplazes 2004). We have detected European-type strains from alveolar hydatid cysts in several dogs in western Canada (K.M.G., E.J.J., unpubl. data). Recent findings of alveolar hydatid cysts in two dogs in Ontario where *E. multilocularis* was not thought to be present (Oscos-Snowball et al. 2014; Skelding et al. 2014) suggest that this parasite may be increasing in distribution in North America.

Only two infections have been reported in humans in central North America, one of which was confirmed as the N2 central North American strain (James and Boyd 1937; Gamble et al. 1979; Yamasaki et al. 2008). In contrast, the global incidence of human alveolar hydatid disease is around 18,000 new cases per year, with most occurring in Asia (91%) and Europe (Torgerson et al. 2010). Little evidence of human infection in North America may be due to a lack of detection and reporting, decreased exposure, or decreased zoonotic potential of native North American haplotypes relative to those in Asia and Europe (Nakao et al. 2009; Jenkins et al. 2012). If introduced European strains have greater zoonotic potential than strains of the parasite long established in central North America, they may have greater public health significance.

Isolates of *E. multilocularis* in deer mice from southwestern SK grouped with the central North American (N2) haplotype, which supports a contiguous central North American population spanning the Canada-US border. Although in-house primers generated shorter sequences (and therefore less chance to detect genetic variability) than published primers (Nakao et al. 2009), we detected six new central North American haplotypes (SK3–SK8) from 10 locations in southwestern SK with no distinct zones of occurrence (Fig. 1). The degree of genetic variability

between the central (N2) and Arctic (N1) North American strains (Nakao et al. 2009; Gesy et al. 2014), and the existence of diversity within the central North American population in the current study, suggests that this is a well-established focus of the parasite and not a recent introduction from the Arctic as previously suggested (Wilson et al. 1995).

No adult cestodes from coyotes were closely related to the larval isolates found in the deer mice, despite the fact these are known definitive and intermediate wildlife hosts in the central North American region (Leiby et al. 1969, 1970). This may simply reflect different sampling locations, as infected rodents came from southwestern SK while the coyotes came from Saskatoon in central SK. Alternatively, coyotes have larger home ranges and dispersal distances than deer mice, and Saskatoon may be near the limit of the distribution of introduced European haplotypes in Canada. It is likely that European and North American haplotypes of *E. multilocularis* exist in a complex mosaic across North America, and our observations simply reflect a relatively small sample size and limited published sequences for comparison.

In summary, we observed genetic diversity of North American strains of *E. multilocularis* in an enzootic region in Canada and found support for establishment of European-type haplotypes in naturally infected wildlife in western Canada. However, a larger-scale survey of wildlife is needed in North America. Understanding baseline distribution and diversity of *E. multilocularis* is paramount because strains may differ in developmental biology, pathogenicity, and zoonotic potential (Nakao et al. 2009; Lymbery et al. 2015). Genetic diversity within a parasite species should be considered when translocating wildlife, when developing country specific guidelines for testing and treatment of imported domestic animals, when evaluating label claims for veterinary drugs, and when considering biocontainment levels for working with parasites of foreign and native origin (Lymbery et al. 2015). Global translocation of hosts (wildlife and domestic dogs) is already altering the host and geographic distribution of *E. multilocularis* around the world.

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LITERATURE CITED

Catalano S, Lejeune M, Liccioli S, Verocai GG, Gesy KM, Jenkins EJ, Kutz SJ, Fuenteabla C, Duignan PJ, Massolo A. 2012. *Echinococcus multilocularis* in urban coyotes, Alberta, Canada. *Emerging Infect Dis* 18:1625–1628.

Davidson RK, Romig T, Jenkins EJ, Tryland M, Robertson LJ. 2012. The impact of globalization on the distribution of *Echinococcus multilocularis*. *Trends Parasitol* 28:239–247.

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

Gamble WG, Segal MS, Schantz PM, Rausch R. 1979. Alveolar hydatid disease in Minnesota. First human case acquired in the contiguous United States. *J Am Med Assoc* 241:904–907.

Gesy K, Hill JE, Schwantje H, Liccioli S, Jenkins EJ. 2013. Establishment of a European-type strain of *Echinococcus multilocularis* in Canadian wildlife. *Parasitology* 140:1133–1137.

Gesy KM, Schurer JM, Massolo A, Liccioli S, Elkin BT, Alisauskas R, Jenkins EJ. 2014. Unexpected diversity of the cestode *Echinococcus multilocularis* in wildlife in Canada. *Int J Parasitol Parasites Wildl* 3:81–87.

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

Jenkins EJ, Peregrine AS, Hill JE, Somers CM, Gesy KM, Barnes B, Gottstein B, Polley L. 2012. Detection of a European strain of *Echinococcus multilocularis* in North America. *Emerg Infect Dis* 18:1010–1012.

König A, Romig T, Thoma D, Kellermann K. 2005. Drastic increase in the prevalence of *Echinococcus multilocularis* in foxes (*Vulpes vulpes*) in southern Bavaria, Germany. *Eur J Wildl Res* 51:277–282.

Leiby PD, Carney WP, Woods CE. 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.

Leiby PD, Lubinsky G, Galaugher W. 1969. Studies on sylvatic echinococcosis. II. The occurrence of *Echinococcus multilocularis* Leuck. 1863 in Manitoba. *Can J Zool* 47:135–138.

Lymbery AJ, Jenkins EJ, Schurer JM, Thompson RCA. 2015. *Echinococcus canadensis*, *E. borealis*, and *E. intermedius*. What's in a name? *Trends Parasitol* 31:23–29.

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.

Oscos-Snowball A, Tan E, Peregrine AS, Foster R, Bronsoiler J, Gottstein B, Jenkins E, Gesy K, Bienzle D. 2015. What is your diagnosis? Fluid aspirated from an abdominal mass in a dog. *Vet Clin Pathol* 44:167–168.

Skelding A, Brooks A, Stalker M, Mercer N, de Villa E, Gottstein B, Peregrine AS. 2014. Hepatic alveolar hydatid disease (*Echinococcus multilocularis*) in a boxer dog from southern Ontario. *Can Vet J* 55:551–553.

Torgerson PR, Keller K, Magnotta M, Ragland N. 2010. The global burden of alveolar echinococcosis. *PLoS Negl Trop Dis* 4:e722.

Wilson JF, Rausch RL, Wilson FR. 1995. Alveolar hydatid disease. Review of the surgical experience in 42 cases of active disease among Alaskan Eskimos. *Ann Surg* 221:315–323.

Yamasaki H, Nakao M, Nakaya K, Schantz PM, Ito A. 2008. Genetic analysis of *Echinococcus multilocularis* originating from a patient with alveolar echinococcosis occurring in Minnesota in 1977. *Am J Trop Med Hyg* 79:245–247.

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