Echinococcus multilocularis and *Echinococcus canadensis* in wolves from western Canada

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SUMMARY

Echinococcus species are important parasites of wildlife, domestic animals and people worldwide; however, little is known about the prevalence, intensity and genetic diversity of *Echinococcus* tapeworms in Canadian wildlife. *Echinococcus* tapeworms were harvested from the intestines of 42% of 93 wolves (*Canis lupus*) from five sampling regions in the Northwest Territories, Manitoba and Saskatchewan, and visually identified to genus level by microscopic examination. Genetic characterization was successful for tapeworms from 30 wolves, and identified both *Echinococcus canadensis* and *Echinococcus multilocularis* in all sampling locations. Mixed infections of *E. canadensis*/*E. multilocularis*, as well as the G8/G10 genotypes of *E. canadensis* were observed. These findings suggest that wolves may be an important definitive host for both parasite species in western Canada. This represents the first report of wolves naturally infected with *E. multilocularis* in North America, and of wolves harbouring mixed infections with multiple species and genotypes of *Echinococcus*. These observations provide important information regarding the distribution and diversity of zoonotic species of *Echinococcus* in western North America, and may be of interest from public health and wildlife conservation perspectives.

Key words: *Echinococcus granulosus*, wolf, Canada, genotype, geographic distribution.

INTRODUCTION

Echinococcus species are cestodes that cycle among domestic and sylvatic animals, with occasional spillover into people. Two zoonotic *Echinococcus* species are distributed in Canada – *Echinococcus canadensis* and *Echinococcus multilocularis*. Previously, *E. canadensis* was known as the sylvatic strain of *Echinococcus granulosus* (or the G8 and G10 cervid genotypes); however, recent molecular evidence based on mitochondrial genes support the nomenclature change (Sweatman and Williams, 1963; Thompson *et al*. 2006; Nakao *et al*. 2007; Moks *et al*. 2008). *Echinococcus canadensis* circulates in a two-host assemblage, utilizing large canids (wolves, coyotes [*Canis latrans*] and dogs [*Canis familiaris*]) as definitive hosts; and ungulates, primarily cervids (moose [*Alces alces*], elk [*Cervus canadensis*], caribou [*Rangifer tarandus*] and deer [*Odocoileus* spp.]) as intermediate hosts (Sweatman, 1952). *Echinococcus canadensis* has a widespread distribution across Canada, and is found in every province and territory except the Maritime Provinces and the island of Newfoundland (Sweatman, 1952; Schurer *et al*. 2013). *Echinococcus multilocularis* is reported only in western Canada and is thought to occur as two geographically and genetically segregated populations (i.e. the Northern Tundra Zone and the North Central Region) (Eckert *et al*. 2001). *Echinococcus multilocularis* predominantly utilizes smaller carnivores (coyotes, dogs, foxes [*Vulpes* spp.], and domestic cats [*Felix catus*]) as definitive hosts and a wide variety of small mammals as intermediate hosts (e.g. voles, mice, lemmings, shrews and muskrats), although aberrant intermediate hosts (such as people, domestic dogs, etc.) do occasionally occur (Rausch, 1995; Jones and Pybus, 2001; Jenkins *et al*. 2012).

Wolves have long been considered the most important definitive host for *E. canadensis*, but with the exception of one experimentally infected animal, wolves infected with *E. multilocularis* have not been reported in North America (Rausch and Richards, 1971; Rausch, 1995, 2003; Craig and Craig, 2005; Jenkins *et al*. 2013). This could be explained by a variety of factors, including the difficulty in harvesting and identifying adult cestodes of *Echinococcus* species (in part due to zoonotic risk), wolf predation preferences, and variable host specificity. The recent advent of molecular tools has facilitated species level differentiation, as well as identification of genotypic variations. The objectives of this study are to report the occurrence and identity of *Echinococcus* cestodes harvested from Canadian wolves, and to better define the geographic and host distribution of these parasites.

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Table 1. Location and occurrence of *Echinococcus* species infection in wolves examined by necropsy (Canada)

Province	Sample size (N)	Location/region	Infection Prevalence[a] No. infected (%)		
			Echinococcus	*E. canadensis*[b]	*E. multilocularis*[b]
Northwest Territories	25	Southern Slave Region	8 (32)	4 (16)	1 (4)
Northwest Territories	19	Northern Slave Region	3 (16)	3 (16)	1 (5)
Northwest Territories	29	Sahtu Region	12 (41)	6 (21)	4 (14)
Saskatchewan	5	Prince Albert National Park	3 (60)	3 (60)	1 (20)
Saskatchewan	1	Key Lake	0 (0)	–	–
Saskatchewan	11	Unknown	11 (100)	8 (73)	3 (27)
Manitoba	3	Riding Mountain National Park	2 (67)	1 (33)	2 (67)
Total	93		39 (42)	25 (27)	12 (13)

[a] Based on morphological identification.
[b] Based on molecular identification of selected cestodes (2–9 per wolf); samples from 9 wolves could not be identified beyond the genus level.

MATERIALS AND METHODS

Wolves were harvested by trappers, hunters and wildlife personnel from Saskatchewan (SK), Manitoba (MB) and the Northwest Territories (NT) for other purposes (2009 to 2011), and intestines were examined under University of Saskatchewan animal care research ethics approval protocol 20090126 (Table 1). The small intestines were ligated, excised and frozen at −80 °C for a minimum of 7 days prior to processing in order to inactivate eggs of *Echinococcus* infective for people (Eckert *et al*. 2001). *Echinococcus* strobilate adults were harvested from the intestines of 39 wolves using the scraping, filtration and counting technique (Gesy *et al*. 2013), morphologically identified to genus level, and stored in 70% ethanol. Freezing and ethanol fixation precluded definitive morphological identification of the adult cestodes to species level. Two to nine individual, intact, adult cestodes were selected from each wolf, and DNA was extracted from individual cestodes (Catalano *et al*. 2012). PCR analysis of all lysed worms was conducted using taeniid-specific primers to amplify a 470 bp region of the NADH dehydrogenase subunit 1 (NAD1) mitochondrial gene of *E. canadensis* (Bowles and McManus, 1993). Similarly, a 395 bp region of NAD1 of *E. multilocularis* was amplified using species-specific primers for any samples that did not amplify using the primers for *E. canadensis* (Trachsel *et al*. 2007). PCR products were resolved by electrophoresis (110 V, 30 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 with positive bands were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA), and sent for sequencing (Macrogen Inc., Seoul, Korea). Forward and reverse DNA sequences were aligned using a Staden Software Package (Pregap 4, Gap 4). The aligned sequences were entered into GenBank™ (National Center for Biotechnology Information) and compared by BLASTn search to previously published sequences for identification at the species and, for *E. canadensis*, at the genotype levels.

RESULTS

Overall, adult cestodes of *Echinococcus* spp. (based on morphological identification) were detected in 39 of 93 wolves (prevalence of 42%). The median intensity of infection was approximately 2420 worms (range: 60, 24 250). *Echinococcus* cestodes were successfully characterized using molecular techniques in 30 of the 39 wolves. Based on NAD1 sequence data, *E. multilocularis* was identified in 12 of the 30 wolves, representing a minimum prevalence of 13% (12 of 93 wolves). Seven of the *E. multilocularis* positive wolves were co-infected with *E. canadensis* G10. The minimum prevalence of *E. canadensis* in these wolves was 27% (25 of 93). Of these, 26% (24 of 93) had *E. canadensis* G10, 5% (5 of 93) had *E. canadensis* G8 (4 had both genotypes).

Sequences from the G10 genotype of *E. canadensis* were most similar (99% identical) to a reindeer isolate from Finland (accession no. AF525297.1), and sequences from the G8 genotype were most similar (99% identical) to a moose isolate from the USA (AB235848.1). Sequences from *E. multilocularis* cestodes were most similar (99–100% identical) to a human liver cyst from Poland (JX266826.1), an M2 European genotype (AJ237640.1), and a European-type haplotype found in a domestic dog from British Columbia, Canada (JF751034.1). Sequences of suitable length and quality were submitted to Genbank™ and assigned accession numbers as follows: *E. canadensis* G8 KC848478-KC848483; *E. canadensis* G10 KC848484-KC848493; *E. multilocularis* KC848462-848477.

Both *E. canadensis* G10 and *E. multilocularis* were found in all of the sample regions (Fig. 1); however, *E. canadensis* G8 was not found in the North Slave

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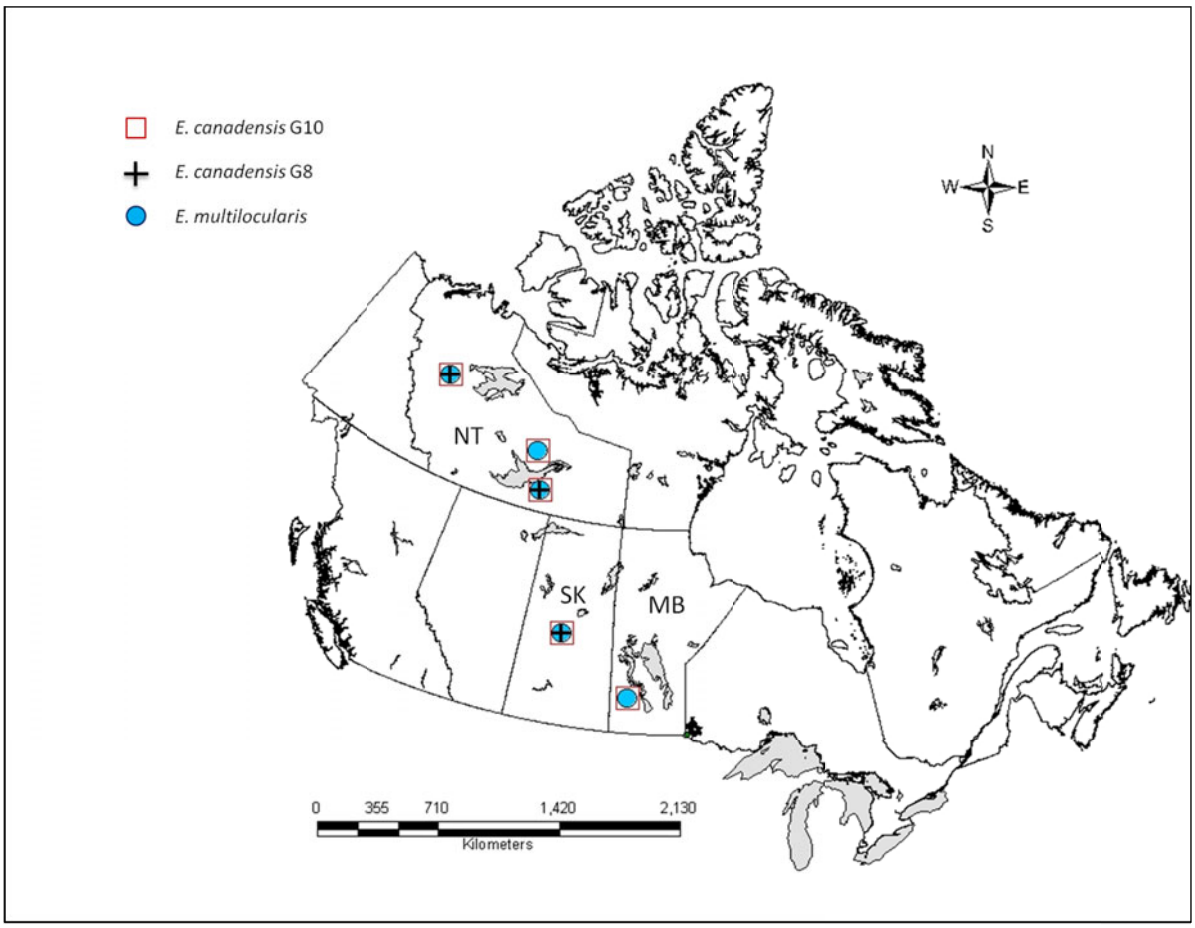

Fig. 1. The occurrence of *Echinococcus multilocularis* and *E. canadensis* (genotypes G8 and G10) in wolves across five sampling regions in Canada ($N = 93$; NT – Northwest Territories, SK – Saskatchewan, MB – Manitoba).

Region of the NT or Riding Mountain National Park (RMNP), MB. Mixed infections of *E. multilocularis* and *E. canadensis* G10 were observed in all locations except the South Slave Region of the NT.

DISCUSSION

To our knowledge, this is the first report of *E. multilocularis* in naturally infected North American wolves (Rausch, 1995, 2003; Craig and Craig, 2005; Jenkins *et al*. 2013). Wolves infected by *E. multilocularis* have previously been reported in Europe, Russia and China (Wang *et al*. 1989; Rausch, 1995; Craig and Craig, 2005). Our study suggests that the significance of wolves for sylvatic transmission of this cestode in North America may be significantly underestimated. Previously, the northern distribution of this parasite was thought to track that of the Arctic fox, which was considered to be the most important definitive host at Arctic latitudes. We found a minimum infection prevalence of 13% of 93 wolves; in contrast, prevalence of *E. multilocularis* in Arctic foxes in mainland regions of Alaska and the western Canadian Arctic is 2–9% (Jenkins *et al*. 2013). Compared with canids from RMNP, our reported median infection intensity of adult *Echinococcus* cestodes is higher than that previously reported in wolves, and is far higher than that of red foxes (Samuel *et al*. 1978). In addition, wolves travel long distances and could contribute to range expansion of *E. multilocularis* (Martinek *et al*. 2001). Wolves are known to consume a wide variety of prey species, including ungulates and rodents (Kuyt, 1972). Tundra wolves utilize rodents as a greater part of their diet than timber wolves, and would be expected to encounter *E. multilocularis* with higher frequency (Pimlott *et al*. 1969; Kuyt, 1972; Choquette *et al*. 1973). Our results show that timber wolves residing in southern regions of western Canada also encounter *E. multilocularis*. This most likely reflects a high prevalence of infection in rodent intermediate hosts as well as maintenance in other definitive hosts, including coyotes, red foxes, dogs and cats.

The current study is the first demonstration of wolves naturally infected with multiple species and genotypes of *Echinococcus*, although mixed infections have previously been reported in dogs (Stefanic *et al*. 2004; Xiao *et al*. 2006; Zhang *et al*. 2006). Mixed infections of *Echinococcus* species and genotypes may be explained by the finding that exposure to larval stages of *Echinococcus* (hydatid cysts) by definitive hosts does not elicit a sufficient immune response to

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prevent a subsequent infection (Jenkins and Rickard, 1985). Presumably, a definitive host could develop mixed infections through the consumption of various intermediate hosts harbouring different species and genotypes of *Echinococcus*. Mixed infections probably occur more frequently than suggested by our results, as this study was limited by the number of adult *Echinococcus* cestodes processed per wolf, and by the number of cestodes for which we successfully amplified DNA. We observed co-infection of wolves with *E. canadensis* G8/G10 genotypes in SK and NT, and co-infection with *E. multilocularis*/*E. canadensis* G10 in MB, SK and NT. Although we did not find the *E. canadensis* G8 strain in a mixed infection with *E. multilocularis*, this likely reflects the relative rarity of this genotype as well as the need for more widespread geographic sampling. Interestingly, mixed infections with *Taenia* spp. were also observed in 13 of the 93 (14%) wolves sampled, suggesting that cross-protective immunity does not occur for other taeniid species (M. Pawlik and E. Jenkins, unpublished results).

These findings of *Echinococcus* in wildlife may cause concern in both the animal and public health sectors. *Echinococcus* infection does not cause significant pathology in definitive hosts, and although hydatid cyst growth in ungulate hosts is usually asymptomatic, pulmonary infections may restrict vital capacity and endurance. Limited evidence is available to demonstrate that infected ungulates are more likely to be removed from herds by hunters or natural predators (Rau and Caron, 1979; Joly and Messier, 2004). In contrast, rodents are seriously compromised by their role as intermediate hosts of *E. multilocularis* (Rausch and Schiller, 1956). *Echinococcus* species are zoonotic, and although people are aberrant dead-end hosts, infection can cause severe long-term health consequences, including death (McManus *et al*. 2003). Cystic hydatid disease associated with *E. canadensis* is thought to be less pathogenic than that associated with the pastoral species in the *E. granulosus* species complex; however, severe clinical disease has been reported in people infected with the G8 strain in Alaska (Castrodale *et al*. 2002). Alveolar echinococcosis caused by *E. multilocularis* is especially dangerous for people, and the western coast of Alaska has been considered a highly endemic focus. This may in part reflect the unique ecology of the disease (especially on islands in the Bering Strait) as well as the possibility of Asian strains of this parasite (Nakao *et al*. 2009). More work is needed to determine the significance of finding European-type strain(s) of *E. multilocularis* in wolves in northern and western Canada, and their relationship to strains from elsewhere in the circumpolar North. The observation of *E. multilocularis* in wolves is an important finding for wildlife managers, veterinarians, and public health personnel in western Canada.

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