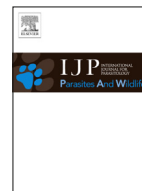




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## Surveillance for *Echinococcus canadensis* genotypes in Canadian ungulates <sup>☆</sup>



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### ABSTRACT

The geographic and host distribution, prevalence and genotypes of *Echinococcus canadensis* in wild ungulates in Canada are described to better understand the significance for wildlife and public health. We observed *E. canadensis* in 10.5% (11/105) of wild elk (wapiti; *Cervus canadensis*) in Riding Mountain National Park, Manitoba, examined at necropsy, over two consecutive years (2010–2011). Molecular characterization of hydatid cyst material from these elk, as well as three other intermediate wildlife host species, was based on sequence of a 470 bp region of the NADH dehydrogenase subunit 1 (NAD1) mitochondrial gene. In moose [*Alces alces*], elk, and caribou [*Rangifer tarandus*] from northwestern Canada, the G10 genotype was the only one present, and the G8 genotype was detected in a muskox (*Ovibos moschatus*) from northeastern Canada. On a search of the national wildlife health database (1992–2010), cervids with hydatid cysts were reported in all provinces and territories except the Atlantic provinces, from which wolves [*Canis lupis*] are historically absent. Of the 93 cervids with records of hydatid cysts, 42% were elk, 37% were moose, 14% were caribou, and 6% were white-tailed and mule deer [*Odocoileus virginianus* and *Odocoileus hemionus*]. In these animals, 83% of cysts were detected in lungs alone, 8% in both lungs and liver, 3% in liver alone, and 6% in other organs. These observations can help target surveillance programs and contribute to a better understanding of ecology, genetic diversity, and genotype pathogenicity in the *Echinococcus granulosus* species complex.

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### 1. Introduction

*Echinococcus granulosus* is a species complex of cyclophyllid cestodes belonging to the Taeniidae family, consisting of at least 10 distinct genotypes (G1–G10), each of which circulates in unique host assemblages (Thompson et al., 2006). Only the sylvatic genotypes (G8 and G10) occur in Canada (Thompson et al., 2006) and have little veterinary significance; however the livestock genotypes (G1–G3), which have a global distribution, are responsible for extensive economic damage due to livestock production losses and human illness (Battelli, 2009). Molecular evidence and older morphological studies suggest that the Holarctic sylvatic genotypes would best be re-classified as a separate species (i.e. *Echinococcus canadensis*) (Sweatman and Williams, 1963; Nakao et al., 2006; Thompson et al., 2006; Moks et al., 2008). *Echinococcus*

*canadensis* is thought to be distributed across Canada except in the Maritime Provinces and the island of Newfoundland where wolves were extirpated (Sweatman, 1952). It is also unlikely to occur in the High Arctic Islands where harsh climate conditions and a low density of intermediate hosts provide natural barriers for successful transmission between wolves [*Canis lupis*] and ungulates (Jenkins et al., 2011).

The North American sylvatic genotypes cycle between definitive canid hosts (such as wolves and coyotes [*Canis latrans*]) and cervid intermediate hosts (mainly caribou [*Rangifer tarandus*], moose [*Alces alces*], and elk [aka. wapiti; *Cervus canadensis*]) (Sweatman, 1952). Domestic dogs with access to raw viscera from infected cervids can also act as definitive hosts (Himsworth et al., 2010), and because of their close proximity to people, should be regularly de-wormed or denied viscera to avoid zoonotic transmission. Infected canids harbor adult tapeworms in their small intestine, shedding gravid proglottids and infective eggs into the environment in feces. These eggs, once ingested by a suitable ungulate intermediate host, penetrate the walls of the small intestine as oncospheres, and eventually develop into unilocular larval cysts in various organs (most often lungs (Sweatman, 1952; Ritcey and Edwards, 1958; Addison et al., 1979)). Neither the definitive nor intermediate wildlife hosts appear to suffer serious adverse

<sup>☆</sup> Note: Nucleotide sequence data reported in this paper are available in the GenBank™.

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effects (Rausch, 1952; Addison et al., 1979); however, several studies have demonstrated that heavily infected cervids are more likely to succumb to predation, either by wolves or by people (Rau and Caron, 1979; Joly and Messier, 2004). This may be a result of decreased pulmonary function, or in the case of intense disseminated infection, poor body condition and decreased stamina.

Surveillance for *E. canadensis* in Canadian wildlife is most often conducted opportunistically when animals are found dead, as part of community hunts, or when a large cull is undertaken. Hydatid cysts recovered from Canadian wildlife and farmed ungulates are often dismissed as incidental findings, and even those recovered from human infections are seldom characterized at the molecular level. Thus, limited information is available regarding the geographical distribution and pathogenicity of the G8 and G10 genotypes. In this paper we present results from a genotypic analysis of hydatid cysts recovered from elk, caribou, moose, and muskox (*Ovibos moschatus*) in Canada, as well as a cross-Canada overview of hydatid cysts detected by pathologists in wild ungulates at the various nodes of the Canadian Cooperative Wildlife Health Centre (CCWHC; [www.ccwhc.ca](http://www.ccwhc.ca)) (Canadian Cooperative Wildlife Health Centre, 2012).

## 2. Materials and methods

### 2.1. Origin of ungulate tissues

Hydatid cysts were recovered in 2010 and 2011 from 105 elk removed from Riding Mountain National Park (RMNP), Manitoba (MB) as a part of a *Mycobacterium bovis* control program (Shury and Bergeson, 2011). In addition, cysts were also obtained from one adult male moose found dead in RMNP in 2011, as well as hunted animals including one elk from RMNP, one caribou from Kugluktuk, Nunavut, and one muskox from Tasiujaq, Quebec. The cysts were recovered by visual inspection and systematic palpation of organ tissue, followed by excision of all cyst-like masses. They were bagged separately, labeled, and placed in cold storage (~5 °C) for one day until they could be transported to the University of Saskatchewan for identification. The caribou and muskox samples were stored at –20 °C prior to shipping. Each hydatid cyst was pierced using a 22 GA needle and drained of the hydatid fluid. One drop of fluid from each specimen was placed on a slide with a cover-slip in order to identify protoscolices under a light microscope. Presence of protoscolices and flame cell activity was used to determine fertility and viability, respectively (Casado et al., 1986). The hydatid liquid and remaining cyst material were placed in 70% ethanol and stored at room temperature.

### 2.2. Molecular characterization

To confirm that the cysts collected were indeed hydatid, DNA was extracted from 200 µL of hydatid fluid using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). Primers were used to amplify a 470 bp segment of the NADH dehydrogenase subunit 1 (NAD1) mitochondrial gene as previously described (Bowles and McManus, 1993; Schurer et al., 2012). PCR products were resolved using electrophoresis (110 V, 30 min) on ethidium bromide stained 1.5% agarose gels, and products were visualized under UV light. PCR products that produced positive bands were purified using ExoSAP-IT (Affymetrix Inc., Santa Clara, CA), and sent for sequencing at the National Research Council Plant Biotechnology Institute (Saskatoon, SK). Sequences were aligned using the Staden Software Package (Pregap 4, Gap 4) and compared to other sequences stored in GenBank™ (National Center for Biotechnology Information).

### 2.3. CCWHC search

The CCWHC maintains a national database of wildlife disease occurrences investigated by staff at the five Canadian veterinary

colleges (AB, SK, ON, QC, PEI) and the Animal Health Centre (BC Ministry of Agriculture). In general, whole carcasses or animal tissues were submitted for diagnostic examination to the CCWHC by biologists, conservation officers, and hunters. In 2011, we searched the database using the terms 'Echinoc', 'granulosus', and 'hydati', (to include both English and French phrases) in any of the comments or morphological diagnosis fields. The results were limited to ungulates and the years 1992–2010.

### 2.4. Statistical analysis

Data was entered into a Microsoft™ Excel spreadsheet, checked for errors and analyzed using SPSS (version 19; Chicago, Illinois, USA). Dichotomous outcome variables were entered into 2 × 2 contingency tables, and the statistical significance between proportions were determined by Fisher's Exact Test at the 5% level.

## 3. Results

### 3.1. Occurrence

The overall prevalence of hydatid cysts in the sample of elk from RMNP was 10.5% (11/105) (Table 1). The prevalence did not differ significantly by sex, with 14.6% in the 48 adult males collected in 2010, and 7% in 57 adult females in 2011 ( $p$ -value = 0.338). The number of cysts per infected elk ranged from one to four, with all cysts found in lung tissue except one in a spleen (Table 2). There were very few intact protoscolices in the hydatid fluid; half of the animals were infected with sterile cysts, and no protoscolices exhibited flame activity. In contrast, protoscolix density was high in the 7 cysts present in the moose from RMNP.

### 3.2. Molecular characterization

PCR products of the NAD 1 locus were successfully amplified and sequenced in all 15 samples. The samples recovered from cervids were all identified as the *E. canadensis* G10 genotype, most closely related to GenBank accession Nos. AF525297.1 (Lavikainen et al., 2003) and DQ144041.1 (Thompson et al., 2006). The cyst recovered from the muskox was most closely related to the G8 genotype (GenBank accession No. EU151429.1) (Moks et al., 2008).

### 3.3. Historical records

In total, 93 reports of cystic hydatid infection were retrieved in our search of the CCWHC database. These included 39 elk, 34 moose, 13 caribou, 3 white-tailed deer (*Odocoileus virginianus*), 3 mule deer (*Odocoileus hemionus*) and 1 unknown species. The majority of tissue submissions originated from the western prairies (Saskatchewan 33%, Alberta 18%, Manitoba 2%), followed by the northern territories (Northwest Territories 15%, Yukon Territories 5%, Nunavut 1%), central Canada (Ontario 18%, Quebec 3%), and finally British Columbia (4%) in the Pacific west. There were no reports from Atlantic Canada. Reports spanned a geographic range between 44°N to 68°N and 73°W to 135°W (Fig. 1). The vast majority of hydatid cysts were found in lung alone (83%), followed by 3% in the liver alone, 2% in the kidney, and 1% each in the spleen and skeletal muscle. Disseminated infection occurred in 9 animals (8% lung and liver; 1% lung, liver, kidney and spleen; 1% lung and heart). Only moose and caribou harbored cysts in organs other than the liver and lungs, and moose and elk had the highest number of cysts per animal.

**Table 1**  
Canadian prevalence of *Echinococcus canadensis* in moose (*Alces alces*), caribou (*Rangifer tarandus*), elk (*Cervus canadensis*), and deer (*Odocoileus* spp.).

Prevalence % (N)	Location	Province	Citation
<i>Moose</i>			
30 (96)	Moose Mountain PP <sup>a</sup> , Prince Albert-Nipawin area, Hudson Bay	SK, NT	Harper et al. (1955)
68 (100)	Wells Gray PP	BC	Ritcey and Edwards (1958)
71 (17)	Lake of the Woods	ON	Sweatman and Williams (1963)
43 (14)	Kapuskasing	ON	
40 (43)	N/A	SK, AB	
17 (53)	Elk Island NP <sup>b</sup>	AB	
52 (62)	North & west AB	AB	Samuel (1976)
0 (22)	Cypress Hills PP		
15 (39)	Elk Island NP		
67 (54)	Chapleau Crown Game Preserve	ON	Addison et al. (1979)
58 (45)	Mont Tremblant NP	QC	Rau and Caron (1979)
42 (114)	Reserve La Verendrye	QC	Frechette (1986)
32 (76)	Reserve des Laurentides	QC	
0 (50)	Reserve Matane	QC	
44 (580)	Southwest QC	QC	McNeill and Rau (1987)
0 (16)	Eastern ON	ON	Hoeve et al. (1988)
47 (224)	Southwest QC	QC	Messier et al. (1989)
73 (51)	N/A	AB	Pybus (1990)
<i>Caribou</i>			
21 (14)	Wholdaia Lake, Northern SK	SK, NT	Harper et al. (1955)
21 (14)	N/A	SK	Sweatman and Williams (1963)
20 (517)	Reindeer Depot	NT	
1 (159)	Northern Labrador	NL	Parker (1981)
4 (488)	North-central Canada	MB, SK, NT	Thomas (1996)
<i>Elk</i>			
16 (280)	Elk Island NP	AB	Sweatman and Williams (1963)
11 (359)	Cascade and Red Deer valleys	AB	Flook and Stenton (1969)
16 (949)	Bow Valley		
38 (193)	Jasper NP		
21 (94)	N/A	AB	Pybus (1990)
11 (105)	Riding Mountain NP	MB	This study
<i>Deer (White-tailed)</i>			
0.3 (353)	Sleeping Giant PP	ON	Sweatman (1952)
7 (28)	Moose Mountain PP	SK	Harper et al. (1955)
44 (9)	Lake of the Woods	ON	Sweatman and Williams (1963)
28 (7)	Moose Mountain PP	SK	
0 (147)	N/A	AB	Pybus (1990)
0 (263) <sup>c</sup>			

<sup>a</sup> PP = Provincial Park.

<sup>b</sup> NP = National Park.

<sup>c</sup> Mule deer.

## 4. Discussion

### 4.1. Occurrence

A review of Canadian literature (1952–present) suggests that ungulate species are not equally suitable as intermediate hosts for *E. canadensis*. Prevalence and intensity of infection appears to be low in muskoxen, white-tailed deer and mule deer (Table 1). In contrast, moose, elk and caribou appear to be highly competent hosts, with higher prevalence and intensity of infection in areas co-inhabited by coyotes or wolves (Sweatman, 1952; Sweatman and

Williams, 1963; Flook and Stenton, 1969; Samuel, 1976; Parker, 1981; Pybus, 1990; Thomas, 1996). In moose and caribou the intensity of infection, as defined by the number and size of cysts, is positively correlated with age (Ritcey and Edwards, 1958; Samuel, 1976; Rau and Caron, 1979; Messier et al., 1989; Thomas, 1996), and possibly gender (females > males) (Flook and Stenton, 1969; Thomas, 1996). The overall apparent prevalence of cystic hydatid infection in the RMNP elk was approximately similar to that reported in elk by other studies conducted in Western Canada (Table 1). The scarcity of protoscolices in RMNP elk support previous suggestions that elk are less suitable as intermediate hosts of *E. canadensis* than moose (Sweatman and Williams, 1963). Our results do not support previous reports of higher infection in females than males; however, our sample size was small.

### 4.2. Molecular characterization

We did not detect the livestock genotypes (G1–3) of *E. granulosus* in wildlife, which could support the belief that these genotypes are not present in Canada. Alternately, ungulates are likely poor hosts for these genotypes, but so few cervid isolates have been characterized genetically that it is difficult to draw conclusions at the present time. Our results failed to identify mixed infections in the animals sampled; however we did not amplify DNA from all individual cysts from each animal. Molecular identification is important for sterile cysts as the differences in viability among genotypes in different host species is not yet known. It also serves as a method of definitive diagnosis, as identification based on gross or histological examination in the absence of protoscolices is difficult. No immediate conclusions can be drawn from these data regarding the proposed new taxonomic status of *E. canadensis*; however this molecular characterization of hydatid cysts serves as a point of comparison for future biogeographical studies of hydatid infection in intermediate hosts.

### 4.3. Historical records

The records retrieved from the CCWHC database over the last few decades confirm the ongoing transmission of *E. canadensis* in most of northern, western, and central Canada. Many factors influence the prevalence and distribution of sylvatic parasites over time, including climatic variation, migration or extirpation of host species and land use changes. To our knowledge, no data have been published in the last several decades that adequately describe the distribution of *E. canadensis* in Canadian ungulates. Surveillance to detect emergence is especially important in areas of newly established host assemblages, such as the island of Newfoundland, where coyotes and moose now intermingle (McGrath, 2004). Our results based on passive surveillance suggest that *E. canadensis* transmission is not occurring in Atlantic Canada; however, active surveillance is needed to confirm this conclusion.

The historical data indicate that the majority of hydatid cysts found in ungulates were located in lungs, which supports the findings of previous reports (Sweatman, 1952; Ritcey and Edwards, 1958; Addison et al., 1979) suggesting that surveillance could focus on examining lung tissues. The database search identified geographic locations in northern Ontario, British Columbia, Nunavut and the Northwest Territories where *E. canadensis* had not previously been reported by the literature (Fig. 1).

Several factors presented limitations in this study. We have no reports of *E. canadensis* in domestic livestock, as the database focuses on wildlife surveillance; however it is thought that the sylvatic genotype rarely infects domestic ungulates (with the exception of farmed reindeer and elk) (Sweatman and Williams, 1963; Thompson and Lymbery, 1988; Thompson et al., 2006). The data obtained in each report is limited by the quality of tissue

**Table 2**  
Description of hydatid cysts found in the lung tissues of wild elk (*Cervus canadensis*), muskox (*Ovibos moschatus*), moose (*Alces alces*), and caribou (*Rangifer tarandus*) in Canada. Elk and moose were from Riding Mountain National Park, Manitoba.

ID #	Year	Host species	# Cysts	Protoscolex density <sup>a</sup>	Genotype	Accession # (GenBank)
1281	2010	Elk	1	0	G10	KC505418
1300	2010	Elk	4	0	G10	KC505419
1235	2010	Elk	4	0	G10	KC505417
1284	2010	Elk	1	0	G10	KC505416
1394	2010	Elk	1	0	G10	KC505415
1037	2010	Elk	1	0	G10	NS <sup>e</sup>
1156	2010	Elk	2	A-0 B-2	G10	KC520777
101	2011	Elk	1	0	G10	KC520779
1435	2011	Elk	1 <sup>b</sup>	0	G10	NS
1444	2011	Elk	1	0	G10	KC520780
1292	2011	Elk	1	10	G10	KC520776
1325	2011	Elk	2	A-0 B-1	G10	KC520778
OM-06	2010	Muskox <sup>d</sup>	2	0	G8	NS
76095	2011	Moose	7	20–200 (range)	G10	KC520775
102 <sup>c</sup>	2011	Caribou <sup>c</sup>	1	0	G10	KC520781

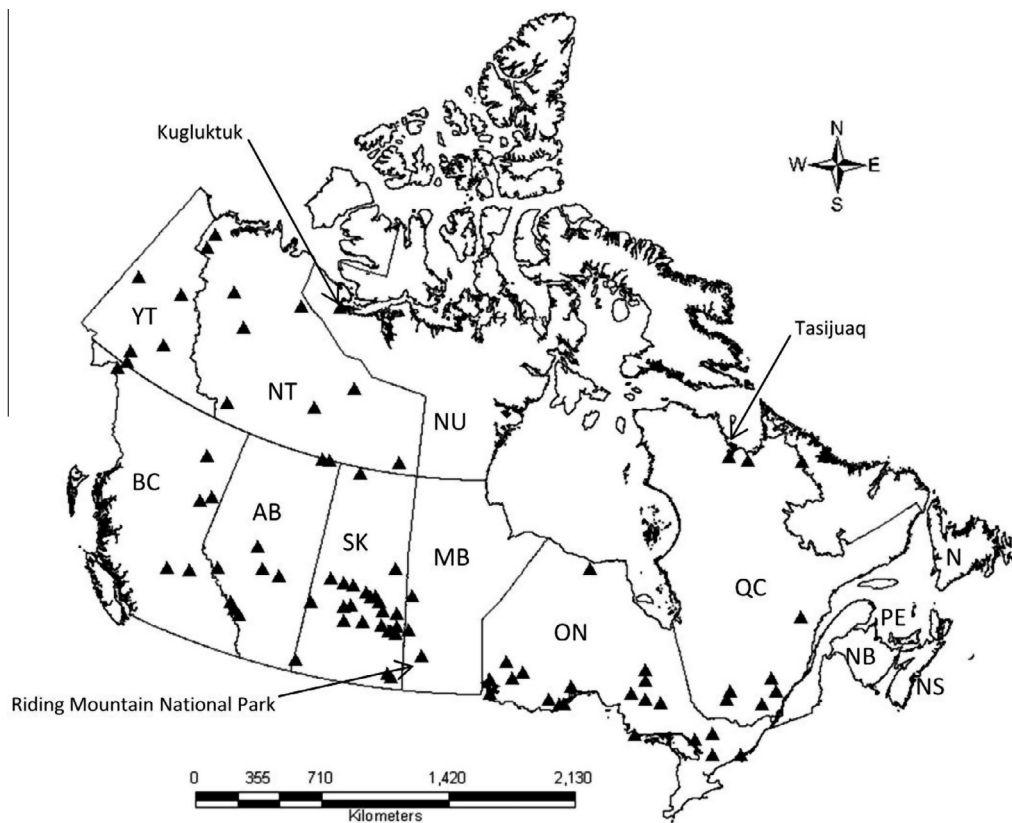
<sup>a</sup> Number of protoscolexes/mL hydatid fluid (A, B used in the case of multiple cysts from the same animal).

<sup>b</sup> Spleen tissue.

<sup>c</sup> Tasiujaq, Quebec.

<sup>d</sup> Kugluktuk, Nunavut.

<sup>e</sup> NS = Not submitted.



**Fig. 1.** Geographic locations of *Echinococcus canadensis* cysts recovered from wild ungulate intermediate hosts in the literature (1952–present) and the CCWHC database (1992–2010) [YT: Yukon Territory; NT: Northwest Territories; NU: Nunavut; BC: British Columbia; AB: Alberta; SK: Saskatchewan; MB: Manitoba; ON: Ontario; QC: Quebec; NB: New Brunswick; PE: Prince Edward Island; NS: Nova Scotia; NL: Newfoundland and Labrador].

submitted, whether the pathologist had access to the entire carcass, and whether the pathologist reported the cysts, often considered as incidental findings. Most CCWHC records did not state the infection intensity (# of cysts per animal), fertility, or viability. We are unable to report the prevalence of hydatid disease in Canadian wildlife, as the total number of whole carcass ungulates examined

during the study period is unknown. As well, sampling bias, as previously described, and low sample size numbers would make any estimate unreliable. The absence of reports from Nunavut and northern Labrador could be due to the lack of proximity and the relative difficulty in transporting tissue samples to any of the CCWHC nodes.

## 5. Conclusions

This is the first cross-Canada review of *E. canadensis* in wild ungulates to be published since 1963 (Sweatman and Williams, 1963). Human echinococcosis remains a public health concern at northern latitudes and in some Indigenous communities where people live in close proximity to areas co-inhabited by moose and wolves (Gilbert et al., 2010; Himsworth et al., 2010). Hydatid infection in people was historically endemic to certain Canadian populations (Webster and Cameron, 1967); however, changing risk factors, the advent of widespread anthelmintic use in domestic dogs, and public health education have decreased the risk of infection (Rausch, 2003; Jenkins et al., 2013). Most recently, the annual overall incidence rate of cystic hydatid disease in Canadians was estimated to be 0.72 cases per million people; however, this rate is likely to be an underestimate because definitive diagnosis of clinical cases is difficult (Gilbert et al., 2010). Historically, autochthonous cases of human echinococcosis in Canada were believed to be less serious than cases caused by the imported livestock genotype. However, this medical paradigm was challenged in 1999 when two Alaskan patients diagnosed with hepatic echinococcosis (G8 genotype) experienced serious sequelae, including one fatality (Castrodale et al., 2002; McManus et al., 2002). Historical data of cystic hydatid disease records are useful for both veterinary and medical professionals as they help define the potential distribution, and the risk of infection. Transmission and distribution of *E. canadensis* may increase as a result of rapid climate and landscape change, in combination with increased globalization of travel and trade, suggesting that national surveillance of this parasite will continue to be important for both human and animal health (Jenkins et al., 2013). This is especially true for areas where canids and ungulates have only recently come into close proximity.

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