ECHINOCOCCUS GRANULOSUS GENOTYPE G8 IN MAINE MOOSE (*ALCES ALCES*)

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ABSTRACT: During a 2012 survey of harvested moose (*Alces alces)* in Maine (USA), an incidental finding of hydatid cysts was found in 39% (21 of 54) of lung sets examined. Cytology of cyst contents was consistent with *Echinococcus granulosus*. The G8 genotype was identified based on PCR and DNA sequencing of a 470 base pair region of the NADH dehydrogenase subunit 1 (NAD1) mitochondrial gene. The hydatid cysts were the northern, or cervid genotype and this is the first confirmed report of *E. granulosus* in Maine moose. The Atlantic regions of the northern USA and Canada were not previously thought to be endemic regions for *E. granulosus*. It is presumed that either domestic dogs or eastern coyotes (*Canis latrans*) are the definitive host.

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Moose (*Alces alces*) populations are in general decline in much of the northeastern United States, but recent population estimates in Maine are reasonably stable based on aerial surveys across core moose range where density ranges from 0.4–4 moose/km^2 (unpublished data, Maine Department of Inland Fisheries and Wildlife [IFW]; Kantar and Cumberland 2013). Outside of Alaska, this regional population is the largest in the United States occupying remote commercial, boreal forestland in northern Maine to predominantly hardwood forests in Massachusetts. Given the substantial ecological, economic, and cultural importance of moose in the region, periodic studies of moose health are warranted to predict potential threats to the moose population, and to monitor for zoonotic disease or other diseases transferrable between wildlife and livestock.

Echinococcus granulosus is a parasitic cestode ("tapeworm") with 2 hosts; a carnivore (usually Canidae) definitive host, and an intermediate ungulate host (Eckert et al. 2001). In the definitive host, the tapeworm resides in the small intestine and causes little pathology. The adult tapeworm releases eggs into the feces of the definitive host and these eggs may persist for long periods in the environment (Eckert et al. 2001). After ingestion of these eggs by the intermediate host, immature forms of the tapeworm migrate into host tissues, developing into cystic structures containing protoscolices (immature heads) of the tapeworm. The tapeworm life cycle is completed when a suitable definitive host consumes cyst-containing tissue of an intermediate host. The definitive host then becomes infected with the tapeworm, which develops to the adult stage within the host's small intestine.

At least 10 genotypes of *E. granulosus* have been identified using molecular methods, and circulate in unique host assemblages around the world. The G8 and G10

strains are associated with the "northern" or "sylvatic" biotype of *E. granulosus*, whose definitive host is a wild canid such as a wolf (*Canis lupis*) or coyote (*C. latrans*) and whose intermediate host is usually a wild cervid such as elk (*Cervus canadensis*), deer (*Odocoileus* spp.), caribou (*Rangifer tarandus*) or moose. This is the first published report of *Echinococcus granulosus* in Maine moose and also the first report of the G8 genotype in the northeastern United States.

STUDY AREA & METHODS

During the 2012 Maine moose hunting season, a partial survey of harvested moose was conducted in collaboration with IFW to determine the prevalence of a novel *Dictyocaulus* spp. lungworm observed in preceding seasons (A. B. Lichtenwalner, unpublished data). In order to most efficiently collect lung samples from harvested moose, one hunter check-in site was staffed during the fall hunt. Hunters returning from Wildlife Management Districts (WMD) 2, 3, 4, 5, and 6 were expected to use this check point for confirmation of their permit and data collection by the IFW (Fig. 1). During the previous year's hunt (2011), these WMDs yielded 309 ± 85 moose each with an overall success rate of 76% (# killed/# permits). During the 2012 hunt, these WMDs yielded 384 ± 144 moose with a success rate of 86% (Fig. 1, 3; <http://www.maine.gov/ifw/hunting_trapping/hunting/harvest.htm>). In total, lung sets (except a single case with one lung) from 54 moose were examined for cysts; 41 were collected at the hunter check-in site and 13 were collected by IFW staff and delivered to the University of Maine Animal Health Lab (UMAHL), including 3 from WMD 1.

Hunters provided the permit number, seal number, general location where the animal was shot, gender, and estimated age of the moose, along with a bag containing the trachea and lungs. The tissues were collected from the hunters within approximately one day of death. Tissues were transported on ice to the UMAHL where they were visually inspected and dissected using routine biosafety precautions. The lungs were flushed with a saline solution to recover any lungworms present in the airways, followed by dissection along the airways to recover lungworms. Fluid was aspirated from selected pulmonary cysts using a 20 gauge needle and syringe. The cyst contents were spun down at 1500 RPM for 5 min. Aliquots of the sediment were examined as wet mounts, and placed into either 10% formalin for histology, or 95% ethanol. The ethanol-fixed cyst sediment was shipped to Western College of Veterinary Medicine at the University of Saskatchewan, Saskatchewan, Canada for DNA extraction and genotyping.

The sediment was re-suspended in approximately 1 mL of 70% ethanol, and DNA was extracted as previously described (Schurer et al. 2013). Briefly, primers were used to amplify a 470 base pair region of the NADH dehydrogenase subunit 1 (NAD1) mitochondrial gene (Bowles and McManus 1993). Electrophoresis (110V, 30 min) using 1.5% agarose gel and RedSafe nucleic acid staining solution (ChemBio Ltd, Hertfordshire, United Kingdom) was conducted to resolve PCR products, and bands were visualized under UV light. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc, Valencia, California, USA) and sent for sequencing (Macrogen Inc., Seoul, Korea). A Staden Software Package (Pregap 4, Gap 4) was utilized to align DNA sequences which were ultimately submitted to GenBank™ (National Center for Biotechnology Information), and identified to the genotype level by comparison to reference sequences.

RESULTS

Pulmonary cysts, consistent with hydatid cysts, were found in 39% (21 of 54) of

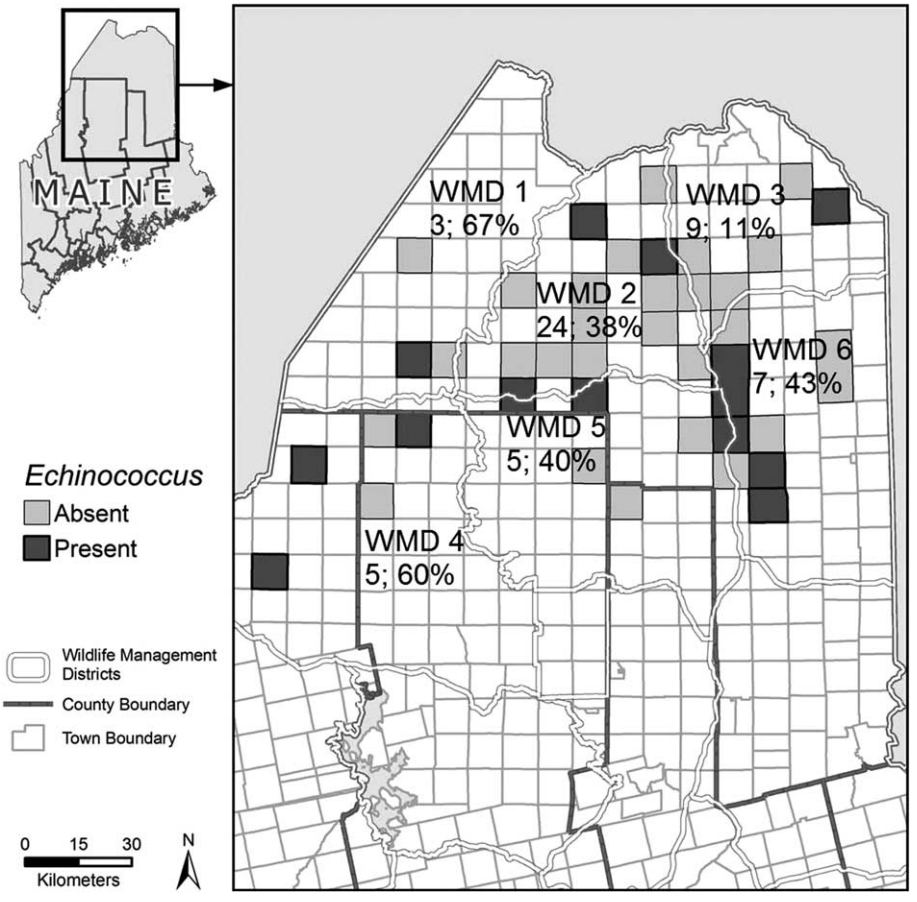

Fig. 1. Map of Wildlife Management Districts (WMD) and townships in the northern Maine study area. Areas from which unaffected moose lungs were collected are shaded grey; areas from which moose lungs were positive for echinococcus-type cysts are shaded black. The number of lung sets sampled and % positive samples are listed under each WMD (n; %).

moose lung samples. Moose with cysts were from 16 townships representing all 6 WMDs; effectively, the geographic spread included the entire study area stretching east to west across northern Maine (Fig. 1); only 1 township had >1 case. Although exact numbers of cysts were not measured, the relative amount was assessed as none, few (~10 or fewer), moderate (11–100), or many (>100) cysts. Of the 21 positive moose, 15 had a few cysts (14 adult females, 1 male calf) and 6 had many cysts (4 adult females, 2 unknown). In some lungs, 100s of cysts were present. The subpleural and interstitial cysts were pale white to yellow, firm to the touch, and ruptured easily during handling.

When observed as a fresh mount, the cytology of the cyst contents was consistent with *Echinococcus granulosus*. Fresh and fixed cytology samples showed clearly defined protoscolices, numerous calcareous granules, a thin cyst wall, and granular cyst fluid debris (Fig. 2, 3). PCR amplification and DNA sequencing of the NAD1 locus identified cyst fluid samples as the G8 strain

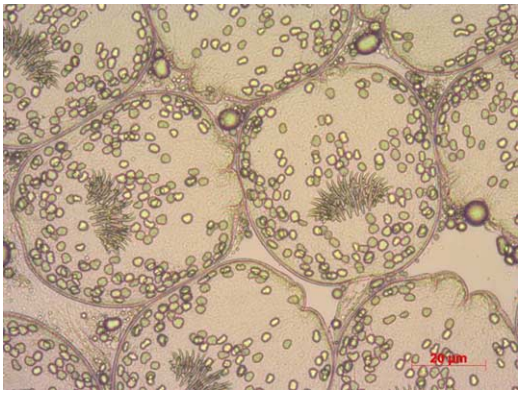

Fig. 2. Fresh mount of aspirate from representative lung cyst. The unstained, unfixed material was imaged at 400x total magnification. Protoscolices with calcareous granules can be seen within the cyst fluid.

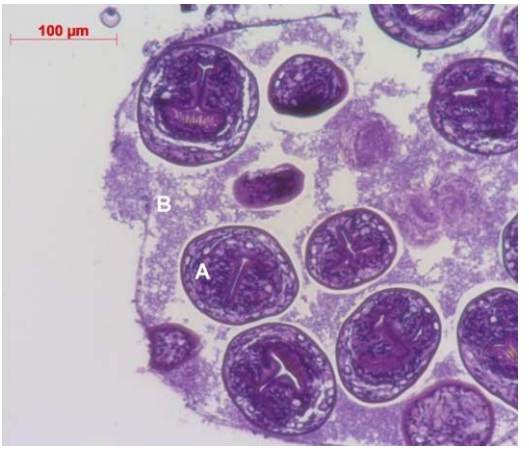

Fig. 3. Cyst aspirate material after formalin fixation, embedding and staining (hematoxylin and eosin). The protoscolices (A) are contained within a brood capsule (B). Photo captured at 100X total magnification.

of *E. granulosus* and were most closely related to the G8 genotype identified in a moose from Minnesota (GenBank™ accession number AB235848.1, Nakao et al. 2007). Sequences from moose in the current study were submitted to the NCBI GenBank™ database (Accession nos: KC839819, KC839820 and KC839821).

DISCUSSION

This is the first report of *Echinococcus granulosus* in Maine, and is unusual since this parasite was not thought as endemic to the northeastern United States due to the current absence of wolves (http://www.fws.gov/midwest/wolf/aboutwolves/WolfPopUS.htm). In a 1941 evaluation of 20 ill moose in Maine, *E. granulosus* was not found (Sweatman 1952). The sylvatic form of *E. granulosus* is present across most of Canada, where it has been reported at prevalences as high as 67% in moose of northern Ontario (Addison et al. 1979).

In a summary report concerning *E. granulosus* in North America, reported sylvatic cases (of wolves, moose, caribou, mule deer and coyotes) were reported in Alaska, Canada, northern Minnesota, and northern California, but not in Maine (Eckert et al. 2001). Further, *E. granulosus* was not reported in Maine moose by state wildlife biologists working during the last 20 years (L. Kantar and K. Morris (retired), IFW; H. Gibbs, University of Maine; pers. comm.). In the current study, only a small percentage (1–5% of the total moose killed during 2012 by WMD) of moose was sampled, but rates of 11–67% were detected, suggesting that *E. granulosus* may be widely distributed in the Maine moose population.

The pathology of *E. granulosus* in the moose host is unclear. In most lungs only a few cysts were found, and cysts appeared to be surrounded by well-aerated lung tissue. Despite 100s of cysts in specific lungs, the lung tissues appeared relatively normal. In general, these lung cysts did not appear to be highly pathogenic in the intermediate host. Certainly large numbers of cysts might be expected to reduce lung capacity and possibly impede mobility and escape from predators. As in this study, a larger and more quantitative study of *E. granulosus* in Canadian moose suggested that abnormally high cyst numbers occur in a small percentage of

affected moose (Messier et al. 1989). Although cysts occur in other internal organs (Addison et al. 1979, Eckert et al. 2001), we only investigated lungs in this study.

Both G8 and G10 are found in North American wildlife (Thompson et al. 2006, Schurer et al. 2013), and G8 was identified in a road-killed moose in Minnesota (Bowles et al. 1994). Recently, wild elk and mule deer in Idaho and Montana have been identified with *E. granulosus* cysts. Examination of grey wolves in these regions revealed the adult worm in their intestinal tracts (Foreyt et al. 2009); however, the genotype of the ungulate cysts was not reported.

It is not established that this report represents a true range expansion of *E. granulosus*, or if it has been simply undetected previoulsy. Identifying *E. granulosus* G8 in Maine moose suggests that either wild coyotes or possibly domestic dogs served as the definitive host for this parasite, as wolves are not present in Maine. Coyotes could serve as sylvatic definitive hosts in regions of the northeastern USA and Canada where wolves have previously been extirpated or were historically absent (Sweatman 1952), thus enabling the local life cycle. The parasite could have been introduced by wildlife translocations or anthropogenic movement of infected domestic dogs, similar to how *Echinococcus* species were introduced in regions of North America and elsewhere (Davidson et al. 1992, Hoberg et al. 1994, Lind et al. 2011, Jenkins et al. 2012). Surveys of coyotes and other canid populations may be warranted in the WMDs of origin to establish the source of infection.

The G8 strain of *E. granulosus* identified in the current study is genetically and biologically distinct from the pastoral biotype associated with genotypes G1-G3 in sheep and buffalo elsewhere in the world. The sylvatic biotype (G8 and G10 strains) has been associated with human disease in North America, but relatively rarely and with mild pathology compared to the pastoral biotype (Lamy et al. 1993, Himsworth et al. 2010, Nakao et al. 2010). For example, the G8 genotype was identified in a 1999 report of cystic hydatid disease in an Alaskan woman (Castrodale et al. 2002, McManus et al. 2002), while the G10 genotype was the most likely identification for a neural hydatid cyst identified in a child in Saskatchewan, Canada in 2008 (Himsworth et al. 2010). Human infection occurs due to ingestion of the tapeworm eggs acquired from the feces of the definitive canid host; most people become infected with *E. granulosus* through cohabitation with, or sharing contaminated environments with infected domestic dogs.

Moose pose no direct risk of *E. granulosus* infection to hunters or people in contact with moose carcasses. However, in regions endemic for sylvatic *E. granulosus*, offal from moose carcasses should be buried or burned to prevent scavenging by wild and domestic canids, and cooked or frozen before feeding to dogs. People should avoid direct contact with carnivore fecal material, and veterinarians should advise regular tapeworm treatment for domestic dogs at risk of exposure to infective *E. granulosus* cysts through scavenging or diet. We advise further study to assess the distribution, ecology, and overall effect of *E. granulosus* on Maine moose.

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