SHORT REPORT: MOLECULAR GENETIC CHARACTERIZATION OF AN UNUSUALLY SEVERE CASE OF HYDATID DISEASE IN ALASKA CAUSED BY THE CERVID STRAIN OF ECHINOCOCCUS GRANULOSUS

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Abstract. Distinct Echinococcus granulosus life cycle patterns have been described in North America: domestic and sylvatic. Gene sequences of the sylvatic E. granulosus indicate that it represents a separate variant. Case-based data have suggested that the course of sylvatic disease is less severe than that of domestic disease, which led to the recommendation to treat cystic echinococcosis patients in the Arctic by careful medical management rather than by aggressive surgery. We recently reported the first two documented E. granulosus human cases in Alaska, with accompanying severe sequelae. Here we describe the results of molecular genetic analysis of the cyst material of one of the subjects that supported identification of the parasite as the sylvatic (cervid) strain and not the domestic (common sheep strain), which was initially thought to be implicated in these unusually severe Alaskan cases.

Echinococcosis is a zoonotic infection caused by adult or larval stages of cestodes belonging to the genus Echinococcus (family Taeniidae). Two distinct types of Echinococcus granulosus life cycle patterns have been described in northern North America and Eurasia: domestic (pastoral) and sylvatic. The former involves dogs as definitive hosts and domestic ungulates, mainly sheep, as intermediate ones. The latter life cycle occurs in the higher latitudes and involves wolves or sled dogs and cervids, such as moose and reindeer. Humans become infected incidentally via ingestion of eggs as a result of direct contact with infected canids that shed proglottids or indirect contact with canid feces containing eggs.

Considerable morphologic and biologic variation has been demonstrated between populations of E. granulosus in different geographic settings and in different host assemblages and, as a result, a number of distinct strains of E. granulosus are now recognized. Differences include features, such as morphology, biochemistry, physiology, pathogenicity, developmental patterns, and infectivity to humans and domestic animals. Our understanding of the nature and extent of variation within the genus Echinococcus has evolved rapidly in recent years through analysis of the nuclear and mitochondrial (mt) genomes of representative isolates of the various strains. To date, nine genotypes (G1-G9) of E. granulosus have been identified using genetic data, and this categorization follows very closely the pattern of strain variation emerging based on biologic characters. Based on data from a limited number of moose cysts, the gene sequences of the sylvatic E. granulosus (cervid strain) indicate that it represents a distinct variant (designated the G8 genotype).

Presence of different strains may account for local variation in patterns of echinococcosis pathology altering the public health significance of the disease. An unanswered question remains: do differences in Echinococcus morphology and genotype correlate with clinical differences in the course of human or animal disease? Case-based data from Canada and Alaska suggested that the course of sylvatic disease was somewhat less severe than that of domestic disease, which had not been documented in Arctic regions. Most cases reported were asymptomatic, usually pulmonary cysts, discovered incidentally in older persons during tuberculosis screening programs. These findings led to the recommendation to treat cystic echinococcosis patients in the Arctic conservatively by careful medical management rather than by aggressive surgery. Several follow-up case reviews and summaries supported conservative management of sylvatic disease.

We recently reported the first two documented E. granulosus human cases in Alaska with accompanying severe sequelae. Here we describe the results of molecular genetic analysis of the cyst material of one of the subjects where DNA extraction was successful. This analysis supported identification of the parasite as the sylvatic (cervid) strain. In addition, we report on new mt DNA sequences obtained for several moose isolates of E. granulosus including the four analyzed previously and an additional sample that we show also conforms to the G8 genotype.

The aspirated contents of four individual cysts of E. granulosus, collected from a road-killed moose in Minnesota in the United States, were fixed in 70% ethanol and dispatched to the Brisbane, Australia, laboratory in 1993. The material was kept at −20°C until required for analysis. Details of earlier genetic characterisation of the four cysts, including its designation as a new genotype (G8) of E. granulosus, can be found in Bowles et al. The contents of a further cyst, taken from the lung of another road-killed moose from Fairbanks, in the interior of Alaska, were similarly fixed in ethanol, transported to Brisbane in 2000 and stored at −20°C.

Cyst contents from two patients from Alaska with severe hydatid disease were dispatched in 2000 to the Brisbane laboratory for analysis. The complete medical history, laboratory findings including diagnostic confirmation of E. granulosus infection, and exposure history have been detailed for the two patients. Genetic analysis of the cystic material was not possible for one of the patients as the tissue sample had been preserved in formalin. Successful analysis was achieved using cystic material obtained surgically from the liver of the other subject, a 17-year-old Native Alaskan woman, admitted to a local hospital in southeastern Alaska in July 1999. The case represented the first documented instance in Alaska of dis-
seminated cystic *Echinococcus* in the peritoneal cavity. The patient was born and lived continuously in southeastern Alaska. Her family kept small indoor dogs, which reportedly did not spend any appreciable time outdoors. For the 5 years before becoming ill, the patient lived in a village close to a National Park and Preserve where wolves and moose roam freely. On several occasions during that time period, she traveled to western Washington State to visit relatives who lived nearby several small farms with livestock. As a consequence, she was exposed to potential hosts of domestic cycle *E. granulosus*, and she may have been infected during this period by the domestic cycle genotype. Furthermore, the sequences obtained for *cox2*, *atp6*, and *nad3* were identical for the human and five moose isolates. The *nad1* sequence obtained for the moose isolates collected in 1993 and that obtained in 2000 differed in only two (one silent) positions (positions 276 and 421; see Figure 5 in Bowles et al.3). The *cox1* sequence obtained previously for all four moose isolates was ambiguous at a number of positions. We therefore reanalyzed the *cox1* sequence for the four cysts by plasmid cloning of the *cox1* PCR products and sequencing of the cloned fragments. The four isolates produced nonambiguous sequence (GenBank Accession no. AY056610) that was identical to that obtained for the recently collected moose and the human isolates. Thus, based on the almost complete identity in the five mt genes analyzed, we conclude that the 5 moose isolates and the human sample were infected with the G8 (cervid strain) genotype of *E. granulosus*. Inspection of the known *cox1* sequences indicated an *EcoR1* restriction site (GAATTTC, at positions 240–245; see Figure 4 in Bowles et al.3) that is unaffected by the restriction enzyme.

![Figure 1. Polymerase chain reaction (PCR)-restriction fragment polymorphism analysis of the *cox1* gene. Lane 1, DNA size ladder (sizes at 2652, 800 and 350 bp are shown at left-hand side); Lanes 2, 3, 5, 7 are PCR products subjected to digestion with the restriction endonuclease EcoR1. Undigested PCR products and fragments resulting from EcoR1 digestion are arrowed. Note the G8 *cox1* PCR fragment is unaffected by the restriction enzyme.](image-url)

### Table 1

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**Note:** G1–G8 are genotypes of *Echinococcus granulosus*. *Em* = *Echinococcus multilocularis*, *Ev* = *Echinococcus vogeli*, *Tc* = *Taenia crassiceps.*

**Pairwise numbers of differences among *Echinococcus* strains and species in the alignment used for construction of Figure 2. Nucleotides are above the diagonal and amino acids below**
al. 3) that was present in the majority of the known E. granulosus genotypes but not in the G8 genotype, thereby providing a PCR-Restriction fragment length polymorphism (RFLP) approach for its rapid discrimination from the other forms (Figure 1).

The G8 genotype is very distinct from all others, and in particular is genetically distant from the G1 (sheep) strain that is known to cause severe disease and which was initially thought to be implicated in the Alaskan cases. The extent of these differences is shown in Table 1 and Figure 2. Data used in preparation of the figure and table were the aligned concatenated sequences of cox1, atp6, nad1, and nad3. Sequence from Taenia crassiceps was used to provide an outgroup. Bootstrap values are shown at relevant nodes.

In summary, several reports from Alaska and Canada have documented that E. granulosus causes benign echinococcosis. This contrasts with the complicated and often serious disease found in other parts of the world where the domestic sheep dog strain of E. granulosus is generally thought to be responsible. Two unusually severe presentations of E. granulosus occurred in Alaska in 1999. Molecular genetic analysis of the cyst material of one of the subjects supported identification of the causative agent as the sylvatic (cervid) strain and not the domestic (common sheep strain), which was initially thought to be implicated.

Acknowledgments: The authors thank L. J Beller, J. F. Wilson, P. M. Schantz, L. H. Fallico, and F. D. Sacco for their help in provision of the hydatid materials.

Financial support: This research was supported by the National Health and Medical Research Council of Australia.

New nucleotide sequence data reported in this paper have been submitted to the GenBankTM database with the accession numbers: AF422147, AF422148, AY056610, AY05661, AY056611-15.

REFERENCES


