

Perspective Piece

Demonstration that a Case of Human Alveolar Echinococcosis in Minnesota in 1977 was Caused by the N2 Strain

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Abstract. Alveolar echinococcosis is the most serious helminthic zoonosis of the northern hemisphere affecting humans. The causative agent, *Echinococcus multilocularis*, occurs in geographically distinct strains that can be distinguished based on sequence variations of mitochondrial genes and nuclear microsatellite targets. This report finds that the human case of Alveolar echinococcosis occurring in Minnesota in 1977 was caused by the N2 haplotype described previously; the N2 haplotype is distinct from European and Asian isolates of the parasite and is restricted to the central region of North America.

Echinococcus multilocularis is a tapeworm whose lifecycle includes a canid definite host and a small mammal intermediate host.¹ Accidental ingestion of *E. multilocularis* eggs by humans (i.e., an aberrant host) can lead to alveolar echinococcosis (AE), the most serious helminthic zoonosis of the northern hemisphere, with an incidence of more than 18,000 cases per year² and a mortality rate > 90% if left untreated.^{1,3}

The parasite has a wide geographical distribution across the northern hemisphere and it exists in geographically distinct strains that can be distinguished based on sequence variations of mitochondrial genes⁴ or based on polymorphism of microsatellites.^{5,6} It is possible to distinguish between isolates obtained from North America and Europe only using specific markers,^{4,5} as not all markers do not allow such a distinction.⁶ Given the recent detection of the European isolate of *E. multilocularis* in Canada,⁷ questions have been raised on what is the extent of the distribution of this strain in North America and how long these European isolates have been present in this continent. Certainly, the presence of allochthonous strains in North America and the fact that the incidence of human AE cases is different for different strains, impose to put more effort in characterizing the origin of infections in humans. Similarly, the detection of human cases from North American strains would pose serious questions both on the pathogenicity of this autochthonous strain and on the possibility that AE might have been overlooked.

Based on sequence variation of mitochondrial genes, two North American isolates have been described by Nakao and co-workers⁴: the N1 haplotype that was found in voles from Alaska (St. Lawrence Island), and the N2 haplotype that was identified in samples originating from Indiana (five adult parasites harbored by a red fox) and South Dakota (adult parasite, laboratory strain). In addition to the endemic region of St. Lawrence Island, Alaska,⁸ only three cases of presumed locally acquired AE in human patients have been described in North America.^{9–11} The first case was a fisherman from Manitoba, Canada,¹¹ who presented with an abdominal mass in 1928, the second case was a 56-year-old female from

Minnesota that presented for abdominal pain in 1977,⁹ and the third case was an immunosuppressed 49-year-old patient from Alberta, Canada in 2013.¹⁰

Yamasaki and co-workers¹² sequenced the mitochondrial gene *cox1* of a metacestode originating from the human patient diagnosed with AE in Minnesota in 1977. The DNA was extracted from formalin fixed paraffin embedded tissue and the *cox1* gene was amplified through polymerase chain reaction. To account for the fact that the specimen had been fixed in formalin, multiple small regions were amplified using multiple primer pairs resulting in amplicons varying in size from 100 to 216 base pairs and a continuous sequence of 1,608 base pairs. The aim of the current report was to compare this sequence with the sequence data published by Nakao and co-workers⁴ to determine which geographical isolate the human case was most closely related to. The Basic Local Alignment Search Tool (BLAST) program was used to align the sequence of the human isolate in question (AB374425.1; *E. multilocularis* mitochondrial *cox1* gene for cytochrome *c* oxidase subunit 1, complete cds) and the sequence of the North American, central region isolate (AB461419.1; *E. multilocularis* mitochondrial *cox1* gene for cytochrome *c* oxidase subunit 1, complete cds, country: USA: Indiana). Figure 1 depicts the nucleotide substitutions of *cox1* that differ between Asian, European, and North American haplotypes and shows the substitutions of the N2 haplotype (numbering is based on AB461419.1). The sequence from the Minnesota case and the sequence from Indiana were found to be 100% identical revealing that the human case in 1977 was caused by the N2 haplotype.

Therefore, in accordance with the finding by Yamasaki and colleagues¹² that reported almost identical sequences between a South Dakota isolate retrieved from a fox and the Minnesota human case, we can now confirm that those sequences belong to what has been defined by Nakao et al. as the N2 Central Region haplotype.

Assessing the strain of human AE cases in North America has become increasingly relevant because European haplotypes have recently been found in wild carnivores⁷ and domestic dogs in Western and Central Canada (British Columbia, Ontario, and Saskatchewan^{13,14}; Audrey Tataryn, personal communication). The pathogenicity of the European strain of *E. multilocularis* for humans is not disputed. Given the apparent low number of human AE cases in North

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