Molecular evidence for local acquisition of human alveolar echinococcosis in Saskatchewan, Canada

Janna M. Schurer, Center for One Health, University of Global Health Equity, Kigali, Rwanda; Cummings School of Veterinary Medicine at Tufts University, North Grafton, 01536, USA

Polly Tsybina, Department of Medicine, University of Saskatchewan, Saskatoon, S7N 5E5, Canada

Karen M. Gesy, Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, S7N 5B4, Canada

Temitope U. Kolapo, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, S7N 5B4, Canada

Stuart Skinner, Department of Infectious Disease, University of Saskatchewan, Saskatoon, S7N 5E5, Canada

Janet E. Hill, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, S7N 5B4, Canada

Emily J. Jenkins, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, S7N 5B4, Canada

Corresponding author: Janna M. Schurer: Telephone: 250 789 125 405, Email: jschurer@gmail.com

Summary: Molecular analysis of metacestode tissue obtained from a human alveolar echinococcosis case in Saskatchewan, Canada demonstrated closest similarity to E3/E4 European strains. Lack of travel history outside North America suggested autochthonous transmission.

Footnote Page

1) The authors state no conflict of interest.
2) This work was supported by the Natural Sciences and Engineering Research Council of Canada; the United States National Centre for Veterinary Parasitology; and the Western College of Veterinary Medicine Wildlife Health Research Fund. These funding sources do not have grant numbers.
3) This work has not been presented at any meetings.
4) Correspondence: Janna M. Schurer: Telephone: 250 789 125 405, Email: jschurer@gmail.com
5) No author has changed his/her affiliation since the completion of this study.

ABSTRACT:

Alveolar echinococcosis (AE) is a life-threatening parasitic disease caused by the zoonotic cestode *Echinococcus multilocularis.* Our goals were to confirm infection, identify species and analyze biogeographical origin of metacestode tissues from a suspected human AE case in Saskatchewan, Canada. We conducted PCR targeting the nad1 mitochondrial gene for *E. multilocularis* and the rrns ribosomal RNA gene for *E. granulosus* and conducted haplotype analysis at the nad2 locus. Our analysis confirmed AE and indicated that sequences matched infected Saskatchewan coyotes and European E3/E4 haplotypes. The patient had no travel history outside North America. This suggests autochthonous transmission of a European-type strain.

Keywords: *Echinococcus multilocularis*, North America, zoonosis, One Health

INTRODUCTION:

Alveolar echinococcosis (AE) is an emerging public health concern in North America (NA), Europe and Asia due to increased prevalence in people and/or animals, increased diagnosis in aberrant hosts, and detection in new geographic locales. The causative agent is *Echinococcus multilocularis.* Adult cestodes reside in the small intestines of definitive canid hosts, including coyotes, wolves, foxes, and domestic dogs [1]. Intermediate rodent hosts, such as voles and deer mice, are infected with AE, characterized by space-occupying metacestodes that invade the liver [1]. People develop AE due to ingestion of parasite eggs and might experience symptoms after 5-15 years that include abdominal discomfort, fatigue and weight loss progressing to liver failure, inter-organ spread, and even death if infection is not diagnosed early and treated appropriately [2,3].

Less than ten autochthonous human cases AE cases have ever been reported in Canada; however, gaps in species identification and patient histories likely contribute to under-reporting [1,4]. Between 2002 and 2011, the Canadian Institute for Health Information reported 16 AE cases and 251 unspecified echinococcosis cases, for which *Echinococcus* species [5]. Travel histories of these patients were not reported, making it impossible to know whether these infections were locally acquired. *E. multilocularis* infection is not notifiable for people or animals at the federal level in Canada or the USA; however, it is provincially reportable in Ontario and annually notifiable to the World Organization for Animal Health [6].

The biogeography of *E. multilocularis* is important because aberrant host predilection (including zoonotic potential) may differ among strains, and transmission is strongly influenced by local ecology [7]. In 2009, a British Columbia (BC) dog was diagnosed with locally-acquired, European-strain AE for the first time in Canada [8]. Since then, AE has been documented in numerous dogs and non-human

primates in various locations across the country, suggesting that *E. multilocularis* is emerging as a parasite of medical and veterinary importance. In this paper, we demonstrate the utility of molecular confirmation of suspected echinococcosis cases and provide evidence for North American transmission of a European-type strain causing AE in people.

METHODS:

Patient history was obtained through hospital records and in-depth interviews between the attending physician and patient. Informed consent was obtained for publishing this case history.

In 2018, fresh metacestode tissue obtained from a suspected human echinococcosis case was sent to the Zoonotic Parasite Research Unit at the University of Saskatchewan for molecular identification, as part of a long-standing research collaboration. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA), with the manufacturer's instructions modified to include a second AW2 buffer wash for enhanced PCR amplification. Samples were analyzed by two simplex PCRs targeting two loci (NADH dehydrogenase subunit 1 (nad1) and the small subunit ribosomal RNA (rrnS)) to differentiate *E. multilocularis* from *E. granulosus* sensu lato, respectively [9]. PCR products were resolved by electrophoresis on a 1.5% agarose gel, viewed under UV light, purified (QIAquick PCR Purification Kit, Qiagen Inc., Valencia, CA), and sequenced in both directions (Macrogen Inc., Seoul, Korea) using PCR primers. Raw sequences were edited and assembled using Staden Software Package v1.5 and then compared to previously reported sequences by BLAST alignment to the non-redundant nucleotide collection of GenBank (National Center for Biotechnology Information).

To identify metacestode haplotype and to analyze biogeographical origin, a second PCR was conducted at the nad2 locus (NADH dehydrogenase subunit 2) [10]. PCR products were purified, sequenced, and

compared to *E. multilocularis* sequences from Saskatchewan coyotes and deer mice, the index BC dog (Accession numbers: MT250266, KC582628-33, KC549993, and JF751036.1), and other haplotypes stored in GenBank. Alignment was performed with CLUSTALw and viewed in an alignment editor (AliView) to create an 855 bp alignment with data in all positions. A haplotype network was created using PopART (http://popart.otago.ac.nz/index.shtml). The human nad2 sequence was submitted to GenBank (Accession number: MT250265).

RESULTS:

In 2015, a 70-year old Metis male resident of Saskatchewan presented to primary care with abdominal pain. Medical history included gastroesophageal reflux disease, hypertension, and osteoarthritis. Initial investigations revealed splenomegaly and retroperitoneal lymphadenopathy. Within months, the patient developed right upper quadrant pain, night sweats, nausea and vomiting. An abdominal CT revealed a 6 cm by 5 cm liver mass biopsied in October 2015. The pathology showed coagulative necrosis and lymphocytic infiltrate compatible with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Fungal elements detected by biopsy led to a diagnosis of invasive candidiasis, and fluconazole therapy began.

The patient's symptoms initially resolved, but by February 2017, the mass had nearly doubled in size (11.2 cm x 10.2 cm). The patient reported abdominal pain, nausea vomiting, malaise, and dyspnea. Pathology on a second liver biopsy was consistent with hydatid disease and chronic lymphocytic leukemia/small lymphocytic lymphoma. AE was confirmed by a second pathologist at the Mayo Clinic. ELISA on sera for antibody to *E. granulosus (sensu lato*) from the National Reference Centre for Parasitology was positive.

In May 2018 the patient began mebendazole therapy and underwent a radical resection (central hepatectomy/extended left hepatectomy, cholecystectomy, and hepaticojejunostomy to the right hepatic duct). Albendazole was continued after the surgery and the patient was initially doing well. A follow up CT in August 2018 showed no signs of AE recurrence. In February 2019, the patient was diagnosed with bowel ischemia and several intra-abdominal abscesses unrelated to echinococcosis, and passed away from complications of septic shock shortly thereafter.

Patient history gives no clear exposure route to *E. multilocularis*. Many years prior, the patient had travelled within Saskatchewan, to northern BC and to northern Ontario. He had travelled to Minnesota, USA, but never outside NA. Potential risk factors for AE included immunosuppression (due to CLL/SLL), owning dogs and hunting. He had no memory of direct contact with wild canids. To his knowledge, none of his dogs had been infected with *Echinococcus* spp.

PCR of metacestode tissue at the nad1 locus confirmed the pathologist's diagnosis of AE. The aligned sequence was 99% similar (with 99% coverage) to a Polish human isolate (Accession: MH986751) and to the index BC dog. At the nad2 locus, the human isolate was 100% identical to isolates from two Saskatchewan coyotes (SKcoy139, SKcoy147) and the index BC dog over the 855 bp region. The human, coyote and dog isolates had five single nucleotide polymorphisms: T vs G at position 42, G vs T at position 157, A vs G at position 234, A vs G at 246, and T vs A at position 252 relative to the 855 bp alignment used for this network. The haplotype network shows that these isolates grouped with E3/E4 strains originally reported from a red fox in France (Accession number: AB461404; Figure 1).

DISCUSSION:

We report the first confirmed human case of AE in the Canadian province of Saskatchewan and provide evidence for local transmission. Heightened awareness among human and animal clinicians is warranted to ensure timely and accurate diagnosis of AE cases. As well, improved capacity for surveillance and in-country diagnostics in North America is needed to ensure that laboratory tests have high sensitivity and specificity to locally endemic strains.

Prior to the index canine AE case in 2009, the known geographic distribution of *E. multilocularis* was limited to two regions in NA – the western coastal regions of Alaska and Canadian Arctic, and the southern Canadian Prairies and northcentral United States [1]. Recent reports of infected wolves, coyotes, and red foxes outside of these areas demonstrate that range expansion has occurred [1]. Since 2009, AE in aberrant animal hosts have emerged across Canada, including 26 dogs, three lemurs, and one Goeldi's monkey [11-12; T. Kolapo and E.J. Jenkins, manuscript in preparation]. Domestic dogs with AE act as sentinels for human infection but cannot infect people unless they also harbour adult cestodes in their intestinal tract and are shedding infectious eggs into shared environments. Increased detection of AE in dogs and of *E. multilocularis* in wild canids strongly suggests that risk of human infection is increasing in NA, and this is reflected by at least seven locally-acquired human cases in AB and one in the USA since 2013 [4,13]. These human cases were most closely related to European-type strains [4,13].

Haplotype differences among *E. multilocularis* isolates have been used to hypothesize geographic origin. Previously, it was suggested that only two North American (N1, N2) and two Asian haplotypes (A2, A4) were endemic to Canada and the USA. However, European-strain haplotypes have more recently been reported in coyotes in BC, Alberta, and Saskatchewan, and the N2 haplotype and six additional related

haplotypes have been reported in deer mice in Saskatchewan [1,4]. This case describes a person with no travel history outside NA who was infected with a haplotype 100% identical to isolates obtained from Saskatchewan coyotes, and more closely related to European haplotypes (E3/E4) than the N2 strain. Further research is needed to clarify how zoonotic risk, host predilection and clinical features differ among *E. multilocularis* haplotypes.

AE prognosis is improved when the infection is detected early, however, there is currently no international consensus on a single gold standard test for definitive diagnosis in people [3]. Tools to detect AE include abdominal imaging (e.g. ultrasound), serology (e.g. various ELISAs, dot immunogold filtration assays), histology, and DNA sequencing of metacestode material [14]. Although used rarely, one important advantage of molecular identification is that it allows for species specific diagnosis, and this allows clinicians to account for differences in patient prognosis and management [14]. In this case, serology was non-specific and suggested cystic echinococcosis, which differs significantly from AE. However, obtaining parasite samples is problematic as it requires biopsy or surgery. Molecular identification also requires appropriate laboratory infrastructure and skilled molecular technicians, and ideally, fresh or frozen tissues, instead of formalin-fixed biopsies or histopathological specimens. In Canada, AE is rare in people and may not be considered a differential diagnosis for most patients presenting with what initially appears to be liver neoplasia or, in this case, fungal granulomas [1]. This study shows the value of a One Health approach where medical and veterinary clinicians exchange information about emerging pathogens observed in sentinel species and discuss new tools for their detection. Such collaborations provide the opportunity to integrate datasets, enhance knowledge of pathogen biogeography, and ultimately to improve patient experiences. Our finding of a European *E. multilocularis* strain signals the need for investments in infrastructure to enhance surveillance, laboratory testing, and clinician awareness in NA.

FUNDING:

This work was supported by the Natural Sciences and Engineering Research Council of Canada; the United States National Centre for Veterinary Parasitology; and the Western College of Veterinary Medicine Wildlife Health Research Fund.

ACKNOWLEDGEMENTS:

We thank the patient who provided tissue samples and Michelle Sniatynski for her laboratory assistance.

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Figure 1 *Echinococcus multilocularis* haplotype network based on statistical parsimony, showing the position of the human isolate sequence in the European cluster. Network is based on the mitochondrial gene nad2 (855 bp alignment). Haplotypes (A1-A10, N1, N2 and E1-E5) are named per (6). Blue: Saskatchewan deer mice (8em, 21em, 74em), coyotes (SK1, SKcoy139, SKcoy147) and a domestic dog from British Columbia (BC dog). Red: Newly identified human case (HL). Unlabeled, white circles: hypothetical haplotypes as represented by a single nucleotide change from adjacent sequences.