Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Massolo A, Klein C, Kowalewska-Grochowska K, et al. European *Echinococcus multilocularis* identified in patients in Canada. N Engl J Med 2019;381:384-5. DOI: 10.1056/NEJMc1814975

SUPPLEMENTAL APPENDIX 1

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Human cases diagnostics

All cases were confirmed according to WHO criteria by imaging, tissue histopathology, serology and PCR. Serology and PCR were performed at the Institute of Parasitology, University of Berne, Switzerland, whereas epidemiological investigation and other diagnostics were carried out at the Alberta Provincial Laboratory for Public Health. Serologic testing utilized in-house Em2 ELISA and Em18 ELISA, confirmed by in house Western Blot. Multiplex PCR was carried out on formalin preserved core needle biopsies or resected tissue blocks. Each patient was interviewed about travel history, dog ownership and exposure, and potential environmental exposure.

Genotyping

DNA was extracted from adult *Echinococcus multilocularis* specimens collected from gastrointestinal tracts of coyotes and from dog feces using the E.Z.N.A stool DNA kit (Omega Bio-tek, Norcross, GA), and from formalin fixed paraffin embedded human and dog liver tissue specimens using the E.Z.N.A.® FFPE DNA Kit (Omega Bio-tek). DNA was quantified via spectrophotometry using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Ottawa, ON) and stored at -20°C.

Genotyping mitochondrial DNA single nucleotide polymorphisms (SNPs) was carried out as previously described by Nakao *et al.*[2], targeting *cob*, *nad2* and *cox1* genes and numbering SNPs based on their location within the PCR product.

Droplet Digital PCR was carried out on one of the human samples (1-2013) whose formalin fixed paraffin embedded tissues yielded minute amounts of DNA only. A primer/probe pair targeting a 69 bp region of the mitochondrial gene cob (GenBank ID AB374426.1) was designed using Primer Express Software (Thermo Fisher Scientific,

Ottawa, ON). Resulting sequences were as follows: forward primer 5'-ACCTTGGCATCAGATGTCTTATTG-3', reverse primer 5'-ACCAACGGCAAACTATCAACAA-3', probe 5'(FAM)-CTGCCACTGTCCTT-3'. Using DNA previously isolated from adult specimens of *E. multilocularis* confirmed to be of European and North American haplotype as described by Nakao *et al.*[2], it was confirmed that the primer/probe pair resulted in a positive PCR outcome using both haplotypes. Droplet digital PCR was positive for all five technical replicates, with an average of 286±26 (SEM±SE) positive droplets corresponding to about 2.7±0.1% of positives over the total amount of droplets produced. A no-template (negative) control yielded no positive droplets.

We then developed a strain-specific probe to characterize the main parasite strain (European vs. North American). A primer/probe pair targeting a 125 bp region of the mitochondrial gene nad2 (GenBank ID AB374427.1) was designed using Primer Express Software (Thermofisher Scientific). The targeted region contained a SNP that differs between the European and the North American strains. Resulting sequences were as follows: forward primer 5'- TTGTTGAGCTATGTAATAATGTGTGGAT -3', reverse primer 5'- CATAAATGGAAACAAACCAAACTTCA -3', EU_probe 5'(FAM)-CTGTGCTATTAGTCTC -3', NA_probe 5'(FAM)-TCTGTGCTATTGGTCTC. Using DNA previously isolated from adult specimens of *E. multilocularis* confirmed to be of European and North American haplotype as described by Nakao *et al.*[2], it was confirmed that each primer/probe pair resulted in a positive PCR outcome for its respective strain.

ECA was present in 66/77 analyzed specimens and presented a unique single nucleotide polymorphism (SNP) at position 235 of the *cob* gene (GenBank accession number: BankIt2217849 Seq1 MK843307); EAB, present only in 4 coyotes from Southern AB,

displayed an additional SNP at position 547 of *cox1* (GenBank accession number: BankIt2217849 Seq2 MK843308); ESK, present in 7 coyotes from Southern Saskatchewan, displayed the same SNP as ECA plus an additional SNP at position 1122 of *cox1* (GenBank accession number: BankIt2217849 Seq3 MK843309).

Data analysis

Haplotype network was calculated using Hamming distance by means of the R-package *pegas 0.10* on the SNPs observed in *nad2*, *cob* and *cox1* MtDNA genes. Phylogenetic tree inferred by partitioned Bayesian analysis using MrBayes 3.2.6 performed on the concatenated mtDNA, using *Echinococcus granulosus* as an outgroup.

Three partitions corresponding to *cob*, *nad2* and *cox1* were considered. Nucleotide substitution schemes were not specified, allowing MrBayes to move across different schemes as part of MCMC sampling (reversible jump MCMC). Four 1 million generation MCMC chains were run, producing 10,000 trees, 2,500 of which were treated as burn-in. The average standard deviation of split frequencies was 0.004. The tree was plotted using the R-package *phangorn 2.3.1*.

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