Am. J. Trop. Med. Hyg., 82(4), 2010, pp. 643–645
doi:10.4269/ajtmh.2010.09-0686

Short Report: Emergence of Sylvatic *Echinococcus granulosus* as a Parasitic Zoonosis of Public Health Concern in an Indigenous Community in Canada

Chelsea G. Himsworth,* Emily Jenkins, Janet E. Hill, Mandiangu Nsungu, Momar Ndao, R. C. Andrew Thompson, Catherine Covacin, Amanda Ash, Brent A. Wagner, Athena McConnell, Frederick A. Leighton, and Stuart Skinner

Department of Veterinary Pathology, and Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; Northern Inter-Tribal Health Authority, Prince Albert, Saskatchewan, Canada; National Reference Centre for Parasitology, Montreal General Hospital, Montreal, Quebec, Canada; World Health Organization Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia, Australia; Division of Infectious Diseases, Royal University Hospital, Saskatoon, Saskatchewan, Canada

Abstract. Within a remote Canadian Indigenous community, at least 11% of people had antibodies against *Echinococcus granulosus* and *E. granulosus* eggs were detected in 6% of environmentally collected canine fecal samples. Dog ownership, hunting, and trapping were not risk factors for seropositivity, suggesting that people are most likely exposed to *E. granulosus* through indirect contact with dog feces in the environment. In this situation, human exposure could be most effectively curtailed by preventing consumption of cervid viscera by free-roaming dogs.

Echinococcus granulosus is a zoonotic cestode (family *Taeniidae*) of worldwide distribution.[1] In Canada, the G8 and G10 forms (cervid strains) are endemic and cycle between wild cervids, primarily moose (*Alces alces*), and wild carnivores, primarily wolves (*Canis lupis*).[2,3] Domestic dogs may become infected through consumption of *E. granulosus* larvae within the organs of affected cervids and subsequently shed eggs in their feces.[3] Dogs are the primary source of infection for humans,[1] who subsequent to the accidental ingestion of *E. granulosus* eggs, may develop the larval metacestode (hydatid) in internal organs.[1,3]

In Canada, indigenous persons are overrepresented among those with autochthonous hydatid disease.[4–6] Many aboriginal Canadians live in remote communities in which hunting of wild cervids for food is commonly practiced.[5,6] These communities often maintain large populations of free-roaming dogs that may be fed or scavenge upon discarded cervid offal potentially infected with *E. granulosus*.[5–7] This combination of factors means that persons living in rural indigenous communities are more likely to be exposed to the parasite compared with persons residing in urban centers or communities without the aforementioned hunting practices and cultural associations with dogs.

In 2008, a six-year-old girl from an indigenous community in northern Saskatchewan was diagnosed with a cerebral cystic hydatid. This finding raised concerns within the community about the prevalence of and risk factors associated with exposure to *E. granulosus* and resulted in initiation of a joint human and animal health investigation. The goals of this study were to ascertain the seroprevalence of exposure to *E. granulosus* within the members of this community, to measure the prevalence of *E. granulosus* eggs in environmentally collected canine feces, and to determine the risk factors associated with exposure and infection in people and dogs.

A total of 110 individuals from the community (population = 1,300) volunteered to provide blood samples and responded to a questionnaire after providing written, informed consent. For those participants < 16 years of age, consent was provided by a parent or guardian. Serum samples were sent to the National Reference Center for Parasitology (Montreal, Quebec, Canada) where they were tested for antibodies against *E. granulosus* by using an in-house developed IgG enzyme-linked immunosorbent assay (ELISA).[8,9] Samples were considered immunoreactive (positive) if the optical density was ≥ 0.35. All persons with positive serologic results were informed and, with their consent, will be screened for the presence of hydatid cysts by thoracic radiography and abdominal ultrasound. The results of this screening are not currently available.

One block was randomly selected within each of the three distinct neighborhoods that comprise the main community. All yards on that block were surveyed on foot and any canid feces found were collected in individual plastic bags. Fecal samples were also collected from around the community landfill based on the researchers' suspicion that domestic dogs might frequent the landfill to scavenge on garbage. A total of 155 samples were collected from the four study sites, of which 153 contained sufficient fecal material to divide into two subsamples of at least five grams.

One set of 153 subsamples was fixed in 70% ethanol and sent to the World Health Organization Collaborating Center for the Molecular Epidemiology of Parasitic Infections (Murdoch, Western Australia, Australia). Each sample underwent direct examination by light microscopy and, for those samples in which taeniid eggs were observed, DNA was extracted using the Maxwell 16 DNA extraction kit (Promega Corporation, Madison, Wisconsin) according to the manufacturer's directions. Polymerase chain reaction (PCR) for *E.granulosus* was performed on extracted DNA as described.[10]

The remaining 155 samples were analyzed at the University of Saskatchewan (Saskatoon, Saskatchewan, Canada) by using quantitative fecal flotation.[11] Taeniid eggs were isolated from samples in which they were observed by using a modified flotation technique, and DNA was extracted from eggs using the QIAGEN DNeasy Blood and Tissue kit (Qiagen Inc., Valencia, CA). A PCR for a 253-basepair segment of the *E. granulosus* NADH dehydrogenase subunit 1 (ND1) mitochondrial gene was performed on extracted DNA using primers JH0156 5'-TGT GCT GGT TGG GGT AGC-3' and JH0157 5'-CAG CCT CCC CGT AAT CAA AT-3'. These primers amplify the region corresponding to nucleotides 361–613 of *E. granulosis* ND1 and were designed for the purposes of this study based

*Address correspondence to Chelsea G. Himsworth, Department of Veterinary Pathology, University of Saskatchewan, 52 Campus Drive, Saskatoon S7N 5B4, Saskatchewan, Canada. E-mail: chelsea.himsworth@usask.ca

on published sequences for cervid strains of *E. granulosis* (GenBank accession no. DQ144041). Briefly, extracted DNA (2 µL) was used as template in PCRs (50 µL total volume) containing 1× reaction buffer (10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris HCl, pH 8.75, 0.1% Triton X-100, 0.1 mg/mL bovine serum albumin), 2.5 units of Taq DNA polymerase (UBI Life Sciences, Saskatoon, Saskatchewan, Canada), 2 mM $MgSO_4$, 200 nM dNTPs, and 20 pmol each of forward and reverse primers. The PCRs were incubated at 95°C for 3 minutes followed by 40 cycles at 95°C for 30 seconds, 60°C for 60 seconds, and 72°C for 30 seconds. Negative controls, including an extraction blank and a no-template control, were included with all assays. All PCR products obtained were sequenced by using the amplification primers.

Age and sex analyses for human participants were performed by using the Wilcoxon rank sum test and chi-square test, respectively, and risk factors were assessed by odds ratio (OR).[12] All calculations were performed using STATA/IC 10.0 (StatCorp Lp, College Station, TX) with a significance level of $P < 0.05$.

The University of Saskatchewan's Committee on Animal Care and Supply and Biomedical Research Ethics Board reviewed and approved the animal and human study protocols, respectively.

Serologic results were available for 106 of 110 participants, of which 12 (11%) had antibodies against *E. granulosus*. These 12 persons (henceforth referred to as reactors) were from eight households, of which five households had one reactor, two had two reactors, and one had three reactors. There was no significant difference in age or sex distribution between reactors and non-reactors. Other than the child with the cerebral hydatid, only one other reactor reported having symptoms compatible with hydatid disease (abdominal pain).[4]

Nine (6%) of 153 dog fecal samples examined at the World Health Organization Collaborating Center contained taeniid eggs, of which four contained *E. granulosus* as evaluated by PCR. Taeniid eggs were identified in 17 (11%) of 155 fecal samples examined at University of Saskatchewan, of which 7 contained *E. granulosus* as assessed by PCR and confirmed by sequencing (GenBank accession no. GQ332570). These seven sequences were identical, with the exception of a G/A polymorphism at position 192, and the consensus sequence generated was 100% identical to the *E. granulosus* G10 reference strain.[13] Amalgamation of results from both institutions resulted in the detection of *E. granulosus* in 9 (6%) of 155 samples. *Echinococcus granulosus* egg-containing samples originated from all three sampled neighborhoods, but not the landfill.

Questionnaire results were available from 106 participants belonging to 35 households. Fifteen households (43%) reported currently owning at least one dog and an additional seven (20%) reported dog ownership within the past six years. One of those 22 households reported providing anthelmintic treatment of their dog(s). There was no significant association between seropositivity and dog ownership (OR = 2.6, 95% confidence interval [CI] = 0.5–25.4). Twenty (91%) of 22 households with past or present dog ownership reported that the dog(s) was allowed to roam freely around the community. Twenty-six (41%) of 58 responders reported observing wild canids (wolves or coyotes [*Canis latrans*]) around the community.

Seventeen (16%) persons from 15 (43%) households reported hunting wild cervids (including moose, deer [*Odocoileus* spp.] and elk [*Cervus canadensis*]) and 3 (3%) persons from 3 (9%) households reported trapping wild carnivores (including wolf and coyote). There was no significant association between seroreactivity and hunting or trapping (OR = 0.7, 95% CI = 0.07–3.9 and OR = 0.0, 95% CI = 0.0–7.8, respectively) or having a hunter or trapper within the household (OR = 1.35, 95% CI = 0.3–6.6 and OR = 0.5, 95% CI = 0.01–3.8 respectively).

Of the 17 hunters, 2 reported having observed *E. granulosus* cysts in the organs of hunted cervids, 5 had never seen cysts, 9 were unsure, and 1 did not respond. Six of 17 hunters responded to a question regarding disposal of remains from hunted animals, of which 3 reported leaving them out in the open, and 3 reported disposing of them at the landfill. During the canid fecal collection component of this study, researchers noted cervid remains around residential areas and in the landfill. Twenty (43%) of 46 responders reported having observed domestic dogs scavenging on cervid remains.

Overall, at least 11% of participants in this study had antibodies against *E. granulosus*. However, the *E. granulosus* IgG ELISA appears to have low sensitivity in Canada, even in patients with clinically apparent hydatid disease.[4,5] This finding may be related to the fact that people infected with the cervid strain of *E. granulosus* are less likely to produce detectible antibodies then those infected with the sheep strain.[5] Additionally, the ELISA used by the National Reference Center for Parasitology uses antigen from the sheep rather than the cervid strain, which could further impact its sensitivity in the detection of autochthonous *E. granulosus* exposure and infection in Canada.

Echinococcus granulosus eggs were detected in canid feces collected throughout the community. Although wild canids were occasionally observed, most of these samples likely emanated from domestic dogs. Previous studies have identified dog ownership as a risk factor for the development of echinococcosis.[14] A similar association was not detected in this study, which is not surprising given that most dogs in this community are free-roaming. Therefore, one infected dog may interact with persons from multiple households or contaminate multiple properties. Additionally, in this situation, the detached relationship between dogs and community members suggests that persons are most likely exposed to *E. granulosus* through indirect contact with canine feces in the in environment rather than through direct contact with infected dogs.

Traditional practices, including hunting and trapping, were not significantly associated with seroreactivity. Harvest and/or consumption of infected cervids does not pose a direct *E. granulosus*-related zoonotic risk to humans. However, consumption of cervid viscera by domestic dogs may lead to infection and shedding of eggs, and is likely a risk factor for this community as a whole.[1]

All of the conditions required for autochthonous hydatid disease are present within this community. These conditions include hunting of wild cervids within an *E. granulosus*-endemic area and consumption of potentially infected cervid offal by domestic dogs, resulting in canine infection and subsequent human exposure.

This project demonstrates that *E. granulosus* continues to present a health risk to indigenous communities in Canada. In this context, risk would be most effectively curtailed by preventing consumption of infected cervid viscera by domestic dogs.

Received November 16, 2009. Accepted for publication December 21, 2009.

Acknowledgments: We thank the Department of Veterinary Microbiology, University of Saskatchewan, for technical support.

Financial support: This study was supported by the Division of Infectious Diseases, Royal University Hospital and the Western College of Veterinary Medicine Interprovincial Graduate Fellowship for Veterinarians.

Authors' addresses: Chelsea G. Himsworth and Frederick A. Leighton, Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, E-mails: chelsea.himsworth@usask.ca and ted.leighton@usask.ca. Emily Jenkins, Janet E. Hill, and Brent A. Wagner, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, E-mails: emily.jenkins@usask.ca, janet.hill@usask.ca, and brent.wagner@usask.ca. Mandiangu Nsungu, Northern Inter-Tribal Health Authority, Prince Albert, Saskatchewan, Canada, E-mail: mnsungu@nitha.com. Momar Ndao, National Reference Centre for Parasitology, Montreal General Hospital, Montreal, Quebec, Canada, E-mail: momar.ndao@mcgill.ca. R. C. Andrew Thompson, Catherine Covacin, and Amanda Ash, World Health Organization Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary and Biomedical Sciences, Murdoch University, Western Australia, Australia, E-mails: a.thompson@murdoch.edu.au, c.covacin@murdoch.edu.au, and A.Ash@murdoch.edu.au. Athena McConnell and Stuart Skinner, Division of Infectious Diseases, Royal University Hospital, Saskatoon, Saskatchewan, Canada, E-mails: Athena.McConnell@saskatoonhealthregion.ca and Stuart.Skinner@saskatoonhealthregion.ca.

REFERENCES

1. Moro P, Schantz PM, 2006. Cystic echinococcosis in the Americas. *Parasitol Int 55:* S181–S186.
2. Thompson RC, Boxell AC, Ralston BJ, Constantine CC, Hobbs RP, Shury T, Olson ME, 2006. Molecular and morphological characterization of *Echinococcus* in cervids from North America. *Parasitology 132:* 439–447.
3. Jenkins DJ, Romig T, Thompson RC, 2005. Emergence/re-emergence of *Echinococcus* spp.: a global update. *Int J Parasitol 35:* 1205–1219.
4. Somily A, Robinson JL, Miedzinski LJ, Bhargava R, Marrie TJ, 2005. Echinococcal disease in Alberta, Canada: more than a calcified opacity. *BMC Infect Dis 5:* 34.
5. Finlay JC, Speert DP, 1992. Sylvatic hydatid disease in children: case reports and review of endemic *Echinococcus granulosus* infection in Canada and Alaska. *Pediatr Infect Dis J 11:* 332–336.
6. Wolfgang RW, Poole JB, 1956. Distribution of *Echinococcus* disease in northwestern Canada. *Am J Trop Med Hyg 5:* 869–871.
7. Unruh DH, Kink JE, Eaton RD, Allen JR, 1973. Parasites of dogs from Indian settlements in northwestern Canada: a survey with public health implications. *Can J Comp Med 37:* 25–32.
8. Coltorti E, Fernandez E, Guarnera E, Lago J, Iriarte J, 1988. Field evaluation of an enzyme immunoassay for detection of asymptomatic patients in a hydatid control program. *Am J Trop Med Hyg 38:* 603–607.
9. Coltorti EA, 1986. Standardization and evaluation of an enzyme immunoassay as a screening test for the seroepidemiology of human hydatidosis. *Am J Trop Med Hyg 35:* 1000–1005.
10. Trachsel D, Deplazes P, Mathis A, 2007. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitology 134:* 911–920.
11. Cox DD, Todd AC, 1962. Survey of gastrointestinal parasitism in Wisconsin dairy cattle. *J Am Vet Med Assoc 141:* 706–709.
12. Petrie A, Watson P, 2006. *Statistics for Veterinary and Animal Sciences*. Second edition. Ames, IA: Blackwell, 55–173.
13. Lavikainen A, Lehtinen MJ, Meri T, Hirvela-Koski V, Meri S, 2003. Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of *Echinococcus granulosus*. *Parasitology 127:* 207–215.
14. Campos-Bueno A, Lopez-Abente G, Andres-Cercadillo AM, 2000. Risk factors for *Echinococcus granulosus* infection: a case-control study. *Am J Trop Med Hyg 62:* 329–334.