# SHORT COMMUNICATIONS

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## *Echinococcus* Species Infections among Wild Canids in Pennsylvania, USA

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ABSTRACT: *Echinococcus* species are zoonotic tapeworms that can impact the health of wildlife, domestic animals, livestock, and humans. Two species of interest in North America are *Echinococcus multilocularis* and *Echinococcus canadensis* (*Echinococcus granulosus* sensu lato). The primary wildlife definitive hosts for *E. multilocularis* and *E. canadensis* are similar, including red foxes (*Vulpes vulpes*), gray foxes (*Urocyon cinereoargenteus*), coyotes (*Canis latrans*), and wolves (*Canis lupus*). These two *Echinococcus* spp. use different intermediate hosts, including small mammals for *E. multilocularis* and artiodactylids for *E. canadensis*. Although historically absent from much of the eastern US, recent reports in new US states (e.g., Virginia, Vermont, Maine, Missouri) highlight the need for *Echinococcus* spp. surveillance in this region. During 2019–2020, 308 gastrointestinal tracts were collected from wild canids in Pennsylvania and microscopically screened for adult *Echinococcus* species. Two coyotes (2/155) were co-infected with both *E. multilocularis* and *E. canadensis* as determined by molecular confirmation. No red foxes ($n$=137) or gray foxes ($n$=16) were positive. These data indicate both *Echinococcus* species are present in Pennsylvanian coyotes, highlighting the need to better understand the ecological and epidemiological consequences for human and animal health.

*Key words:* Canid, *Echinococcus canadensis*, *Echinococcus multilocularis*, Pennsylvania, zoonotic disease.

*Echinococcus* species are zoonotic tapeworms of veterinary and public health concern. Two species in North America are *Echinococcus multilocularis* and *Echinococcus canadensis* (part of the *Echinococcus granulosus* sensu lato). Currently, *E. granulosus* sensu lato is composed of 10 genotypes (G1–10) with G8 and G10 recognized as *E. canadensis* (Cerda et al. 2018; Dell et al. 2020). In the US, red foxes (*Vulpes vulpes*), gray foxes (*Urocyon cinereoargenteus*), coyotes (*Canis latrans*), and wolves (*Canis lupus*) are the primary wild definitive hosts for *Echinococcus* spp. Domestic dogs (*Canis familiaris*) can also serve as definitive hosts (Schurer et al. 2013; Cerda et al. 2018). The *E. multilocularis* life cycle includes small mammals (i.e., rodents and insectivores) as intermediate hosts, and *E. canadensis* uses both wild and domestic artiodactyld species such as white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus*), and sheep (*Ovis* sp.; Schurer et al. 2013; Cerda et al. 2018).

*Echinococcus* spp. infections in canids are generally restricted to the intestinal tract and not associated with overt disease. Recently in North America, however, alveolar echinococcosis (parasitic lesions in organs that are filled with developing protoscolices) has been reported in domestic dogs (Peregrine et al. 2012; Zajac et al. 2020). In intermediate hosts, larval tapeworm cysts form in a variety of organs (e.g., liver or lungs) resulting in morbidity and mortality. *Echinococcus* spp. are potential threats to wildlife, livestock, and human health. Further, these parasites threaten livestock production systems, especially in European and Asian countries, where production losses can total $1.5–2 billion USD annually (Cerda et al. 2018).

In North America, *Echinococcus* spp. are primarily reported from northern regions of the midwest and western US, Alaska, and Canada (Massolo et al. 2014); however, *E. multilocularis* was recently reported in eastern



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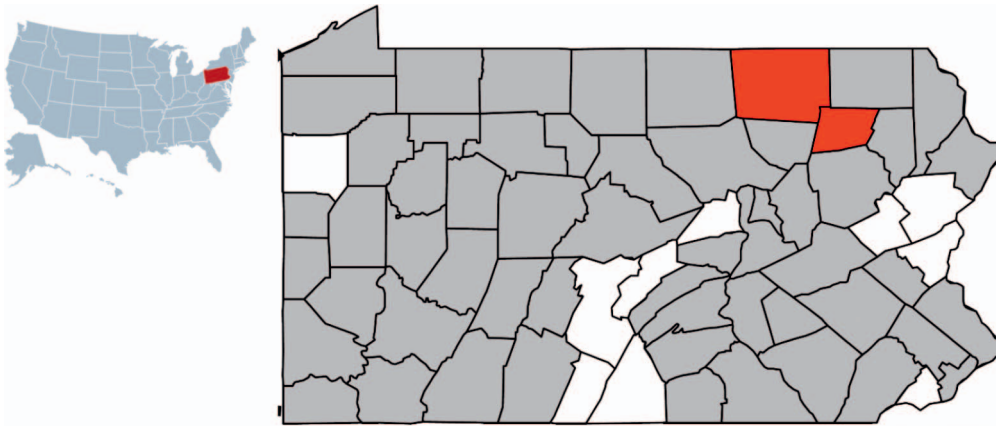

FIGURE 1. Map of Pennsylvania, USA, showing counties from which wild canid samples for *Echinococcus* spp. surveillance were submitted, January 2019–October 2020. Counties with no samples submitted are white, counties with samples submitted are gray ($n$=308), and counties where *Echinococcus* spp. were detected in this study are in red ($n$=2). For further detail on number of samples submitted per county and by canid species see Supplementary Material Table S1.

US states: a dog in Virginia, and a human in Vermont, and *E. canadensis* was reported in moose and coyotes in Maine and translocated elk in Tennessee (Catalano et al. 2012; Schurer et al. 2018; Dell et al. 2020; Zajac et al. 2020). These recent detections suggest that the distribution of *Echinococcus* spp. are more widespread than originally thought, emphasizing the need for increased surveillance in the eastern US. Our study tested wild canids from Pennsylvania for *Echinococcus* spp. to determine geographical presence and the definitive host species involved in transmission.

Between January 2019 to October 2020, 308 gastrointestinal tracts (GITs; stomach to rectum) were collected from coyotes, red foxes, and gray foxes from 48 counties in Pennsylvania, USA (Fig. 1, Supplementary Material Table S1). Samples were collected at check stations from organized recreational predator hunts, and fresh road-killed animals. No animals were euthanized specifically for this study. All samples were frozen at –20 C and shipped to the Southeastern Cooperative Wildlife Disease Study, University of Georgia (UGA; Athens, Georgia, USA) where sample processing occurred following biosafety protocols as described (Gesy et al. 2013). All methods were reviewed and approved by UGA's International Animal Care and Use (A2020 11-010-Y2-A3) and biosafety committees. The GITs were screened for adult *Echinococcus* spp. by scraping and washing small and large intestinal contents into a sieve and visually inspecting the contents for *Echinococcus* spp. as described (Gesy et al. 2013), with one modification: the mucosa of examined intestines were scraped to dislodge parasites as opposed to shaken in jars. This method was chosen because the scraping and counting technique (SCT) is considered the gold standard; however, Gesy et al. (2013) found that including a filtration step does not reduce the sensitivity and reduces the time to screen samples. Feces were collected from the large intestine and colon during this process. Any *Echinococcus* spp. suspects were preserved in 70% ethanol and identified to genus by morphology (i.e., 2–6 proglottids and scolex) using a dissecting microscope. Morphology was used to identify suspect worms only to genus level, as freeze–thaw led to some degradation of cestodes. To identify species, DNA was extracted from a subset of *Echinococcus* spp. (15 individual worms from a suspect positive sample) using DNeasy Blood and Tissue extraction kits (Qiagen, Germantown, Maryland, USA) to identify species and confirm positive *Echinococcus*

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TABLE 1. Primers used for molecular species identification of *Echinococcus* cestodes from the gastrointestinal tracts of wild canids in Pennsylvania, USA, January 2019–October 2020.

| Target species | Region | Amplicon size (base pairs) | Primer | Sequence (5′–3′) | Reference |
|---|---|---|---|---|---|
| *Echinococcus* genus | *COI* | 366 | JB 3 | TTTTTTGGGCATCCTGAGGTTTAT | Bowles et al. (1992) |
| | | | JB 4.5 | TAAAGAAAGAACATAATGAAAATG | |
| *Echinococcus multilocularis* | *nad2* | 126 | Nad 234F | TTGTTGAGCTATGTAATAATGTGTGGAT | Santa et al. (2018) |
| | | | Nad 234R | CATAAATGGAAACAAACCAAACTTCA | |
| *Echinococcus canadensis* | *cox1* | 143 | Cox 143F | ATGAGGTGTTGGGTTCCTATAGG | Santa et al. (2018) |
| | | | Cox143R | ACAATCATCAACCCAACGCA | |
| *Echinococcus granulosus* | *nad1* | 298 | EgNDI1 | AGTCTCGTAAGGGCCCTAACA | Bart et al. (2006) |
| | | | EgNDI2 | CCCGCTGACCAACTCTCTTTC | |
| *Echinococcus granulosus* | *cox1* | 298 | EgCOI1 | TTTTTTGGCCATCCTGAGGTTTAT | Bart et al. (2006) |
| | | | EgCOI2 | TAACGACATAACATAATGAAAATG | |
| *Echinococcus multilocularis* | *rrn*S | 200 | EM-H15 | CCATATTACAACAATATTCCTATC | Stieger et al. (2002) |
| | | | EM-H17 | GTGAGTGATTCTTGTTAGGGGAAG | |

sp. infection. Genus-wide and species-specific PCRs were used (Table 1). Amplicons were gel purified (Qiagen) and submitted to GENEWIZ (Azenta Life Sciences, South Plainfield, New Jersey, USA) for bidirectional sequencing. Consensus sequences were generated using Geneious Prime (Dotmatics, San Diego, California, USA).

In addition to intestinal sieving, a random subset of fecal samples ($n$=139) were PCR tested to compare the two methods. Fecal samples from the two animals detected as infected with *Echinococcus* spp. by morphology and PCR were included. For these two positive canids, three subsets of the feces were tested via molecular analysis to confirm species of *Echinococcus*. If only one sample was collected from a county, it was included. Before DNA extraction, 1 g of feces was frozen for 24 h at –80 C then heated at 105 C for 10 min to fracture eggshells. The DNA was extracted using a miniStool kit (Qiagen). The same PCR protocols were used to test feces as to test individual worms (Table 1). Negative water controls were included for the DNA extraction and PCR to ensure no contamination occurred during molecular analyses. Positive controls were *E. multilocularis* from a previous study for the conventional PCR analysis and *E. granulosus* and *E. multilocularis* commercially available gene fragments (gBlocks, Integrated Data Technologies Inc., Coralville, Iowa, USA) for RT-PCR. The gBlock for *E. canadensis* does not amplify using the *E. multilocularis* RT-PCR and vice versa.

Two of 155 coyotes (1.29%; 95% confidence interval [CI], –0.5%, 3.09%), both from 2020, each had adult *Echinococcus* spp. Using the intestinal sieving method, one adult male coyote from Bradford County was positive for both *E. multilocularis* (100% nucleotide match; GenBank accession nos. OP068158 and OP081143) and *E. canadensis*. G8 (99% match; nos. OP068161 and OP068166); one adult female coyote from Wyoming county was infected with *E. canadensis* G8 (99% match; nos. OP068160 and OP068164; Fig. 1, Supplementary Material Table S2). *Echinococcus* spp. were not enumerated in either coyote because of high infection intensities. None of the 137 red foxes or 16 gray foxes were positive via GIT sieving or fecal PCR. Two of 139 fecal samples, both from the positive coyotes mentioned, were *Echinococcus* spp. positive by PCR test and yielded co-infections of *Echinococcus* spp.: *E. multilocularis* (100% match; nos. OP068157, OP081141, and OP081142) and *E. canadensis* G8 (99% match; nos OP068159, OP068162, OP068163, and OP068165); see Supplementary Material Table S2.

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The presence of *Echinococcus* spp. in Pennsylvania represents a historically unrecognized disease risk to humans, livestock, domestic animals, and wildlife. *Echinococcus* spp. eggs are relatively persistent, which may lead to human contact and infection in urbanized areas where indirect interactions with wildlife probably increases risk of exposure to humans (Veit et al. 1995). Intestinal *Echinococcus* spp. infections in domestic dogs also may elevate this risk, as humans might be exposed through interactions with their pets (Carmena and Cardona, 2013).

Domestic dogs classically serve as definitive hosts for *Echinococcus* spp. and do not typically develop clinical disease with intestinal infections. However, they do pose a peridomestic source for environmental contamination. Interestingly, there have been recent reports of domestic dogs developing alveolar echinococcosis, suggesting that these hosts can also act as aberrant intermediate hosts (Peregrine et al. 2012; Skelding et al. 2014; Pinard et al. 2019; Zajac et al. 2020). It is not fully understood why some domestic dogs develop alveolar echinococcosis; it has been hypothesized that either ingestion of eggs from infected wild canid feces and subsequent intermediate host-like infection occurs, or they have an existing intestinal infection from ingestion of infected cysts that results in autoinfection (Weiss et al. 2010; Pinard et al. 2019). It is currently unknown if wild canids can also serve as aberrant intermediate hosts.

*Echinococcus canadensis* poses a threat to wildlife and domestic animals that can serve as intermediate hosts. Although *E. canadensis* is traditionally found in sylvatic cycles with wild cervids acting as the intermediate hosts, infections have been reported in domestic muskox (*Ovibos moschatus*) from Quebec, Canada, and sheep from China (Schurer et al. 2013; Hua et al. 2019). When considering wild ruminants and wildlife management, *E. canadensis* infections in cervids could affect declining moose populations and restoration efforts for eastern elk populations (Musante et al. 2010; Schurer et al. 2013). Similarly, North American *E. multilocularis* infections in humans and dogs pose not only a veterinary and human health threat, but also indicate another risk for wildlife populations such as the Allegheny woodrat (*Neotoma magister*; Skelding et al. 2014; Zajac et al. 2020; Polish et al. 2021).

Although only two coyotes were detected to be infected with *Echinococcus* spp., this might be because of the relatively small sample sizes surveyed in the different regions. There is a need for continued surveillance of wild definitive and intermediate hosts to better define geographic prevalence and distribution, and to inform risk communication and preventative measures. Furthermore, the findings of both *Echinococcus* spp. in each infected coyote confirms co-infections in this region. Although such co-infections may not be common, they have also been noted in coyotes and red foxes in Alberta, Canada where they found both singly and co-infected individuals (Santa et al. 2018). Coyotes predate on both cervids and rodent hosts, and therefore may be exposed to both species of *Echinococcus*. It is important to remember that *E. granulosus* may be relatively small in coyotes and mistaken for *E. multilocularis* if no molecular confirmation is conducted (Santa et al. 2018). In the face of changing climate and landscape alteration, surveillance, research strategies, and informed management approaches for this important group of zoonotic cestodes are greatly needed.

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## SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-22-00042.

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