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New Geographic Records for *Trichinella nativa* and *Echinococcus canadensis* in Coyotes (*Canis latrans*) from Insular Newfoundland

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ABSTRACT: Coyotes (*Canis latrans*) rapidly expanded across North America during the 20th century and in 1987 colonized insular Newfoundland, Canada. Their arrival brought the potential for new predator-prey interactions and the potential for transmission of parasites to naïve populations. *Trichinella* spp. and *Echinococcus* spp. are zoonotic parasites not previously reported from the island of Newfoundland. Muscle samples (diaphragm and tongue) from 153 coyotes and feces from 35/153 coyotes were collected. Larvae of *Trichinella* spp. were recovered by muscle digestion from 6/153 coyotes (3.9%) and identified using multiplex PCR and Sanger sequencing as *T. nativa*. Fecal samples were screened for DNA of *Echinococcus* spp. using qPCR, and intestines from positive animals were examined for adult cestodes. No fecal samples were positive for DNA of *E. multilocularis*, and 2/35 (5.7%) samples were positive for *E. canadensis*, of which one was successfully genotyped as the G10 cervid strain. *Echinococcus canadensis* has not previously been reported on the island of Newfoundland, historically the only region of Canada where *Echinococcus* spp. was not known to occur. No species of *Trichinella* have previously been reported on the island. Both parasites are zoonotic, and hunters, trappers, dog owners, and the general public should be aware of these new risks for public health.

Key words: *Echinococcus*, epidemiology, food safety, *Trichinella*, zoonotic disease.

The geographic range of coyotes (*Canis latrans*) has expanded rapidly since the early 1900s and now includes most of North America (Hody and Kays 2018). In the last 40 years, coyotes crossed ice bridges that formed between mainland Canada and the Canadian island of Newfoundland (Blake 2006). The arrival of coyotes on insular Newfoundland was confirmed in 1987 near Deer Lake, Newfoundland and Labrador, Canada, and is considered the most significant terrestrial ecological event to have occurred on the island of Newfoundland in the last century (Blake 2006). Invasion of a new species into an isolated ecological environment can pose the risk of introducing new infectious diseases to which local fauna are naïve.

Trichinella spp. and *Echinococcus* spp. are important zoonoses with public and animal health significance. *Trichinella* spp. are zoonotic muscle-dwelling nematodes transmitted via food-borne routes among carnivores and omnivores, including livestock and people. Although terrestrial wildlife infected with *Trichinella* spp. have not previously been reported from insular Newfoundland (Butler and Khan 1992; Government of Newfoundland and Labrador 2021), coyotes are hosts of *Trichinella* spp. elsewhere in Canada (Appleyard and Gajadhar 2000). Intestinal infections with adult nematodes are short-lived (a few weeks), but encysted muscle larvae remain potentially viable throughout the life of the host (Gottstein et al. 2009), which allows for the possible introduction of *Trichinella* spp. to new areas with coyote movements. In addition, several sylvatic *Trichinella* spp. in Canada are freeze-resistant (*Trichinella nativa*, *Trichinella* T6, and *Trichinella chanchalensis*), enabling the larvae to remain viable longer and increasing the chance of being scavenged from carrion (Pozio 2016; Sharma et al. 2020).

Echinococcus spp. are zoonotic tapeworms that are transmitted to intermediate hosts via consumption of food, water, or soil contaminated with eggs from the feces of primarily canid definitive hosts (Deplazes et al. 2017). In intermediate hosts, they form hydatid cysts (cystic or alveolar echinococcosis) in internal organs, which are infective to the definitive hosts when preyed on or scavenged (Deplazes

et al. 2017). *Echinococcus granulosus* and *Echinococcus multilocularis* are food-borne parasites with global importance in wildlife, livestock, and humans. Coyotes serve as definitive hosts for *Echinococcus canadensis* (G8 and G10 cervid genotypes of the *E. granulosus* species complex) and *E. multilocularis* in Canada, the latter usually found at high prevalence in coyotes and in increasingly more locations (Kolapo 2023). The life cycle of *E. canadensis* involves cervid intermediate hosts, such as moose (*Alces alces*), elk (*Cervus canadensis*), caribou (*Rangifer tarandus*), or deer (*Odocoileus* spp.), while *E. multilocularis* uses a range of rodent intermediate hosts such as arvicoline rodents (lemmings and voles) and deer mice (*Peromyscus maniculatus*) (Deplazes et al. 2017). Within Canada, insular Newfoundland, Nova Scotia, New Brunswick, and Prince Edward Island have previously been considered free of *E. canadensis* due to the absence of wolves (*Canis lupus*) as definitive hosts (Schurer et al. 2013), but over the last decade there have been reports and genetic evidence of Labrador wolves (*Canis lupus labradorius*) in insular Newfoundland (Callahan, unpublished data). Recently *E. canadensis* was documented in moose from Nova Scotia (Priest et al. 2021) and a hydatid cyst, the form seen in intermediate hosts, was found in the lung of a moose harvested in 2018 from Gros Morne National Park, insular Newfoundland (Pollock, provincial veterinarian, pers. comm.). Our study aimed to determine if these parasites were present in coyotes from insular Newfoundland.

As part of a carcass collection program implemented by the Government of Newfoundland and Labrador, Canada, to monitor canid health on the island of Newfoundland, carcasses were solicited from hunters and trappers, legally harvested for purposes other than research, and therefore exempt from university animal ethics approval. Samples were collected from 153 coyotes: 153 diaphragms, 151 tongues, and 35 fecal samples. Samples were frozen promptly after collection and were kept frozen until analysis.

Trichinella spp. larvae were recovered from previously frozen muscle via the double separatory digestion method (Forbes and Gajadhar 1999). Diaphragms were halved and digested in pools of four or five (total of 36 pools), with no more than 20 g of tissue from each individual coyote. The remaining half of each diaphragm in positive pools was then digested individually. For one positive pool, diaphragm tissue was depleted, and 1.36-8.21 g of tongue was used for the individual digests. Larvae were counted using 12–16× magnification on a dissecting microscope.

The DNA was extracted using the Promega DNA IQ kit (Promega, Madison, Wisconsin USA) from five individual larvae and one pool of 10 when possible (one pool had only four larvae). Larvae were identified to species or genotype (Pozio and Zarlenga 2019) based on the unique electrophoretic DNA patterns on multiplex PCR using primers targeting the ITS1, ITS2, and ESV (127–404 bp) regions. A 30 µL reaction mixture contained 15 µL of 2X GoTaq PCR Master Mix (Promega), 2 µL of primer mix, 8 µL of nuclease-free water, and 5 µL of genomic DNA. The thermocycling protocol was the same as Pozio and Zarlenga (2019) with the exception of optimizing annealing temperature of 58 C rather than 55 C.

DNA was extracted from three single larvae from each positive coyote and assayed using primers for the mitochondrial cytochrome b (Cytb) gene (approximately 914 base pairs) (Sharma et al. 2020). The DNA was amplified by conventional PCR with the following conditions: initial denaturation at 98 C for 3 min, followed by 35 cycles of 98 C, 20 s; 50 C, 30 s; 68 C 90 s, with a final extension of 68 C for 5 min. The PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sent for Sanger sequencing to the National Research Council (NRC) in Saskatoon, Saskatchewan, Canada, to confirm multiplex PCR results and to differentiate *T. nativa* from *T. chanchalensis* (Sharma et al. 2020).

Larvae of *Trichinella* spp. were detected in 6/36 pools. When remaining samples were digested individually, only one coyote from each of five pools was positive, and individuals from the remaining pool (which contained only one larva) were negative. Therefore, 6/153 (3.9%) coyotes were positive. The DNA was extracted from larvae from each of the five coyotes, but larvae

from one coyote failed to amplify on multiplex PCR. *Trichinella nativa* was identified in all cases, based on the banding pattern and Sanger sequencing (Pozio and Zarlenga 2019).

DNA was extracted following the protocol by Kolapo, 2023, from feces of 35 coyotes and assayed with quantitative PCR for *Echinococcus* spp. (Kolapo 2023). Melting curve analysis began immediately following the last extension step and temperature increased from 65 C to 95 C by 0.2 C increments with a 5 s hold at each step (Kolapo 2023). To confirm identification as *E. canadensis* and to differentiate between G8 and G10, DNA extracted from positive samples were assayed with primers targeting the ~470 bp ND1 region and a ~446 bp region of COX1 mitochondrial genes on conventional PCR (Bowles et al. 1992; Bowles and McManus 1993). We resolved the PCR products on 1.5% agarose gel electrophoresis, purified them with QIAquick 124 PCR Purification Kit (Qiagen Inc., Valencia, CA), and sent the products to NRC for Sanger sequencing.

We detected DNA of *Echinococcus canadensis*, but not *E. multilocularis*, in 2/35 (5.7%) coyotes. One sample was successfully genotyped as G10, and the COX1 sequences were most similar to *E. canadensis* from a cyst found in an Alaskan reindeer (accession number LC184606.1). One animal was positive for both *Trichinella* sp. and *E. canadensis* (Fig. 1). Using the scraping, filtration, and counting technique (Gesy et al. 2013), attempts to recover *Echinococcus* spp. from the small intestines of two coyotes positive by fecal analysis were unsuccessful. This may be due to low infection intensity, multiple freeze-thaw cycles that may damage adult cestodes and make them difficult to identify microscopically, or the method not being optimized for *E. canadensis* (Karamon et al. 2010).

The introduction to and establishment of parasites in new and isolated geographic regions are dependent upon three primary factors: 1) ease and frequency of movement of hosts between enzootic and nonenzootic regions, 2) presence and density of suitable hosts, and 3) suitable climate and microhabitat for parasite survival and transmission (Priest et al. 2021; Santa et al. 2021). In 2012, Labrador wolves were documented on insular Newfoundland for the first time since their extirpation in the 1930s (Callahan, pers. comm.). The current population of wolves on the island of Newfoundland is unknown, but it is possible that wolves and domestic dogs, as well as coyotes, may play a role in the introduction and maintenance of *E. canadensis* on the island.

Trichinella spp. can infect many terrestrial carnivores and omnivores including, but not limited to, red fox (*Vulpes vulpes*), American marten (*Martes americana*), coyote (*Canis latrans*), lynx (*Lynx canadensis*), and black bear (*Ursus americanus*), all of which are present on Newfoundland Island (Snow 1996). Transmission is facilitated by muscle-dwelling larvae of *T. nativa* that can survive freezing within a carcass in the environment for years, thus increasing the chance of transmission via scavenging (Pozio 2016). Similarly, the eggs of *Echinococcus* spp. are freeze tolerant and can survive for months to years in the environment (Eckert and Deplazes 2004). Insular Newfoundland, like most of Canada, has climactic conditions permissive for *T. nativa* and *E. canadensis* to persist in the environment but was lacking an appropriate predator-prey cycle to establish endemic transmission in local fauna until the arrival of coyotes. While we did not detect *E. multilocularis*, coyotes are increasingly recognized as reservoirs of this important zoonotic parasite in mainland Canada, and this parasite was recently detected for the first time on Prince Edward Island, Canada (Kotwa et al. 2019; Robbins et al. 2022; Kolapo 2023).

Echinococcus canadensis is a public health concern, and provincial authorities may wish to advise hunters and trappers to avoid contact with fecal material when handling and skinning coyote carcasses, traps, and contaminated soil. Drinking unfiltered surface water and eating unwashed produce are also potential risk factors for human exposure to *Echinococcus* spp. (Deplazes et al. 2017). Coyote-human and coyote-domestic animal interactions may pose an increased risk of *Echinococcus* spp. transmission by exposure to shared contaminated environments (Aguirre 2009; Priest et al. 2021). Dog owners in Newfoundland should be aware of this risk and avoid feeding uncooked meat or organs from hunted cervids to their dogs, as well as consult their veterinarian regarding regular deworming of dogs to protect human health. Hydatid cysts in the

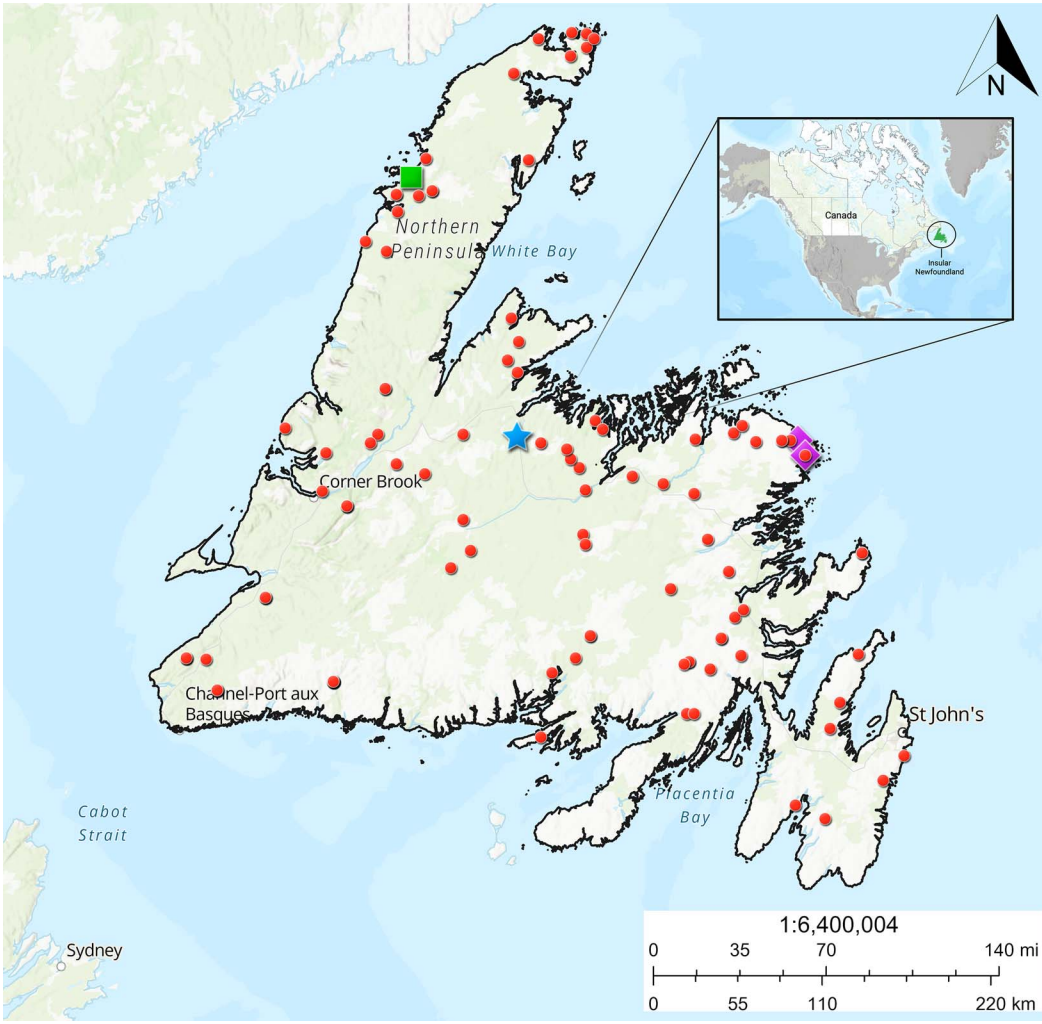

FIGURE 1. Map of the island of Newfoundland, on the east coast of Canada, showing the harvest locations of coyote (*Canis latrans*) carcasses collected as part of a program implemented by the Government of Newfoundland and Labrador, Canada, for canid health monitoring on the island. Square = positive for *Echinococcus canadensis*; diamond = positive for *Trichinella nativa*; star = positive for *E. canadensis* and *T. nativa*; circle = negative for both parasites.

lungs or livers of moose and caribou pose no food safety risk to people, but if consumed by dogs or wild canids they can develop into adult tapeworms and pass eggs in feces that are immediately infective to people (Priest et al. 2021).

The presence of *T. nativa* and *E. canadensis* on the island of Newfoundland poses potential veterinary and public health risks that may not have been previously considered. Coyotes pose a direct risk of transmission of *E. canadensis* to humans. Although *T. nativa* in coyotes does not generally pose a direct public health risk because coyotes are not frequently consumed by people, meat and organs from hunted wild carnivores and omnivores should be cooked to an internal temperature of at least 71 C (160 F) before consumption, as freezing will not inactivate larvae of *T. nativa* (Pozio 2016). Further studies should investigate the transmission cycle and terrestrial wildlife reservoirs of *Trichinella* spp. and *Echinococcus* spp. on the island to better determine regional risks of these parasites for people, dogs, and endemic wildlife.

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