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# *Trichinella spiralis* in Sylvatic Hosts from Prince Edward Island

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ABSTRACT: Larvae of *Trichinella* sp. were found in two of 208 red foxes (*Vulpes vulpes*) and one of 125 coyotes (*Canis latrans*) obtained from trappers from Prince Edward Island (Canada) in 1995 and 1996. A polymerase chain reaction based DNA biotyping method revealed the larvae to be isolates of *Trichinella spiralis*. This is the first verified identification of *T. spiralis* in sylvatic hosts from Canada.

*Key words:* *Canis latrans*, coyote, red fox, survey, trichinellosis, *Trichinella spiralis*, *Vulpes vulpes*.

*Trichinella* spp. are common nematode parasites found worldwide; across North America, the sylvatic cycle is maintained by many species of wild carnivores and omnivores (Smith and Snowdon, 1988). In the provinces of Atlantic Canada, *Trichinella* spp. have been reported in black bears (*Urus americanus*) from Nova Scotia (NS), New Brunswick (NB), Newfoundland (NFLD) and Prince Edward Island (PEI), as well as in wolves (*Canis lupus*) from NFLD (Smith et al., 1976; Smith, 1978; Butler and Khan 1992; Duffy et al., 1994).

In addition to cannibalism, and garbage feeding, sources of infection for swine (*Sus scrofa*) include rodents such as rats (*Rattus* spp.) and other omnivorous mammals (Murrell et al., 1987). *Trichinella* sp. has been isolated from rats in NB, NS, and PEI (Frank, 1951). In Canada, all reported cases in humans diagnosed since 1983 have been caused by eating meat from animals other than domestic swine (Gajadhar et al., 1997).

Around the world eight gene pools of *Trichinella* spp. have been identified from which five species (*T. spiralis, T. nativa, T. britovi, T. pseudospiralis, T. nelsoni*) have been named (Pozio et al., 1992a). Both *T. spiralis* and the sylvatic arctic isolate, *T. nativa*, can infect a variety of wild and domestic animal hosts (Smith, 1985). It is unclear to what extent *Trichinella* spp. gene pools overlap in host range and geographical distribution in Canada. This survey was conducted to assess the prevalence and identify the biotype of *Trichinella* sp. in wild red foxes (*Vulpes vulpes*) and coyotes (*Canis latrans*) from PEI.

Coyotes have only recently become established on PEI, presumably after migration from the mainland. The first report of one caught by a trapper was in 1983 (R. Diblee, pers. comm.). The population has been estimated at 700 to 800 animals (R. Diblee, pers. commun.). The expansion of the coyote population may be to the detriment of red foxes since the number of red foxes harvested by fur trappers has declined from 1,347 animals in 1994 to 995 animals in 1996 (R. Diblee, pers. commun.).

Carcasses of red foxes (n = 208) and coyotes (n = 126) were obtained from trappers across PEI (46°15′N to 63°08′W) during 1995 and 1996. The tongues were removed and stored frozen at approximately −20 C until tested. Thirty g of tissue from each tongue was digested in IL of a digestion solution of 1% HCl and 1% pepsin (American Laboratories Inc, Omaha, Nebraska, USA) in a shaking waterbath at 40 C and 190 rpm for 2 hr. Undigested material was removed by filtration (180 μm). Particulate matter in the filtrate was concentrated by several sediment-decant cycles. The final 50 ml of sediment was examined under a stereo-microscope for the presence of *Trichinella* sp. larvae. Any larvae found were collected and stored in water at −20 C.

In preparation for polymerase chain reaction (PCR) biotyping, crude deoxyribonucleic acid (DNA) from approximately 10

washed *Trichinella* sp. larvae were placed in 20 μl of distilled water, heated to 100 C for 12 min and then centrifuged (3,000 × G, 30s). Amplifications with primers TR1 (GCGATTGAGTTGAACGC) and TR2 (CGACATGAATTGTAAGAC) were performed using a 94 C for 1 min denaturation step, 55 C, 1 min annealing step and a 72 C for 1.5 min reaction step, a modification from that previously described (Zarlenga and Dame, 1992). Forty cycles of this program (480 Thermo-Cycler, Perkin-Elmer Cetus, Norwalk, Connecticut, USA) were run with 2 units of Taq polymerase enzyme (Perkin-Elmer Cetus, Norwalk, Connecticut, USA) per sample. Amplified product was electrophoresed in 3.0% Metaphor agarose (FMC Bioproducts, Rockland, Maine, USA) gel with TBE buffer and stained with ethidium bromide.

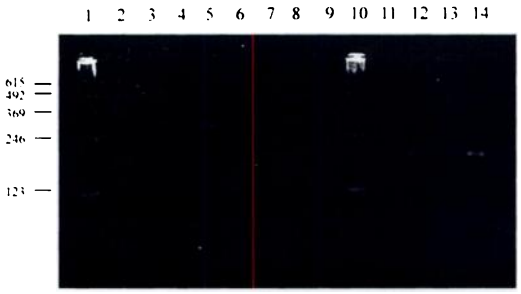


FIGURE 1. Ethidium bromide stained agarose gel of polymerase chain reaction products from reference isolates of *Trichinella* spp. (Lane 2; *T. spiralis*; Lane 3; *T. nativa*, Lane 4; *T. britovi*, Lane 5; *T. pseudospiralis*, Lane 6; *Trichinella* T5, Lane 7; *Trichinella* T6, Lane 8; *T. nelsoni* and Lane 9; *Trichinella* T8) and unknown samples (Lanes 11; red fox, Lane 12; red fox, Lane 13; coyote) using primers TR1/TR2. Lane 14 is a laboratory maintained isolate of *T. spiralis*. Each reaction used nucleic acid extracted from 10 larvae. Lanes 1 and 10 are a 123 base pair molecular weight marker set.

*Trichinella* sp. larvae were collected from the tongues of two of 208 red foxes and one pool of 3 tongue samples of 126 coyotes. Intensity ranged from 0.9 to 1.7 larvae per gram. All larvae were non-motile and in a "comma" shape which is characteristic of dead excysted larvae. Analysis of the PCR-amplified larval genomic DNA showed a single fragment of approximately 175 base pairs which is indicative of *T. spiralis* (Fig. 1). Control DNA samples of reference isolates *T. spiralis* T1 (Reference number ISS248), *T. nativa* T2 (ISS70), *T. britovi* T3 (ISS63), *T. pseudospiralis* T4 (ISS13), *Trichinella* T5 (ISS246), *Trichinella* T6 (ISS334), *T. nelsoni* T7 (ISS29) and *Trichinella* T8 (ISS149) from the World Trichinella Reference Centre, Rome, Italy, generated amplified fragments characteristic of those isolates, thus confirming the identity of our specimens as *T. spiralis* (T1).

*Trichinella* sp. in swine on PEI was last reported in 1975 (Smith et al., 1976). Human cases of trichinellosis have not been reported from PEI since trichinellosis became a reportable human disease in 1971 (Gajadhar et al., 1997). *Trichinella* sp. have been reported in swine (Frank, 1952) as well as in several sylvatic hosts including black bears and wolves in the Canadian Atlantic region (Smith, 1978; Butler and Khan, 1992; Duffy et al., 1994). The technology for biotyping was not available at the time of these findings. Consequently, assumptions that the swine isolates were *T. spiralis* and the wildlife isolates were *T. nativa* are unsubstantiated. We report here the finding of *T. spiralis* in red foxes and a coyote. In the apparent absence of *Trichinella* sp. infections in swine for the last 20 yr, a sylvatic reservoir seems probable.

*Trichinella spiralis* is not freeze-resistant. Therefore it is not usually associated with sylvatic cycles in cold-temperate climate zones (Pozio et al., 1992b). However, *T. spiralis* has been found in human-influenced environments of colder climates, such as farm yards and garbage dumps (Leiby et al., 1988). A sylvatic reservoir for *T. spiralis* in PEI may be associated with human-influenced habitats and hosts such as rats.

Since, sylvatic *Trichinella* spp. has the potential to be transmitted from a wild animal reservoir to pigs (Smith, 1985), identification of *T. spiralis* in foxes and a coyote has important ramifications for animal

health and food safety. These findings emphasize that vigilance must be maintained in surveillance programs for *Trichinella* sp. in swine. Farmers and veterinarians should be kept aware of the risks of raising swine in areas where contact with rats and other wildlife can occur. Hunters and consumers of wild carnivores and omnivores should be made aware of the health risk in consuming raw or undercooked wild game meat.

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