Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Parasitology 132 (2005) 143–146
veterinary parasitology
www.elsevier.com/locate/vetpar

Trichinella nativa in a black bear from Plymouth, New Hampshire

D.E. Hill [a,*], H.R. Gamble [c], D.S. Zarlenga [a,b], C. Coss [a], J. Finnigan [d]

[a] *United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1044, BARC-East, Beltsville, MD 20705, USA*
[b] *Bovine Functions and Genomics Laboratory, Building 1044, BARC-East, Beltsville, MD 20705, USA*
[c] *National Academy of Sciences, Washington, DC, USA*
[d] *The Food Safety Microbiology Unit, New Hampshire Public Health Laboratories, Concord, NH, USA*

Abstract

A suspected case of trichinellosis was identified in a single patient by the New Hampshire Public Health Laboratories in Concord, NH. The patient was thought to have become infected by consumption of muscle larvae (ML) in undercooked meat from a black bear killed in Plymouth, NH in October 2003 and stored frozen at −20 °C fro 4 months. In January 2004, a 600 g sample of the meat was thawed at 4 °C, digested in hydrochloric acid and pepsin, and larvae were collected by sedimentation. Intact, coiled, and motile ML were recovered (366 larvae per gram (lpg) of tissue), which were passed into mice and pigs. Multiplex PCR revealed a single 127 bp amplicon, indicative of *Trichinella nativa*. The Reproductive Capacity Index (RCI) for the *T. nativa*-Plymouth isolate in mice was 24.3. Worm burdens in the diaphragms of two 3-month-old pigs given 2500 ML were 0.05 and 0.2 lpg by 35 days post-inoculation, while 2.2 and 0.75 lpg were recovered from two 3-month-old pigs given 10,000 ML; no larvae were recovered from four 1-year-old pigs given 2500 ML ($n = 2$) or 10,000 ML ($n = 2$). Viable larvae were also recovered from frozen black bear meat harvested at two additional locations, one in southern Ontario, Canada, and one in upstate New York, USA. Multiplex PCR using genomic DNA from these parasite samples demonstrated that both isolates were *T. nativa*. This is the first report of the freeze-resistant species, *T. nativa*, within the continental United States.
Published by Elsevier B.V.

Keywords: *Trichinella nativa*; zoonosis; uncooked meat; *Ursus americanus*

1. Introduction

Sylvatic isolates of the genus *Trichinella* are widespread in the environment due to an expansive host range and worldwide geographic distribution. Though *Trichinella spiralis* is virtually absent from the U.S. pig population (NAHMS, 2000, unpublished), sylvatic isolates pose a risk for zoonotic transmission when wildlife has access to pig barns or non-confined animals. In addition, game animals serve as hosts for *Trichinella* species that can cause human disease if meats are not properly prepared.

Currently, eight sibling species and two genotypes of undetermined taxonomic status have been identified

* Corresponding author. Tel.: +1 301 504 8770; fax: +1 301 504 6273.
E-mail address: dhill@anri.barc.usda.gov (D.E. Hill).

0304-4017/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetpar.2005.05.043

in the genus *Trichinella* (Kapel, 2000; Murrell et al., 2000; La Rosa et al., 2003). Geographic distribution of these isolates has been described (Pozio et al., 1992; Kapel, 1997; Pozio et al., 1998; Pozio, 2001a,b). Two of the sibling species, *Trichinella nativa* and *Trichinella* T6 have been identified as freeze tolerant and are capable of surviving in frozen muscle tissues for extended periods of time at temperatures from −5 to −18 °C (Kapel et al., 1999; Malakauskas and Kapel, 2003). *Trichinella* T6 has been identified in cougar and other carnivores from the Rocky Mountain region of the western continental United States, Pennsylvania, and Ontario, Canada (Dworkin et al., 1996). *T. nativa*, on the other hand, was thought to be confined to the arctic and subarctic zones of the Holarctic region (Murrell et al., 2000) and has not been previously reported within the continental United States or in areas where the January isotherm is higher than −5 °C (Pozio et al., 1998). In this study, we describe an apparent southern geographic extension of the known range of *T. nativa* into the continental United States.

2. Materials and methods

Approximately 1.5 kg of frozen black bear meat was received at the Animal Parasitic Diseases Laboratory, Beltsville, MD, in December 2003. The bear meat had been frozen for at least 6 weeks and was kept frozen at −20 °C until January 2004. Coiled intracellular larvae were noted on compression slides. Pepsin:HCl digestion (Gamble, 1996) of the bear meat revealed live, motile larvae which were washed by centrifugation. Five groups of 5 mice (Swiss-Webster females, Taconic Farms, Germantown, NY) were orally inoculated with 500, 1000, 1500, 2000, and 2500 muscle larvae (ML) each. After 21 days, larvae were recovered from skinned, eviscerated carcasses by pepsin:HCl digestion, enumerated, and photographed using an Olympus BX-2 digital photomicroscope.

The reproductive capacity index (RCI = #ML recovered/#ML inoculated) was determined in two groups of 10 mice inoculated with 50 or 100 ML and digested 35 days later as described above. The RCI index in pigs was determined by inoculating two groups of four pigs (Group 1: 1 year in age; Group 2: 3 months in age) with 2500 or 10,000 larvae. Thirty-five days after inoculation, 100 g samples from tongue, diaphragm, loin, tail (50 g), shoulder, quadriceps, hams, shanks (front), masseter, intercostals, neck, tenderloin, flank, and throat were collected from each pig, weighed, digested, and worm burdens were determined. In addition, serum was collected from each pig at necropsy for ELISA (Gamble et al., 1997).

For multiplex PCR, ML recovered from the naturally infected bear meat, and experimentally infected mice and pigs, were each washed three times in Hanks Balanced Salt Solution, pelleted, and subjected to genomic DNA extraction utilizing a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA). The multiplex PCR was carried out essentially as described by Zarlenga et al. (1999).

During the same approximate time period, viable ML were obtained from two additional frozen black bear meat samples obtained (1) through a custom slaughterhouse in Clinton County, New York, and (2) from an animal shot and field dressed in Timmons, Ont., Canada, prior to transporting the meat to Tennessee. Multiplex PCR was carried out on DNA isolated from each parasite sample as described above.

3. Results

All mice survived the inoculation of 500–2500 larvae recovered from the New Hampshire bear meat. Infection efficiencies determined from mice inoculated with 50 or 100 ML resulted in mean larval worm burdens of 1260 (±735) and 2340 (±1458), respectively, and a mean RCI of 24.3. A single larva only was recovered from throat muscle of one pig in Group 1 (1 year of age) inoculated with 10,000 ML. No other larvae were recovered from Group 1 pigs. Viable ML were recovered from all four pigs in Group 2 (3 months of age) (Table 1). Pigs in Group 2 had a calculated worm burden of 0.05 and 0.2 lpg in animals inoculated with 2500 ML, and 2.2 and 0.75 lpg in animals inoculated with 10,000 ML. All pigs in both groups had high antibody titers to *T. nativa* E/S products in ELISA (Table 2).

Multiplex PCR revealed amplicons of the expected size (127 bp) for *T. nativa* in assays containing DNA from the naturally infected bear and from the experimentally infected pigs (Fig. 1). Multiplex PCR analysis of *Trichinella* isolates from both Clinton

Download English Version:

https://daneshyari.com/en/article/8991033

Download Persian Version:

https://daneshyari.com/article/8991033

Daneshyari.com