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2005

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Gamble, H. Ray; Pozio, E.; Lichtenfels, J. Ralph; Zarlenga, D. S.; and Hill, D. E., "*Trichinella pseudospiralis* from a Wild Pig in Texas" (2005). *Faculty Publications from the Harold W. Manter Laboratory of Parasitology*. 628.

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Trichinella pseudospiralis from a wild pig in Texas

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Abstract

In December 2001, the routine inspection of a wild boar intended for human consumption revealed the presence of *Trichinella* ssp. larvae. Biological, morphological and genetic analyses demonstrated the parasite to be *Trichinella pseudospiralis*. This is the second report of *T. pseudospiralis* in the United States and the first report of the parasite in a food animal species in the U.S.

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Keywords: *Trichinella pseudospiralis*; Wild boar; Meat inspection

1. Introduction

Trichinella pseudospiralis is one of two species of the genus *Trichinella* that do not form a capsule in the host. First reported from Russia (Garkavi, 1972), this species has been found to occur in a wide range of hosts including wild carnivores, carnivorous birds, marsupials, rodents and pigs in Asia, Europe, Australia and North America (Kapel, 2000). Its role in human disease has been reported in New Zealand, Thailand and France (Jongwutiwes et al., 1998; Dupouy-Camet, 2000; Ranque et al., 2000).

The U.S. Department of Agriculture (USDA), Agricultural Marketing Service, administers an

inspection program for *Trichinella* in pork, horsemeat and game meats. Inspectors in this program test tissues from meat intended for export using the pooled sample digestion method. In December 2001, a positive test result was recorded for tissue from a wild boar harvested in the state of Texas. We report here on the identification of this isolate of *Trichinella* as *T. pseudospiralis*.

2. Materials and methods

Diaphragm tissue samples from a wild pure Russian boar (*Sus scrofa*) that had tested positive for *Trichinella* sp. at slaughter were shipped to the USDA's Animal Parasitic Diseases Laboratory in Beltsville, Maryland, USA. The worm burden of infected boar tissue was determined by artificial

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digestion as previously described (Gamble et al., 1999). Recovered larvae were counted and passed into female Swiss Webster mice (100–500 larvae/mouse). Mice were killed at 6 and 26 weeks after inoculation and tissues were examined microscopically by compression of small pieces of diaphragm. Additional tissues were subjected to artificial digestion and recovered larvae were used for morphological and genetic analyses.

Individual male and female muscle larvae, recovered from Swiss Webster mice, were measured as previously described (Gamble et al., 1989). For genetic typing, larvae were tested by a multiplex polymerase chain reaction (PCR) for single larvae, as described by Pozio and La Rosa (2003), using *ExTaq* DNA polymerase from Takara in 50 ml containing 1.5 mM MgCl₂, 200 mM dNTPs, 50 pmol of each primer and 0.5 unit of *ExTaq* DNA polymerase.

For further characterization, two mitochondrial genes [cytochrome oxidase subunit I (CO I) and the large subunit ribosomal-DNA (mt-lsrDNA)] and the expansion segment V (ESV) were studied. Since multiple bands have been observed in some ESV amplification products, individual bands were cloned prior to sequencing. The primer set oTsr1 (5'-CGA AAA CAT ACG ACA ACT GC) and oTsr4 (5'-GTT CCA TGT GAA CAG CAG T) was used to enzymatically amplify the ESV (Zarlenga and Dame, 1992; Zarlenga et al., 1996). Amplifications consisted of 35 cycles, as follows: denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 60 s. The PCR amplified fragments from purified DNA were visualized by low resolution agarose gel electrophoresis (1.5% standard agarose) (IBI). Finer resolution of the polymorphism among non-encapsulated isolates required higher resolution agarose gels (3.0% metaphor agarose) (FMC). The primer set SB5 (forward 5'-GGTATAAGGGAAAGCCGGAA and reverse 5'-CACTCACATTGTAT GCCAAG) was used to amplify a 680 bp repetitive DNA fragment specific for *T. pseudospiralis* according to the published protocol (Wu et al., 1999). A 419 bp region of the CO I gene was amplified by PCR with the primer set L6625 (5'-TTYTGRTTYTTYGGNCAAYCC) and H7005 (5'-ACNACRTARTANGTRTCRTG) (Nagano et al., 1999). A portion (445 bp) of the mt-lsrRNA gene was amplified by PCR using the primer set P753 (5'-WACAATGGTCCTTTCG TACT) and P754 (5'-

TGAGGACATTAAGGTAGC). For the amplification of CO I and mt-lsrRNA genes, PCR was carried out for 35 cycles, each consisting of 30 s at 94 °C, 60 s at 50 °C, and 60 s at 72 °C. Nine *Trichinella* reference strains were used for the comparison: *T. spiralis* (code ISS3), *T. nativa* (code ISS10), *T. britovi* (code ISS2), *T. murrelli* (code ISS35), *T. nelsoni* (code ISS37), *T. papuae* (code ISS572), and, because of the genetic variability of *T. pseudospiralis*, three strains of this species from different zoogeographical regions [one each from the Nearctic region (code ISS470), the Palearctic region (code ISS13) and the Australian region (ISS141)] (Pozio et al., 2001).

3. Results

The infected boar in this study was harvested near Newcastle, Texas (Longitude 98°44'W, Latitude 33°12'N) and was identified as *Trichinella* positive during routine testing using a 1 g sample. Digestion of 23 g of diaphragm tissue from this boar yielded 3300 larvae for a worm burden of 143.5 larvae per gram (LPG) of tissue.

Tissues of infected mice examined 6 and 26 weeks after inoculation showed larvae that were not encapsulated (Fig. 1), indicating they were one of two *Trichinella* species that do not form a capsule in host musculature. Larvae recovered from mice measured 684 ± 29 µm for female worms and 672 ± 29 µm for male worms.

Initial genetic analysis by multiplex PCR analysis indicated the species to be *T. pseudospiralis*. Subsequent analysis of cytochrome oxidase subunit I, the large subunit ribosomal DNA, and expansion segment V genes placed this isolate in the Nearctic population.

The isolate of *T. pseudospiralis* was contributed to the International *Trichinella* Reference Centre as Isolate Code (ISS) 1132.

4. Discussion

In a previous study, we reported *T. pseudospiralis* from a carnivorous bird in Alabama, USA (Lindsay et al., 1995); however, this species has not previously been reported in a food animal species in the United



Fig. 1. *Trichinella pseudospiralis* larvae recovered from a wild boar in Texas, USA. Larvae were passed once in Swiss Webster mice. Tissue is from a mouse diaphragm recovered 26 weeks after inoculation of 100 larvae.

States. This report extends the range of *T. pseudospiralis* in the U.S. and adds to the list of reported host species.

This was the first report of *Trichinella* in wild boar inspected for slaughter since the inception of the testing program in 1996. Other studies conducted in our laboratory have tested additional samples of wild boar from Texas, with negative results for *Trichinella* infection. From July 1997 through June 1998, 226 samples were collected from wild boar harvested by the Texas Department of Parks and Wildlife. Animals were harvested by trapping, ground shooting or aerial

shooting. Testing was performed by digestion of 10–100 g of diaphragm tissue (207 animals) and/or ELISA (187 animals). All results were negative. Animals tested in that study came from Cottle, Dickens, Foard, King, and Motley counties in North central Texas. The positive animal in this study came from Young county, which is very close to the other counties surveyed. The combination of these data suggests that the overall prevalence of *Trichinella* is quite low in wild boar in this region. In contrast, prevalence rates of 2–15% have been reported in feral pigs in New England states (Worley et al., 1994; Gamble, unpublished).

Pigs, horses and feral swine slaughtered for export are tested for *Trichinella* infection by the pooled sample digestion method. This program is administered by the USDA's Agricultural Marketing Service. Currently, five pork facilities, three horse facilities, and one wild game facility are enrolled in the program. Since inception of the program, no positive horses have been identified and there has only been one report of a positive pig, with only 1 larva recovered. This is the first finding of *Trichinella* in feral pigs since the USDA testing program has begun. Further, it is the first report of *T. pseudospiralis* in a food animal species in the U.S. Because *T. pseudospiralis* is a human pathogen (Ranque et al., 2000), care should be taken in inspecting feral pigs and other game meats for this parasite; testing should be performed by artificial digestion and not by compression or trichinoscopy methods which will not detect *T. pseudospiralis*.

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