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Short communication

A *Trichinella murrelli* infection in a domestic dog in the United States

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Abstract

Trichinella murrelli infection was diagnosed in a naturally infected Beagle bitch from VA, USA, where encapsulated larvae were found in histological sections of several skeletal muscles. A laboratory reared dog fed infected muscles resulted in viable muscle larvae that were subsequently infective to Swiss–Webster mice. Multiplex PCR using larvae from the experimentally infected dog demonstrated two distinct bands migrating at 127 bp and 316 bp which together are diagnostic for *T. murrelli*; the isolate was assigned the ISS code: ISS1608 by the International *Trichinella* Reference Centre. This is the first report of *T. murrelli* infection in a companion animal.
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Keywords: *Trichinella murrelli*; Dog; *Canis familiaris*; USA; Natural infection

1. Introduction

Trichinellosis is a serious disease of humans caused by ingestion of undercooked meat harboring parasites of the genus, *Trichinella*. Most species of *Trichinella* have been documented as infectious for humans, though recent reports point to *T. spiralis*, *T. britovi*, and the freeze resistant *T. nativa* found in the higher latitude Holarctic, as the most common etiological agents of human trichinellosis (Pozio and Zarlenga, 2005). Even the non-encapsulated species *Trichinella pseudospiralis*, once believed to circulate only among avian hosts, is now well documented as a human pathogen (Pozio and Zarlenga, 2005). Although the number of reported cases of trichinellosis in the United States that result from pork and pork products has been low in recent years, there has been a resurgence of trichinellosis caused by ingestion of game meats.

Trichinella murrelli is a recently described species (Pozio and La Rosa, 2000) found exclusively in the Nearctic and considered to be the predominant species circulating among sylvatic hosts in temperate North America (Zarlenga et al., 1991; Pozio and La Rosa, 2000). There was one report of an outbreak of trichinellosis in France resulting from the consumption of horsemeat where 2 of 325 infected individuals

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died (Ancelle et al., 1988). It was later concluded that the infection did not originate from France, but from a horse carcass imported from Connecticut in the United States. Although parasites were never recovered from the original source, a human biopsy performed on a chronically ill patient nearly 6 years after the initial outbreak determined that the etiological agent was indeed *T. murrelli* based upon genetic analysis (Dupouy-Camet et al., 2001). Viable *T. murrelli* has been isolated from black bears, raccoons, red foxes, bob cats and coyotes (Pence et al., 2001; Pozio et al., 2001; Pozio and La Rosa, 2000) though there is no apparent limitation to the host range except for avian species and marine animals.

To our knowledge, there is no record of a natural infection of *T. murrelli* in a companion animal. In the present report we document such an infection in an adult Beagle dog and demonstrate its experimental transmission to dogs and mice.

2. Material and methods

2.1. Naturally infected dog

During the course of an investigation of neosporosis in dogs, a 2-year-old abandoned Beagle bitch was found in Case City, VA 23924 (latitude: 36.7715 and longitude: 78.4254), in November 2004. The dog gave birth to five pups on 15 January 2005 (Table 1). Blood samples were obtained from the five pups and the bitch on 8 May 2005 for serologic diagnosis of neosporosis. The present owner decided to donate the bitch and one of the worst affected pups (BB, male) for research. The pup and the bitch were euthanized on 1 June and 8 June 2005, respectively.

Table 1
Antibodies to *Trichinella* sp. in sera of the naturally infected bitch and her pups

Dog I.D.	ELISA OD values[a] (18 May 2005)
Pup 1 (Fella)	**0.464**
Pup 2 (BB)[b]	0.211
Pup 3 (Rascal)	0.286
Pup 4 (Biscuit)	0.261
Pup 5 (Missy)	**0.328**
Bitch (Sally)[c]	**0.762**, **0.740**[c]

[a] Positive cut-off = 0.3; positive control 3.3; negative control 0.049. Figures in bold are interpreted as positive values.
[b] Euthanized 1 June 2005.
[c] Euthanized 8 June 2005.

2.2. Experimental infection in a dog with T. murrelli

A complete necropsy examination was performed on the pup BB and the bitch. Tissues were collected for histology and for bioassay (see later sections). Initially, muscles from the body and brain of the bitch were fed to a laboratory-raised, 4-month-old Hound dog (dog no. FD; Covance Research Products, Cumberland, VA) over a period of 2 days in order to obtain *Neospora* oocysts. Dog no. FD was euthanized in good health 104 days post-inoculation (p.i.). Blood samples were obtained 1 day before feeding canine tissues, 61 days p.i., and on the day of euthanasia (day 104 p.i.).

2.3. Necropsy examination

At necropsy, specimens of brain, spinal cord, eyes, heart, lung, liver, spleen, kidneys, and tongue were obtained in addition to muscles from limbs, ribs, massetter, and loin from all three dogs. Samples of all tissues were fixed in 10% buffered formalin, paraffin-embedded, stained with hematoxylin and eosin (H and E), and examined microscopically. In addition, unfixed muscle squashes from dog no. FD were examined between coverslips and slides for *Neospora* tissue cysts.

2.4. Examination for Trichinella spp.

Upon microscopic examination of muscle tissues from dog no. FD, encapsulated nematode muscle larvae (ML) were found, recovered from muscle tissue, and processed for genotyping. Serum samples from the bitch, all five pups, and dog no. FD were examined by ELISA for the presence of antibody to *Trichinella* with a commercial kit using *T. spiralis* excretory/secretory antigen (Safepath Inc., Carlsbad, CA) following the manufacturers instructions. For the ELISA, dog serum from each of the seven dogs (bitch, five pups, and dog FD) was diluted 1:100 in antibody dilution buffer supplied by the manufacturer before being added to duplicate wells of the antigen coated plate. Anti-dog IgG peroxidase conjugated antibody, diluted 1:800, was used as the second step antibody in

the assay. After adding substrate, plates were read at 450/650 nm using a Vmax ELISA reader (Molecular Devices). Negative dog control sera were included on the plate. A positive cut-off was established as five times the mean + standard deviation of the mean of a set of five *Trichinella* negative dog serum samples.

2.5. Bioassay for Trichinella

Samples of dog no. FD leg muscles weighing 100 g were placed in a small amount of tap water and chopped in a blender. The chopped muscle was combined with 1 L of digestion fluid (1% pepsin and 1% HCl), stirred vigorously for 30 m at 46–48 °C, then filtered through a 200 μm mesh sieve. Larvae were collected from the filtrate by sedimentation and 200 ML were used to inoculate each of five Swiss–Webster mice (Taconic Farms, Germantown, NY). Isolated ML were used also for nucleic acid extraction to perform multiplex PCR as described below. At day 40 p.i., mice were euthanized and the diaphragms examined for the presence of ML. Mouse carcasses containing ML were shipped to the International *Trichinella* Reference Center where the isolate was assigned the ISS code: ISS1608 (www.iss.it/site/trichinella/).

2.6. Genetic characterization of Trichinella

Trichinella larvae were isolated from the leg muscles of dog no. FD as described above. Recovered parasites were extensively washed in purified water then digested with proteinase K/SDS followed by organic extraction (Dame et al., 1987) to purify total nucleic acids. Samples containing DNA were subjected to multiplex PCR as described (Zarlenga et al., 1999). Amplified products were separated on a 2% NuSieve agarose gel which was subsequently stained with ethidium bromide and photographed.

3. Results and discussion

Encapsulated ML were found in histologic sections of tongue (Fig. 1) and bicep of the naturally infected bitch and in the abdominal rectus and bicep of the experimentally infected dog no. FD (Fig. 2). In addition, encapsulated ML were found in unstained

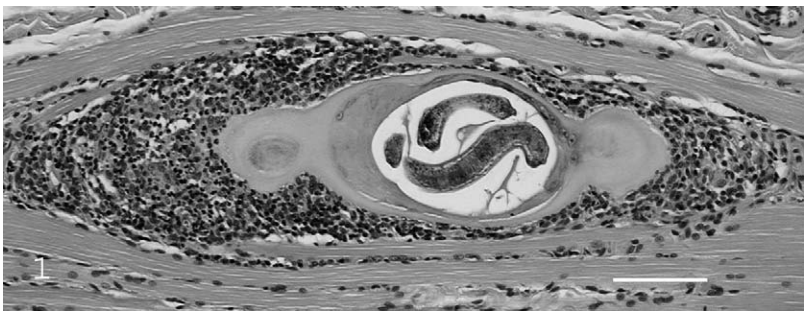

Fig. 1. A *T. murrelli* ML in tongue tissue from the naturally infected bitch surrounded by inflammatory cells. H and E. Bar = 100 μm.

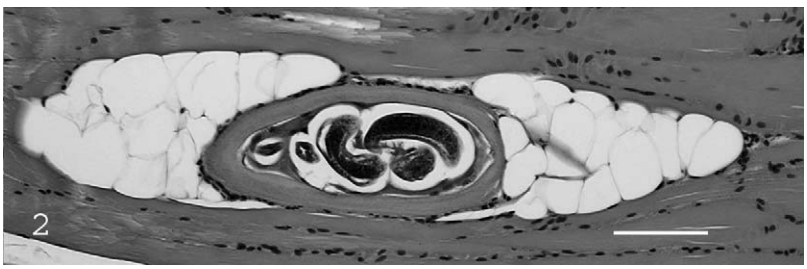

Fig. 2. An encapsulated *T. murrelli* ML in sections of biceps femoris from the experimentally infected dog FD without host reaction. H and E. Bar = 100 μm.

muscle squashes. The ML in the bitch were surrounded by several layers of mononuclear cells whereas larvae in dog no. FD had no inflammatory cell infiltration (Figs. 1 and 2). The inflammatory host reaction in the bitch and the absence of any reaction in the experimentally infected dog no. FD may be related to the duration of infection which was unknown in the bitch, but 104 days in the experimentally infected dog. Also little is known of the inflammatory responses induced by *T. murrelli* and the type and duration of canine immunity to *Trichinella* species other than *T. spiralis*. The lack of an inflammatory response in dog no. FD is likely related to the duration of infection.

Multiplex PCR data clearly demonstrated two distinct bands migrating at 127 bp and 316 bp (data not shown) which together are diagnostic for *T. murrelli*. The possibility of a mixed infection of *T. spiralis* (127 bp) and *T. murrelli* (127 bp and 316 bp) cannot be ruled out because individual larvae were not tested. Mixed infections are known to occur in nature but at relatively low rates (Pozio et al., 1995).

Antibodies to *Trichinella* were found in the sera of the bitch and two of her pups (Table 1). It is unlikely that the pups had maternal antibodies because they were 113 days old. However, the persistence of maternal antibodies in infected offspring of animals is not well studied (Marti and Murrell, 1989).The pup BB that was euthanized had ELISA values below the cut-off values and *Trichinella* was not found in histological sections of numerous muscles. The bitch was a mongrel and may have consumed tissues from any of the wild reservoirs of *T. murrelli*. The owner had fed only cooked or dry dog food to the bitch and the pups. Whether the two pups with *Trichinella* antibodies had persistent infection or only milk acquired antibodies could not be determined. The difference in the inflammatory response surrounding the nurse cell between naturally infected animals and that observed in experimentally infected animals would suggest that the bitch was infected prior to being found by the most recent owner, though contracting the parasite from infected meat fed by that owner has not been ruled out.

This finding raises the question of exposure of hunting dogs to *Trichinella*, especially dogs used for hunting predators such as bears and raccoons, since in North America, these animals can harbor *T. murrelli*, *T spiralis*, *T. nativa*, or *Trichinella* T6. Though not unexpected, these data also expands the host range for *T. murrelli* to include companion animals. *Trichinella* infections in dogs have been documented from numerous countries and there is a report of trichinellosis in humans linked to ingestion of infected dog meat (Berumen-de-la-Torre et al., 2002; Cui and Wang, 2001; Frydas et al., 1995; Mikhail et al., 1994; Oivanen et al., 2005; Ozeretskovskaya et al., 2005). However, little is known of the prevalence of *Trichinella* infections in dogs in the United States, nor its course of infection or predilection sites within the host. Thus, stray dogs, though not a likely source of human infection in North America, can nonetheless act as reservoirs for transmission given their increasing numbers and scavenging characteristics.

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