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Molecular identification of nematode larvae different from those of the *Trichinella* genus detected by muscle digestion

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ABSTRACT

Although larvae of the genus *Trichinella* are the most common parasite species detected in vertebrate muscles using artificial digestion, nematode larvae belonging to other genera are sometimes detected and incorrectly identified as *Trichinella*. However, it is often very difficult to identify these larvae at the species, genus or family level using microscopy because of the absence of specific morphological characters or cuticle damage, and the only means of identification is PCR and sequencing of specific molecular markers (12S mtDNA; COI; 18S rDNA; and ITS1). From 2008 to 2011, 18 nematode isolates not belonging to the genus *Trichinella* were collected from different host species. Eleven of these isolates were successfully identified at the species, genus or superfamily level: larvae from two common kestrels, three hooded crows, a hen harrier and a domestic pig were identified as *Toxocara cati*; larvae from a badger were identified as *Toxocara canis*; larvae from a domestic pig were identified as a free-living nematode of the genus *Panagrolaimus*; larvae from a wild boar were identified as belonging to the *Metastrongylus* genus; and larvae from a rough-legged buzzard were identified as belonging to the superfamily Filarioidea. The recovery of nematodes belonging to genera other than *Trichinella* during routine meat inspection suggests that the persons performing the analyses need to be informed of the possibility of false positives and that a molecular-based identification system that allows for a rapid and reliable response must be adopted (i.e., a DNA barcoding-like system).

1. Introduction

Eight species and four genotypes have been recognized in the genus *Trichinella*, yet these worms cannot be identified at the species or genotype level based on morphological characters (Pozio et al., 2009). Only two clades can be easily identified based on the morphology of the muscle cell-larva complex, in particular, the presence of a collagen capsule ("encapsulated") or the lack of one ("non-encapsulated"). The gold standard method for detecting *Trichinella* sp. larvae in muscle tissues of animals and humans is artificial digestion. However, the detection of nematode larvae outside of their natural niche (i.e., the muscle cell) can lead to an incorrect diagnosis of *Trichinella* infection during routine inspections. In fact, larvae of nematodes living in or migrating to different niches (e.g., gut lumen, liver, lungs, lymphatic or blood vessels) can contaminate muscle tissues and be mistakenly identified as belonging to the genus *Trichinella*. Often the size and shape of these larvae are sufficiently different from *Trichinella* larva, allowing misidentification to be avoided; however, in other cases, a thorough knowledge of the morphology of *Trichinella* larva is needed to make the distinction.

It is well known that the morphological identification of nematode larvae at the species, genus or family level may be difficult or impossible, given the lack of specific morphological characters, which are quite often present only at the adult stage. The objective of the present work was to

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Table 1
Molecular identification of nematode larvae different from those of the genus *Trichinella*, detected in muscle tissues of birds and mammals by artificial digestion.

Host species	No. of animals	Country of origin	Marker	Identification
Common kestrel (*Falco tinnunculus*)	2	Italy	18S	*Toxocara cati*
Hen harrier (*Circus cyaneus*)	1	Italy	18S, 12S	*Toxocara cati*
Hooded crow (*Corvus cornix*)	3	Italy	18S, ITS1	*Toxocara cati*
Rough-legged buzzard (*Buteo lagopus*)	1	Latvia	18S, ITS1	Filarioidea
Badger (*Meles meles*)	1	Italy	18S, 12S	*Toxocara canis*
Domestic pig	1	Italy	18S	*Toxocara cati*
Domestic pig	1	Italy	18S, 12S	*Panagrolaimus* sp.
Wild boar (*Sus scrofa*)	1	Italy	18S, COI	*Metastrongylus* sp.

identify at the species, genus or family level nematode larvae isolated after the artificial digestion of muscle tissues from different *Trichinella* hosts, using short DNA sequences similar to taxon "barcodes" (Hebert et al., 2003; Blaxter, 2004).

2. Materials and methods

From 2008 to 2011, 18 nematode isolates not belonging to the genus *Trichinella* were sent to our laboratory for identification. Four different molecular markers with

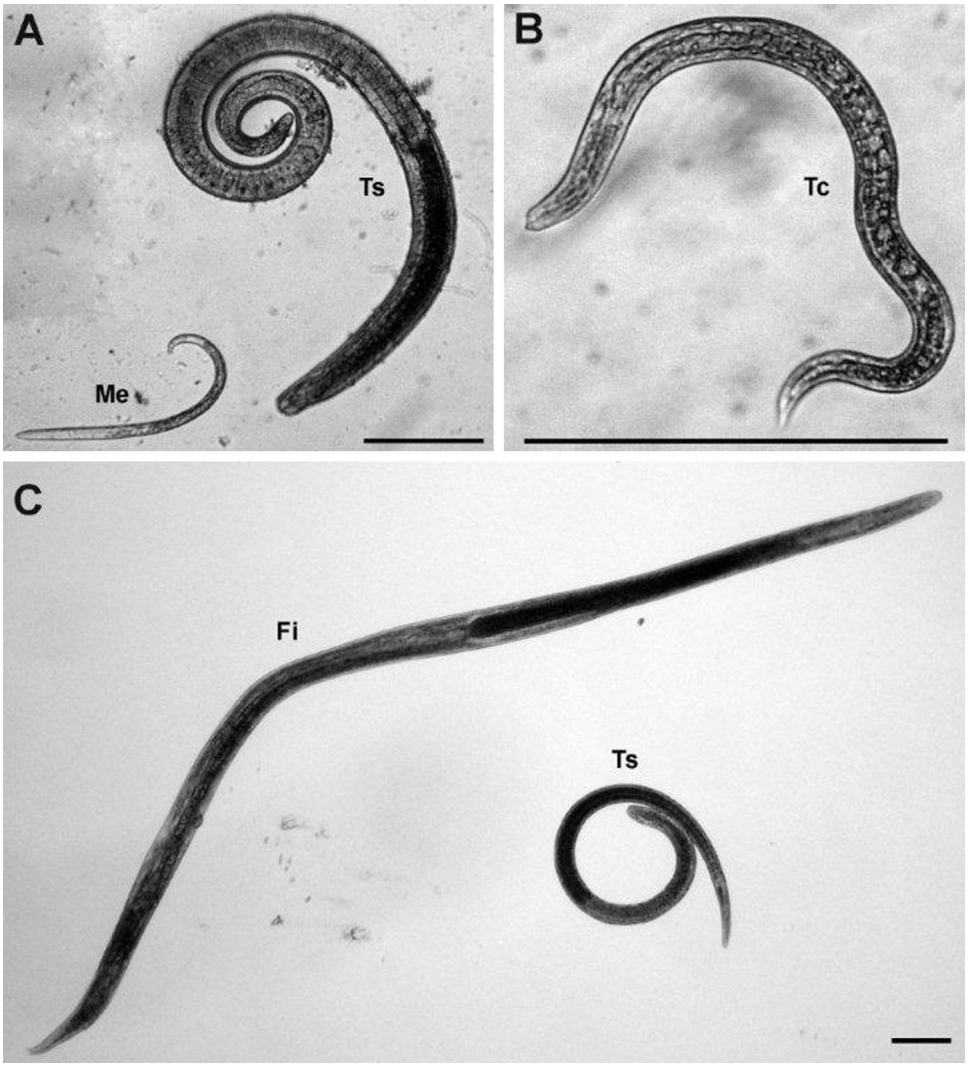

Fig. 1. Nematode larvae detected in muscle samples of mammals and of one bird by artificial digestion. Panel A, larva of the genus *Metastrongylus* (Me) from a wild boar (*Sus scrofa*); panel B, larva of *Toxocara cati* (Tc) from a domestic pig; panel C, larva of the Filarioidea superfamily (Fi) from a rough-legged buzzard (*Buteo lagopus*). Ts, *Trichinella spiralis* reference larva. Scale bars 100 μm.