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Real-time PCR assay for detection of *Trichinella* in meat

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ABSTRACT

Trichinella are a group of widely distributed parasites which have acquired high social relevance due to their involvement in foodborne infections caused by consumption of raw or undercooked food. A TaqMan®-LNA probe Real-time PCR assay targeting the 5S rRNA was developed allowing the simultaneous detection of the 10 species and 3 genotypes of *Trichinella* present in meat tissues. The detection limit employing dilutions of genomic DNA was 2 pg and the determination of the detection limt in terms of ppm was 1 ppm.

The main novelty of this work lies in the fact that it can assure the absence of 10 species and 3 genotypes of *Trichinella* in an only assay. The proposed methodology is rapid, robust, highly sensitive and readily adaptable in routine molecular diagnostic laboratories, and can be employed as molecular screening method in order to assess the food security.

1. Introduction

The trichinellosis is a parasitic disease extended around the world; it is originated for infection with the *Trichinella* genus. The infection is acquired by eating raw or underdcooked meat which is infested with the parasite. The pig meat is considered the first cause of infection to human; however the trichillellosis outbreaks have been also associated with eating other animals like horse, wild board, and deer (Dupouy-Camet, 2000; Kapel, 2000; Kociecka, 2000).

This disease has a first stage characterized by gastro-intestinal alterations as abdominal pain, sickness and diarrhea, that will appear few days after eating the infested meat. Once that the blood and lymphatic systems are invaded with recent born larvae, it is started the systemic phase; this phase is characterized by fever, muscular pain generalist, skin allergenic reactions, hives and SNC, cardiovascular and respiratory alterations (Dupouy-Camet, 2000).

There are several *Trichinella* species that cause this disease in human: *T. spiralis, T. nativa, T. nelsoni, T. britovi, T. pseudospiralis, T. murrelli*, and *T. papuae*. Just as *T. spiralis* is the more frequent and the largest world distribution species, the other species are linked to human populations of limited areas of geographic distribution (Pozio, 2001) (Fig. 1).

Following the European Union (EU) regulations, CE no 2075/2005 the detection of *Trichinella* in meats for human consumes is made by artificial digestion methods in pepsin acid solution or in exceptional cases by trichinoscopic methodology (Kapel, 2005). However, these methodologies are sometimes few efficient, because there have low sensitively. In EEUU, the traditional approach for trichinae control is strict control of processed products to inactivate trichinae and warnings to consumers of the need to cook fresh pork. This approach no longer seems appropriate since trichinae are almost non-existent in EEUU pork. This can be possible for the application of a pilot program since 1997; the voluntary U.S. Trichinae Certification Program (USTCP) became an official USDA program in October 2008, with publication of regulations in the Code of Federal Regulations. For export to the EU, packers must test carcasses using the same methods employed by European meat inspectors (Gamble & Patrascu, 1996; Pyburn, Gamble, Wagstrom, Anderson, & Miller, 2005).

Direct detection methods in combination with more sensitive methods might provide a better diagnostic, more accurate for trichinellosis detection. Furthermore, it would allow the creation of adequate control systems for this important zoonotic disease.

One alternative methodology for diagnostic of trichinellosis is the use of serological test, such as enzymatic immunoassay, ELISA, which has great accuracy detecting of infected animals besides its high sensitivity and its easy handle. However, it was detected numerous cross reactions with antigens of other nematodes, causing false–positive results (Gamble, 1996; Gamble et al., 2004; Gamble, Wisnewski, & Wasson, 1997).

The molecular diagnostic methodologies are an effective tool in parasitic detection, the parasite DNA amplification through

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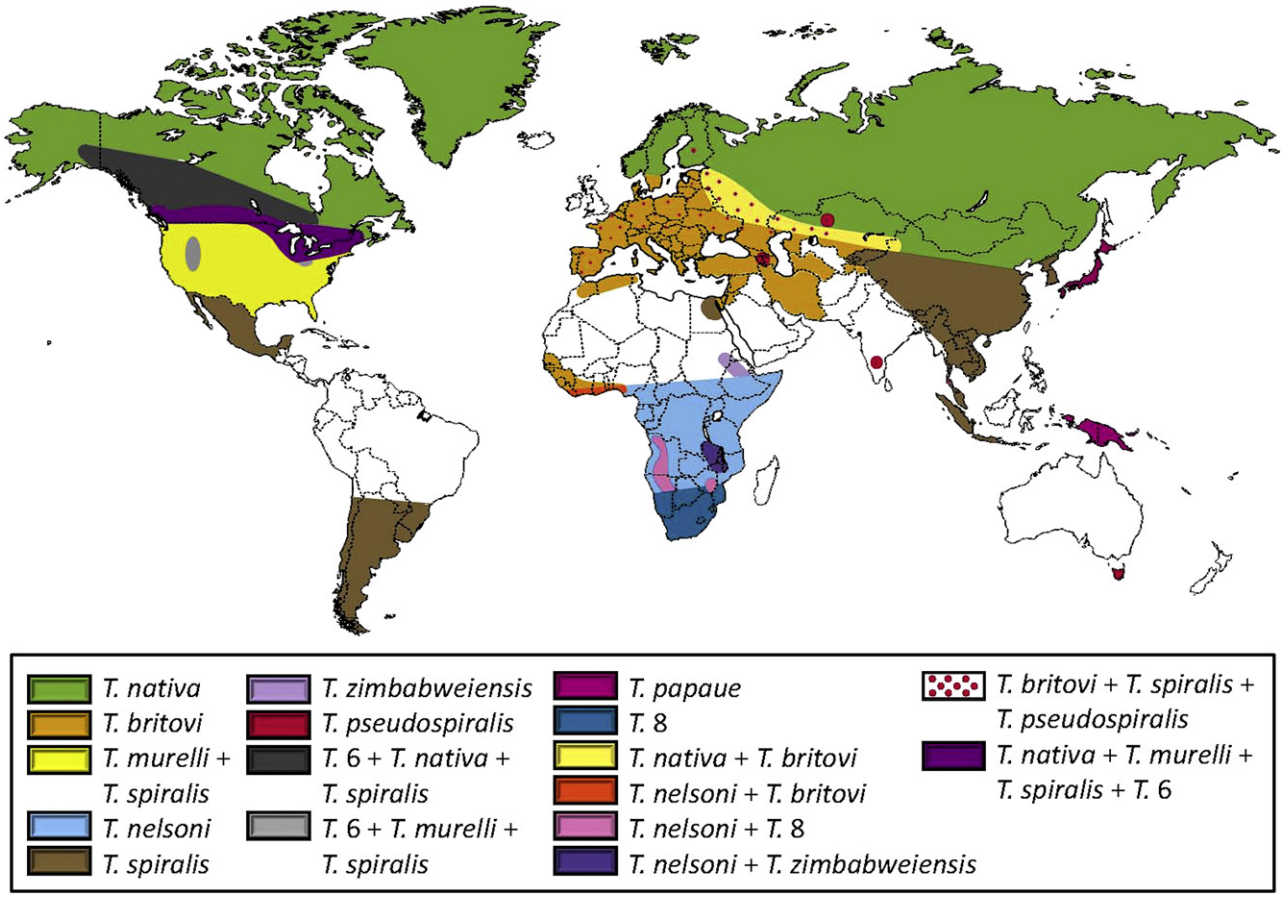

Fig. 1. Distribution map of the *Trichinella* species and genotypes included in the present study.

Polymerase Chain Reaction (PCR) among those is emphasized. This technique provides high sensitivity and specificity (Borsuk, Moskwa, Pastusiak, & Cabaj, 2003; De Bruyne, Yera, Le Guerhier, Boireau, & Dupouy-Camet, 2005; van der Giessen, Fonville, Briels, & Pozio, 2005). The PCR methodologies used for identification of *Trichinella* include Random Amplified Polymorfic DNA (RAPD–PCR) (Bandi et al., 1995; Pozio, Kapel, & Gamble, 1999), PCR-Single-Strand Conformational Polymorfism (SSCP–PCR) (Gasser, Hu, El-Osta, Zarlenga, & Pozio, 2005; Gasser et al., 1998; Wu, Nagano, Pozio, & Takahashi, 1999), PCR-Restriction Fragment Length Polymorfism (PCR-RFLP) (Wu et al., 1999), Multiplex PCR (Blaga et al., 2009; Zarlenga, Chute, Martin, & Kapel, 1999, 2001).

A novel genetic technique for species detection and identification is the application of specific DNA probes with the method of Real-time PCR (RT-PCR). This methodology is based on the specific hybridization of a probe designed for a certain species with the DNA in the samples to be analyzed. Only the DNA complementary to the specific probe will hybridize to it. This technique is acquiring more importance because of its high speed and sensitive and it is a method highly used in microbiology for detection of bacteria, virus and parasites.

In detection of *Trichinella* with RT-PCR must be emphasized a recent work of Guenther et al in 2008 (Guenther et al., 2008), where the development technique allow detect only the tree species of *Trichinella* predominant in Europe, *T. spiralis, T. britovi* and *T. pseudospiralis*. But the globalization makes possible the commercialization of food from multiples origins; it is the reason why detection of European species do not allow to assure the absence of infection in commercial meats. It is necessary to develop effectives methodologies for certification of absence of parasite in meat become to market from all around the World.

The present work develops one RT-PCR methodology for detection of 10 species and 3 genotypes of *Trichinella*, which will allow certify the absence of this parasite on meat, in fast mode and with high sensibility, assuring the quality and safety of meat to supply the international market.

2. Materials and methods

2.1. Samples materials and DNA extraction

Trichinella reference strains used in this study were obtained from European Union Reference Laboratory for Parasites, Department of Infectious, Parasitic and Immunomediated Diseases. Istituto Superiore di Sanitá, (Italy) and Instituto de Salud Carlos III, Centro Nacional de Microbiología (Spain) (Table 1). *Trichinella* larvae were stored at −80 °C until processing for DNA extraction.

Genomic DNA of parasites was isolated and purified from individual larvae according to a standard CTAB phenol–chloroform protocol of Rogers and Bendich with slight modifications (Rogers & Bendich, 1985).

The quality and quantity were determined by measuring the absorbance at 260 nm and the 260/280 nm and 234/260 ratios using a *NanoDrop™ ND-1000* spectrophotometer *(Thermo Scientific)* (Winfrey, Rott, & Wortman, 1997). DNA extractions were appropriately labeled and stored at −80 °C for subsequent tasks.