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Molecular identification of Korean *Trichinella* isolates

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Abstract: Muscle larvae of *Trichinella* isolates from two outbreaks in Korea were analyzed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and multiple-PCR. All of the muscle larvae showed a band similar to that of *T. spiralis* larvae of the reference strain. The two Korean *Trichinella* isolates (isolate code ISS623 and ISS1078) might be classifiable to *Trichinella spiralis.*

Key words: *Trichinella spiralis*, classification, polymerase chain reaction (PCR)

Trichinosis is one of the most wide spread helminthic zoonoses. In Korea, its presence was first confirmed in 1998 as encysted larvae in the biopsied muscle of patient, who ate the raw flesh of a badger (Sohn et al., 2000). Another a small outbreak originating from a wild boar took placed in a mountainous area of Kangwon-do, Korea (Park et al., 2001). In this study, we performed a molecular biological study to identify the two Korean *Trichinella* isolates from two outbreaks at the species level.

Parasites were isolated and maintained in ICR mice. The mouse carcasses, infected with two Korean isolates, were sent to the *Trichinella* Reference Center at the Laboratory of Parasitology of the Instituto Superiore di Sanita in Rome, Italy. Muscle larvae (ML) were collected after artificially digesting the mouse carcass (Pozio, 1987) and washed three times in distilled water. After washing, individual ML were stored in 5 μl of H_2O at -30°C until used.

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For the identification of the ML of the first outbreak (isolate code ISS623), a single ML was placed in 14 ml of Tris-HCl (pH 7.6), overlaid with mineral oil, heated at 90°C for 10 min, treated with 100 mg/ml of proteinase K at 55°C for 3 hr, and heated again at 90°C for 10 min. Polymerase chain reaction (PCR) was carried out using SB2 primers, which are specific for *Trichinella spiralis* (forward 5′-CTCCACTTACGCAAT GCACG-3′ and reverse 5′-ACACCAAACGGCAA CTGCTA-3′) (Wu et al., 1997) and by PCR-RFLP with the primer set Ts43CA (forward 5′-ATGCGAAATA TACATTTTTCTTA-3′ and reverse 5′-TTAGCTGTAT GGGCAAGG-3′), and this was followed by *RsaI* restriction for *T. nativa* and *T. britovi*, in accordance with the protocol of Wu et al. (1999). Muscle larvae from the reference strains of *T. spiralis* (code ISS3), *T. nativa* (code ISS10), and *T. britovi* (code ISS2) were used as controls (La Rosa et al., 1992).

To identify ML originating from the second outbreak (isolate code ISS1078), a 0.1 μl solution of 0.1 M Tris-HCL (pH 7.6) and 1.9 μl of H_2O was added to the larva, overlaid with mineral oil, and heated at 90°C for 10 min. Then, 0.4 μl of proteinase K and 2.6 μl

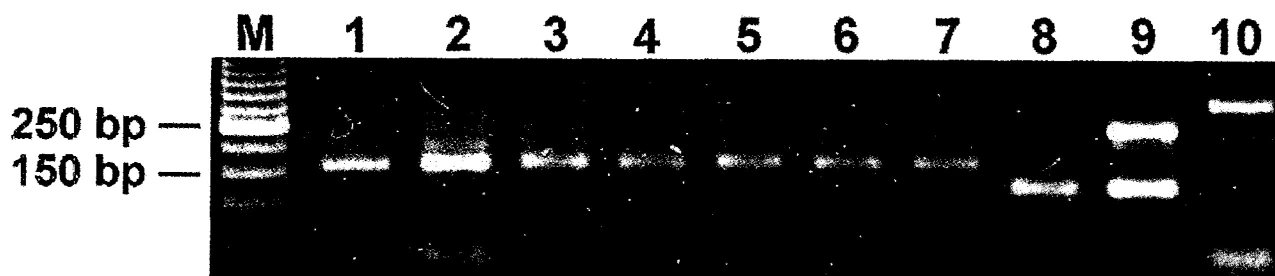

Fig. 1. Electrophoretic patterns after multiplex-polymerase chain reaction amplification of *Trichinella* larvae originating from a badger and from a wild boar in Korea. Lane M, 100 bp ladder; lanes 1-3, larvae from the badger (isolate code ISS623); lanes 4-6, larvae from the wild boar (isolate code ISS1078); lane 7, *Trichinella spiralis* reference larva (code ISS3); lane 8, *T. nativa* reference larva (code ISS10); lane 9, *T. britovi* reference larva (code ISS2); lane 10, *T. pseudospiralis* reference larva (code ISS13).

of H_2O were added to the larva at 48°C for 3 hr and at 90°C for 10 min. PCR was performed using 4 μl of a single larva preparation, 0.1 μl *Taq* DNA polymerase (Takara, Otsu, Shiga, Japan), 5 μl 10X PCR buffer, 4 μl dNTPs, 0.7 μl of primers (I-V primer sets) (Zarlenga et al, 1999), 0.5 μl DMSO, and H_2O up to 50 μl. Amplifications consisted of 35 cycles of: denaturation at 94°C for 30 sec, annealing at 62°C for 20 sec and at 58°C for 30 sec, and elongation at 72°C for 1 min. The MLs of reference strains were used as controls. The ISS numbers of the isolates refers to the code of the *Trichinella* at the Reference Center (Pozio et al., 1989).

All of the ML from the mice infected with two Korean *Trichinella* isolates showed a band similar to that of *T. spiralis* larvae of the reference strain (Fig. 1).

Trichinosis is generally diagnosed by detecting larvae in biopsied muscle and/or by the detection of antibody in the serological test without species differentiation. Recently, rapid and sensitive genotyping tools for *Trichinella* have been developed, and studies on the differentiation of genotypes and species of *Trichinella* have been performed successfully by several investigators (Gasser et al., 1998; Appleyard et al., 1999; Nagano et al., 1999; Wu et al., 1999; Zarlenga et al., 1999). Gasser et al. (1998) identified 7 isolates from mainland China by PCR-based SSCP. Wu et al. (1999) applied PCR-RFLP analysis to identify 5 species, i.e. *T. spiralis, T. nativa, T. britovi, T. pseudospiralis* and *T. nelsoni*, and 3 phenotypes of uncertain taxonomic status (*Trichinella* T5, T6 and T8).

Of the 7 isolates from the mainland China, 5 were identified as *T. spiralis* and the results upon the other two were identical with those of *T. nativa* and *Trichinella* T6 (Gasser et al., 1998). The Japanese isolates from wild animals were identified as *T. britovi* by random amplified polymorphic DNA (Pozio et al., 1996). In the present study, PCR-RFLP and multiple-PCR found that both Korean isolates (ISS623 and ISS1078) showed the same molecular pattern as that of a *T. spiralis* reference strain.

According to the new taxonomic scheme, nematodes belonging to the genus *Trichinella* are divided into 7 valid species, *T. spiralis, T. nativa, T. britovi, T. pseudospiralis, T. nelsoni, T. murrelli* and *T. papuae* (Murrell and Pozio, 2000). Among them, *T. spiralis* has the widest geographical distribution and the largest range of host species. The majority of human infections are caused by this species (Capo and Despommier, 1996; Murrell and Pozio, 2000). However, since the various genotypes or species of *Trichinella* are distributed worldwide including Asia, *Trichinella* isolates should be examined in detail. In this study, we have identified two Korean *Trichinella* isolates infecting human as *T. spiralis*.

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