Short Report: Molecular Identification of *Trichinella papuae* from a Thai Patient with Imported Trichinellosis

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Abstract. Previously, we reported the presence of imported trichinellosis in a Thai worker returning from Malaysia, who presented with progressive generalized muscle hypertrophy and weakness after eating wild boar meat. This work analyzed a partial small subunit of a mitochondrial ribosomal RNA gene of *Trichinella* larvae isolated from the patient. The results showed complete identity with a mitochondrial RNA gene of *Trichinella papuae* (GenBank accession no. EF517130). This is the first report of imported trichinellosis in Thailand caused by *T. papuae*. It is possible that *T. papuae* is widely distributed in the wildlife of Southeast Asia.

Human trichinellosis is an important food-borne parasitic zoonosis that causes ~10,000 human infections per year with a corresponding mortality rate of 0.2%.^{1,2} Humans are infected by eating raw or undercooked meat of various omnivores and carnivores, particularly pork from domestic or wild pigs. In Thailand, the first trichinellosis outbreak was caused by the consumption of raw pork from hill tribe pigs in Mae Hong Son province, in the northern part of Thailand.³ Subsequently, as many as 118 outbreaks were reported from 1962 to 1991, mostly in northern Thailand.⁴ The sources of infection were mainly hill tribe pigs and wild boars, and the infecting parasite in all cases was regarded as *Trichinella spiralis*.⁵ However, species identification confirming the presence of *T. spiralis* was revealed in only one outbreak.⁶ Two more species were later found. In 1994, an outbreak of human trichinellosis caused by *Trichinella pseudospiralis* was reported in Chumporn province in the southern part of Thailand.⁷ In 2006 and 2007, two outbreaks of trichinellosis caused by *T. papuae* were reported in Uthai Thani province in the central part of Thailand.^{8,9} All cases were indigenous in nature.

The first imported case of trichinellosis in Thailand was reported in 2005. A Thai worker with progressive generalized muscle hypertrophy and weakness visited Srinagarind Hospital, Khon Kaen province, in the northeastern part of Thailand¹⁰; however, the species was misdiagnosed and not initially identified. The patient had worked in Malaysia and had a history of eating raw hunt wild boar meat 1 month before onset of illness. Quadriceps muscle biopsy was performed by a compression method, and non-encapsulated *Trichinella* larvae were seen under the microscope (Figure 1). These larvae were isolated and maintained in Swiss mice under hygienic conditions at the Department of Parasitology, Faculty of Medicine, Khon Kaen University, for species identification. The mice were fed with a standard granulated feed and water *ad libitum*. Handling and processing of animals were performed following the guidelines for animal experimentation of the National Research Council of Thailand. The study was approved by both the Animal Ethics Committee of Khon Kaen University (reference no. 0514.1.12.2/1) and the Human Ethics Committee

of Khon Kaen University (reference no. 470655). Informed consent was obtained from the patient. In this study, the worms originally isolated from the patient were subjected to molecular identification, and were found to be *Trichinella papuae* by polymerase chain reaction (PCR) and sequencing.

For recovery of *T. papuae* larvae, the infected mice were killed 2 months after oral inoculation. The infective larvae were regained from mouse muscles by pepsin-HCl digestion, and harvested using a modified Baermann technique.¹¹ *Trichinella* larvae were washed extensively with normal saline solution. For genomic DNA extraction, the larvae were homogenized with disposable polypropylene pestles (Bellco Glass Inc., Vineland, NJ), and then DNA was extracted using a NucleoSpin Tissue Kit (Macherey-Nagel GmbH & Co., Düren, Germany). The DNA was eluted in 100 µL of distilled water and stored at –20°C until used.

A fragment of a small subunit of mitochondrial ribosomal RNA gene was amplified using the genus-specific primers TPα-F (5'-AATAGTGTGCCAGCTATCG-3') and TPα-R (5'-CTTCCAGAAGATCTACTTTGTTCG-3'), which were designed from a highly conserved region within a mitochondrial RNA gene of *Trichinella* (GenBank accession no. EF517130). The PCR was carried out using a GeneAmp PCR System 9700 (Applied Biosystems, Singapore). The reaction was carried out in a 25 µL volume containing PCR

FIGURE 1. Muscle biopsy tissue, compressed between glass slides, showing non-encapsulated *Trichinella* larvae.

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buffer (10 mmol/L Tris-HCl, pH 8.4, 50 mmol/L KCl, 1.5 mmol/L MgCl₂), 200 µmol/L of each deoxyribonucleotide triphosphate, 0.2 µmol/L of each primer, and 0.625 unit of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). The DNA template was initially denatured at 94°C for 5 min. The amplification procedure consisted of 30 cycles at 95°C for 30 sec (denaturation), 55°C for 30 sec (annealing), and 72°C for 30 sec (extension), with a final extension at 72°C for 10 min. Amplified product was run on a 1.5% agarose gel; a 565 bps fragment was then cut

and purified for DNA sequencing, which was performed using the MegaBACE 1000 DNA Analysis System (GE Healthcare, Piscataway, NJ). Sequence alignment was conducted using the multiple sequence alignment program of the National Institute for Agricultural Research (INRA) on the MultAlin homepage.¹² The partial small subunit of a ribosomal RNA gene of *Trichinella* larvae Malaysian isolate showed complete identity with a mitochondrial RNA gene of *T. papuae* (GenBank accession no. EF517130) (Figure 2).

FIGURE 2. Multiple alignment of representative DNA sequence obtained from imported *Trichinella* Malaysian isolate (*Trichinella pa-ma*). Nucleotide sequences from mitochondrial RNA genes of 11 *Trichinella* species and genotypes—*Trichinella papuae*, *Trichinella zimbabwensis*, *Trichinella pseudospiralis*, *Trichinella spiralis*, *Trichinella nelsoni*, *Trichinella nativa*, *Trichinella* sp. T6, *Trichinella murrelli*, *Trichinella* sp. T9, *Trichinella britovi*, and *Trichinella* sp. T8 (GenBank accession nos.: EF517130, EF517131, EF517124, GU339146, EF517127, DQ159093, EF517126, EF517125, EF517129, DQ159092, and EF517128, respectively)—were used for comparison. Dots represent sequence identity with *Trichinella* Malaysian isolate. Gaps are represented by dashed lines.

FIGURE 3. Multiple alignment of partial DNA sequence of cytochrome c oxidase subunit I (COI) gene obtained from four isolates of *Trichinella papuae*. An imported *Trichinella* Malaysian isolate (*Trichinella*_pa-ma) was aligned with three isolates of *T. papuae* in GenBank (accession nos.: EF601546, DQ007899, and GQ180179). Dots represent the conservation of the base among all the examined isolates. Horizontal bars represent the position of polymerase chain reaction (PCR) primers.

For examination of intraspecies differences existence between geographical isolates, the partial cytochrome oxidase subunit I (COI) region was amplified by specific primers Tri-COIF (forward: 5'-GTTTATATCCTAGTACTACC-3') and Tri-COIR (reverse: 5'-GCGTTTGATAGTCTAACTCC-3'),⁸ which were designed from partial COI sequences of mitochondrial DNA from *T. papuae* (GenBank accession no. DQ007899). The PCR reaction was done similar to the above except the annealing condition at 50°C for 30 sec was performed. After sequencing, the partial COI sequence *T. papuae* Malaysian isolate revealed complete identity with *T. papuae* Papua New Guinea isolate (accession no. EF601546)¹³ and isolate from Thailand (accession no. GQ180179),⁹ but single nucleotide different with another isolate from Papua New Guinea (accession no. DQ007899).¹⁴ This result revealed no intraspecies difference with Thailand isolate (Figure 3).

One encapsulated (*T. spiralis*) and two non-encapsulated (*T. pseudospiralis* and *T. papuae*) species have been identified in Southeast Asia.¹⁵ *Trichinella papuae* was first described in 1999 from domestic and sylvatic swine and farmed salt-water crocodiles of Papua New Guinea.¹⁶⁻¹⁸ Outbreaks of human trichinellosis caused by *T. papuae*, however, were subsequently reported in Thailand.^{8,9} Here, we validated the molecular evidence from our previous study of *T. papuae* isolated from a Thai worker returning from Malaysia.¹⁰ This was the first imported trichinellosis case in Thailand caused by *T. papuae*. It is possible that *T. papuae* is distributed in the wildlife of Southeast Asia. People in Singapore who

had visited neighboring Malaysian islands in 1998 acquired trichinellosis.¹

Prolonged progressive generalized muscle hypertrophy and weakness in a patient, together with a history of consumption of raw wildlife meat, should alert physicians to the possibility of trichinellosis caused by *T. papuae*.

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