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Short Communication

Molecular identification of *Gnathostoma spinigerum* (Nematoda: Gnathostomatidae) as causative agent of human gnathostomiasis in Thailand

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Abstract. A 43-year-old male residing in Prachin Buri Province, Thailand, was admitted to the Siriraj University Hospital of Mahidol University, Thailand, in July 2014 with right eyelid swelling and serpiginous lesion for three weeks. A nematode specimen was accidentally recovered from his upper right eyelid area. The body of the worm was cylindrical and measured 11.0 × 1.4 mm. The head bulb had eight circles of transverse spines. Anterior half of the body was covered with rows of comb-like pointed spines. The tail part was rounded dorsally and flattened ventrally and no copulatory spicule was observed at the posterior end. It was morphologically identified as an immature female worm of gnathostome species. Sequence analysis for partial cytochrome *c* oxidase subunit I (*COI*) gene revealed this recovered nematode as *Gnathostoma spinigerum*.

Human gnathostomiasis caused by infection with a spirurid nematode of the genus *Gnathostoma*, is an important emerging foodborne parasitosis worldwide including Thailand (Daengsvang, 1980; Herman & Chiodini, 2009). Currently, a total of 13 species of *Gnathostoma* have been described, including five species (*G. spinigerum*, *G. dorolesi*, *G. hispidum*, *G. nipponicum* and *G. binucleatum*) which cause diseases in humans (Miyazaki, 1991; Nawa, 1991; Nawa *et al.*, 2015). Clinical presentations of gnathostomiasis are cutaneous and/or visceral larva migrans, often associated with peripheral blood eosinophilia. Occasionally, serious clinical manifestations such as ophthalmitis, blindness, ocular or subarachnoid hemorrhage, eosinophilic meningitis or myeloencephalitis occur when the larva penetrates deep into vital organs like eyes and/or central nervous system (Daengsvang 1980; Herman & Chiodini, 2009; Nawa *et al.*, 2015).

Humans are accidental hosts of *Gnathostoma* parasites and get infected by eating raw or improperly cooked meat or fish containing living infective third-stage larva(e) (Miyazaki, 1991; Nawa *et al.*, 2015). Within humans, *Gnathostoma* larvae rarely develop into mature stage and migrate through subcutaneous tissue and internal organs to produce migratory swelling in the skin and other symptoms based on the affected organ (Miyazaki, 1991, Nawa *et al.*, 2015). Definitive diagnosis of gnathostomiasis is made by recovery of the worm from the patient's skin lesions, eyeball or surgical excision but this is a rare occurrence. In the majority of cases, diagnosis is based on patient's history and

clinical pictures together with detection of antibody to a diagnostic protein of 24 kDa component or its recombinant surrogate (Chaicumpa, 2010; Intapan *et al.*, 2010; Janwan *et al.*, 2013).

In Thailand, many cases of gnathostomiasis have been reported. The annual number of patients presenting with larva migrans caused by *Gnathostoma* nematode has also been observed (Daengsvang, 1980; Lertanekawattana *et al.*, 2004; Nontasut *et al.*, 2005; Niranvichaiya & Chairatchaneeboon, 2016). However, the number of the gnathostomiasis cases with molecular confirmation is rather rare (Jongthawin *et al.*, 2015). We report here an additional case of parasitologically confirmed gnathotomiasis caused by *G. spinigerum*. The identification of the causative species was confirmed further by sequence analysis of the partial cytochrome *c* oxidase subunit I (*COI*) gene.

The present gnathostomiasis case was a 43-year-old male residing in Prachin Buri Province, Thailand, who admitted to the Siriraj University Hospital, Mahidol University in July 2014. He presented with a history of progressive right-side headache for 4 weeks. One week later he developed generalized seizure of his extremities associated with right eyelid swelling and blurred vision. Physical examination revealed a slightly raised erythematous serpiginous cutaneous track on his right upper eyelid. Chemosis and mild conjuctival injection were detected. His visual acuities were 3/60 and 6/6. Peripheral blood examination showed WBC count of 9,240 cells/μL with 14% eosinophils. CT scan of his brain demonstrated thin subdural hematoma at right temporo-parietal area, minimal subarachnoid hemorrhage, intra-parenchymal hematoma at right occipital lobe 2x3 cm in size. CT angiography showed no aneurysm or arteriovenous malformation. On the fourth day of admission, a nematode worm was accidentally removed by the patient when wiping his right eyelid area with tissue paper. The body of this worm measured 11 mm in length and 1.4 mm in width (Figure 1). The head bulb had eight circles of transverse spines. Anterior half of the worm body was covered with rows of comb-like pointed cuticular spines. The tail part was rounded dorsally and flattened ventrally and no copulatory spicule was observed at the posterior end. It was morphologically suggested as an immature female worm of *Gnathostoma* parasite. The worm was preserved in absolute ethanol and kept at -40°C for DNA extraction.

In this study, the molecular method was carried out according to Ando *et al.* (2006) using a portion of the cytochrome c oxidase subunit I (*COI*) gene. The *Gnathostoma* worm recovered from the patient was sequenced and compared with different worldwide *Gnathostoma* species from GenBank. A comparison was made on 380 nucleotides of a portion of the mitochondrial *COI* gene between the present *Gnathostoma* worm and several populations of *G. spinigerum* from Japan, Indonesia and China. The results revealed 99% identity with *COI* sequences of other *G. spinigerum* obtained from GenBank (Figure 2).

The phylogenetic relationship between *G. spinigerum* (isolates from Japan, Indonesia and China) and three other *Gnatostoma* species (*G. hispidum*, *G. dorolesi* and *G. nipponicum*) was compared with the recovered *Gnatostoma* sample. The phylo-genetic tree based on partial *COI* gene sequences was reconstructed by the neighbour-joining (NJ) and maximum likelihood (ML) methods using MEGA 6.0 (Tamura *et al.*, 2013). Both methods

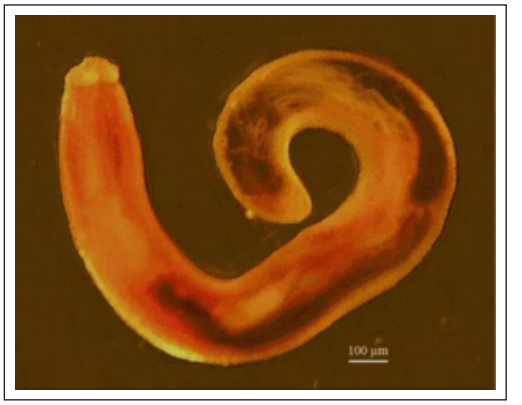

Figure 1. An immature female worm of *Gnathostoma spinigerum* collected from the upper eyelid of patient in this study.

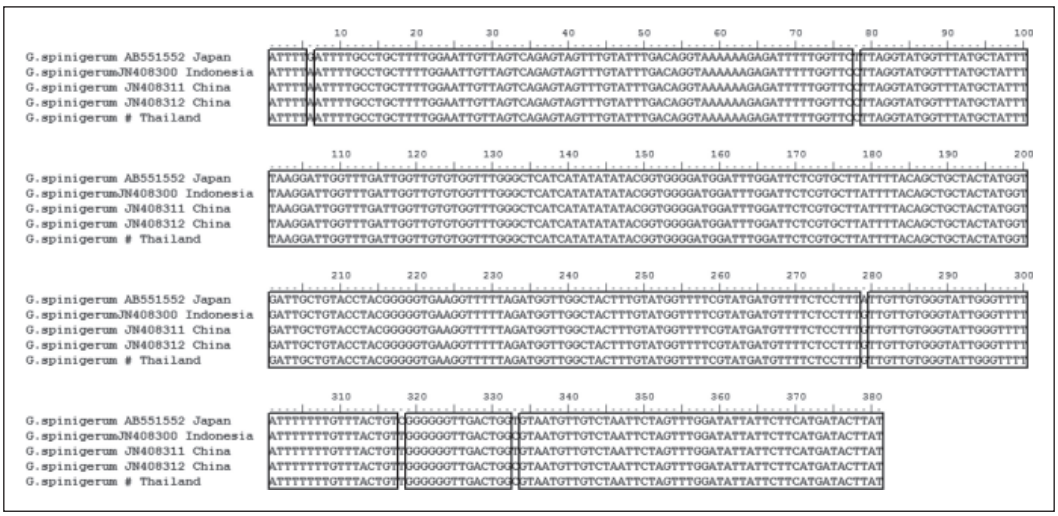

Figure 2. Comparison of 380 nucleotides of a portion of *COI* mitochondrial gene between the present *Gnathostoma* worm and *Gnatostoma spinigerum* from Japan, Indonesia and China.

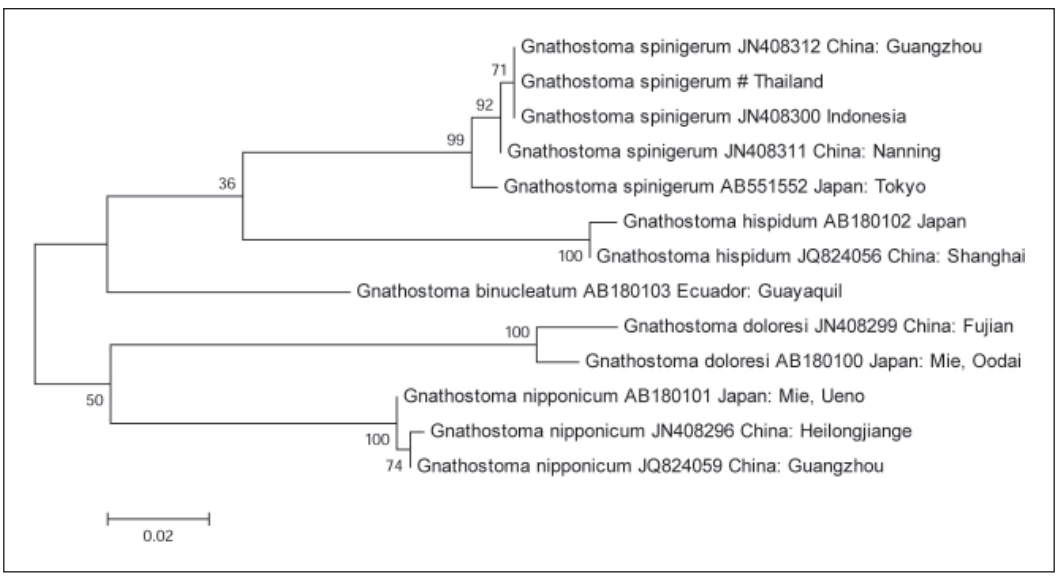

Figure 3. Phylogenetic tree of the present *Gnathostoma spinigerum* and *Gnathostoma* species based on partial *COI* nucleotide sequences reconstructed by the Maximum Likelihood (ML) method using MEGA 6.0. Note: *G. spinigerum* # Thailand = *G. spinigerum* from the present case in Thailand.

produced identical phylogenetic tree and revealed this recovered nematode to be *G. spinigerum* (Figure 3).

Over the years, immature worms of *Gnathostoma* species recovered from infected patients have been identified morphologically based primarily on the body sizes, number of row of the cephalic hooklets and the shape and distribution of cuticular spines or the feature of body cuticular spines (Daengsvang, 1980). However, morphological identification of young adults detected from humans is difficult as immature worms have overlapping morphological features among the five pathogenic species of *Gnathostoma*.

Over a decade, molecular genetic information such as DNA sequencing analysis has been reported to be an accurate tool for the identification of *Gnathostoma* at both the species and isolates levels (Almeyda-Artigas *et al*., 2000; Ando *et al*., 2006). The nucleotide region of the ITS2 sequence can be used for interspecies identification, and the partial *COI* gene is useful for intraspecies variation of *G. spinigerum* populations (Almeyda-Artigas *et al*., 2000; Ando *et al*., 2006). DNA sequencing analysis can clarify the misidentification based on the morphological features of *Gnathostoma* species that cause human diseases.

Morphological identification of recovered *Gnathostoma* worms from patients is difficult and there is very limited information on molecular identification of *Gnathostoma* species recovered from human cases in Thailand (Jongthawin *et al*., 2015). The present report adds to the molecular identification of *Gnathostoma* species in Thailand.

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REFERENCES

Almeyda-Artigas, R.J., Bargues, M.D. & Mas-Coma, S. (2000). ITS-2 rDNA sequencing of *Gnathostoma* species (Nematoda) and elucidation of the species causing gnathostomiasis in the Americas. *Journal of Parasitology* **86**: 537-544.

Ando, K., Tsunemori, M., Akahane, H., Tesana, S., Hasegawa, H. & Chinzei, Y. (2006). Comparative study on DNA sequences of ribosomal DNA and cytochrome c oxidase subunit 1 of mitochondrial DNA among five species of gnathostomes. *Journal of Helminthology* **80**: 7-13.

Chaicumpa, W. (2010) Immunodiagnosis of gnathostomiasis. *Siriraj Medical Journal* **62**: 79-83.

Daengsvang, S. (1980). A Monograph on the Genus *Gnathostoma* and Gnathostomiasis in Thailand. Tokyo, Japan: Southeast Asian Medical Information Center (SEAMIC), International Medical Foundation of Japan.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.

Herman, J.S. & Chiodini, P.L. (2009). Gnathostomiasis, another emerging imported disease. *Clinical Microbiology Reviews* **22**: 484-492.

Intapan, P.M., Khotsri, P., Kanpittaya, J., Chotmongkol, V., Sawanyawisuth, K. & Maleewong, W. (2010). Immunoblot diagnostic test for neurognathostomiasis. *American Journal of Tropical Medicine & Hygiene* **8**: 927-929.

Janwan, P., Intapan, P.M., Yamasaki, H., Laummaunwai, P., Sawanyawisuth, K., Wongkham, C., Tayapiwatana, C., Kitkhuandee, A., Lulitanond, V., Nawa, Y. & Maleewong, W. (2013). A recombinant matrix metalloproteinase protein from *Gnathostoma spinigerum* for serodiagnosis of neurognathostomiasis. *Korean Journal of Parasitology* **51**: 751-754.

Jongthawin, J., Intapan, P.M., Sanpool, O., Sadaow, L., Janwan, P., Thanchomnang, T., Sangchan, A., Visaetsilpanonta, S., Keawkong, W. & Maleewong, W. (2015). Three human gnathostomiasis cases in Thailand with molecular identification of causative parasite species. *American Journal of Tropical Medicine & Hygiene* **93**: 615-618.

Lertanekawattana, S., Waikagul, J., Pilomvarakorn, S. & Saepeung, K. (2004). Gnathostomiasis : two cases admitted to Nong Khai hospital, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **35** (Suppl1): 100-103.

Miyazaki, I. (1991). Gnathostomiasis. In *An Illustrated Book of Helminthic Zoonoses*. ed. Miyazaki, I. Tokyo: International Medical Foundation of Japan. pp 368-409.

Nawa, Y. (1991). Historical review and current status of gnathostomiasis in Asia. *Southeast Asian Journal of Tropical Medicine and Public Health* **22** (Suppl. 2): 217-219.

Nawa, Y., Maleewong, W., Intapan, P.M. & Diaz-Chamacho, S.P. (2015). *Gnathostoma*. eds. Xiao, L., Ryan, U., Feng, Y. In *Biology of Food Borne Parasites*. New York, NY: CRC Press Taylor and Francis Group. pp 405-426.

Ngarmamonpirat, N., Waikagul, J., Petmitr, S., Dekumyoy, P., Rojekittikhun, W. & Anantapruti, M.T. (2005). Analysis of sequence variation in *Gnathostoma spinigerum* mitochondrial DNA by single-strand conformation polymorphism analysis and DNA sequence. *Parasitology International* **54**: 65-68.

Niranvichaiya, S. & Chairatchaneeboon, M. (2016). Cutaneous gnathostomiasis: A case report from Thailand. *Siriraj Medical Journal* **68**: 114-116.

Nontasut, P., Claesson, B.A., Dekumyoy, P., Pakdee, W. & Chullawichit, S. (2005). Double-dose ivermectin vs albendazole for the treatment of gnathostomiasis. *Southeast Asian Journal of Tropical Medicine & Public Health* **36**: 650-652.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729.