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▣ BRIEF COMMUNICATION

# Molecular Detection of *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, and *Necator americanus* in Humans in Northeastern and Southern Thailand

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**Abstract:** The 2 principal species of hookworms infecting humans are *Necator americanus* and *Ancylostoma duodenale*. Case studies on zoonotic hookworm infections with *Ancylostoma ceylanicum* and/or *Ancylostoma caninum* are known mainly from Asian countries. Of these 2 zoonotic species, only *A. ceylanicum* can develop to adulthood in humans. In the present study, we report a molecular-based survey of human hookworm infections present in southern and northeastern Thailand. Thirty larval hookworm samples were obtained from fecal agar plate cultures of 10 patients in northeastren Thailand and 20 in southern Thailand. Partial ITS1, 5.8S, and ITS2 regions of the ribosomal DNA genes were amplified using PCR. The amplicons were sequenced, aligned, and compared with other hookworm sequences in GenBank database. The results showed that, in Thailand, *N. americanus* is more prevalent than *Ancylostoma* spp. and is found in both study areas. Sporadic cases of *A. ceylanicum* and *A. duodenale* infection were seen in northeastern Thailand.

Key words: *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, *Necator americanus*, human, molecular detection

Human hookworm infections commonly cause socioeconomic and public health problems, with approximately 1 billion persons infected worldwide [1]. Hookworm infections cause iron deficiency anemia, resulting in mental retardation and growth insufficiency in children [2]. *Ancylostoma duodenale* and *Necator americanus* are the 2 common species that cause human infections [3]. The former is common in the Middle East, Northern Africa, India, Australia, and Europe, while *N. americanus* is widespread in the western hemisphere, sub-Saharan Africa, eastern Asia, and southeast Asia [4]. In addition, zoonotic hookworms such as *Ancylostoma ceylanicum*, *Ancylostoma braziliense*, and *Ancylostoma caninum* have been reported as potentially significant public health threats in many areas [5]. Hookworm infections are still highly prevalent in Thailand [6,7] and are generally diagnosed by finding larvae/eggs in fecal preparations. Morphological identification of hookworm larvae to species is difficult and molecular identification is a useful tool in this regard. Zoonotic hookworm disease caused by *A. ceylanicum* was detected by copro-molecular methods in central Thailand [6,8] and Lao PDR [9]. A molecular approach to identify the causative species in other parts of Thailand is still lacking. Here, we report molecular identification of hookworm species that infect humans in the northeastern (NE) and southern Thailand. This genetic data is important as a part of continuing investigations into epidemiology of hookworms in Thailand.

The study was conducted between 2011 and 2013. Hookworm larvae were collected from 30 fecal samples using the agar plate culture technique [10]. Ten fecal samples were collected from patients in Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, NE Thailand and 20 samples from rural villagers, Nakhon Si Thammarat, southern Thailand. A single larval hookworm from each fecal sample was kept in 95% ethanol until used for DNA extraction. Oral or written

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informed consent was obtained from patients and legal guardians. This study was approved by the Khon Kaen University Ethics Committee for Human Research (HE551247), Khon Kaen, Thailand.

DNA was extracted separately from each larva using a Genomic DNA Mini Kit (Macherey-Nagel GmbH & Co., Düren, Germany) according to the manufacturer's instructions. The partial ITS1, full length 5.8S gene, and partial ITS2 ribosomal DNA regions were amplified from each larva using PCR and the primers RTHW1F (forward): 5´-GAT GAG CAT TGC WTG AAT GCC G-3´ and RTHW1R (reverse): 5´-GCA AGT RCC GTT CGA CAA ACA G-3´ [8]. PCR conditions were as follows; initial denaturation at 94℃ for 5 min, followed by 35 cycles of 95℃ for 30 sec (denaturation), 65℃ for 30 sec (annealing), and 72℃ for 30 sec (extension), and a final extension at 72℃ for 10 min. The reaction was carried out in a 25 µl volume containing PCR 1x FastStart High Fidelity Reaction Buffer (Roche Applied Science, Mannheim, Germany), 1.8 mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide triphosphate, 0.2 µM of each primer, and 0.625 units of FastStart High Fidelity Enzyme Blend (Roche Applied Science), respectively. The PCR product was run on a 1% agarose gel, cut out, and purified for DNA sequencing, which was performed using the MegaBACE™ 1000 DNA Analysis System (GE Healthcare, Piscataway, New Jersey, USA). The specific primers above were used as sequencing primers. The nucleotide sequences were analyzed by BLAST-N search via NCBI and the DNA alignment using Clustal-W [11].

Amplicon sizes were approximately 485 bp (typical of *N. americanus*) or 380 bp (typical of *Ancylostoma* spp.). Sequences showed extremely high similarities (99-100%) with hookworm sequences in the GenBank database. Of the 10 samples from NE Thailand, 6 were *N. americanus* (Khon Kaen, n=3; Mukdaharn, n=1; Roi ET, n=1; Loei, n=1) (different from AF217891 at a single base), 3 were *A. ceylanicum*, (Khon Kaen, n=2; Mahasarakham, n=1) (identical with AB501355), and 1 was *A. duodenale* (Loei) different from AB501348 at 2 bases). All 20 samples from Nakhon Si Thammarat, southern Thailand, were *N. americanus* (different from AF217891 at a single base) (n=20). All sequences obtained in this study were deposited in the GenBank data base under the accession no. KC896796-KC896825 (Table 1).

In the present study, molecular analysis was used to confirm human infections with 2 species of human hookworms, namely, *N. americanus* and *A. duodenale* found in NE and southern Thailand. In addition, 1 species of animal hookworm, namely, *A. ceylanicum*, was found in NE Thailand. In NE Thailand, *N. americanus* was the main hookworm identified, but *A. duodenale* and *A. ceylanicum* were also found. In southern Thailand, only *N. americanus* was detected. Our results complement previous reports from the central part of Thailand [6,7]. A survey of gastrointestinal parasites of dogs and humans in communities in Bangkok revealed *A. ceylanicum* and *A. caninum* in dogs and *N. americanus* and *A. ceylanicum* in humans [6]. Recently, a cohort study to identify the incidence and risk factors of hookworm infections was conducted in a rural community, central Thailand. *N. americanus* was the most common hookworm identified there. *A. duodenale* and *A. ceylanicum* were also detected [7]. We confirmed that the principal hookworm species infecting humans in Thailand is *N. americanus*. However, *A. duodenale* and *A. ceylanicum* infections were also detected in NE Thailand. The finding of the predominance of *N. ameri-*

**Table 1.** Hookworm sequences deposited in Genbank database

| Parts of Thailand | Provinces | Sequence ID | Accession no. | Hookworm species |
|---|---|---|---|---|
| Northeastern | Khon Kaen | THA FHWKK4 | KC896796 | *N. americanus* |
| | | THA FHWKK6 | KC896798 | *A.ceylanicum* |
| | | THA FHWKK7 | KC896799 | *N. americanus* |
| | | THA FHWKK9 | KC896801 | *N. americanus* |
| | | THA HWKK3 | KC896805 | *A.ceylanicum* |
| | Loei | THA FHWKK8 | KC896800 | *A.duodenale* |
| | | THA HWKK1 | KC896803 | *N. americanus* |
| | Mahasarakham | THA FHWKK5 | KC896797 | *A.ceylanicum* |
| | Roi Et | THA FHWKK10 | KC896802 | *N. americanus* |
| | Mukdahan | THA HWKK2 | KC896804 | *N. americanus* |
| Southern | Nakhon Si Thammarat | THA HWS1 | KC896806 | *N. americanus* |
| | | THA HWS2 | KC896807 | *N. americanus* |
| | | THA HWS4 | KC896808 | *N. americanus* |
| | | THA HWS5 | KC896809 | *N. americanus* |
| | | THA HWS6 | KC896810 | *N. americanus* |
| | | THA HWS7 | KC896811 | *N. americanus* |
| | | THA HWS8 | KC896812 | *N. americanus* |
| | | THA HWS9 | KC896813 | *N. americanus* |
| | | THA HWS10 | KC896814 | *N. americanus* |
| | | THA HWS11 | KC896815 | *N. americanus* |
| | | THA HWS12 | KC896816 | *N. americanus* |
| | | THA HWS13 | KC896817 | *N. americanus* |
| | | THA HWS15 | KC896818 | *N. americanus* |
| | | THA HWS16 | KC896819 | *N. americanus* |
| | | THA HWS17 | KC896820 | *N. americanus* |
| | | THA HWS18 | KC896821 | *N. americanus* |
| | | THA HWS19 | KC896822 | *N. americanus* |
| | | THA HWS20 | KC896823 | *N. americanus* |
| | | THA HWS21 | KC896824 | *N. americanus* |
| | | THA HWS22 | KC896825 | *N. americanus* |

*canus* in southern Thailand is consistent with the study in Peninsular Malaysia [12], where *N. americanus* was more common than *A. ceylanicum*, whereas *A. duodenale* infection was not found. In contrast, a study in Lao PDR found that *A. duodenale*, and the animal hookworms, *A. caninum* and *A. ceylanicum* were slightly more prevalent than *N. americanus* [8]. Thailand, Lao PDR, and Malaysia are neighboring countries. The geographical differences in the species of hookworms causing human infections might possibly be associated with parasite behavior, ethnicity, climate, temperature, and environmental factors [13,14].

In conclusion, our study revealed evidence of both human and animal hookworms among people in various areas of Thailand.

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