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Molecular Identification of Human Hookworm Infections in Economically Disadvantaged Communities in Peninsular Malaysia

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Abstract. Species identification of human hookworm infections among eight communities in rural areas of Peninsular Malaysia was determined during 2009–2011. Fecal samples were examined by microscopy and subsequently, the internal transcribed spacer 2 and 28S ribosomal RNA region of *Necator americanus* and *Ancylostoma* spp. were sequenced. Overall, 9.1% (58 of 634) were identified positive by microscopy for hookworm infection, and 47 (81.0%) of 58 were successfully amplified and sequenced. Sequence comparison found that *N. americanus* (87.2%) was the most predominant hookworm identified, followed by *Ancylostoma ceylanicum* (23.4%). No *A. duodenale* infection was detected in this study. Detection of *A. ceylanicum* in humans highlighted the zoonotic transmission among humans living near dogs. Thus, implementation of effective control measures for hookworm infections in future should seriously consider this zoonotic implication.

INTRODUCTION

Human hookworm infections have widespread socioeconomic and public health implications. Globally, an estimated 600 million persons are infected, resulting in up to 135,000 deaths annually.[1] Human infection is primarily caused by two species of hookworm (*Ancylostoma duodenale* and *Necator americanus*).[2] Geographic distribution of *A. duodenale* infections is mainly in the Middle East, northern Africa, India, Australia, and Europe, and *N. americanus* is more common in the Western Hemisphere, sub-Saharan Africa, eastern Asia, and southeast Asia.[3]

Clinically, infection in human causes iron-deficiency anemia, which may result in mental retardation and growth deficiencies, particularly in children.[4,5] Besides the two human species, intestinal zoonotic infections with canine and/or feline hookworm such as *A. ceylanicum*, *A. caninum*, and *Ancylostoma braziliense* have also been reported in many parts of the world.[6–8] More recently, zoonotic ancylostomiasis caused by *A. ceylanicum* was detected by using copro-molecular diagnostic tools in rural communities in Thailand[9,10] and Laos.[11]

Accurate diagnosis by precise identification and differentiation of species involved is essential in monitoring the efficacy of mass treatment and effective control of hookworm infection. Currently, most diagnosis and research conducted on the epidemiology of human hookworm infection greatly relies on the use of a conventional method for the detection of eggs in fecal samples. The benefits of this method are mainly technical simplicity and low cost. Although microscopy is limited and hampered because *N. americanus* eggs are morphologically indistinguishable from *Ancylostoma* spp. and other strongylid nematodes, including *Trichostrongylus* spp. and *Oesophagostomum* spp., microscopy is still the gold standard technique for rapid diagnosis.

Frequently, mass treatment with anthelminthic drugs is performed without identification of the causative species of infection. Given that a clinical manifestation such as severity of anemia differs according to the hookworm species involved[12] and the route of infection for each hookworm species also differs from species to species (e.g., *N. americanus* infection is mainly by skin penetration, and *Ancylostoma* spp. infections are more common by ingestion of infective third-stage larvae), species identification is paramount in designing appropriate and effective prevention and control strategies. Moreover, if a zoonotic hookworm is prevalent, the control target and strategies formulated also need to encompass animal hosts.

Although hookworm infection is still highly prevalent, especially in rural and remote areas of Peninsular Malaysia,[13–15] information on the species of hookworm present in humans is lacking. Because of the importance of accurate identification of hookworm infection, this study was conducted as part of an ongoing epidemiologic investigation to provide genetic data on the species of hookworm infecting humans in Peninsular Malaysia.

METHODS

Study area and population. The study was carried out during April 2009–April 2011 in eight villages in West Malaysia, which have been recognized as geohelminth-endemic areas.[14] The villages were Pos Iskandar (3.06°N, 102.65°E), Sungai Layau (1.53°N, 104.10°E), Bukit Serok (2.91°N, 102.82°E), Gurney (3.43°N, 101.44°E), Sungai Bumbun (2.85°N, 101.42°E), Kuala Pangsun (3.21°N, 101.88°E), Sungai Miak (3.52°N, 101.90°E), and Kemensah (3.21°N, 101.77°E) (Figure 1).

Each village had a small population, and the number of residents in each village was estimated to be 80–100 inhabitants. A total of 634 villagers, 2–82 years of age (276 males and 358 females) participated in this study. These communities lived in poor and socioeconomically deprived circumstances where overcrowding, poor environmental sanitation, low level of education, and poor provision of safe water are widespread. All houses have untreated tap water originating from a nearby river and there are no household-based sanitation facilities. The environmental condition of the village is generally poor with limited provision of latrine facilities therefore encouraging defecation in and around bushes or nearby rivers. Children usually defecated indiscriminately around their houses without parental supervision. In addition, it has been observed that it was common for villagers to walk barefooted while outdoors. The villagers also kept dogs, cats, monkeys, rabbits, and birds, and most of these domestic animals were left to roam freely.

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