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Rapid Detection and Identification of Human Hookworm Infections through High Resolution Melting (HRM) Analysis

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Abstract

Background: Hookworm infections are still endemic in low and middle income tropical countries with greater impact on the socioeconomic and public health of the bottom billion of the world's poorest people. In this study, a real-time polymerase chain reaction (PCR) coupled with high resolution melting-curve (HRM) analysis was evaluated for an accurate, rapid and sensitive tool for species identification focusing on the five human hookworm species.

Methods: Real-time PCR coupled with HRM analysis targeting the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA as the genetic marker was used to identify and distinguish hookworm species in human samples. Unique and distinct characteristics of HRM patterns were produced for each of the five hookworm species. The melting curves were characterized by peaks of 79.24±0.05°C and 83.00±0.04°C for *Necator americanus*, 79.12±0.10°C for *Ancylostoma duodenale*, 79.40±0.10°C for *Ancylostoma ceylanicum*, 79.63±0.05°C for *Ancylostoma caninum* and 79.70±0.14°C for *Ancylostoma braziliense*. An evaluation of the method's sensitivity and specificity revealed that this assay was able to detect as low as 0.01 ng/µl hookworm DNA and amplification was only recorded for hookworm positive samples.

Conclusion: The HRM assay developed in this study is a rapid and straightforward method for the diagnosis, identification and discrimination of five human hookworms. This assay is simple compared to other probe-based genotyping methods as it does not require multiplexing, DNA sequencing or post-PCR processing. Therefore, this method offers a new alternative for rapid detection of human hookworm species.

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Introduction

Hookworms are blood feeding intestinal nematodes that infect almost 600 million people worldwide, resulting in up to 135,000 deaths annually [1]. *Necator americanus* and *Ancylostoma duodenale* are two most common species causing infection in humans. In general, mixed infections of these hookworms are common in many endemic areas especially among people in tropical and subtropical countries with low socioeconomic status. Besides the two human species, canine and/or feline hookworms such as *Ancylostoma ceylanicum*, *Ancylostoma caninum* and *Ancylostoma braziliense* can also cause infections to human. Recently, zoonotic ancylostomiasis caused by *A. ceylanicum* has been occasionally reported in rural communities in Malaysia [2], Thailand [3] and Laos PDR [4]. The gravest consequences are manifested in children and women of childbearing age [5] displaying chronic intestinal blood loss which may result in iron-deficiency, anaemia and hypoalbuminemia [6,7]. The most deleterious effects of hookworm infections include impaired physical, intellectual and cognitive development of children, increased mortality in pregnant women and their infants and reduced work capacity of adolescents and adults [5–9].

Accurate diagnosis and genetic characterization of hookworms are essential for the formulation of effective control measures. Currently, most research conducted on the epidemiology of hookworm and other intestinal nematodes has relied on the use of conventional microscopy for the identification of eggs in faeces and third-stage larvae (L3) through the coproculture technique. The benefits of this method are mainly due to technical simplicity and low cost. However, utilization of microscopy is limited by the fact that most of the nematode eggs are morphologically indistinguishable from those of other species, and it is laborious, time-consuming and requires relatively skilled personnel. Thus, there is a crucial need for a practical, highly sensitive and specific diagnostic and analytical tool, particularly one based on the polymerase chain reaction (PCR) [10] to address key epidemiology and population genetic questions to underpin surveillance, treatment and control programme.

Following extensive evaluation of the specificity of genetic markers of hookworm such as first (ITS-1) and second (ITS-2)