

Epidemiological and Genetic Data Supporting the Transmission of *Ancylostoma ceylanicum* among Human and Domestic Animals

Romano Ngui¹, Yvonne A. L. Lim^{1*}, Rebecca Traub², Rohela Mahmud¹, Mohd Sani Mistam³

1 Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, **2** School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia, **3** Department of Orang Asli Development, Ministry of Rural and Regional Development, Kuala Lumpur, Malaysia

Abstract

Background: Currently, information on species-specific hookworm infection is unavailable in Malaysia and is restricted worldwide due to limited application of molecular diagnostic tools. Given the importance of accurate identification of hookworms, this study was conducted as part of an ongoing molecular epidemiological investigation aimed at providing the first documented data on species-specific hookworm infection, associated risk factors and the role of domestic animals as reservoirs for hookworm infections in endemic communities of Malaysia.

Methods/Findings: A total of 634 human and 105 domestic canine and feline fecal samples were randomly collected. The overall prevalence of hookworm in humans and animals determined via microscopy was 9.1% (95% CI = 7.0–11.7%) and 61.9% (95% CI = 51.2–71.2%), respectively. Multivariate analysis indicated that participants without the provision of proper latrine systems (OR = 3.5; 95% CI = 1.53–8.00; $p = 0.003$), walking barefooted (OR = 5.6; 95% CI = 2.91–10.73; $p < 0.001$) and in close contact with pets or livestock (OR = 2.9; 95% CI = 1.19–7.15; $p = 0.009$) were more likely to be infected with hookworms. Molecular analysis revealed that while most hookworm-positive individuals were infected with *Necator americanus*, *Ancylostoma ceylanicum* constituted 12.8% of single infections and 10.6% mixed infections with *N. americanus*. As for cats and dogs, 52.0% were positive for *A. ceylanicum*, 46.0% for *Ancylostoma caninum* and 2.0% for *Ancylostoma braziliense* and all were single infections.

Conclusion: This present study provided evidence based on the combination of epidemiological, conventional diagnostic and molecular tools that *A. ceylanicum* infection is common and that its transmission dynamic in endemic areas in Malaysia is heightened by the close contact of human and domestic animal (i.e., dogs and cats) populations.

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* E-mail: limailian@um.edu.my

Introduction

Hookworms are one of the most common parasitic nematodes that inhabit the small intestine of humans and animals such as dogs and cats. The two primary species of hookworm infecting humans are *Ancylostoma duodenale* and *Necator americanus* [1], with *A. duodenale* occurring mainly in the Middle East, North Africa, India, Australia and Europe, whilst *N. americanus* in the Americas, Sub-Saharan Africa, East Asia and Southeast Asia [2]. The socioeconomic and public health impact of human hookworm infections are extensive, infecting an estimated 600 million people worldwide and resulting in up to 135,000 deaths annually [3]. Infection in human causes iron-deficiency anemia which may result in mental retardation and growth deficiencies, particularly in children [4,5].

Canine and feline hookworm species are also able to cause zoonotic disease in humans. For example, cutaneous larva migrans (CLM) or ‘creeping eruptions’ is a hypersensitivity reaction caused

by migrating nematode larvae, of which *Ancylostoma braziliense* is the most frequently implicated aetiological agent in humans [6,7]. Another canine hookworm, *Ancylostoma caninum* is the leading cause of human eosinophilic enteritis (EE) and an outbreak of 150 cases was reported between 1988 and 1992 in Australia [8–10]. Cases have also been reported in the United States [11], Egypt [12], the Philippines, South America and Israel [13].

Ancylostoma ceylanicum however, is the only species of animal hookworm known to produce patent infections in humans. This has been demonstrated both experimentally [14,15] and naturally. Natural infections with *A. ceylanicum* have been reported in Dutch servicemen returning from West New Guinea, who suffered heavy infection with concurrent anemia [16], whilst light infections have been mostly reported from humans in the Philippines [17], Taiwan [18], Thailand [19] and India [20]. More recently, zoonotic ancylostomiasis caused by *A. ceylanicum* was reported in temple and rural communities in Thailand [21,22] and rural communities in Laos PDR [23] using copro-molecular diagnostic