Review Article

Zoonotic Ancylostomiasis: An Update of a Continually Neglected Zoonosis

Katharina Stracke,[1,2]* Aaron R. Jex,[1,3] and Rebecca J. Traub[3]

[1]*The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia;* [2]*The Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Australia;* [3]*Faculty for Veterinary and Agricultural Sciences, The University of Melbourne, Melbourne, Australia*

Abstract. Hookworm infections are classified as the most impactful of the human soil-transmitted helminth (STH) infections, causing a disease burden of ~4 million disability-adjusted life years, with a global prevalence of 406–480 million infections. Until a decade ago, epidemiological surveys largely assumed *Necator americanus* and *Ancylostoma duodenale* as the relevant human hookworm species implicated as contributing to iron-deficiency anemia. This assumption was based on the indistinguishable morphology of the *Ancylostoma* spp. eggs in stool and the absence of awareness of a third zoonotic hookworm species, *Ancylostoma ceylanicum*. The expanded use of molecular diagnostic assays for differentiating hookworm species infections during STH surveys has now implicated *A. ceylanicum*, a predominant hookworm of dogs in Asia, as the second most common hookworm species infecting humans in Southeast Asia and the Pacific. Despite this, with the exception of sporadic case reports, there is a paucity of data available on the impact of this emerging zoonosis on human health at a population level. This situation also challenges the current paradigm, necessitating a One Health approach to hookworm control in populations in which this zoonosis is endemic. Here, we have summarized the available research studies and case reports on human *A. ceylanicum* infections in Southeast Asia and the Pacific after 2013 using a systematic review approach. We summarized eight research articles and five clinical case studies, highlighting the importance of future in-depth investigation of zoonotic *A. ceylanicum* infections using sensitive and cost-effective diagnostic tools.

Hookworm infections in humans occur predominantly in countries with low socioeconomic status located in tropical and subtropical areas of the world.[1] Symptomology is measured in disability-adjusted life years (DALYs), which include the years a human host has lived with the infection (years lost due to disability (YLD)) and the years that are lost because of early death (years of life lost (YLL)).[2] The global infection prevalence for any hookworm in 2016 was around 450.68 million (1,297 million in 1994),[3] with a disease burden of around 1.8 million DALYs in 2015.[4,5] Primary symptoms associated with infection include iron-deficiency anemia caused by intestinal blood loss within the small intestine,[6,7] which particularly impacts pregnant women[8] and children.[9] For hookworms, as well as other soil-transmitted helminths (STHs), disease burden has decreased significantly over the last two decades.[3] This decrease can be attributed principally to targeted mass drug administration (preventive chemotherapy) in populations at risk of infection[10–12] and integrated intervention programs targeting improved access to safe water, sanitation, and hygiene[13,14] and development of molecular high-sensitivity diagnostic methods (improved infection prevalence estimation)[15] and socioeconomic development.[16] The WHO-recommended and -approved diagnostic tool for STH detection is the Kato–Katz thick smear.[17] However, this microscopy-based diagnostic method has important limitations: innately low diagnostic sensitivity in regard to hookworm detection,[18] the requirement for rapid sample screening to avoid over-clearance of hookworm eggs following slide preparation,[19] and the inability of microscopy-based diagnostics to identify human hookworms to a species level.[20] These factors together suggest an underestimation of

the true species-specific prevalence and intensity of human hookworms.

Hookworm infections in humans have largely been attributed to *Necator americanus* and *Ancylostoma duodenale*.[6] However, contrary to this belief, recent molecular studies have unequivocally demonstrated that *Ancylostoma ceylanicum* is highly endemic, comprising the second most common species of hookworm, after *N. americanus*, in many parts of Southeast Asia and the Pacific and estimated to infect ~100 million people.[21] This situation challenges the current hookworm paradigm. Despite the global efforts to meet the WHOs 2020 road map for eliminating morbidity associated with STH infections, *A. ceylanicum* is not included as a causal agent of human hookworm infection and therefore not included within this WHO framework.[11,22] This is despite a growing body of evidence to demonstrate that its prevalence in certain restricted areas or countries outcompetes that of *A. duodenale*.[23]

Importantly, *A. ceylanicum* is a zoonosis and transmissible to humans from animals. This species comprises the predominant hookworm of dogs and cats throughout Southeast Asia and the Pacific, which act as reservoirs for human infections.[21,24,25] This situation contrasts that of *N. americanus* and *A. duodenale*, which are both specific to humans. In 2013, a review by Traub highlighted the history of the reported distribution and overlooked public health significance and the impact of *A. ceylanicum* infections in humans in Southeast Asia and the Pacific.[21] The review hypothesizes that mass anthelmintic programs may contribute to the re-emergence of *A. ceylanicum* infections in humans unless a One Health approach toward its control is undertaken.[21] In this review, we aim at summarizing and updating information spanning the 6 years since the last major review by Traub, on the prevalence, distribution, and impacts of *A. ceylanicum* on human health.

Here, we conducted a systematic review, searching the publicly available database Google Scholar on June 14, 2019

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* Address correspondence to Katharina Stracke, The Walter and Eliza Hall Institute of Medical Research, 1G, Royal Parade, Parkville 3052, Australia. E-mail: stracke.k@wehi.edu.au

with no restrictions in terms of scientific journal or author for publications in English only. We limited the output to novel original research articles published from 2013 to 2019 using the following search term in the title: "*Ancylostoma ceylanicum*." Publication titles and abstracts of 70 peer-reviewed articles were screened and relevant articles included in this systematic review, as well as two other known case reports.[26,35] Review articles were excluded, with only original research articles included within the context of this mini-review. A list of all included publications is found in Tables 1 and 2.

Since 2013, *A. ceylanicum* infections in humans continue to be identified across Southeast Asia and the Pacific, including Thailand, Myanmar, India, Cambodia, and the Solomon Islands (Table 1, Figure 1). The proportion of *A. ceylanicum* infections among all hookworm-positive samples was relatively high in some regions (16.7–46.0%). Five of 13 publications describe individual cases of returning travelers or personnel from Southeast Asia and the Pacific, highlighting the relevance of this emerging zoonosis to the field of travel medicine (Table 2). Most of the aforementioned patients had a severely increased eosinophil count, which is a known immune response to hookworm infections.[33] Symptoms ranged from abdominal pain to weight loss, fever, diarrhea, vomiting, and other generalized symptoms (dizziness, tightness of chest, and sweats). Interestingly, only one of these clinical cases presented with anemia (Table 2), which is likely due to the general health status of travelers from developed countries compared with that of the local community. Although these were sporadic case reports from developed countries, there is no attention to ascertain morbidity caused on an endemic population scale. Moreover, these "rare" singular occurrence findings highlight the neglect of other symptomatic or subclinical cases in the country of origin.

The inconsistency in diagnostic tools used to estimate infection prevalence may additionally contribute to this underestimation.[39] In the context of some studies that used a traditional microscopy-based gold standard diagnosis tool, only hookworm-positive samples were further investigated for species-specific infections using molecular tools.[25,30] Not only does this lead to an underestimation of overall hookworm prevalence,[40] but it also underestimates the

proportion of *A. ceylanicum* infections even further,[25] owing to the relatively lower egg-shedding intensities of *A. ceylanicum*–infected individuals than those infected with *N. americanus*.[41]

Despite the increased availability and application of molecular diagnostic assays,[40] there remains a lack of large-scale mapping of *A. ceylanicum* in areas other than Southeast Asia and the Pacific, with a clear lack of available data in regard to infection prevalence and intensity, particularly in human populations. This lack of knowledge is partly due to the application of microscopy-based diagnostic tools that are not able to differentiate between hookworm species.[42] Apart from Southeast Asia and the Pacific, *A. ceylanicum* has also been reported in 3% of canines in Tanzania.[43] To date, the global distribution of cases in humans reflects that of its distribution in dogs. During the previous 6 years after publication of Traub's 2013 review, the focus of STH research has been on molecular biological areas such as genomics, transcriptomics, and diagnostics.[44] At the same time, diagnostic tool application is shifting from invasive endoscopy[38] to molecular laboratory–based techniques such as semi-nested polymerase chain reaction (PCR)-RFLP, quantitative PCR (qPCR), and multiplexed-tandem qPCR.[28,45] Moving toward these advanced molecular biology–based diagnostic tools using PCR systems can prove beneficial in increasing general knowledge of hookworm infection prevalence, the species-specific burden they cause and examining effects of coinfections within hosts.[46] Currently, however, novel diagnostic techniques are limited to research settings and still need to be completely transferable to the endemic field in regard to resources, expertise, and simple feasibility in remote settings.[47]

As evidently shown within the literature, *A. ceylanicum* continues to present itself as an increasingly emergent zoonosis, with a potentially wider than previously assumed geographical distribution. For the majority of countries, there is a paucity of epidemiological information available, which would serve as the basis for monitoring and diagnosing infections with this zoonosis for both clinical and population settings. Of the 13 epidemiological studies on *Ancylostoma ceylanicum* retrieved from Google Scholar (since 2013) (70 total hits), nearly all used molecular-based screening to further identify hookworms to a species level. Traub hypothesized the

TABLE 1
Summary of publicly available data on the proportion of hookworm infections attributed to *A. ceylanicum* in humans worldwide published between 2013 and 2019

Author and year	Reference number	Study country	Diagnostic tools used	Proportion of hookworm-positive samples	Type of infection
Koehler et al., 2013	26	Australia	PCR-coupled SSCP	18.2% (2/11)	Mono-infection
Phosuk et al., 2013	27	Thailand	Agar plate culture + PCR	10% (3/30)	Mono-infection
Inpankaew et al., 2014	25	Cambodia	PCR + Sanger sequencing for *Ancylostoma* spp.	46.0% (57/124)	Mono-infection
				3.2% (4/124)	*N. americanus*/*A. ceylanicum*
				1.6% (2/124)	*A. duodenale*/*A. ceylanicum*
				0.8% (1/124)	*N. americanus*/*A. duodenale*/ *A. ceylanicum*
George et al., 2015	28	India	Semi-nested PCR-RFLP	4.9% (2/41)	Mono-infection
Aung et al., 2017	29	Myanmar	PCR	27.3% (3/11)	Mono-infection
Bradbury et al., 2017	30	Solomon Islands	Kato–Katz + PCR	16.7% (11/66)	Mono-infection
				1.5% (1/66)	*N. americanus*/*A. ceylanicum*
Papaiakovou et al., 2017	31	Timor-Leste	Semi-nested PCR-RFLP	95.5% (21/22)	*Ancylostoma* spp.
		Argentina	PCR	0% (0/8)	*Ancylostoma* spp.
O' Connell et al., 2018	32	Thailand (Myanmar refugees)	Semi-nested PCR-RFLP, qPCR	17.4% (83/476 baseline)	–
				4.7% (6/128, follow-up)	
				0% (0/29, follow-up)	

A. ceylanicum = *Ancylostoma ceylanicum*; *N. americanus* = *Necator americanus*. Systematic review findings are summarized chronologically.

TABLE 2
Summary of *A. ceylanicum*–positive clinical cases from 2013 to 2018

	Yoshikawa et al.[34]				Brunet et al.[35]	Speare et al.[36]	Kaya et al.[37]	Ngui et al.[38]
Age (years), gender	25, male	47, male	26, male	26, male	33, male	26, male	47, male	58, female
Country	Malaysia	Laos	India	Papua New Guinea	Myanmar	Solomon Islands	Thailand/Laos	Malaysia
Tourist	Yes				Yes	No	Yes	No
Symptoms	Abdominal pain, watery diarrhea			Asymptomatic	Fever, weight loss, dyspnea, abdominal pain, bloody diarrhea	Abdominal pain, peripheral eosinophilia	Intermittent diarrhea, extreme eosinophilia, fever, abdominal pain, watery diarrhea, vomiting	Melena, discolored stool, dizziness, tightness of chest, cold sweats
Eosinophil count/μL (range, 30–500 or < 6%)	3,000	20,470	7,050	1,570	59%	6.20×10^9/L	43%, 74%	1%
Anemia	No	No	No	No	No	No	No	Yes

emergence of *A. ceylanicum* owing to multiple factors, including wide-scale mass deworming programs, resulting in the disproportional increase in infective larvae in the environment compared with that of *N. americanus* larvae, exacerbated by the lack of an "allergy-based" elimination of any new incoming larvae of "unnatural" hookworms. If this is due to differential benzimidazole drug efficacy for different hookworm species remains unclear.

There is a clear need for further in-depth investigation of *A. ceylanicum* infection prevalence, intensity, and morbidity
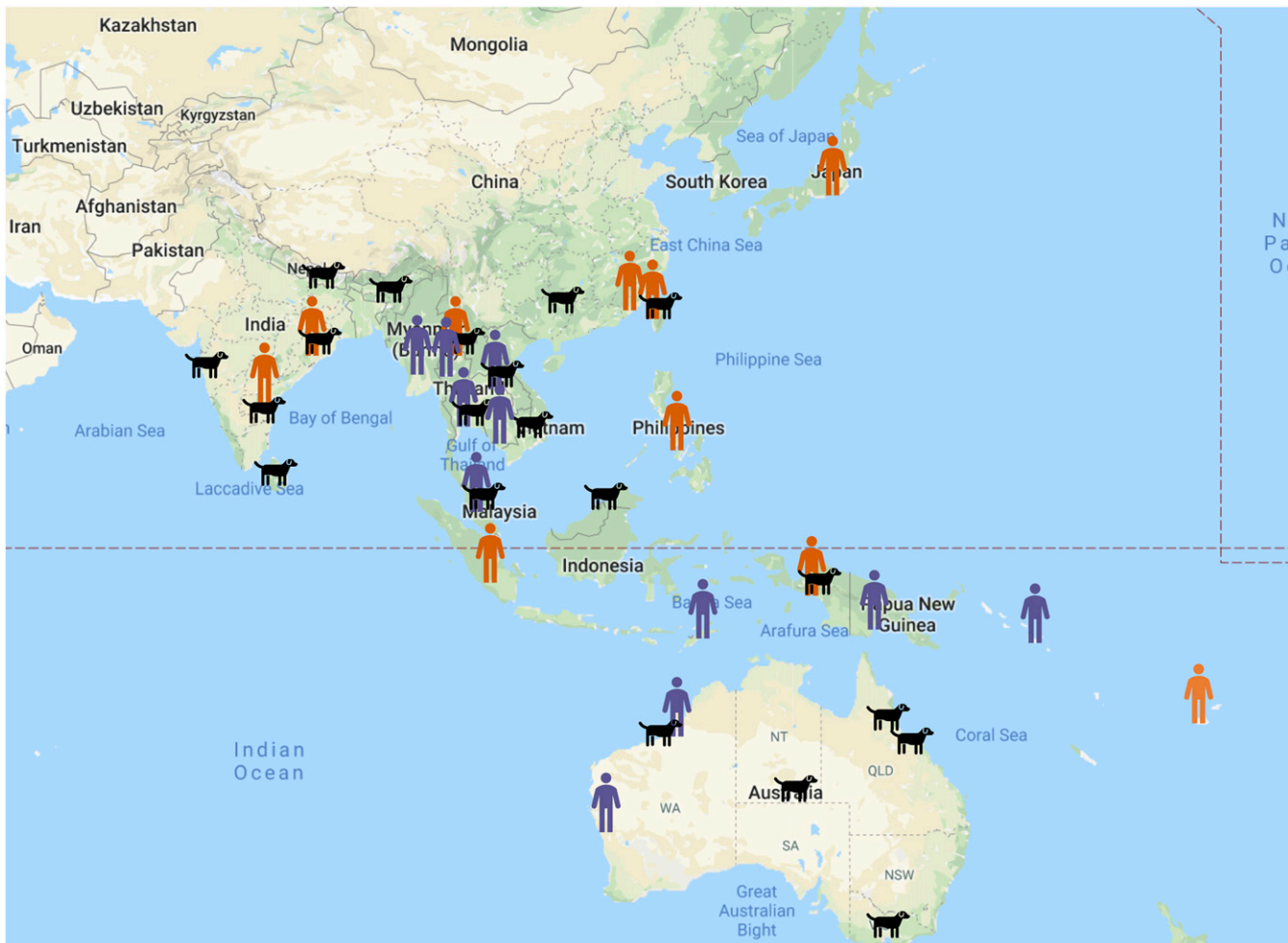

FIGURE 1. Known distribution of *Ancylostoma ceylanicum* infections in Southeast Asia and the Pacific. Human infections from 2013 or earlier are depicted in orange, human infections after 2013 to date are depicted in purple, and all canine infections are highlighted in black. Not depicted here are studies of canine *A. ceylanicum* infections from Madagascar, Kenya, Tanzania, and South Africa.

data in human hosts worldwide. These data will aid in mapping a more detailed picture of STH infections, inform governmental agencies about targeted treatment programs (including One Health approaches), and ultimately contribute to an eradication of not only hookworm infections but also helminth infections more broadly.

Authors' addresses: Katharina Stracke, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, and The Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Australia, E-mail: stracke.k@wehi.edu.au. Aaron R. Jex, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, and Faculty for Veterinary and Agricultural Sciences, The University of Melbourne, Melbourne, Australia, E-mail: jex.a@wehi.edu.au. Rebecca J. Traub, Faculty for Veterinary and Agricultural Sciences, The University of Melbourne, Melbourne, Australia, E-mail: rebecca.traub@unimelb.edu.au.

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