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Case Report

Rare case of human *Ancylostoma ceylanicum* infection in Bangladesh

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ABSTRACT

The zoonotic hookworm species *Ancylostoma ceylanicum* has drawn more attention recently because of its potential impact on public health. Although *A. duodenale* and *Necator americanus* are more common, *A. ceylanicum* is still known to play a major role in human infections, particularly in regions where close human-animal interactions are prevalent. While there has been a notable increase in documenting the presence of *A. ceylanicum* in the Asia-Pacific area, bottlenecks remains in understanding its epidemiology in Bangladesh. This report highlights the first documented case of *Ancylostoma ceylanicum* infection isolated and identified in a 15-year-old girl experiencing frequent diarrhea and weakness, residing in an urban tea garden area in Sylhet, Bangladesh. Microscopic examination of stool samples revealed the presence of hookworm eggs and subsequent culture led to the observation of larvae. Molecular investigation by amplifying Internal Transcribed Spacer (ITS1+) regions of the ribosomal deoxyribonucleic acid (rDNA) confirmed the infection as *A. ceylanicum*. The identification of *Ancylostoma ceylanicum* in a human host in Bangladesh carries significant implications for global health. The careful measurement of eggs and larvae, coupled with molecular analysis, serves as an appropriate diagnostic strategy for confirming the infections. This finding emphasizes the emergence of *A. ceylanicum* as a zoonotic infection in endemic regions and calls for increased awareness among healthcare professionals and the general public.

Introduction

Soil-transmitted helminths, including hookworms, roundworms, and whipworms, pose a substantial public health challenge because of their widespread among the Neglected Tropical Diseases (NTDs). Hookworm infections are prevalent in tropical and subtropical regions, with particularly high endemicity observed in the Asia-Pacific region. Humans naturally host three species of hookworms: *Necator americanus, Ancylostoma duodenale*, and *Ancylostoma ceylanicum*, embodying a delicate dance between nature and our existence [1]. The significance of *A. ceylanicum* lies in its capacity to cause infections in diverse hosts, indicating its potential for zoonotic transmission. Multiple studies demonstrated *A. ceylanicum*'s zoonotic potential by documenting human infections in areas where dogs and cats are common [2,3]. This zoonotic aspect adds a layer of complexity to hookworm epidemiology, necessitating a deeper understanding of the role of *A. ceylanicum* in human health. Given that helminthiasis is common in Bangladesh and there are close human-animal relationships, the significance of *A. ceylanicum* is particularly important. Existing literature predominantly focuses on *A. duodenale* and *N. americanus*, with limited exploration of the prevalence, clinical manifestations, and transmission dynamics of *A. ceylanicum* in human populations in this region. A study by Ngui et al. [3] emphasizes the importance of identifying and characterizing zoonotic hookworm infections, particularly in regions with close human-animal interactions. By identifying *A. ceylanicum* in humans from Bangladesh, this study aimed to advance knowledge of zoonotic hookworm infections and pave the way for targeted interventions and improved control strategies.

Methods

Fecal samples were collected from a 15-year-old girl residing in an urban tea garden in Sylhet, Bangladesh. The patient presented with recurrent diarrhea and weakness, prompting an investigation into potential parasitic infections. The formal ether sedimentation technique was employed for the initial examination of the stool sample [4]. Microscopic examination of the concentrated sediment revealed the presence of hookworm eggs. To further characterize the infection, the Baermann method was applied to isolate larvae from the charcoal-cultured

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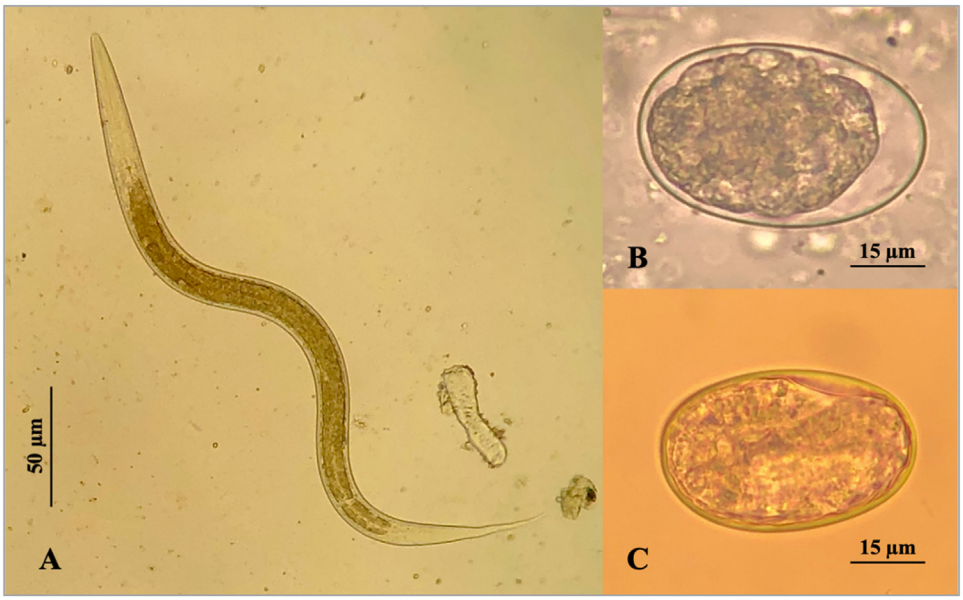

Figure 1. Microphotographs of *Ancylostoma ceylanicum*, (a) cultured larvae, (b, c) egg.

samples [5]. The isolated larvae were examined microscopically for morphological identification and then confirmed by amplifying Internal Transcribed Spacer (ITS1)+ region of the ribosomal deoxyribonucleic acid (rDNA). The primer AF (5′-GACTGCGGACTGCTGTAT-3′) and its complementary primer AR (5′-AAGTTCAGCGGGTAGTCA-3′) were designed according to Liu et al. [6]. DNA sequencing utilized a BigDye terminator kit (version 3.1, Applied Biosystems, Foster City, California, USA), and the resultant products were directly sequenced using a DNA sequencer (ABI3730XL, Applied Biosystems). Subsequently, the polymerase chain reaction (PCR) products underwent sequencing at Cosmogentech (South Korea). Sequences obtained were aligned using Clustal W and Bioedit software version 7.1. Analysis of sequencing data was conducted using the Basic Local Alignment Search Tool (BLAST) programs and databases available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and compared with sequences in the GenBank database. Phylogenetic analysis was performed to evaluate the genetic relationship of the isolated larvae with relevant hookworms in GenBank. Multiple sequence alignments were carried out using Geneious software version 9 (Biomatters, New Zealand), and the program Muscle within MEGA7 software facilitated the multiple alignments.

Result and discussion

The eggs (n = 10) with typical hookworm morphology; these eggs had a mean (±SD) length of 49.3 µm (±4.4 µm) and width of 27.8 µm (±2.3 µm) (Figure 1). The distinctive features, such as the thin-shelled appearance and characteristic morula stage, were consistent with the known morphological attributes of *A. ceylanicum* eggs [7]. The infectious larvae (n = 10) measured 651.1 ± 3.8 µm (633-669 µm) in total body length, 23.7 ± 0.4 µm (18-29 µm) in body width, 149.7 ± 5.1 µm (146-162 µm) in esophagus, and 75.1 ± 1.7 µm (72-79 µm) in tail, with mean ± SD (range) values (Figure 1). These morphometric traits are consistent with the previously reported typical traits of *A. ceylanicum* larvae [7,8].

PCR analysis was conducted to amplify the ITS+ regions of DNA that extracted from the isolated larvae. Within ITS+ regions, the PCR with the AF and AR primers yielded 415 bp of product, and sequence analysis with Basic Local Alignment Search Tool (BLAST) showed that the present specimen was 99.7% identical to *Ancylostoma ceylanicum* (KU996385). The phylogenetic tree constructed by maximum likelihood method revealed a distinct clustering of the *A. ceylanicum* sequence (Figure 2) with other hookworm isolates available in the NCBI database, supporting the accurate identification of the isolated hookworm.

The morphometric findings of *A. ceylanicum* larvae and eggs in this rare human infection case align with previously reported measurements for this species. The characteristic features, such as the size of larvae, width, and morphology of eggs, were consistent with the known features of *A. ceylanicum* [7]. Morphometric analysis is crucial in distinguishing different hookworm species, and the observed measurements provide additional confirmation of the accurate identification of *A. ceylanicum* in the studied case. The rarity of human *A. ceylanicum* infections in Bangladesh, as evidenced by the limited reports in the literature, underscores the significance of these morphometric findings. Morphological identification is often the initial step in diagnosing helminthic infections. This case contributes valuable data to the limited pool of morphometric information on *A. ceylanicum* in human infections, particularly within the context of Bangladesh, where information on this specific hookworm species is scarce.

The molecular findings from the PCR analysis targeting the ITS+ regions provided additional confirmation of the identity of the isolated *A. ceylanicum*. The high sequence homology with known *A. ceylanicum* sequences in the National Centre for Biotechnology Information GeneBank further supports the specificity of the molecular assay for detecting this particular hookworm species. The phylogenetic analysis reinforced the genetic distinctiveness of *A. ceylanicum*, showcasing a close relationship with other *A. ceylanicum* isolates and clear separation from other hookworm species. The molecular confirmation of *A. ceylanicum* in a human host in Bangladesh is particularly noteworthy. While this zoonotic hookworm species has been reported in the environment and other animals in the region [4], human infections are seldom documented. The molecular findings underscore the potential for zoonotic transmission of *A. ceylanicum* in areas with close human-animal-environment interactions, such as urban tea gardens in Bangladesh. A study by Inpankaew et al. [9] underscores the importance of molecular techniques for accurate identification of zoonotic hookworm species, emphasizing their role in understanding transmission dynamics. The integration of molecular tools in this study will enhance the precision of species identification, contributing to a more nuanced understanding of *A. ceylanicum* infections in the context of Bangladesh. This is particularly relevant in regions where multiple hookworm species coexist, as accurate identification is crucial for tailoring treatment strategies and implementing effective control measures [10].

The life cycle of *A. ceylanicum* is direct, devoid of any intermediate hosts, with dogs, cats, and humans serving as the primary definitive hosts. In the context of Bangladesh, where helminthic infections are prevalent and often linked to socioeconomic factors and environmental

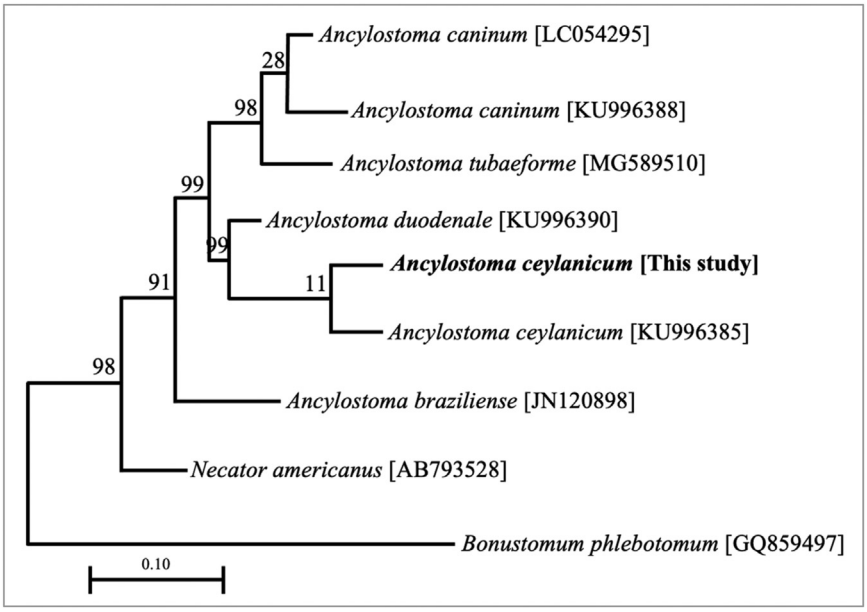

Figure 2. Phylogenetic tree of *Ancylostoma ceylanicum* isolates reconstructed by maximum-likelihood method based on the ITS+ sequences, using *Bonustomum phlebotomum* (GenBank™ Accession Nos. GQ859497) as the outgroup. The reference sequences are available in the GenBank by their accession numbers. Numbers at the branch nodes indicate percentage bootstrap support for 1000 replicates.

conditions, the rarity of human *A. ceylanicum* infections is significant [11]. The findings emphasize the need for increased awareness among healthcare professionals and researchers regarding the potential for zoonotic transmission. Globally, the identification of *A. ceylanicum* in a human host adds to the understanding of the geographical distribution of this zoonotic hookworm. This case report contributes to the growing body of evidence indicating the potential emergence of *A. ceylanicum* as a human pathogen, necessitating vigilance in monitoring and controlling zoonotic infections.

While this study provides crucial insights into human *A. ceylanicum* infection, several limitations warrant consideration. An in-depth clinical history that includes particular animal exposures is lacking, which makes it difficult to investigate potential risk factors and transmission pathways. Furthermore, the reliance on a cross-sectional design restricts the study's capacity to capture the dynamic aspects of the infection over time. While molecular techniques were employed for species confirmation, the potential cross-reactions with closely related hookworm species introduce uncertainties in the accuracy of species differentiation. These limitations highlight the need for cautious interpretation and emphasize the necessity for further research with larger sample sizes.

To sum up, the combined morphometric and molecular findings provide a comprehensive understanding of a rare case of human *A. ceylanicum* infection in Bangladesh. The morphometric data contribute to the broader understanding of helminth diversity, while the molecular analysis offers insights into the genetic characteristics of this zoonotic hookworm. These findings have implications for both local health practices in Bangladesh and global efforts in the surveillance and control of zoonotic helminth infections.

Declaration of competing interests

There is no conflict of interest declared by any of the authors.

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Ethical approval

This study protocol was reviewed and approved by the Sylhet Agricultural University Research System and the Department of Parasitology, Sylhet Agricultural University, Bangladesh. The corresponding author personally obtained written consent for publication from the parents of the patient.

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Author contributions

TCN wrote the first and final drafts of this manuscript. All other authors made significant contributions to laboratory experiments, analysis, and the revision of the manuscript. The final version of this manuscript was vetted and approved by all authors.

Availability of data and materials

The datasets and resources used in this current study are available from the corresponding author upon reasonable request.

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