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CASE REPORT

A Case of Zoonotic *Ancylostoma ceylanicum* Infection in a Suburban Area of Selangor, Malaysia

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Abstract

Introduction The animal hookworm, *Ancylostoma ceylanicum*, is a dominant hookworm species of dogs and cats. However, it has increasingly been found infecting humans in Southeast Asia.

Purpose We report an autochthonous case of *A. ceylanicum* in a suburban area of Selangor, Malaysia. A 66-year-old Indian lady who is an avid gardener presented with chronic diarrhea of 4 months' duration.

Methods The patient was examined clinically and colonoscopy was performed. Adult parasites obtained via colonoscopy were subjected to microscopy and molecular investigations.

Results Clinical examinations were unremarkable, and blood investigation revealed normochromic normocytic anemia. Stool occult blood was positive but negative for ova, cyst and adult parasites. Colonoscopy performed showed multiple diverticulae and worm infestation from the terminal ileum to sigmoid colon. Morphological examination on the adult worms showed the specific characteristics of *Ancylostoma* species. Molecular investigations further confirmed the nematode as *Ancylostoma ceylanicum*. She was treated with albendazole 400 mg daily for 3 days with symptomatic improvements sustained 3 months later. It is suspected that the patient had ingested or contacted soil contaminated with filariform larvae while gardening.

Conclusion Information on the *A. ceylanicum* infection in humans, especially in urban and suburban areas, is limited, necessitating further epidemiological and clinical studies.

Keywords Zoonotic hookworm · *Ancyclostoma* · *Ceylanicum* · Colonoscopy · Ancylostomiasis · Malaysia

Introduction

The animal hookworm, *Ancylostoma ceylanicum*, is a dominant hookworm species of dogs and cats and is the second most common hookworm infecting humans in southeastern Asia [1, 2]. In 1966, investigations on hookworm infections in dogs, cats, and man carried out in Taiwan showed *A. ceylanicum* found in 2 of 12 aborigines and 3 of 128 Chinese of the general population [3]. Other sporadic reports included one case in Philippines [4], 16 positive out of 183 persons in Calcutta, India [5], 2 cases in Thailand's temple communities [6] and 29.6% of the population in Cambodia (2012) [2]. In 2017, molecular identifications of human hookworms from certain parts of rural Lower Myanmar identified three sequences with high similarity of *A. ceylanicum* [7]. Recently. US-bound refugees living in Myanmar–Thailand border camps revealed that the prevalence of *N. americanus* hookworm was 25.4%, *A. duodenale* 0%, and *A. ceylanicum* 5.4% [8]. Besides Asia, *A. ceylanicum* is considered an emerging public health risk in tropical north Australia where the infection has been detected in domestic dogs in an indigenous community and present in soil samples at popular tourist locations [9]. Also, *A. ceylanicum* is endemic in the East Malaita region of Solomon Island, a Pacific Island nation located near to the east of Papua New Guinea [10].

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Intestinal helminths infection in Malaysia had been reported with a wide prevalence of 0.7% up to 92.7% depending on the cohort area and demographics [11, 12]. Species identifying human hookworm infections among eight economically disadvantaged communities in rural areas of Peninsular Malaysia were determined molecularly and *N. americanus* was the most predominant hookworm identified, followed by *A. ceylanicum* [13]. In another study, molecular analysis was performed on 634 human and 105 domestic canine and feline fecal samples in Malaysia and revealed that most hookworm-positive individuals were infected with *N. americanus, A. ceylanicum* contributed 12.8% of single infections and 10.6% mixed infections with *N. americanus*. As for cats and dogs, more than half were positive for *A. ceylanicum*, followed by *Ancylostoma caninum* and *Ancylostoma braziliense* [14].

Although most hookworm studies were concentrated on rural area in Malaysia, recent reports indicate increasing prevalence among the urban population [15, 16]. Here, we report an autochthonous case of *A. ceylanicum* in a suburban area of Selangor, Malaysia.

Case Report

A 66-year-old Indian lady, who is an avid gardener, presented with chronic diarrhea (Bristol Type 6 and 7) for the last 4 months before she visited our center. There was no mucus or blood in the stool detected macroscopically. She has type 2 diabetes mellitus (HbA1c 9.6%) and hypertension. She has no family history of gastrointestinal pathology and is a non-smoker. Clinical examinations were unremarkable, and investigations revealed normochromic normocytic anemia (hemoglobin 10.3 g/dL) with no eosinophilia. Stool occult blood was positive but negative for ova, cyst and adult parasites. Colonoscopy was performed to rule out colorectal carcinoma. It showed multiple diverticulae and worm infestation from the terminal ileum to the sigmoid. Ten worms were visualized, with three extracted for identification (Fig. 1). Morphological examination of the adult worms showed the specific characteristics of *Ancylostoma* species (teeth) (Fig. 2). For species identification, the adult worm was subjected to a polymerase chain reaction (PCR) targeting the internal transcribed spacer-2 (ITS-2) ribosomal RNA gene [17]. In brief, the worm was mechanically grounded with a sterile scalpel using a vortex. The homogenate was then digested with proteinase K, followed by genomic DNA extraction using a commercial kit (MachereyNagel, Neumann-Neander, Duren, Germany). DNA amplification was performed, and the positive amplicon was subjected to DNA sequencing. Homology search using the National Center for Biotechnology Information (NCBI) reference sequences with Basic Local Alignment Search Tool (BLAST) confirmed the species as *A. ceylanicum* with 99.7% identity to the reference sequences deposited in the GenBank (GenBank accession number JN164659.1, JF960369.1, LC036567.1). Our observed consensus ITS-2 sequence was available in GenBank under the accession number MT925626. She received albendazole 400 mg once daily for 3 days with symptomatic improvements and hemoglobin returning to a healthy 12.6 g/dL at 3 months and 13.2 g/dL at 6 months.

Fig. 1 Colonoscopic view of the adult worm, *Ancylostoma ceylanicum*, in the present case

Discussion

Ancylostoma ceylanicum is a hookworm of cats and dogs that have long been known to have established patent enteric infections in humans and has been considered the most neglected among all human hookworm species [18]. However, it is still impossible to differentiate eggs of all hookworm to species level based on the light microscopic techniques during direct fecal examination [19]. With the advent of PCR methods, differentiation between all hookworm species in humans, dogs, and cats is now feasible.

Ancyclostoma ceylanicum has been experimentally tested on human volunteers. In Thailand, the main signs and symptoms of the human volunteer include itching, urticaria-like skin lesions on the arm, becoming nodular in 1 day and developing blebs with edema. This symptom disappeared on the 6th day; there was irritation of the throat and dry cough at night after 12 days; hookworm eggs first appeared in the feces 32 days after the infection. The eosinophil count rose to a maximum of 58% on the 28th day, while chest X-rays remained negative. Post-treatment with tetrachloroethylene was given and revealed a total of 87 adults *A. ceylanicum* [20]. Another human volunteer study was published after a year where three human volunteers were infected orally and another three percutaneously with *A. ceylanicum* obtained

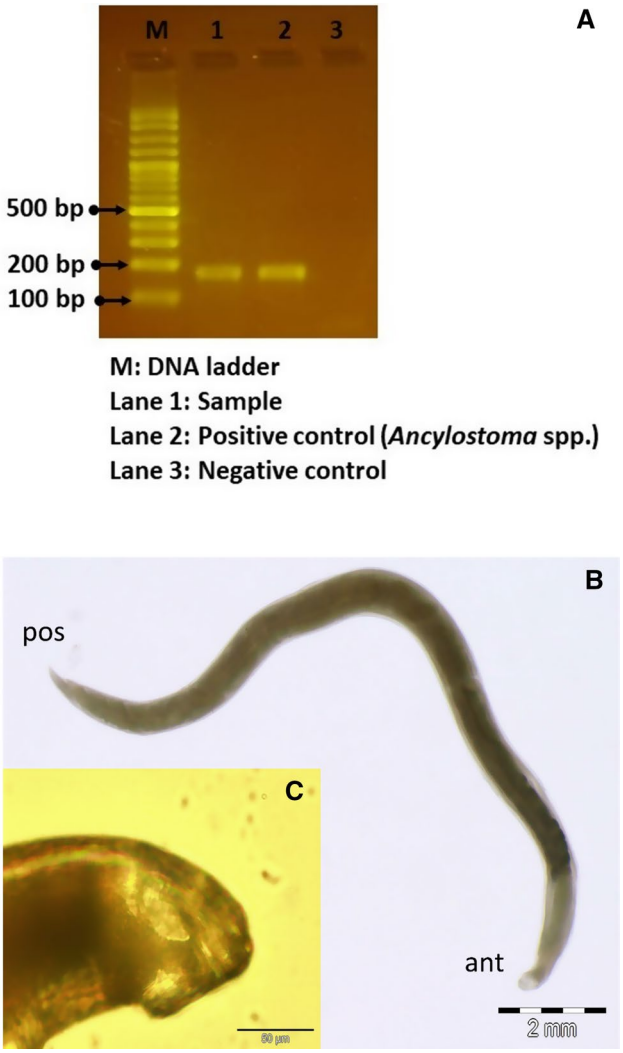

Fig. 2 **A** Gel electrophoresis image suggestive of *Ancylostoma* species. **B** The extracted *Ancyclostoma ceylanicum* adult worm via colonoscopy. C) The mouthpart of the extracted hookworm under light microscopy. ant = anterior; pos = posterior

from a cat. Oral infections became apparent in 18–26 days, at which time the two subjects in whom the adult worms were counted, 43% and 61%, had matured into the adult stage, respectively. However, less than 5% of larvae given percutaneously developed and the creeping eruption was not seen, indicating the more effective way of hookworm maturation in human hosts could be the oral route [21]. Heavy infection can result in bloody diarrhea and iron deficiency anemia, but unlike the other human hookworms, it can also cause severe enteritis and cognitive impairment [22]. Our patient experienced chronic diarrhea and anemia with colorectal carcinoma and helminths infestation low on the list, inexplicably resulting in inadequate social history.

Molecular diagnostic investigations can be used to confirm the diagnosis and allowed the identification of *A. ceylanicum*. The cytochrome c oxidase subunit 1 (cox 1) sequence of *A. ceylanicum* was phylogenetically analyzed. The results demonstrated that isolates of *A. ceylanicum* were divided into two distinct clusters, which might suggest potential haplotype-linked differences in zoonotic, epidemiological and pathobiological characteristics [23]. Recently, a novel, species-specific, real-time PCR-based assay for detecting *A. ceylanicum* has been developed to identify the species of hookworms [24].

In Malaysia, a case of A. *ceylanicum* infection was detected by endoscopy in a 58-year-old woman who lives in a rural area where uncontrolled populations of stray and semi-domesticated dogs are common [25]. Similarly, a Japanese patient who returned from a visit to Thailand and Lao People's Democratic Republic (PDR) was diagnosed with *A. ceylanicum* infection [26]. Another 25-year-old Japanese biologist who stayed in a rainforest in Malaysia for 4 weeks collecting spiders developed abdominal pain and diarrhea in the third week. A total of 11 adult worms were expelled after treatment with pyrantel pamoate and were identified as *A. ceylanicum* based on morphological characteristics and DNA sequences of the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene [27]. A recent study on hookworm infections among migrant workers in Malaysia was conducted. The identified PCR amplicons revealed 81.0% identified as *Necator americanus*, 16.7% as *Ancylostoma* spp. and 2.4% as mixed infections of both species. All eight *Ancylostoma* spp. were confirmed to be *Ancylostoma duodenale,* albeit no *A. ceylanicum* was identified through this study [28].

It is suspected that our patient had ingested or contacted soil contaminated with filariform larvae while gardening in an area where stray cats and dogs are commonly seen. It is known that both *A. ceylanicum* and *A. braziliense* are found abundantly among dogs and cats in Malaysia [29]. However, a molecular survey had identified *A. ceylanicum* as being the predominant species among urban stray dogs. In contrast, rural dogs had a higher prevalence of *A. caninum* than *A. ceylanicum*, while both species demonstrated equal distribution among dogs in shelters [30]. Furthermore, a molecular survey on helminth eggs excreted in the faeces of stray cats, dogs, and soil samples collected from Klang Valley, a central-west region of Peninsular Malaysia, indicated that *A. ceylanicum* was dominant in both faecal and soil samples in the area [31].

Hookworm infection is well associated with iron deficiency anemia. Its infection can be easily treated with a common anthelminthic drug such as albendazole [32]. Although albendazole is still an effective treatment as presented in the current case, there is a need to acknowledge that gastrointestinal nematodes are becoming more resistant to the available antihelmintic drugs. Also, there is no new anthelmintic on the market for quite some years [33]. Nevertheless,

vaccine using recombinant antigens of *A. ceylanicum* has been explored on animal models, and the immunization with a vaccine cocktail resulted in a 33.5% worm burden reduction in hamsters [34].

More zoonotic hookworm species can cross species and mature into adults other than their regular hosts, and it is expected that emerging infectious diseases will be of zoonotic origin [35]. For example, in South Korea, Jung et al. [36] reported a 60-year-old man who underwent a colonoscopy and recovered a juvenile female worm with three pairs of teeth. DNA sequencing showed 100% identity with *A. caninum*, a species of dog hookworm. A systematic review approach on research studies and case reports on human *A. ceylanicum* infections in Southeast Asia and the Pacific has been published. Therefore, a One Health approach to zoonotic hookworm control in populations in places where this zoonosis is still endemic is strongly recommended [37].

Conclusion

The epidemiology of *A. ceylanicum* is poorly known, especially in urban and suburban areas in Peninsular and Malaysian Borneo, necessitating further epidemiological and clinical studies. Furthermore, on a larger scale, the "hotspots" of zoonotic hookworm infection need to be identified in Asia and Pacific regions, and appropriate prevention and control measures should be implemented to counter the disease emergence soon.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval Not applicable.

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