



Endoscopic Detection and Molecular Confirmation of *Ancylostoma ceylanicum* with Atypical Gastrointestinal Manifestations: A Case Series

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Abstract

Background Hookworm is a widely known soil-transmitted helminth (STH) traditionally linked to iron-deficiency anemia caused by chronic blood loss in the small intestine. The classical human pathogens are *Ancylostoma duodenale* and *Necator americanus*. However, emerging evidence indicates that *Ancylostoma ceylanicum*, a hookworm of cats and dogs, is a significant cause of human infection, particularly in Southeast Asia and other tropical regions. Its detection in human populations signals an evolving epidemiological landscape, challenging the previous notion that zoonotic hookworms have a negligible impact on human health.

Case Series This series presents three atypical cases that challenge classic paradigms of hookworm disease. In all cases, adult worms were retrieved from the colon by endoscopy and subsequently identified by molecular analysis. The first case is a young man with profound eosinophilia and watery diarrhea. Adult hookworms were found ectopically in the colon, an atypical site, demonstrating the worm's potential for aberrant migration and a severe systemic immune response. The second case is an elderly male with multiple chronic conditions whose refractory anemia was unexpectedly attributed to a colonic infection discovered during a routine exam, underscoring hookworm as a treatable cause of anemia in geriatric populations. The third case is a young female with colitis-like symptoms. Colonoscopy revealed widespread superficial ulceration and live worms, highlighting the mimicry of inflammatory bowel disease.

Conclusion Collectively, these cases emphasize the diagnostic value of endoscopy in identifying hookworm infection, its diverse clinical manifestations beyond chronic anemia, and the requirement for the consideration of hookworm infection in the differential diagnosis of eosinophilia, unexplained colitis, and refractory iron deficiency across a wide demographic spectrum.

Keywords *Ancylostoma ceylanicum* · Zoonotic hookworm · Colonoscopy · Eosinophilia · Atypical presentation

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Introduction

Ancylostoma ceylanicum, traditionally known as a zoonotic hookworm affecting dogs and cats, has become an important human pathogen, especially in Southeast Asia and other tropical areas. Studies now identify *A. ceylanicum* as the third leading human hookworm, alongside *Ancylostoma duodenale* and *Necator americanus* [1]. Transmission to humans occurs through environmental exposure, primarily in areas where stray dogs and cats are prevalent. Research indicates a direct link between domestic animal infections and zoonotic transmission, mainly through contaminated soil or feces, raising significant public health concerns [2]. Epidemiological evidence suggests that these infections are not only endemic in areas with high poverty rates but also manifest in urban settings where stray dogs and cats are prevalent, indicating a complex interplay between animal and human health within shared environments [2].

Clinically, *A. ceylanicum* causes symptoms in humans similar to those of traditional human hookworms (*A. duodenale* and *N. americanus*), including gastrointestinal symptoms, iron-deficiency anemia, and eosinophilia [3, 4]. The parasite's ability to exploit humans as definitive hosts, together with the complex immune response, poses ongoing challenges for transmission modeling and the development of treatments [5]. Its zoonotic nature underscores the need for improved surveillance and diagnostic methods, particularly in endemic areas where human and animal infections coexist [6–10]. Notably, Heo et al. previously reported ectopic localization of *A. ceylanicum* in the terminal ileum and sigmoid colon [11], further supporting the atypical presentations described in this series.

This report details cases of human *A. ceylanicum* infections acquired in urban Kuala Lumpur, the capital city of Malaysia, aiming to expand understanding of its clinical features, epidemiology, and management. This case series presents three uncommon cases of ectopic colonic infection, challenging the typical view of hookworm as a small-intestinal pathogen. These cases cover a wide demographic range, from a young woman with colitis resembling inflammatory bowel disease, to an older man with multiple health issues and refractory anemia, to a young male with severe eosinophilia, highlighting the varied and often overlooked clinical presentations of this emerging zoonosis. Overall, these cases emphasize the importance of colonoscopy and molecular testing in diagnosing unexplained gastrointestinal issues, anemia, or eosinophilia, and aim to increase awareness of this significant and evolving public health concern.

Case Series

Case 1

A 34-year-old male with no significant past medical history presented with a one-month history of persistent, watery diarrhea and an unintentional 2 kg weight loss. He reported engaging in regular outdoor activities but no recent travel to underdeveloped countries or to unsanitary places. Physical examination was notable only for mild, diffuse abdominal tenderness. Initial laboratory workup revealed profound peripheral eosinophilia (64%). Routine stool microscopy for ova and parasites was performed using both the direct wet mount and the formalin-ether concentration techniques. No hookworm ova were detected. Given the marked eosinophilia and persistent symptoms despite negative stool examination, a colonoscopy was performed. The procedure revealed multiple live, motile adult worms attached to the mucosa of the ascending and transverse colon, with associated mucosal erythema and edema. No features of inflammatory bowel disease were seen. A total of 7 worms (5 male, 2 female) were retrieved endoscopically. Microscopic examination confirmed adult *Ancylostoma* by characteristic morphological features, including a male copulatory bursa with multiple spicules and a well-developed buccal capsule with paired ventral teeth (Fig. 1a, b). One male and one female worm were examined in detail morphologically, while a single worm was used for molecular identification. Numerous typical hookworm ova were also observed within a ruptured uterus of a female worm (Fig. 1c).

Definitive speciation by bursal morphology was inconclusive. Therefore, molecular identification was performed. Genomic DNA was extracted from a homogenized worm specimen using a commercial kit (1st BASE, Apical, Malaysia). The internal transcribed spacer-2 (ITS-2) region of ribosomal DNA was amplified by PCR. PCR amplification (Fig. 2) and subsequent sequencing of the target region were conducted. A consensus sequence derived from forward and reverse reads was used for species assignment. 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* (99–100% identity across all three cases). A definitive diagnosis of *A. ceylanicum* infection with colonic involvement was made. The clinical presentation, watery diarrhea, weight loss, and extreme eosinophilia, was attributed to direct colonic mucosal irritation and a systemic immune response to the parasites. The patient was treated with a single 400 mg dose of albendazole, with a repeat dose scheduled for two weeks later to ensure eradication of the worm. Supportive care includes oral rehydration

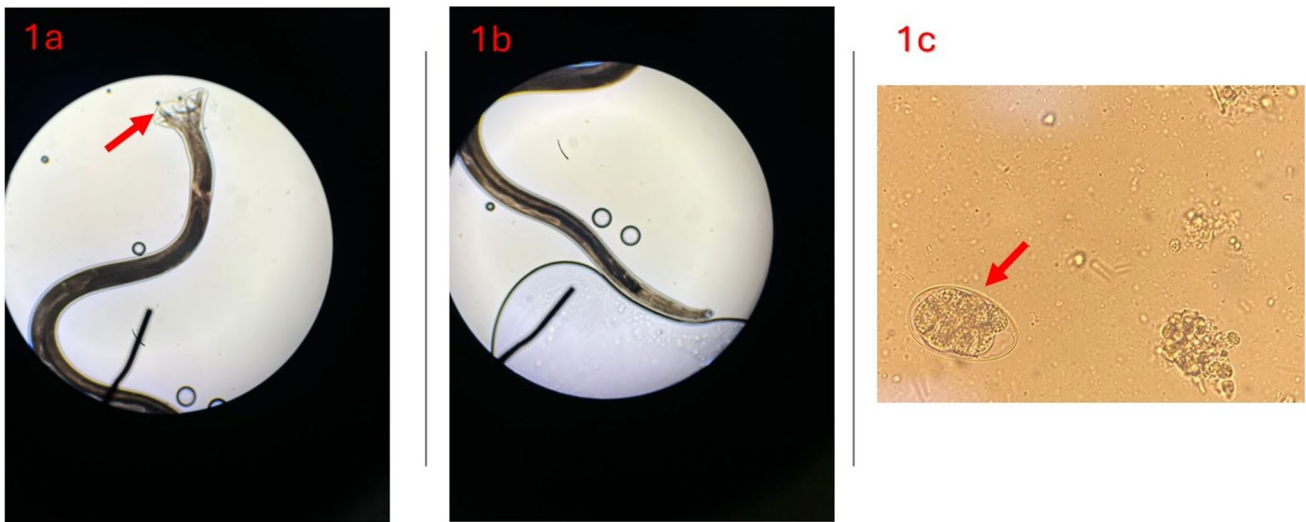


Fig. 1 Microscopic examination of retrieved adult *Ancylostoma* worms in case 1. **a, b** male worm showing the characteristic copulatory bursa with multiple spicules. **c** female worm with a ruptured uterus containing numerous typical hookworm ova

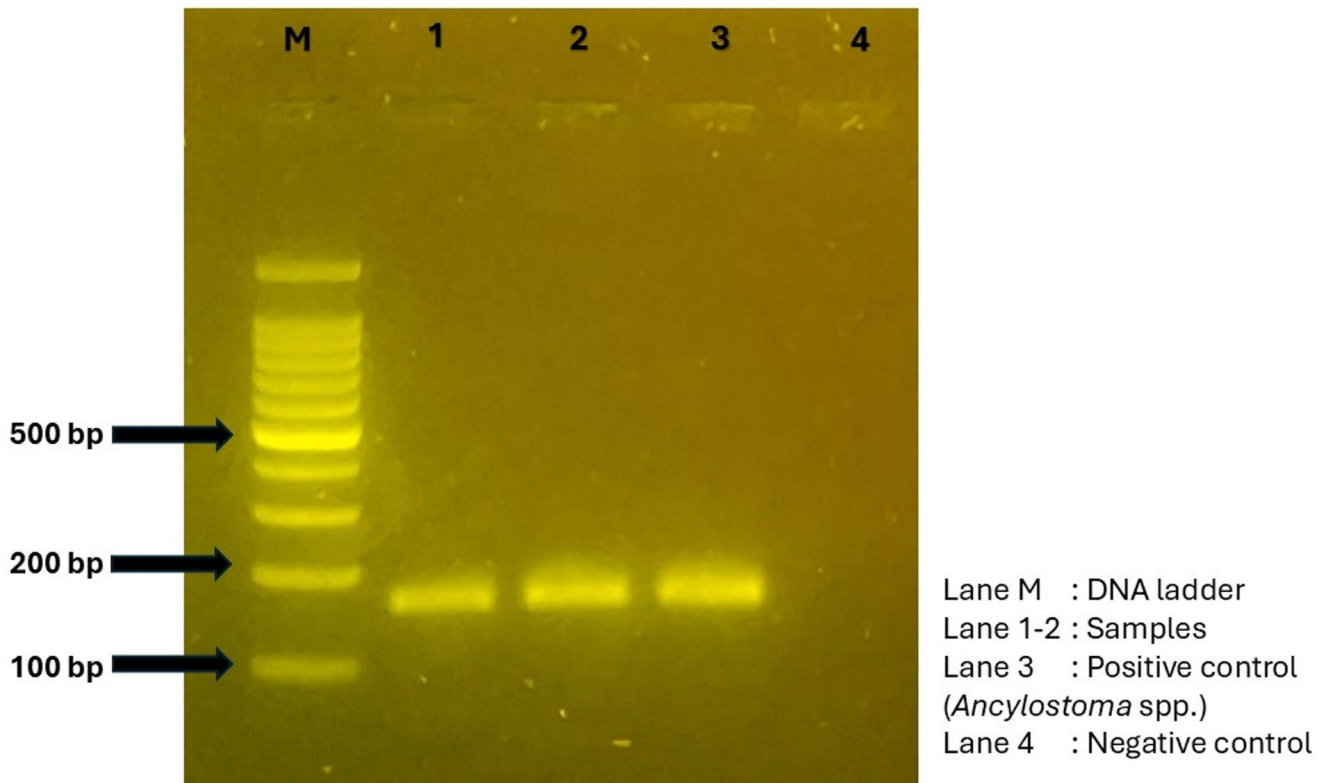


Fig. 2 PCR amplification products visualized by agarose gel electrophoresis, confirming successful amplification of target DNA regions for molecular identification

and nutritional support to address weight loss and fluid loss from diarrhea.

Case 2

A 73-year-old male with a complex medical history, including Type 2 diabetes mellitus, hypertension, a history of treated pulmonary tuberculosis, Stage 4 chronic kidney disease (CKD 4), and Barrett’s esophagus, presented with

chronic constipation and symptomatic anemia (hemoglobin 10.6 g/dL). The anemia had been previously ascribed to his CKD, chronic disease state, and possible gastrointestinal blood loss. As part of his management, he underwent endoscopic surveillance for Barrett's esophagus and a colonoscopy for constipation. While the upper endoscopy was unremarkable for acute bleeding, the colonoscopy revealed a significant, unexpected finding. There were 4 live, motile worms (2 males, 2 females) attached to the mucosa of the cecum and the ascending colon, with associated erythema and superficial erosions (Fig. 3). The remainder of the colon was normal.

All four were examined morphologically, and one was used for molecular analysis, confirming *A. ceylanicum*, consistent with the prior case (Case 1). The final diagnosis was *A. ceylanicum* infection, identified as the primary cause of

his iron-deficiency anemia. His constipation was attributed to multifactorial causes, including age, medications, and immobility from a known right-sided hemiparesis. Management was tailored to his comorbidities. Given his Stage 4 CKD, a carefully monitored single dose of albendazole 400 mg was administered without immediate repetition. Intravenous iron sucrose was administered to expedite the correction of anemia, circumventing the limitations associated with oral absorption tolerability. Care was coordinated with the Nephrology and Hematology team. Follow-up with a repeated full blood count and stool examination was scheduled to monitor treatment efficacy and confirm parasitological cure.

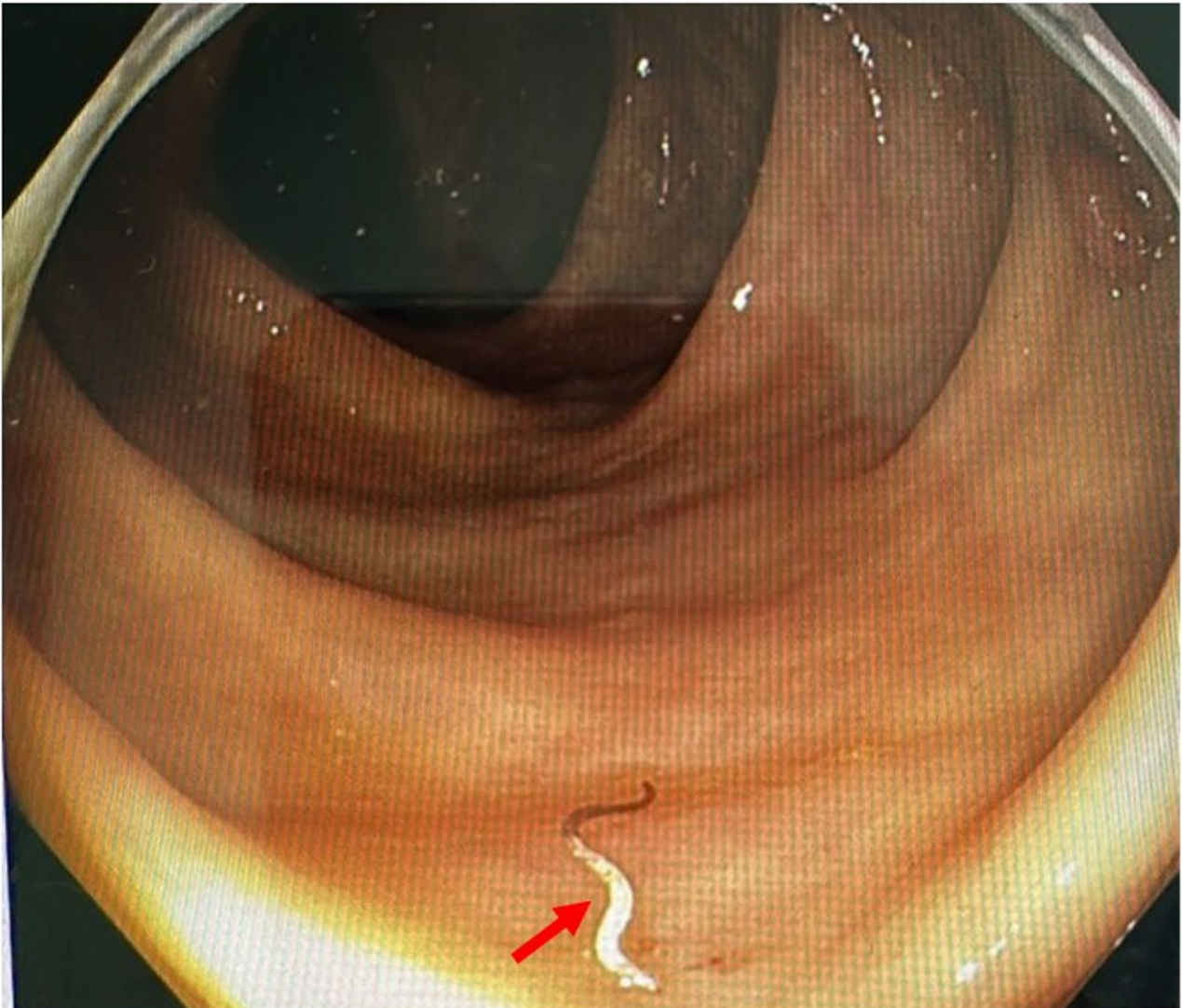


Fig. 3 Colonoscopic findings in case 2 show live, motile worm attached to the mucosa of the cecum and ascending colon, with associated erythema and superficial erosions

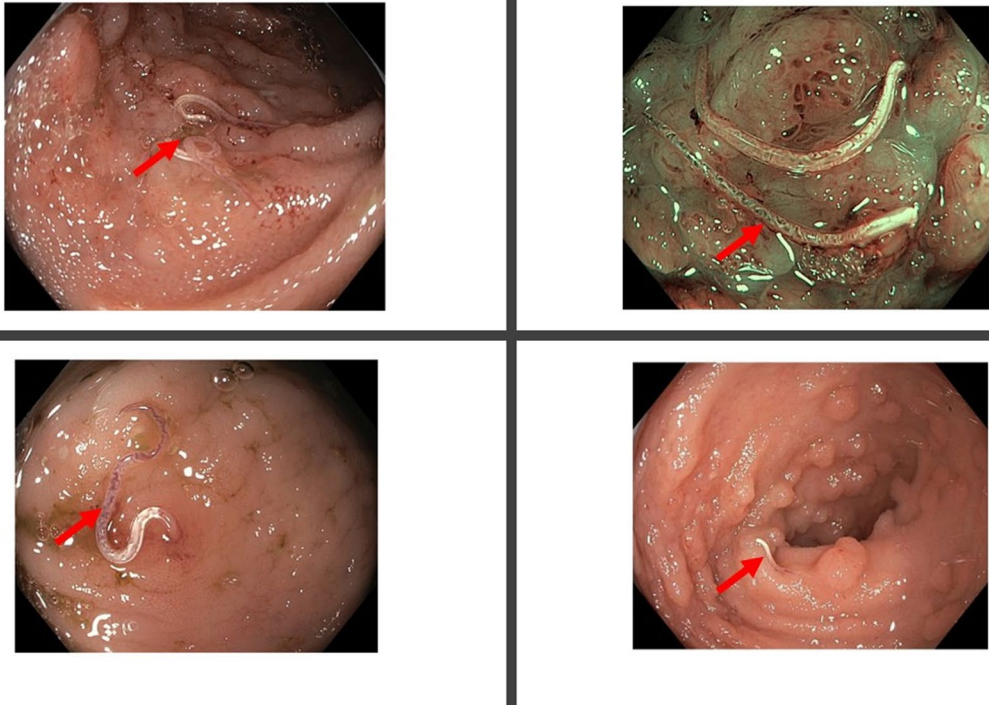


Fig. 4 Endoscopic image from case 3 showing live, motile worms attached to the colonic mucosa, with surrounding superficial ulcerations

Case 3

A 21-year-old female university student presented with chronic gastrointestinal symptoms, including abdominal discomfort, altered bowel habits, and possible anemia signs such as fatigue. She underwent a diagnostic colonoscopy to rule out inflammatory bowel disease. The procedure revealed extensive mucosal abnormalities in the right colon and terminal ileum, with abnormal findings at the hepatic flexure, ascending colon, cecum, and ileocecal valve. Multiple superficial 1 mm ulcers were seen in the cecum and ascending colon, and the terminal ileum showed a nodular appearance consistent with lymphoid hyperplasia or prominent Peyer's patches, indicating chronic immune stimulation. Endoscopy also captured 6 live, motile worms (4 males, 2 females) attached to the colonic and ileal mucosa (Fig. 4). Molecular analysis of one worm identified *A. ceylanicum*. The patient was prescribed a standard anthelmintic regimen of albendazole 400 mg once daily for three days. She was also prescribed ferrous fumarate, folic acid, and vitamin B complex, along with pantoprazole for stomach protection. A follow-up was planned for one month to evaluate symptom improvement.

Discussion

This case series presents three distinct clinical presentations of *A. ceylanicum* infection that collectively challenge traditional paradigms of hookworm disease and underscore the emerging role of this zoonotic species as a significant human pathogen [1–11]. By documenting cases that deviate from classic symptomatology, including colonic localization, variable eosinophilic response, and occult anemia in elderly multimorbid patients, this series extends the recognized demographic and clinical spectrum of hookworm infection. Furthermore, it highlights the critical diagnostic and epidemiological value of integrating endoscopy with molecular confirmation for accurate species identification and informed public health surveillance.

Hookworm infection, traditionally linked to *A. duodenale* or *N. americanus*, is generally considered a disease of the small intestine, where adult worms attach to the jejunal mucosa, causing chronic blood loss and iron-deficiency anemia [3, 4]. We discover that adult hookworms can also inhabit the colon, an ectopic and often overlooked site. Cases 1 and 3 showed extensive colonic involvement with superficial ulcers, whereas Case 2 had an infection limited to the cecum and ascending colon. These findings challenge the conventional understanding that hookworms reside only in the small intestine. Colonic localization may explain atypical symptoms such as watery diarrhea (Case 1) rather

than classic anemia, as direct mucosal irritation in the colon can affect fluid absorption and transit [12]. Furthermore, the endoscopic findings in Case 3 revealed superficial ulcers and nodular lymphoid hyperplasia, which closely resembled inflammatory bowel disease (IBD). This highlights the importance of including hookworm infection in the differential diagnosis of colitis and ileitis, particularly in endemic regions [13].

Our finding of colonic involvement aligns with several recent case reports from Asia and South America. Wu et al. identified hookworm in the colon using capsule endoscopy in a patient with acute infection [14]. Wang et al. described a healthy adult with severe eosinophilia and diarrhea, and colonoscopy revealed colonic hookworms [15]. Chen et al. similarly reported a case of colonoscopic detection of hookworm infection in Taiwan [16]. Most recently, Ramirez-García et al. documented colonic hookworm infection in the Peruvian Amazon and provided a literature review highlighting the diagnostic value of endoscopy [17]. Together with our cases, these reports suggest that colonic localization may be more common than previously recognized and should be considered when evaluating patients with unexplained gastrointestinal symptoms, even in the absence of anemia.

Eosinophilia is a hallmark of the T-helper-2 (Th2)-mediated immune response to tissue-invasive helminths, and its presence should prompt strong consideration of parasitic infection [18], even when initial stool microscopy is negative, a well-recognized limitation due to intermittent ova shedding and suboptimal sample handling [19]. Case 1 exemplified this with extreme peripheral eosinophilia (64%), reflecting a vigorous systemic response to migrating larvae and adult worm antigens. In contrast, the absence of eosinophilia in Cases 2 and 3 illustrates the variable host immune response to chronic hookworm infection, which may be modulated by factors such as parasite burden, host genetics [3, 4], or underlying immunosuppressive conditions, including chronic kidney disease [20] and diabetes mellitus [21]. Accordingly, clinical suspicion for hookworm should remain high even in the absence of eosinophilia, particularly in elderly or multimorbid patients.

Hookworm infection is typically seen as a disease affecting children and young adults in resource-limited areas [3, 4]. However, Case 2 is a 73-year-old man with multiple comorbidities, including Stage 4 CKD, diabetes, and heart failure, in whom a *A. ceylanicum* infection was incidentally found during a routine colonoscopy for constipation. This case highlights an often-overlooked at-risk group, including elderly individuals, where parasitic infections are frequently missed because anemia and fatigue are attributed to chronic underlying conditions. In this patient, refractory anemia, initially linked to CKD, chronic inflammation, and

possible angiodysplasias, is ultimately explained by a colon hookworm burden. The lack of diarrhea and the presence of constipation differ from typical symptoms, showing that atypical gastrointestinal signs do not exclude significant parasitic infections. These findings support emerging research reporting intestinal worm infections in older populations and emphasize that, in older patients with multifactorial anemia, careful investigation for reversible causes, including hidden gastrointestinal blood loss from hookworm, is essential [22].

In all three cases, the diagnosis was made by direct endoscopic visualization, followed by the removal of live adult worms and molecular identification. This method was essential because routine stool microscopy was negative in Cases 1 and 3, despite worm burdens, an often-encountered challenge due to intermittent egg shedding, low larval density, or sample processing issues [23]. Colonoscopy not only provided immediate, clear confirmation of the ongoing infection but also allowed therapy during the same procedure. This was especially important in Case 2, where chronic constipation and multifactorial anemia masked a treatable parasitic cause. These cases highlight that when clinical suspicion is high, especially with unexplained eosinophilia, persistent anemia, or colitis-like symptoms, endoscopy is a vital diagnostic tool, providing direct visualization and facilitating prompt, targeted treatment [24].

Molecular analysis of the worms, performed by PCR and DNA sequencing, confirmed *A. ceylanicum* in all three cases. This step is crucial because species-level identification cannot rely solely on morphology, given the subtle differences in buccal capsule and bursal structures among *Ancylostoma* species [25]. While species identification does not impact individual treatment, since albendazole is effective against all common human hookworm species, it has significant epidemiological importance. It helps us understand transmission patterns, reservoir hosts, and zoonotic sources, which are vital for public health surveillance and control strategies [26]. Managing these cases also highlights the necessity for personalized, context-aware treatment plans. In healthy young patients, standard albendazole 400 mg (either a single dose or a three-day course) was adequate. In contrast, the elderly patient with Stage 4 CKD needed careful dose adjustments and close monitoring to prevent toxicity, emphasizing that antiparasitic therapy must be adapted to renal function and overall health. Additionally, intravenous iron sucrose was chosen over oral iron in this patient to address poor absorption and ensure quick correction of anemia caused by hookworm-related blood loss and CKD. This multidisciplinary team approach, involving gastroenterology, nephrology, and hematology, demonstrates the comprehensive management necessary for complex elderly patients.

Conclusion

This case series expands on the diverse presentations of hookworm disease caused by *A. ceylanicum*, revealing that it can manifest differently than typical cases. It includes colonic infections mimicking inflammatory bowel disease, severe eosinophilia with unusual gastrointestinal symptoms, and hidden anemia in an elderly patient with multiple health issues. These cases emphasize that hookworm infections should be considered in patients of all ages and across diverse clinical settings. Endoscopy is essential for diagnosis when non-invasive tests are inconclusive, especially when clinical suspicion is strong. Recognizing *A. ceylanicum* as an emerging human pathogen highlights the need for increased awareness among healthcare providers and public health officials.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interests.

Consent for Publication Written informed consent for publication of clinical details and any accompanying images was obtained from the patients.

Ethical Approval Ethical approval is not required for this case report, as it is part of routine clinical care. Any potential identifiers of the patient, such as the patient's name, specific residence, and card number, were not included in the case report to maintain anonymity.

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