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Combined morphological and molecular approaches to the clinical diagnosis of *Necator americanus* infection: a case report

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Abstract

Background Hookworm infection remains of considerable importance to public health. However, because critical cases caused by hookworm infection are rarely observed in China, accurate and prompt diagnoses are difficult to achieve in clinical practice. In this study, we describe how we combined morphological and molecular approaches to achieve the clinical diagnosis of hookworm infection.

Case presentation A 75-year-old Chinese woman who presented with dizziness, poor appetite, poor sleep, and weakness in her limbs was diagnosed with chronic atrophic gastritis and was positive for *Helicobacter pylori*, iron deficiency anemia with a hemoglobin concentration of 35 g/L, and left atrial enlargement. However, after symptomatic treatment, the patient did not improve. Upper gastrointestinal endoscopy revealed the presence of live nematodes in the descending portion of the patient's duodenum. Fecal examination via saturated brine flotation revealed hookworm eggs. Further verification via semi-nested reverse transcription-polymerase chain reaction assay confirmed provided confirmation that the hookworm species was *Necator americanus*. Albendazole was used for antihelminthic treatment. Through follow-up visits, we found that the antihelminthic treatment was successful and that her anemia was cured.

Conclusion In this study, a combination of morphological and molecular approaches were used to make a definite diagnosis of severe iron deficiency anemia caused by *Necator americanus* infection in a patient. The results presented here provide suitable guidance for the clinical diagnosis of hookworm infection and a powerful tool for the identification of hookworms.

Keywords Morphological and molecular approach, Clinical diagnosis, *Necator americanus*, Chronic atrophic gastritis, Severe anemia, Case report

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Introduction

Hookworm is one of the most prevalent soil-transmitted helminths (STH), and hookworm infections result in an estimated 2.1 million disability-adjusted life years (DALYs) lost, accounting for more than US $100 billion in global economic losses [1–4]. Hookworm infection in humans is usually caused by the species *Ancylostoma duodenale* and *Necator americanus* [5, 6]. These two species differ in terms of geographical distribution [2, 7], egg production, density-dependent fecundity effects, and their contribution to morbidity [8]. However, although performing accurate species identification of hookworms for clinical etiological diagnoses is still difficult, the application of molecular biotechnology has been shown to be useful.

Humans frequently become infected with hookworms through skin penetration following contact with soil or vegetation contaminated with third-stage larvae (L3) [9, 10]. Hookworms parasitize the small bowels of humans and release anticoagulative substances and enzymes to facilitate blood sucking; therefore, gastrointestinal symptoms and iron deficiency anemia (IDA) are the principal clinical symptoms of hookworm infection [11]. When many hookworms infest the intestines, patients generally present with severe IDA due to chronic massive blood loss [12]. The mean hemoglobin concentration can be reduced to 98 g/L over 3 months [1], even declining to 53 g/L in neonates [12–14].

In this study, we report a case involving a patient with severe IDA caused by *Necator americanus* infection who presented with a very low hemoglobin concentration of 35 g/L and was initially diagnosed with chronic atrophic gastritis and myelodysplastic syndrome (MDS).

Case presentation

The patient was a 75-year-old Chinese woman who was a farmer from Yongzhou City, Hunan Province, China. A total of 2 months prior, the patient presented with symptoms of dizziness, poor appetite, poor sleep, and weakness in the limbs without any obvious cause. The patient gradually worsened and was diagnosed with chronic atrophic gastritis with *Helicobacter pylori* (Hp) positivity, hypoproteinemia, and IDA. Moreover, left atrial enlargement and left ventricular diastolic dysfunction were diagnosed via echocardiography.

The results of routine blood tests were as follows: a red blood cell (RBC) count of 1.84×10^{12}/L, hematocrit level of 13%, platelet count of 500×10^{9}/L, hemoglobin level of 35 g/L (critical value), mean corpuscular volume of 70.7 fL, total protein level of 44.6 g/L, eosinophil ratio of 5.3%, erythrocyte sedimentation rate of 25 mm/h, immunoglobulin (Ig)E level of 1282.2 IU/ml, serum Fe level of 3.85 μmol/L, and positive fecal occult blood test result. Her bone marrow cytomorphologic examination revealed active proliferation of the granulocyte, megakaryocyte, and myeloid cell series. A flow cytometry examination did not indicate the abnormal immunophenotypes indicative of acute leukemia, MDS, lymphoma, or myeloid tumors. Gastrointestinal endoscopy showed live nematodes in the descending portion of the patient's duodenum. The patient was treated with leukocyte-reduced red blood cells to correct severe anemia and the antihelminthic albendazole. After treatment, her hemoglobin level was confirmed to be 77 g/L. During follow-up visits, the patient successfully underwent antihelminthic treatment, and her anemia was cured.

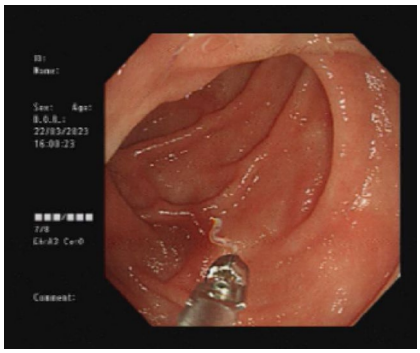


Fig. 1 Gastrointestinal endoscope showing cylindrical worms feeding on the small intestine mucosa in the duodenum

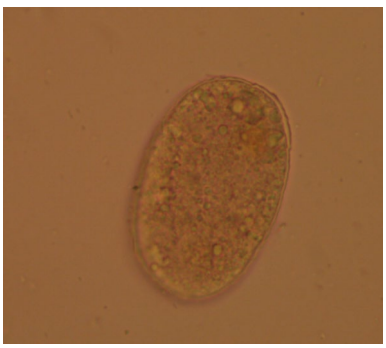


Fig. 2 Stool examination by saturated brine flotation showing hookworm eggs

Imaging and morphological examination

To rule out gastrointestinal disease, the patient underwent gastrointestinal endoscopy. Live nematodes were discovered feeding on the mucosa of the descending portion of the patient's duodenum (Fig. 1). Hookworm eggs were found in the stool via saturated brine flotation (Fig. 2). Therefore, the patient ultimately was diagnosed with severe IDA caused by hookworm infection.

Molecular identification of the hookworm species

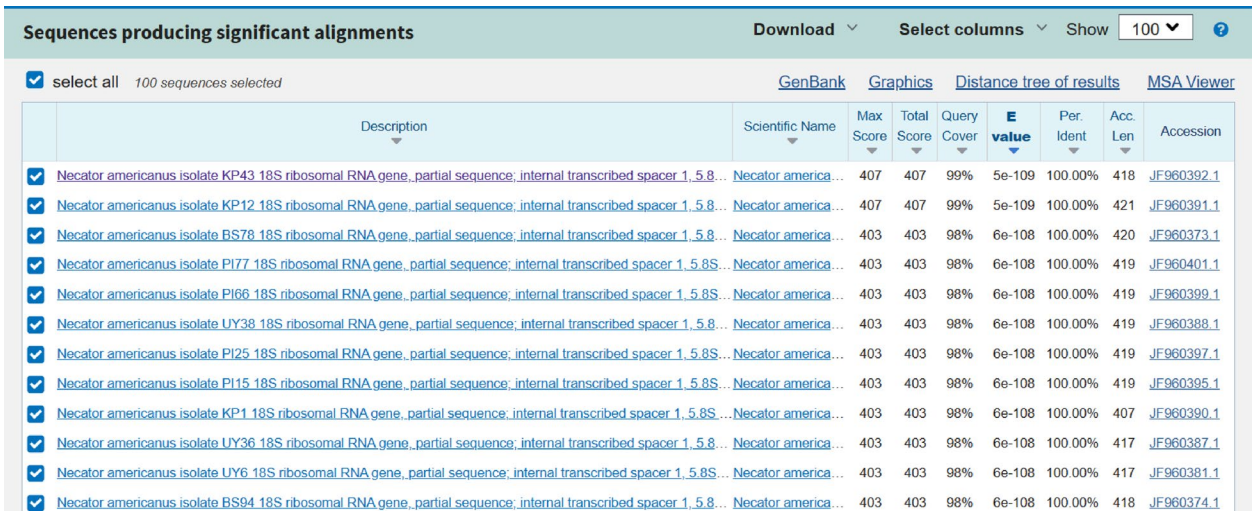
Semi-nested reverse transcription-polymerase chain reaction (RT-PCR) was performed to identify the species of hookworm. Total DNA was extracted from fecal samples via the Mol Pure® Stool DNA Kit (18820ES50) by Yi Sheng Biotechnology (Shanghai, China). The primers (forward primer NC1: ACGTCTGGTTCAGGGTTCTT; reverse primer NC2: TTAGTTTCTTTTCCTCCGCT) were designed to amplify the second internal transcribed spacer (ITS2) and 28S RNA region of the ribosomal DNA of the hookworm. The amplification condition for the first round of the semi-nested RT-PCR included denaturation at 95 ℃ for 3 minutes; followed by 35 cycles: denaturation at 95 ℃ for 10 seconds, annealing at 55 ℃ for 20 seconds, and extension at 72 ℃ for 30 seconds; and a final extension at 72 ℃ for 5 minutes. The amplified PCR product was used for the second step of the semi-nested PCR. The primer NC2 was used as the common reverse primer, whereas the first forward primer AD1: CGACTT TAGAACGTTTCGGC was used for *A. duodenale*, and the second forward primer NA: ATGTGCACGTTATTC ACT was used for *N. americanus*. The amplification conditions included denaturation at 95 ℃ for 3 minutes; followed by 35 cycles: denaturation at 95 ℃ for 10 seconds, annealing of AD1 at 60 ℃ for 20 seconds, annealing of NA at 55 ℃ for 20 seconds, and extension at 72 ℃ for 30 seconds; and a final extension at 72 ℃ for 5 minutes. The PCR products were electrophoresed in a 1% agarose gel and visualized under ultraviolet (UV) light.

The first round of the semi-nested PCR was performed in a 10-μL reaction volume containing 5 μL of Premix Taq, 0.6 μL of extracted DNA, 0.2 μL of each primer (10 pmol), and 4 μL of water. For the second round, the PCR mixture contained 15 μL of Premix Taq, 1.8 μL of extracted DNA, 0.6 μL of each primer (10 pmol), and 12 μL of water for a final volume of 30 μL.

The product of the semi-nested RT-PCR was submitted to Bioengineering (Shanghai, China) for DNA sequencing, and the results were compared via BLAST algorithms and databases from the National Center for Biotechnology Information database (Fig. 3). The DNA sequencing results were as follows: TTGTTTACT AAC GTATGATAGCGGTGCATACTGTATGACATGAAC ATATCGTTGTTCACTGTTTAATCGCTCTCGCGA CTTATGAGCGTGGTTGAACGGAGACAATGTGAA GGACAACGATGTTCGCCATGTGGATGTGTCATT TGCAATGCAACCTGAGCTCAGGCGTGATTACCC GCTGAACTTAAGCATATCATTTAGCGGAGGAAA AGAAACTAAATG. Finally, we confirmed that the hookworm species was *N. americanus*.

Discussion

Hookworm infection is one of the neglected tropical diseases (NTDs) that are still widespread and impose a substantial burden in low-income and middle-income countries, despite the implementation of mass drug administration (MDA) programs [15–18]. Owing to social and economic development, the popularization of sanitary toilets, an improved supply of clean water, and the implementation of control measures, the prevalence of hookworm infection in China has decreased drastically [19]. According to the national parasite surveys in 1988–1992, 2001–2004 [20], and 2014–2016 [21], the rate of hookworm infection decreased from 17.2% to 2.6%. In 2020, the rate of hookworm infection was 0.51% [22]. Hunan Province has also made significant progress



Sequences producing significant alignments Download Select columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Necator americanus isolate KP43 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Necator america...	407	407	99%	5e-109	100.00%	418	JF960392.1
Necator americanus isolate KP12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Necator america...	407	407	99%	5e-109	100.00%	421	JF960391.1
Necator americanus isolate BS78 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Necator america...	403	403	98%	6e-108	100.00%	420	JF960373.1
Necator americanus isolate PI77 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	Necator america...	403	403	98%	6e-108	100.00%	419	JF960401.1
Necator americanus isolate PI66 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	Necator america...	403	403	98%	6e-108	100.00%	419	JF960399.1
Necator americanus isolate UY38 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Necator america...	403	403	98%	6e-108	100.00%	419	JF960388.1
Necator americanus isolate PI25 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	Necator america...	403	403	98%	6e-108	100.00%	419	JF960397.1
Necator americanus isolate PI15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	Necator america...	403	403	98%	6e-108	100.00%	419	JF960395.1
Necator americanus isolate KP1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ...	Necator america...	403	403	98%	6e-108	100.00%	407	JF960390.1
Necator americanus isolate UY36 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Necator america...	403	403	98%	6e-108	100.00%	417	JF960387.1
Necator americanus isolate UY6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	Necator america...	403	403	98%	6e-108	100.00%	417	JF960381.1
Necator americanus isolate BS94 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Necator america...	403	403	98%	6e-108	100.00%	418	JF960374.1

Fig. 3 DNA sequence alignment with GenBank showing the hookworm species as *N. americanus*

in controlling hookworms, and the rate of hookworm infection decreased from 22.86% in 1994 to 0.43% in 2020 [22]. Although the rate of hookworm infection remains low, the risk remains. Hookworm disease is easily misdiagnosed because the clinical symptoms such as gastrointestinal symptoms and anemia are atypical [23]. The long-term effects of anemia may produce ventricular dilation and edema, ultimately triggering heart failure [24]. Thus, identification and subsequent correction of these conditions are very important in this patient group [25]. Although the patient had no obvious gastrointestinal bleeding, her hemoglobin concentration was exceptionally low at just 35 g/L, which is much lower than that typically observed in patients with hookworm infection. The underlying reasons might be related to the patient's living environment or dietary and lifestyle habits. In addition, the patient did not receive anthelmintic treatment in a timely manner.

Species identification of hookworms is essential for treatment and control in national surveillance. In poor tropical and subtropical areas, many patients have extensive hookworm infestations, but very few of them show symptoms of overt bleeding from the gastrointestinal tract, leading to the infections being ignored [26]. In addition, it is not easy to identify the species of hookworm accurately through routine stool testing alone [13]. In this case, semi-nested RT-PCR was used to amplify the internal transcribed spacer of the hookworm, and the sequencing results confirmed that the hookworm species was *Necator americanus*. The patient's hemoglobin level was confirmed to be 77 g/L and the patient's symptoms of dizziness, poor appetite, poor sleep, and weakness in the limbs disappeared after treatment with albendazole and leukocyte-reduced red blood cells. During follow-up visits, we found that the antihelminthic treatment was successful and that the patient's anemia was cured.

Obscure upper gastrointestinal bleeding (UGIB) is defined as bleeding in the upper gastrointestinal tract without an identifiable source [27]. Patients with obscure upper gastrointestinal bleeding are often diagnosed with IDA, and their symptoms do not significantly improve when anti-anemia treatment is administered. Hence, the timely detection of the cause of IDA in patients is extremely important for treatment and prognosis. Capsule endoscopy (CE) [28], esophagogastroduodenoscopy, and double-balloon endoscopy (DBE) [29] are common methods of examining the upper gastrointestinal tract. Because most hookworm-infected individuals present with chronic occult bleeding, hookworm infection should be considered in the differential diagnosis of patients with IDA in low-income and middle-income countries. Further progress and developments in virtual endoscopy, such as the incorporation of computed tomography (CT) and magnetic resonance imaging, are expected in the future [30], which will provide new treatment methods for UGIB.

Conclusion

In this study, combined morphological and molecular approaches were used to make a definite diagnosis of severe iron deficiency anemia caused by *Necator americanus* infection in a patient. The results presented here provide suitable guidance for the clinical diagnosis of hookworm infection and a powerful tool for the identification of hookworms. Special attention should be given in high-endemic areas, especially among older people.

Abbreviations

STH	Soil-transmitted helminths
DALY	Disability-adjusted life years
IDA	Iron deficiency anemia
MDS	Myelodysplastic syndromes
Hp	Helicobacter pylori
RBC	Red blood count
NTDs	Neglected tropical diseases
MDA	Mass drug administration
RT-PCR	Reverse transcription-polymerase chain reaction
DNA	Deoxyribonucleic acid
UGIB	Upper gastrointestinal bleeding
CE	Capsule endoscopy
DBE	Double-balloon endoscopy

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None

Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by AS, XZ, MX, and YL. The first draft of the manuscript was written by XL and revised by SH and XW. All authors commented on previous of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

The informed consent from the study participants was obtained.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors have no relevant competing interests to declare.

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References

1. Chapman PR, *et al*. Experimental human hookworm infection: a narrative historical review. PLoS Negl Trop Dis. 2021;15(12):e0009908.
2. Loukas A, *et al*. Hookworm infection. Nat Rev Dis Primers. 2016;2:16088.
3. Fleitas PE, *et al*. Mapping the global distribution of *Strongyloides stercoralis* and hookworms by ecological niche modeling. Parasit Vectors. 2022;15(1):197.
4. Soong XDAL. Emerging and re-emerging zoonoses are major and global challenges for public health. Zoonoses. 2021. https://doi.org/10.15212/ZOONOSES-2021-0001.
5. Bai C, Yang Y. The prevalence of human hookworm disease and advances in its treatment. Chin J Pathog Biol. 2020;15(7):865–9.
6. Xu FF, *et al*. Hookworm infection in central China: morphological and molecular diagnosis. Parasit Vectors. 2021;14(1):537.
7. Jourdan PM, *et al*. Soil-transmitted helminth infections. Lancet. 2018;391(10117):252–65.
8. Malizia V, *et al*. Appropriateness of the current parasitological control target for hookworm morbidity: a statistical analysis of individual-level data. PLoS Negl Trop Dis. 2022;16(6):e0010279.
9. Bharti B, Bharti S, Khurana S. Worm infestation: diagnosis, treatment and prevention. Indian J Pediatr. 2018;85(11):1017–24.
10. Ding X, *et al*. Establishment of a simple and rapid nucleic acid detection method for hookworm identification. Pathogens. 2023;12(4).
11. Bosch F, *et al*. Diagnosis of soil-transmitted helminths using the Kato–Katz technique: what is the influence of stirring, storage time and storage temperature on stool sample egg counts? PLoS Negl Trop Dis. 2021;15(1):e0009032.
12. Ravindranath A, Sarma MS, Jain D. Melena in neonates: endoscopic surprise. Gastroenterology. 2020;159(6):e3–4.
13. Li B, *et al*. Chronic hemorrhagic anemia caused by hookworm infection: a case report. Acta Parasitol. 2023;68(1):288–92.
14. Kalousova B, *et al*. Adult hookworms (*Necator* spp.) collected from researchers working with wild western lowland gorillas. Parasit Vectors. 2016;9:75.
15. Nath TC, *et al*. Molecular evidence of hookworms in public environment of Bangladesh. Sci Rep. 2023;13(1):133.
16. Ma L, *et al*. A case of severe hookworm infection. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2022;47(6):814–22.
17. Clements ACA, Alene KA. Global distribution of human hookworm species and differences in their morbidity effects: a systematic review. Lancet Microbe. 2022;3(1):e72–9.
18. Bartsch SM, *et al*. Modeling the economic and epidemiologic impact of hookworm vaccine and mass drug administration (MDA) in Brazil, a high transmission setting. Vaccine. 2016;34(19):2197–206.
19. Ying-Dan C, *et al*. Status and working principals of soil-transmitted nematodiasis during new period in China. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi. 2019;31(1):23–5.
20. Coordinating Office of the National Survey on the Important Human Parasitic Diseases. A national survey on current status of the important parasitic diseases in human population. Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi Chin J Parasitol Parasit Dis. 2005;23:332–40.
21. Ying-dan C, *et al*. National survey on the current status of important human parasitic diseases in China in 2015. Chin J Parasitol Parasit Dis. 2020;38(1):5.
22. Mizhen Z, *et al*. Epidemiological analysis of soil-transmitted nematode infections in China in 2020. Chin J Parasitol Parasit Dis. 2023;41(3):331.
23. Li R, *et al*. A half-century studies on epidemiological features of ancylostomiasis in China: a review article. Iran J Public Health. 2019;48(9):1555–65.
24. Silverberg DS, Wexler D, Iaina A. The importance of anemia and its correction in the management of severe congestive heart failure. Eur J Heart Fail. 2002;4(6):681–6.
25. van Veldhuisen DJ, *et al*. Anemia and iron deficiency in heart failure: mechanisms and therapeutic approaches. Nat Rev Cardiol. 2011;8(9):485–93.
26. Wei K-Y, *et al*. Hookworm infection: a neglected cause of overt obscure gastrointestinal bleeding. Korean J Parasito. 2017;55(4):391–8.
27. Singh S, Mohan BP, Adler DG. Management of obscure gastrointestinal bleeding. Am J Gastroenterol. 2024. https://doi.org/10.14309/ajg.0000000000002950.
28. Liao YJ, *et al*. Clinical application and feasibility of capsule endoscopy in children at a medical center in central Taiwan. J Formos Med Assoc. 2024. https://doi.org/10.1016/j.jfma.2024.06.012.
29. Ohmiya N. Management of obscure gastrointestinal bleeding: comparison of guidelines between Japan and other countries. Dig Endosc. 2020;32(2):204–18.
30. Tanabe S. Diagnosis of obscure gastrointestinal bleeding. Clin Endosc. 2016;49(6):539–41.

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