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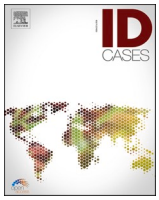

Case report

The first human case of rhabditiasis in Bangladesh

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ABSTRACT

Rhabditiasis, caused by *Rhabditis* nematode, has long been recognized as a veterinary concern, however, human infection is exceedingly rare. For the first time in Bangladesh, this study confirmed human rhabditiasis infecting a 12-year-old child. The identification was made based on morphometric features and confirmed by amplifying the mitochondrial *cox*1 gene. During microscopical examination of stool samples, heavy infection with several developmental stages (larvae, adult males, and females) of nematodes was observed. Following morphometric analysis, the nematode was identified as *Rhabditis* sp. The features used in confirming the species were elongated tail, bulbous enlargement of mid-esophagus, and presence of adult males passed in the stool. The results of the phylogenetic analysis showed that isolates of *Rhabditis* sp. belonged to distinct clades alongside *S. stercoralis*.

Introduction

The genus *Rhabditis* is one of the free-living nematodes in the family Rhabditiae. Most species are considered to be largely saprophytic and are found in soil and decayed organic matter; however, opportunistic infections have been reported in humans [1] and animals [2]. *Rhabditis* larvae have been reported in human urine, feces, and vaginal swabs, although in most cases, the clinical relevance of the presence of the nematodes remained obscure [3]. The exact prevalence of rhabditiasis in Bangladesh is unknown. However, as with other countries, the infection is generally rare in humans and typically occurs in individuals who have had prolonged contact with contaminated soil or decaying plant matter and immunocompromised people are more likely to get infections. Human infections with *Rhabditis* have been reported in several countries around the world, including China, Korea, Japan, Brazil, Germany, and Iran [4]. However, information on infection in Bangladesh is missing. The rich biodiversity of Bangladesh along with the complex ecosystem and climate change, has contributed to the emergence and reemergence of several infectious diseases in both animals and humans. With a significant population reliant on agriculture and livestock for their livelihood, the intricate interplay between animals, humans, and the environment underscores the importance of understanding and monitoring emerging zoonotic diseases in this region. Here, we present the first human case of *Rhabditis* infection in Bangladesh.

Methods

Collection of samples and study location

Fresh stool tool samples were collected from a 12-years-old child living in an urban slum of Sylhet (24°89′17′N, 91°88′33′E), Bangladesh, for three consecutive days. Prior to sample collection, a pre-tested questionnaire was used to collect epidemiological data. About 5 g of feces samples were collected each time in a screw-capped container.

Microscopy and morphological examination

Collected samples were examined on the same day of collection. Parasitological assessment was done at the Department of Parasitology, Sylhet Agricultural University, Bangladesh, using a modified formalin ether sedimentation technique. Observed worms were collected using stereomicroscope (Olympus ACH-1X, Japan) and preserved in fixatives (formalin and ethanol). For morphological observation, the worms were placed in lactophenol, and measurements were made with a light

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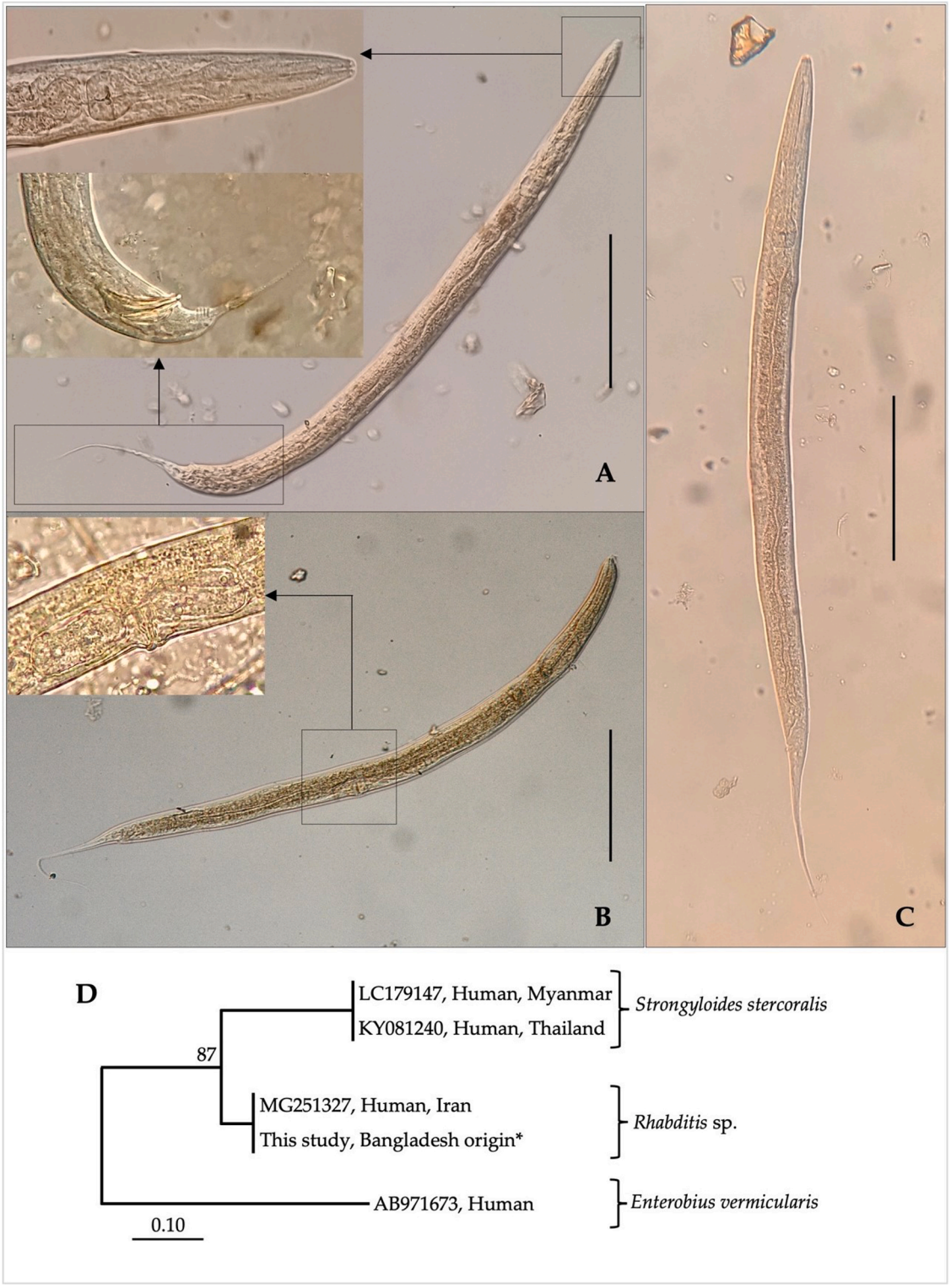

Fig. 1. Photomicrographs of *Rhabditis* sp. (size of scale bar: 200 μm): (A) male (sidebar shows buccal cavity, esophagus, spicules, and tail), (B) female (sidebar shows opening of vulva and eggs), (C) larva, (D) phylogenetic tree based on *cox*1.

microscope (Olympus BX-53, Japan). Unless otherwise stated, measurements are given in millimeters (mm).

Molecular analysis

Molecular analysis was carried out at the Chungbuk National University, South Korea. Isolated individual nematode was washed in phosphate-buffered saline (PBS), and genomic DNA was extracted using QIAamp DNA mini-Kit (Qiagen, Valencia, USA). Polymerase chain reactions (PCR) based on the mitochondrial *cox*1 were performed with primers *cox*F (5′TGGTTTGGGTACTAGTTG-3′) and *cox*R (5′-GATGAGCTCAAACTACACA-3′). Amplification was carried out in a final reaction mixture containing 30 μL of PCR mix, including 1 μL of each primer (10 pmol), 1 μL of each sample DNA, 6 μL of 5X PCR Master Mix

Table 1
Morphometry of *Rhabditis* sp. with differential diagnosis.

Differential criteria	*Rhabditis* sp.	*Rhabditis* sp.	*Rhabditis elongata*	*Strongyloides stercoralis*
References	Present specimen	Ahn et al. (1985)	Lee et al. (1978)	Little (1966)
Locality	Bangladesh	Korea	Korea	-
Host	Human	Human	Human	Human
Male				
Body length	878.3–911.7	1006–890	810–1337	810–1000
Body thickness	35.4–39.2	48–49	25–37	40–50
Length of esophagus	176.8–186.1	176–209	165–210	110–125
Length of spicule	37.4–40.1	73–76	34–40	35–40
Female				
Body length	991.6–1011.1	1176–1419	972–1599	920–1700
Body thickness	39.4–43.2	79–82	38–54	52–85
Length of esophagus	181.8.– 197.1	258–291	190–225	125–150
Distance of vulva from mouth	496.2–505.3	771–802	506–729	470–820
Tail	240.1–252.8	70–87	210–265	80–130
Egg size (n = 5)	46–49 × 24–31	66 × 56	-	-

* Description based on free-living specimens (value presented in μm).

(ELPIS biotech, South Korea), and 20 μL of nuclease-free water. A negative control (distilled water) was applied in each run. The thermal PCR profile included an initial denaturation step at 95 °C for 15 min, followed by 30 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s, respectively. The final extension was carried out at 72 °C for 10 min. The PCR products were run on a 1.5 % agarose gel and visualized using a UV transilluminator. DNA sequencing was performed by Cosmogenetech, South Korea. The obtained sequences were assembled with Geneious program 9.0 (Biometer, New Zealand) and aligned using ClustalW multiple alignments implanted MEGA7. The phylogenetic tree was constructed using the maximum likelihood algorithm with 1000 bootstrap replications.

Results

A 12-year-old child presented frequent diarrhea and abdominal pain during an uncertain period. The child was administered anthelmintics 3 months before as part of a nationwide chemotherapy program. Although the boy was asymptomatic at the visit, the coprological study revealed a heavy infection with nematodes. Microscopic examination showed several developmental stages of nematodes, including adult (male, females) and larvae (Fig. 1, A,B,C). As the observation was surprising, additional stool samples were collected for another two consecutive days and similar results were observed. The larvae (n = 10) were measured 0.524–0.782 long by 0.023–0.034 wide and characterized by the club-shaped esophagus and long pointed tail. The adult female (n = 10) was measured 0.991–1.011 in length and 0.039–0.043 in breadth (Table 1). The body has a cylindrical shape, tapers gradually to become a long and fine tip. Tail pointed and measured 0.241–0.268 in length from the level of the anus to the end. The oral cavity consists of a relatively long canal and the esophagus measuring 0.181–0.197 in length. Vulva opens at about the middle of the body; situated at a distance of 0.496–0.505 from the mouth. The oval-shaped eggs (n = 5) measuring 46–49 × 24–31 μm were observed in the female uterus. Adult males (n = 10) measured 0.878–0.991 in length and 0.035–0.039 in breadth. Males have indistinguishable anterior structures with females, however, esophagus was shorter than that of the female, measuring 0.176–0.186 in length. Male has two spicules, parallel and nearly equal in size, measuring 0.037–0.041 long. The prolonged tail measures 0.2510–0.273 in length. A band-like structure was seen at the posterior part of male. Partial *cox*1 sequences of 451 bp fragments of *Rhabditis* isolates in the present study showed 99.8 % similarity with publicly available *Rhabditis* sp. from NCBI database. According to the phylogenetic analysis, isolated *Rhabditis* sp. was located solely in a separate clade from *S. stercoralis* (Fig. 1, D).

Discussion

Rhabditis nematode can be confused with *Strongyloides* during routine examination and might be misdiagnosed with commonly occurring strongyloidiasis [4,5]. Therefore, careful observation is necessary to diagnose and distinguish these two species. *Rhabditis* species can be distinguished from *Strongyloides* or hookworm larvae by the long, attenuated tails and bulbous enlargement of the mid-esophagus of *Rhabditis*. Morphologically, the buccal capsule of *Rhabditis* is longer than that of *Strongyloides*. Tails of *Rhabditis* are finely tapered, with a whip-like tip, while the tail of *Strongyloides* is relatively smaller, never exceeding 15 % of the total body length [4,6]. While spicules in *Strongyloides* are comparatively less curve with broad and bluntly rounded anterior end [7]. Rhabditoids generally have curved spicules with narrow anterior ends. Presence of adult males and adult females along with larvae could be considered as another important point. In strongyloidiasis, only parasitic female and larvae can be found; the male of *Strongyloides* is free-living and can only be present in nature. In the case of *Rhadditis*, the eggs closest to the vulva contain full-grown larvae, which are ready to escape from the egg immediately ejected by the mother [6]. That's why we did not observe any eggs during the fecal examination. It can also be considered as a differential point to diagnose rhabditiasis [8].

The origin of the infection has remained uncertain. The patient had a history of frequent contact with soil and animals. During observation of the surrounding household environment, we found openly defecated human and animal feces distributed scattered along the river blank. The community people used untreated river water for their daily activities and lived in close proximity to animals including free-range animals including pigs, and dogs. After consulting with a clinician, the boy was treated with a single dose of ivermectin, and his stool tested negative for parasites on a follow-up examination at 4 weeks. The clinical relevance and pathogenicity of the nematodes in human have remained unclear. So far, cases of rhabditiasis have been reported to infect the digestive system, urinary system, vaginal swabs, and ear canal [9]. There might have been an association between *Rhabditis* infection with immunodeficiency, however, further investigation is needed (Meamar et al., 2007).

Conclusion

Taking the data together, this study presents the first human case of rhabditiasis infection in Bangladesh, employing microscopy and molecular analysis. Rhabditiasis needs a differential diagnosis with strongyloidiasis, and further attention should be given to any potential opportunistic infections in individuals with immunodeficiency. Data provided in this study may promote and improve our understanding of rhabditiasis as an emerging public health threat.

Ethical approval

This study protocol was reviewed and approved by Parasite Research Center, Chungbuk National University, International Parasite Resource Bank, South Korea, and Department of Parasitology, Sylhet Agricultural University, Bangladesh (2020EC-59).

Ethical approval and consent to participate

This study protocol was reviewed and approved by Parasite Research Center, Chungbuk National University, International Parasite Resource Bank, South Korea, and Department of Parasitology, Sylhet Agricultural University, Bangladesh (2020EC-59). Informed consent for participation

was not applicable in this study.

Consent

None.

Competing interests

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

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Authors' contributions

TCN wrote the first and final drafts of this manuscript. JUB, HP, DL, and KSE, all made significant contributions to the revision of the manuscript. The final version of this manuscript was vetted and approved by all authors.

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