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Ancylostoma ceylanicum hookworm infection in Japanese traveler who presented chronic diarrhea after return from Lao People's Democratic Republic

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ABSTRACT

Ancylostoma (*A.*) *ceylanicum*, one of the most common species of hookworms infecting dogs and cats, also causes patent infections in humans and is now considered to be the second most common hookworm species infecting populations in southeast Asia. A Japanese patient who returned from a visit to Thailand and Lao People's Democratic Republic (PDR) was presented with intermittent watery diarrhea with eosinophilia. Hookworm eggs were found in feces samples, and adult worms were confirmed to be present in the jejunum with capsule endoscopy and double balloon enteroscopy. A diagnosis of *A. ceylanicum* infection was made based on the morphology of the adult worms along with findings of a PCR-based molecular study using larvae obtained from a fecal sample culture. The infection was considered likely to have been obtained during a 1-month stay in a Laotian village, where the patient had eaten local food, worn sandals on bare feet, and lived as a local native villager, though he had stayed in modern hotels during the visit to Thailand.

1. Introduction

Traveler's diarrhea is the most frequently encountered travel-related illness and can be caused by a wide range of pathogens, including bacteria, viruses, and parasites. Hookworms are one of the most common parasitic nematodes that infect both humans and animals, and such infection is an etiological factor in chronic diarrhea [1]. The two primary species of hookworm found in humans are *Ancylostoma* (*A.*) *duodenale* and *Necator* (*N.*) *americanus*, which infect approximately 600 million individuals throughout the world [2]. Furthermore, *A. ceylanicum*, *A. braziliense*, and *A. caninum* are hookworms found in cats and dogs that are also able to cause zoonotic disease in humans, such as creeping eruption, eosinophilic enteritis, localized myositis, erythema multiforme, and ophthalmological manifestations. Among these zoonotic hookworms, *A. ceylanicum* is known to cause patent infections in humans, and natural infection with that seems to be endemic and widely distributed throughout Asian countries, especially in southeast Asia [3–6], where a high prevalence of infection with *A. ceylanicum* has been reported in domestic stray as well as community raised dogs and cats. As a result, this zoonotic hookworm is emerging as a commonly encountered human hookworm in cases seen in southeast Asia [7,8]. We recently treated a case of *A. ceylanicum* hookworm infection in a Japanese patient who returned from a visit to Thailand and Lao People's Democratic Republic (PDR), and was presented with abdominal pain, vomiting, and diarrhea with eosinophilia.

2. Case

In October 2015, a 47-year-old man with no medical history visited us because of intermittent diarrhea with extreme eosinophilia. In July, he had visited Bangkok, Thailand and its suburbs for 2 weeks, then went on to Lao PDR and spent 4 weeks in a rural Laotian village near Vang Vieng. On August 20, day 24 of living in the village, he experienced an abrupt onset of fever, abdominal pain with watery diarrhea, and vomiting, and required treatment for dehydration by a local doctor. The symptoms did not completely disappear, and he returned to Japan and visited a local clinic at the end of August, where he was treated with hydration and antibiotic drugs. Laboratory examination showed increased eosinophiles (43%) with normal leukocyte count (7600 cells/μL), but any pathogenic organisms were not detected in stool cultures. The patient recovered within 1 month, except for

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intermittent watery diarrhea, for which he visited us at the beginning of October.

We performed laboratory tests, which showed leukocytosis (27,900 cells/μL) with hypereosinophilia (74%) and unembryonated hookworm ova in feces (Fig. 1A). No protozoa potentially causative of an intestinal infection were observed in microscopic and molecular examinations of feces samples, while a Harada-Mori filter paper test-tube culture of a fecal sample demonstrated actively moving infective larvae (Fig. 1B). Although conventional upper gastrointestinal and colon endoscopic examinations had negative findings, capsule endoscopy demonstrated thread-like worms in the jejunum (Fig. 1C). In addition, double-balloon intestinal endoscopy confirmed nematode worms, some of which had hooked their head into the mucosa and were sucking blood (Fig. 1D). Three worms (2 females, 1 male) were removed and identified as *A. ceylanicum* by their characteristic mouthparts (Fig. 1E) and the copulatory bursa of the male adult worm (Fig. 1F). No histological evidence of infection with *Strongyloides stercoralis* was found in any of the biopsied samples obtained from the stomach, or small and large intestines. Furthermore, for confirming species identification, DNA extracted from an adult worm using a DNeasy Blood & Tissue Kit (QIAGEN) was subjected to a PCR assay specific for the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene. Using an ABI Prism 3100-*Avant* Genetic analyzer (Applied Biosystems), the *cox1* region was shown to have a 100% sequence identity with the *A. ceylanicum* sequence (GenBank accession number KC247727.1, [9]). In addition, DNA samples extracted from larvae formed in culture were also subjected to PCR specific for ribosomal internal transcribed spacer (ITS)-1 and ITS-2. No amplified product was shown to correspond to *N. americanus* and the amplified DNA sequence data revealed *A. ceylanicum*. Although nucleotide substitutions are expected to be identified at several points in a case of mixed infection with *A. duodenale* (GeneBank accession numbers AJ001594, AJ001679), all of the points showed only *A. ceylanicum* (GeneBank accession number DQ381541.2, [10]), suggesting a mono-infection.

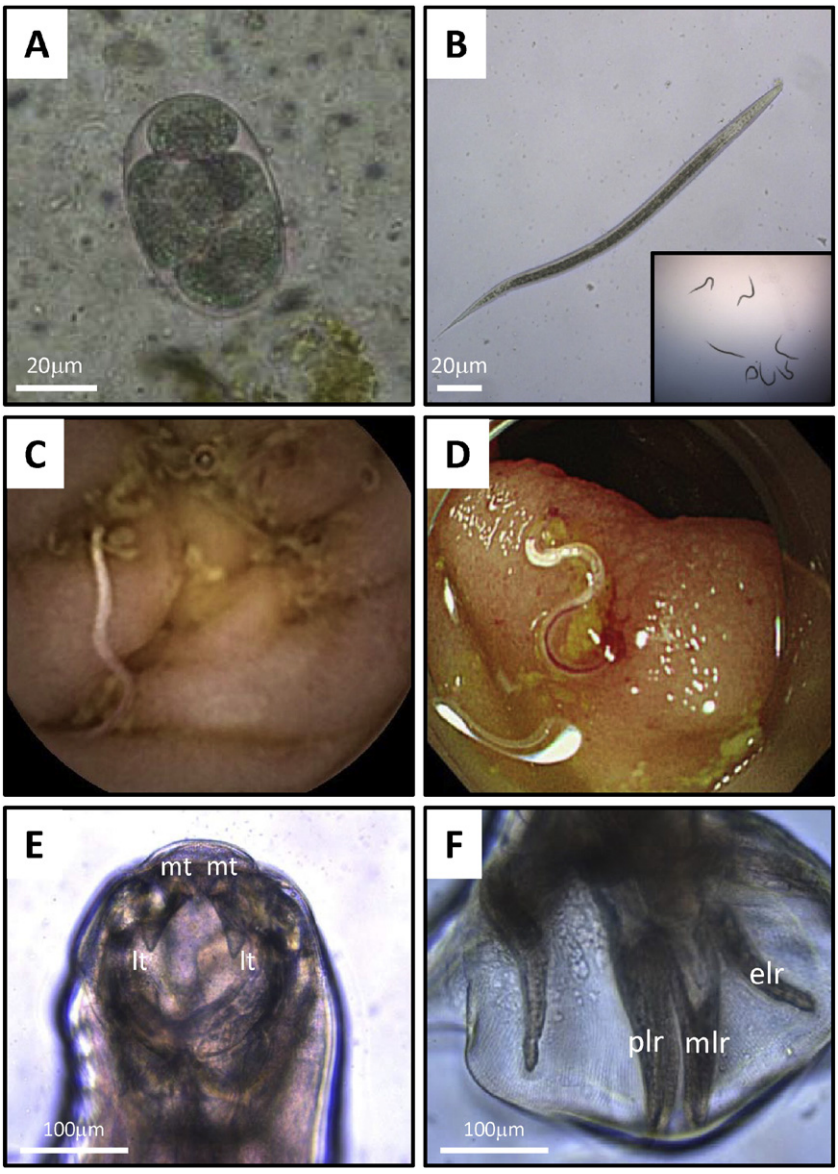

Fig. 1. A: Hookworm egg in early segmentation stage found in feces sample after formalin-ethyl acetate sedimentation. B: Infective larva, with 7 living larvae shown in inset, from Harada-Mori culture. C: Hookworm in jejunum, shown by capsule endoscopy. D: Hookworm withdrawing blood, shown by intestinal endoscopy. E: Buccal capsule. Two pairs of double teeth are shown, with the lateral tooth (lt) large and median tooth (mt) quite small. F: Copulatory bursa of male hookworm. The mediolateral ray (mlr) and posterolateral ray (plr) are parallel, and quite close together, though separated from the externolateral ray (elr).