## RESEARCH NOTE

# A COMPLICATED CASE OF STRONGYLOIDIASIS PRESENTING WITH INTESTINAL LYMPHADENOPATHY OBSTRUCTION: MOLECULAR IDENTIFICATION

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**Abstract.** *Strongyloides stercoralis* is an intestinal nematode, which can cause complications in immune-compromised hosts. We present a rare case of intestinal obstruction due to mesenteric lymphadenopathy, a complication due to strongyloidiasis, developing in a male subject chronically receiving corticosteroid for pemphigus vulgaris. DNA was extracted from biopsied lymph nodes containing nematode larvae and PCR amplified using primers specific for *S. stercoralis* 18S rDNA. Nucleotide sequence of the amplicon showed identity with that of *S. stercoralis* deposited in GenBank. To the best of our knowledge, this is the first report of a diagnosis of strongyloidiasis from biopsied samples using molecular techniques.

**Keywords:** *Strongyloides stercoralis*, intestinal obstruction, mesenteric lymphadenopathy, molecular identification, strongyloidiasis

## INTRODUCTION

*Strongyloides stercoralis* is a common human parasite worldwide (Lim *et al*, 2004). *S. stercoralis* has a free-living cycle and parasitic cycle (CDC, 2013a). Adults of both sexes live in the soil and lay eggs that hatch to release rhabditiform larvae, which develop in the soil into mature adults to complete the life cycle, or can develop into filariform larvae, infective to humans.

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Filariform larvae can penetrate any area of the skin that is in contact with soil, after which they migrate through the dermis to enter the vasculature (CDC, 2013a). The larvae circulate with the venous blood until they reach the lungs, where they break into the alveoli and ascend the bronchial tree. The worms then are swallowed with bronchial secretions and pass into the small intestine, where they embed in the jejunal mucosa and mature. Parasitic female *S. stercoralis* can lay fertile eggs by parthenogenesis and therefore do not require males to reproduce (CDC, 2013a). The eggs hatch within the small intestine and rhabditiform larvae migrate into the lumen and they are passed in the stool.

A critical feature of *S. stercoralis* infection is that some rhabditiform larvae sporadically develop within the intestine into infective filariform larvae, which are able to re-infect (auto-infect) the patient, thereby increasing the parasite burden and permitting prolonged colonization, especially in individuals receiving corticosteroids or who are immunocompromised (Marcos *et al*, 2008; CDC 2013a, b). Strongyloidiasis can exist for many decades at a subclinical level (Elliott, 2010).

There have been case reports of intestinal obstruction (Al-Bahrani *et al*, 1995; Dash *et al*, 2012), ileus (Yoshida *et al*, 2006) and duodenal obstruction (Harish *et al*, 2005; Juchems *et al*, 2008; Cruz *et al*, 2010; Hindy *et al*, 2011) due to this parasite. However, mesenteric lymphadenopathy caused by *S. stercoralis* is a rare occurrence (Ramdial *et al*, 2006).

Here, we report a case of complicated strongyliodiasis presenting with mesenteric lymphadenopathy causing intestinal obstruction, in which the causative the parasite was identified using PCR-based assay and DNA sequencing.

## MATERIALS AND METHODS

### Case

The subject was a 49-year-old Thai male with a history of pemphigus vulgaris, confirmed by tissue histology three years previously and who had been treated regularly since then with 60 mg of prednisolone/day at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon, Thailand.

One and a half months ago the subject presented with bloating and fullness of abdomen without any constipation or difficulty in passing stool. On physical examination, the patient was seen to be obese, with a cushingoid appearance. *Strongyloides stercoralis* larvae were found in his stool using a formalin ethyl acetate concentration technique (Anamnart *et al*, 2010). Ivermectin 200 μg/kg/day was prescribed for two consecutive days and no *S. stercoralis* larva was found subsequently.

His symptoms improved in a few days but kept recurring. The patient reported a low-grade fever and abdominal fullness for ten days prior to admission in June 2012 at a local hospital for symptomatic treatment with antipyretic and pancreatin drugs for four days before referral to Srinagarind Hospital. His clinical signs became more progressive with further distension of the abdomen, vomiting of ingested food and lower quadrant abdominal pain but still able to pass small round hard stools. On admission, the patient had a temperature of 38 $^{o}$C and mild pale conjunctivae. The abdomen showed symmetrical globular distension, absence of bowel sound, mild generalized tenderness without rebound tenderness and guarding, and no rectal shelf.

### Laboratory and clinical studies

Blood picture was as follows: hemoglobin 8.9 g%, hematocrit 26.6%, leukocyte count 11,660/mm$^3$, and neutrophils 82%. Liver function test showed hypoalbuminemia (1.5 g%) but other standard values were within normal limits. Plain film x-ray (abdomen supine) (Fig 1A) demonstrated marked dilatation of the small bowel, suggestive of intestinal obstruction. Computerized tomography scan of his upper abdomen (Fig 1B) revealed an abrupt change in caliber and a fluid filled loop of small intestine, thought to be the distal ileum. Multiple mesenteric lymph node enlargements were seen with minimal ascites. Partial small intestinal obstruction was suspected and the patient

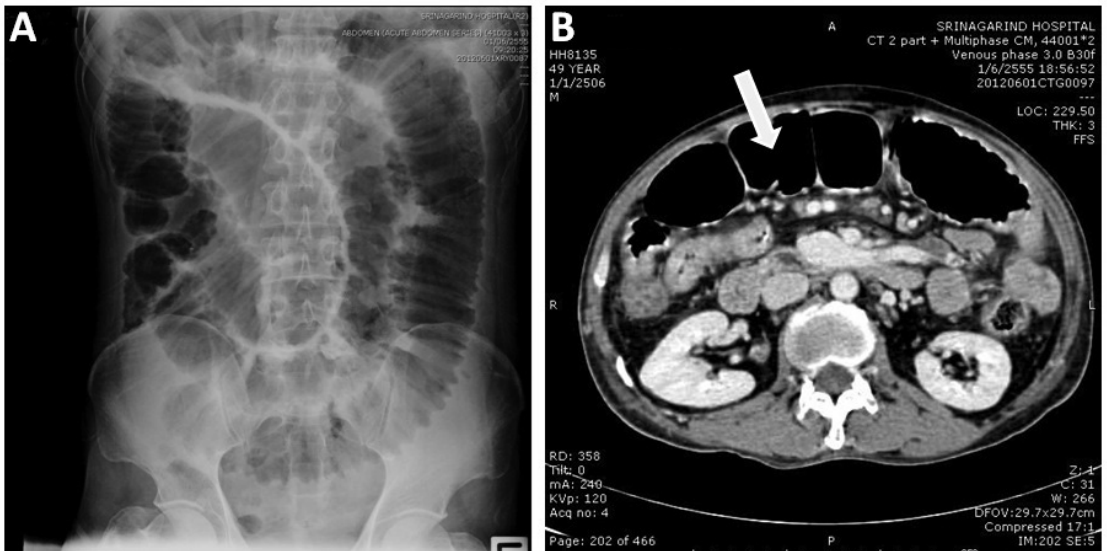


Fig 1–Acute abdomen supine x-ray film (A) revealed abrupt change in caliber and a fluid filled loop of the small intestine, thought to be the distal ileum. Multiple mesenteric lymph node enlargements were seen with some ascites. In panel B, showing axial computerized tomography scan of upper abdomen revealed abrupt change in caliber and a fluid-filled loop of the small intestine, thought to be the distal ileum. Multiple mesenteric lymph node enlargements were seen with minimal ascites. Arrow indicates transitional zone of small bowel dilatation at distal ileum.

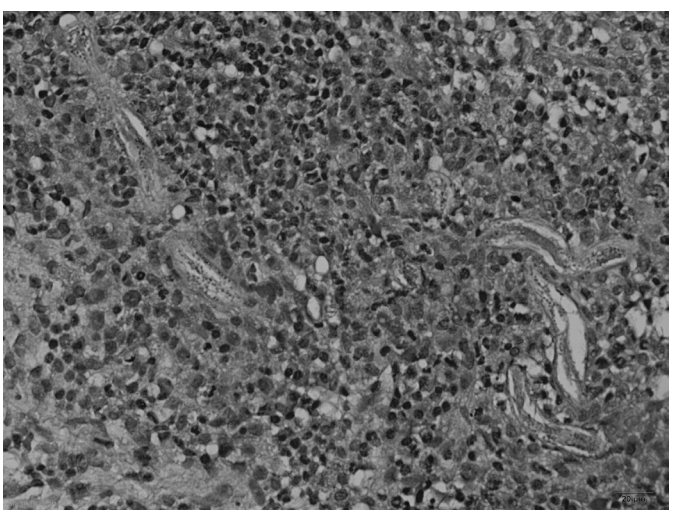

Fig 2–Histopathology section of mesenteric lymph node illustrating many nematode larvae in the subcapsular lymphatic sinus of a mesenteric lymph node (Hematoxylin and eosin staining; 40x magnification).

was initially treated in a conservative manner including nil per os, nasogastric decompression, and intravenous hydration, but clinical condition deteriorated. Laparotomy was performed, demonstrating multiple lymphadenopathy along the mesentery with minimal ascites. There was dilatation of the proximal jejunum of about 120 cm in length due to obstruction from lymphadenopathy. Biopsies of mesenteric lymph nodes and retrograde decompression without resection or entry into the intestinal lumen were performed. Hematoxylin and eosin stained

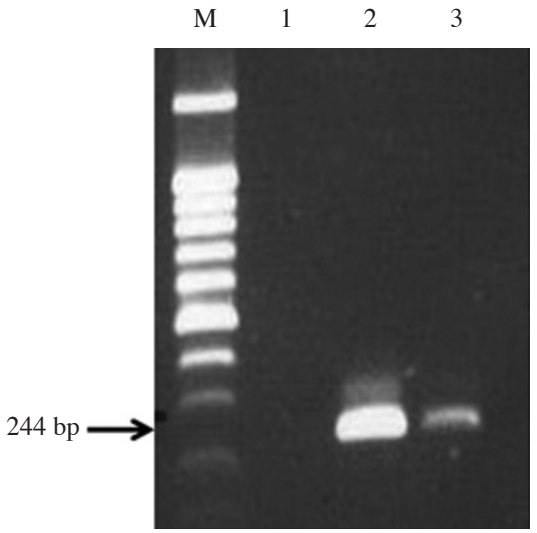


Fig 3–Ethidium bromide staining pattern of 1.0% agarose gel-electrophoresis of amplicon from extracted DNA of formalin-fixed paraffin-embedded mesenteric lymph node sections. PCR was performed using primers specific to *Strongyloides stercoralis* 18S rDNA. Lane M, 100 bp DNA size markers; lane 1, negative control (distilled water); lane 2, *S. stercoralis* positive DNA control plasmid ($10^7$ copies/μl); lane 3, extracted DNA.

sections revealed many nematode larvae in the subcapsular sinus of the mesenteric lymph nodes (Fig 2).

**PCR-based assay and DNA sequencing**

DNA was extracted from unstained serial sections of formalin-fixed, paraffin-embedded lymph nodes attached on glass slides using a DEXPAT kit (TaKaRa Bio, Shiga, Japan) as reported previously (Koonmee *et al*, 2011) and used as DNA template for PCR using *S. stercoralis* (GenBank accession no. AF279916) 18S rDNA-specific primers SS-F (5′-ACCG-TAAACTATGCCTACTAG -3′) and SS-R (5′-AACCACTAAATCATGAAAGAGCT -3′). The 25-μl reaction mixture contained 10X FastStart High Fidelity Reaction buffer containing 18 mM $MgCl_2$ (Roche Applied Science, Mannheim, Germany), 200 μM dNTPs, 0.2 μM each primer, 1 μl of extracted DNA solution and 0.625 U FastStart High Fidelity Enzyme Blend (Roche Applied Science). Thermocycling (conducted in GeneAmp PCR System 9700; Applied Biosystems, Singapore) conditions were as follows: 94°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 54°C for 30 seconds and 72°C for 45 seconds; with a final step at 72°C for 10 minutes. Amplicon was separated by 1% agarose gel-electrophoresis and a 244 bp (Fig 3) was excised and sequenced using MegaBACE™ 1000 DNA Analysis System (GE Healthcare, Piscataway, NJ). In addition, for confirmation the amplicon was cloned in *Escherichia coli* JM109 by insertion into pGEM-T Easy vector (Promega, Madison, WI) according to the manufacturer's instructions and sequenced in both directions as described above.

The study was approved by the Human Ethics Committee of Khon Kaen University (Reference no. HE561085). Oral informed consent was obtained from the patient.

## RESULTS

The DNA sequence (GenBank accession no. KM387397) was identical with that of *S. stercoralis* 18S rDNA from various geographical localities (Fig 4) confirming that the parasite obtained from the patient was that of *S. stercoralis* larvae.

## DISCUSSION

The gold standard for diagnosis of strongyloidiasis is agar plate culture for stool and examination under the microscope (CDC, 2013b). Duodenal aspiration

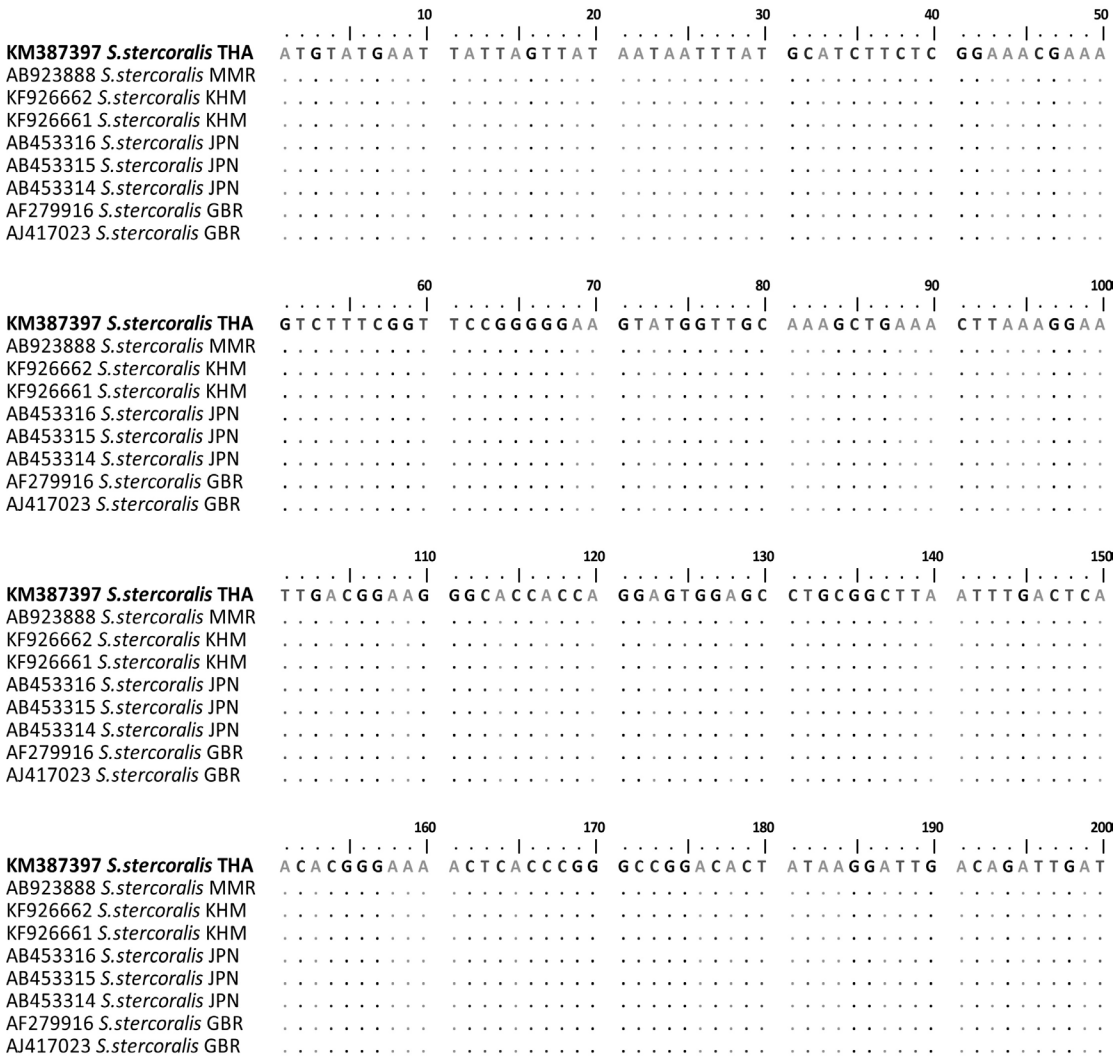


Fig 4–Multiple alignment of partial DNA sequences of *Strongyloides stercoralis* 18S small subunit ribosomal RNA gene, obtained from the present study (KM387397) and eight *S. stercoralis* sequences from GenBank from various geographical localities. Dots represent the conservation of the base among all the examined isolates. Country code (ISO 3166-1 alpha-3 codes) was presented (THA, Thailand; MYR, Myanmar; KHM, Cambodia; JPN, Japan; GBR, United Kingdom).

is more sensitive than stool examination, and duodenal biopsy may reveal parasites in gastric crypts and duodenal glands, or eosinophilic infiltration into the lamina propria (CDC, 2013b).

In this case, the patient had been receiving corticosteroid treatment regularly for three years and likely had a pre-existing infection with *S. stercoralis*. We clearly demonstrated using PCR and DNA sequencing of putative *S. stercoralis* in formalin-fixed, paraffin-embedded

sections that strongyloidiasis was present with intestinal obstruction, due to mesenteric lymphadenopathy. This is the first such case proved using molecular techniques.

Retrospectively, the patient had progressive and intermittent subacute abdominal symptoms of bloating and fullness prior to the acute event. These symptoms may be a prognostic feature of presumptive *S. stercoralis* infection, and strongyloidiasis should be considered when taking care of patients presenting with such abdominal symptoms. Preventive measures, such as periodic stool examination for the parasite, may be beneficial and may eventually prevent more serious complications in immunocompromised patients due to long-term corticosteroid treatment.

## ACKNOWLEDGEMENTS

This research was funded by grants from the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission and the Khon Kaen University, Thailand. WM and PMI were supported by The Thailand Research Fund (TRF) Senior Research Scholar grant, number RTA5580004 and the Faculty of Medicine, Khon Kaen University Grant, number TR57201. The authors acknowledge the support of Khon Kaen University Publication Clinic, Research and Technology Transfer Affairs, Khon Kaen University and Prof Yukifumi Nawa for assistance in preparing the manuscript.

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