

# Diagnosis of *Strongyloides stercoralis* by morphological characteristics combine with molecular biological methods

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**Abstract** Strongyloidiasis is one of the neglected tropical diseases caused by infection with the nematode *Strongyloides* genus and distributed worldwide. Strongyloidiasis can be fatal in immunosuppressed patients induced hyperinfection or disseminated strongyloidiasis. Unfortunately, until now, due to the unspecific clinical symptom in infected individuals and the low sensitivity diagnosis of strongyloidiasis, many patients were misdiagnosed every year. Furthermore, the larvae of the *Strongyloides stercoralis* (*S. stercoralis*) is similar to other nematodes such as hookworm, *Trichostrongylus* increased the difficulty of diagnosis. In this case, the patient is a 63-year-old male person, who had a nearly 30 years medical history of asthma and emphysema, and 4–5-year medical history of diabetes. The sputum examination found some parasite larvae, then we identify the larvae using clinical observation and morphological characteristics combine with examined cytochrome oxidase subunit 1 (COX1) and 18S rRNA genes by PCR, sequence analysis and finally classified by phylogenetic analysis, the larvae were diagnosed as *S. stercoralis*. Our results showed that diagnosis with strongyloidiasis by morphological characteristics combine with molecular biological methods can improve the

sensitive of diagnosis and provide a final diagnosis for the disease in the clinics.

**Keywords** Strongyloidiasis · *Strongyloides stercoralis* · COX1 · 18S rRNA · Morphological characteristics · Molecular biological methods

## Introduction

Strongyloidiasis, one of the neglected tropical diseases but widespread parasitic disease, is caused by infection with the nematode *Strongyloides stercoralis* and to a lesser extent by the zoonotic species *Strongyloides fuelleborni* (Beknazanova et al. 2016; Rodpai et al. 2016). *S. stercoralis* is common recognized parasite spread in tropical and subtropical areas, where heat and humidity favor the growth of helminths in general. According to conservative estimates, over 370 million people spread the world were infected (Bisoffi et al. 2013). *S. stercoralis* is a soil-transmitted helminth (STH); it has some peculiarities character which different from the other STHs. *S. stercoralis* has the ability to develop two distinct reproductive cycles: one asexual cycle inside the human host allowing autoinfection and the other involving sexual reproduction into the soil (Duvignaud et al. 2016). *S. stercoralis* infect human by filariform larvae through intact skin penetration; the larvae can either penetrate the intestinal mucosa and remain in the human organism (Ericsson et al. 2001).

*S. stercoralis* infection can cause gastrointestinal symptoms, skin problems and dermatitis or respiratory symptoms, including abdominal pain, diarrhea, nausea, vomiting, pruritus, dermatitis, cough, asthma, and dyspnea (Hochberg et al. 2001; Mascarello et al. 2011). Strongyloidiasis can be fatal in a few days or weeks in immunosuppressed patients hyperinfection or disseminated strongyloidiasis (Requena-

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Méndez et al. 2013; Greaves et al. 2013; Rodrigues et al. 2007), most of patients finally died of the *S. stercoralis* disseminated hyperinfection complicated by systemic lupus erythematosus (SLE), malignant tumor, leukemia, tuberculosis, diabetes, immunodeficiency and HIV infection (Meamar et al. 2007; Richter et al. 2006). The larvae rapidly increase in number and disseminate throughout the human body often causing bacteremia by intestinal translocation, and treatment is often ineffective at this stage (Mejia and Nutman 2012). Therefore, it is essential to diagnose and treat the infection at an earlier stage and complete eradication of the infection eliminates the future risk of a fatal complication (Krolewiecki et al. 2013). Unfortunately, the diagnosis of strongyloidiasis remains particularly low sensitivity due to the intermittent excretion of larvae in the feces. Examination of several stool samples and sputum, concentration techniques, improve performance of microscopy (Knopp et al. 2008), which nevertheless remains insufficiently sensitive.

Furthermore, the *S. stercoralis* larvae is similar to those of other nematodes such as the larvae of *Toxocara canis*, *Toxascaris leonine*, *Trichostrongylus*, hookworm, and *Ascaris lumbricoides*, increasing the difficulty of diagnosis. Thus, further work is necessitated to improve the sensitive of diagnosis. In the present case report, we diagnosed a patient who infected with *S. stercoralis* by morphological characteristics combine with molecular biological methods, providing a more sensitive diagnosis method for the disease in the clinics.

## Case report

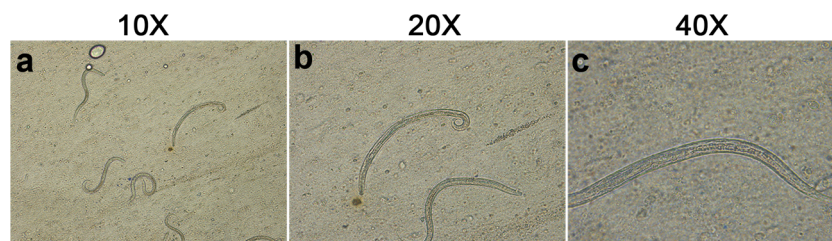
A 63-year-old male patient had a 30-year history of asthma and emphysema, 4–5-year history of diabetes. The patient is a farmer who was originated from a rural area in Guangdong Province, China. There are a dog mother and a group of little dog in his home. He presented at The First Hospital Affiliated to Guangzhou Medical University for general chest malaise, fever and cough since 23 days before. Complete medical examinations, including blood pressure, temperature and pulse rate, and examination of the head and chest, were done in The First Hospital Affiliated to Guangzhou Medical University. The clinical examination revealed that the pulse rate was regular, fever, blood pressure was normal, and the head was unremarkable. His condition deteriorated after admission and

then arrival to the emergency ICU. The patient felt dyspnea and sputum examination found that larvae infection, but failed to identify its accurate species. Then, the sputum specimen containing larvae was sent to the Department of Parasitology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China, for further identification.

The sputum was first observed by microscope for identification. Under the microscope, the sputum specimen revealed the presence of larvae. The larvae were small and linear, bodies were transparent, tails were short, one end was obtuse and the other end taper (Fig. 1). The characteristics of the larvae were very similar to the larvae of *S. stercoralis*, *Toxocara canis*, *Toxascaris leonine*, *Trichostrongylus*, hookworm, and *A. lumbricoides*. Thus, we hypothesized that the larvae might be a member of nematodes, but incapable to identify its accurate species. Therefore, in addition to morphological observation, other methods still need to apply for identification.

To discriminate the larvae species, the genomic DNA was isolated from larvae using Hipure Tissue DNA Mini Kit (Magen, China) according to the manufacturer's recommended protocol. Conventional PCR was conducted using the Green Master mix 2× (Promega, USA) and the primers for identifying COX1 gene of the larvae. The primers were designed according to the sequences of COX1 gene from *S. stercoralis*, *Toxocara canis*, *Toxascaris leonine*, *Trichostrongylus*, hookworm, and *A. lumbricoides*. Then, the PCR products were analyzed by 1.0% agarose gel electrophoresis, after staining with ethidium bromide. These results indicated that the larvae were *S. stercoralis*. The forward primer (5'-GTGGTTTTTGTAATTGAATGGTT-3') and reverse primer (5'-ACCAGTTATACCACCTATAGTAA-3') for *S. stercoralis* COX1 gene were used and the following PCR cycling conditions were used for amplification: initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 50 s, 53 °C for 50 s, 72 °C for 50 s and a final extension at 70 °C for 10 min. Furthermore, 18S rRNA gene was PCR amplified using the Green Master mix 2× (Promega, USA) and the *S. stercoralis* forward primer (5'-TCGTCCACCTTGGTGACTCT-3') and reverse primer (5'-CTTCCGCGAACTCGGTGATA-3'). The PCR consisted of 35 cycles at 95 °C for 50 s, 52 °C for 50 s, and 72 °C for 50 s with an initial denaturation (95 °C for 5 min) and a final extension (72 °C for 10 min). 18S rRNA PCR product was analyzed by 1.0% agarose gel electrophoresis, after staining with ethidium bromide.

**Fig. 1** *S. stercoralis* under the microscope, recovered from the sputum of the patient. Magnified ×10 (a), ×20 (b), and ×40 (c)



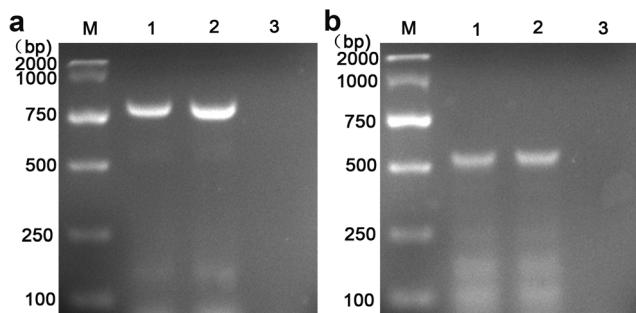


**Fig. 2** Representative results of electrophoresis of COX1 and 18S rRNA PCR products from larvae sample. DNA was extracted from samples and PCR was performed as described in Materials and Methods. **a** Lane M: DL2000 DNA marker, lanes 1 and 2: COX1 PCR product, and lane 3: negative control (no DNA). **b** Lane M: DL2000 DNA marker, lanes 1 and 2: 18S rRNA PCR product, and lane 3: negative control (no DNA)

In the PCR analyses of COX1 and 18S rRNA, species-specific products coinciding with the species-specific primers were successfully detected (Fig. 2). COX1 and 18S rRNA PCR results indicated that the larvae were *S. stercoralis* larvae.

To confirm the PCR results, further exploration was used to identify the larval based on genes sequenced. The amplified PCR products of the larvae COX1 and 18S rRNA genes were electrophoresed on a 1% agarose gel and the expected band was excised, purified using Agarose Gel DNA Extraction Kit (TakaRa, Japan), then the purified DNA was sequenced by company (Invitrogen). The sequences of COX1 and 18S rRNA were used for homology search by the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The BLAST results indicated that the sequences of COX1 and 18S rRNA had high homology with published *S. stercoralis* COX1 gene

(Accession No. KU962143.1) and 18S rRNA gene (Accession No. LC085481.1), respectively. The alignments produced a score = 1425 bits (1580), expect = 0.0, identities = 790/790 (100%), gaps = 0/790(0%), and strand = Plus/Plus for COX1 and a score = 865 bits (958), expect = 0.0, identities = 483/484 (99%), gaps = 1/484 (0%), and strand = Plus/Plus for 18S rRNA. Taken together, this data indicate that the larvae were *S. stercoralis* larvae.

To assign the sequences of COX1 and 18S rRNA to the species/genotype level, phylogenetic analysis was performed by comparison of the obtained sequences with those reference sequences downloaded from GenBank. Multiple sequence alignment was performed by CLUSATLW with default parameters, and phylogenetic trees of COX1 and 18S rRNA was reconstructed with MEGA7.0.14 using neighbor joining method and edited in Figtree v1.4.2. Bootstrap values were used to assess the robustness of clusters using 1000 replicates. Our phylogenetic analyses showed that both the observed COX1 and 18S rRNA sequences were clustered closer to *S. stercoralis* reference sequences (Figs. 3 and 4). In summary, all results confirmed that the larvae in this case were *S. stercoralis*.

## Discussion

*S. stercoralis* remains one of the neglected tropical diseases. Strongyloidiasis is distributed worldwide and constituted a medically important and socially neglected problem (Beknazarova et al. 2016). Humans infected third-stage

**Fig. 3** Neighbor-joining phylogenetic tree of COX1 in the sequence of case product and related species using MEGA7.0.14 and edited in Figtree v1.4.2. Multiple sequence alignment was performed by CLUSATLW with defaults

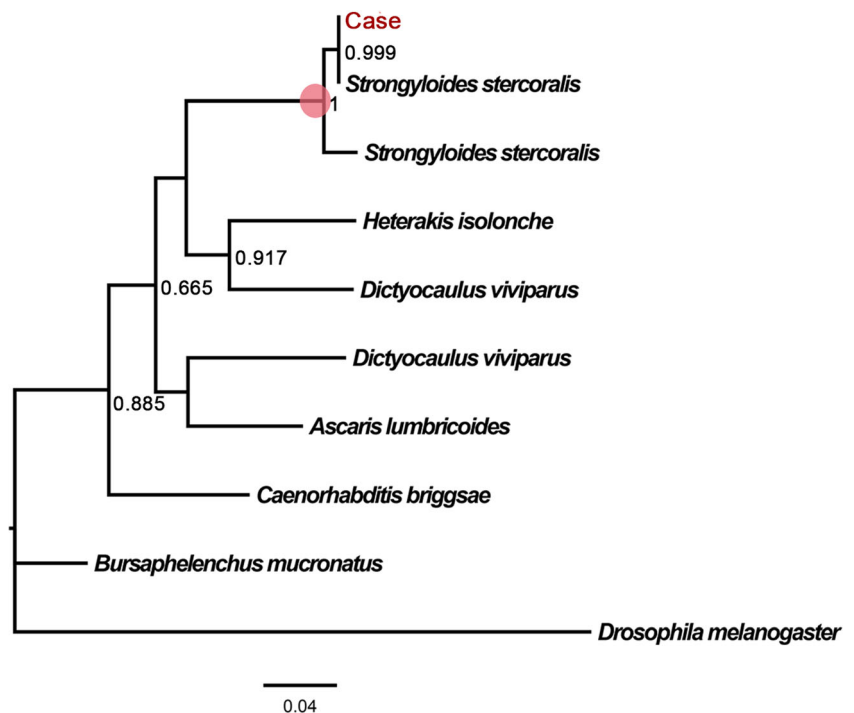
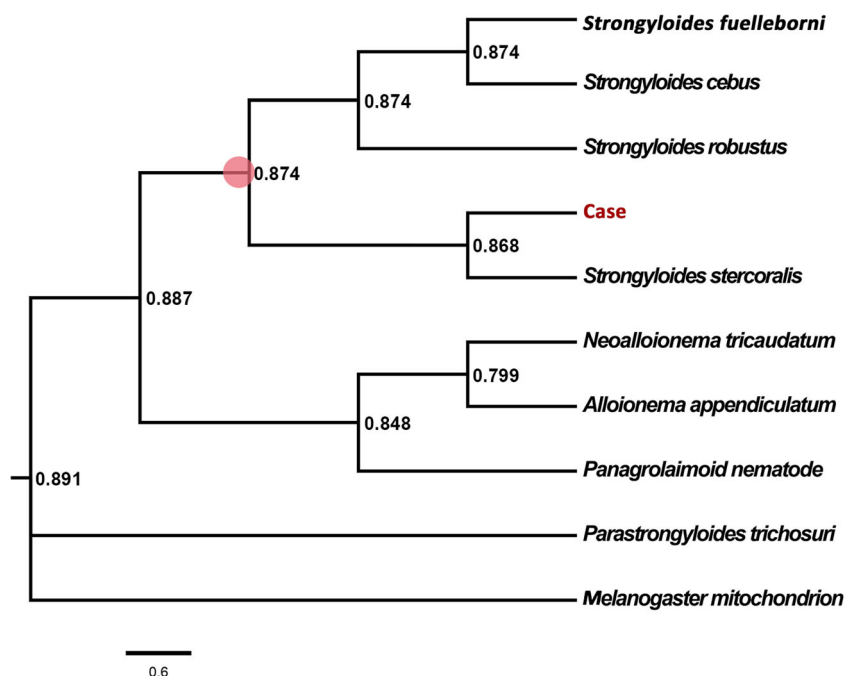


**Fig. 4** Neighbor-joining phylogenetic tree of 18S rRNA in the sequence of case product and related species using MEGA7.0.14 and edited in Figtree v1.4.2. Multiple sequence alignment was performed by CLUSATLW with defaults



filariform larvae of *S. stercoralis*, third-stage filariform larvae is transmitted from the soil and penetrates the skin and then transport to the respiratory system via the bloodstream, migrate along with swallowed sputum and enter the gastrointestinal tract, matures in the duodenum and upper jejunum (Kishimoto et al. 2008). Strongyloidiasis can be fatal by the larvae rapidly increase in number and causing bacteraemia by intestinal translocation (Buonfrate et al. 2015). Larvae is similar to those of other nematodes such as the larvae of *Toxocara canis*, *Toxascaris leonine*, *Trichostrongylus*, hookworm, and *A. lumbricoides* which also can be infected in the lung, increasing the difficulty of diagnosis. The diagnosis of strongyloidiasis remains particularly low sensitivity by examination of several stool samples and sputum. Therefore, sensitivity diagnosis is critical in patients at high risk due to *S. stercoralis* infection.

Herein, we confirmed a case of Strongyloidiasis caused by *S. stercoralis* in a 63-year-old male. In this case, the patient is a farmer who has 30-year history of asthma, history of emphysema, 4–5-year diabetes, and has the history of contact with dogs. We found the larvae in the sputum specimens and identified under microscope. The larvae were small and linear, bodies were transparent, tails were short, one end was obtuse and the other end taper (Fig. 1). We presumed that these kinds of larvae might be the larvae of *S. stercoralis*, *Toxocara canis*, *Toxascaris leonine*, *Trichostrongylus*, hookworm, *A. lumbricoides*, but unable to identify its accurate species. To identify the larvae species, we examined the COX1 and 18S rRNA genes of the larvae by PCR amplification, and the PCR results indicated

that the larvae were *S. stercoralis* larvae (Fig. 2). Then, the amplified PCR products of the larval COX1 and 18S rRNA genes were sequenced and the sequences were used for homology search using the BLAST program against GenBank database. The BLAST results indicated that the sequences of COX1 and 18S rRNA had high homology with published *S. stercoralis* COX1 gene (Accession No. KU962143.1) and 18S rRNA gene (Accession No. LC085481.1), respectively. The phylogenetic analyses showed that both the observed COX1 and 18S rRNA sequences were clustered with *S. stercoralis* reference sequences (Fig. 3 and Fig. 4). The clinical observation and morphological characteristics combine with PCR findings, we confirmed the patient was infected with *S. stercoralis*.

## Conclusions

In this case, the patient was a 63-year-old male has 30-year history of emphysema, 4–5-year diabetes, immunocompetent and immunocompromised. Because the patient delayed to diagnose *S. stercoralis* infection and had high risk complications, after arrival to the emergency ICU, the patient was dead. Therefore, it is essential to diagnose and treat the infection at an earlier stage. In our study, we identified the Strongyloidiasis by clinical observation and morphological characteristics combine with molecular biological methods, providing a final diagnosis method for the disease in the clinics.

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### Compliance with ethical standards

**Ethical approval** The study was reviewed by Human Research Ethics Committee of the Sun Yat-sen University in China. Written informed consent was obtained from the patient.

**Consent for publication** Written informed consent was obtained from the patients for the publication of this report.

**Competing interests** The authors declare that they have no competing interests.

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