RESEARCH ARTICLE

Molecular characterization of hookworm spp. isolated from food handlers, Khartoum, Sudan: A cross-sectional study

[version 1; peer review: 2 approved with reservations]

Tarig A. Gamar ¹, Hassan H. Musa², Hisham N. Altayb³,
Mohamed H. Mohamed ⁴, Adam D. Abakar⁵

¹Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, University of Sciences and Technology, Khartoum, Sudan

²Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan

³Department of Microbiology, Faculty of Medical Laboratory Sciences, Sudan University of Science and Technology, Khartoum, Sudan

⁴International University of Africa, Khartoum, Sudan

⁵Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan

V1 First published: 24 May 2018, 7:662
<https://doi.org/10.12688/f1000research.14683.1>

Latest published: 24 May 2018, 7:662
<https://doi.org/10.12688/f1000research.14683.1>

Abstract

Background: Hookworms infect the intestines, cause an itchy rash, respiratory and gastrointestinal problems, and eventually iron deficiency (anaemia) due to the ongoing loss of blood. The objectives of this study were to assess the prevalence and molecular characterization of hookworms isolated from food handlers attending the Public Health Laboratories in Khartoum state, Sudan, for annual check-ups, and to assess the efficiency of PCR as molecular probe for hookworm infection.

Methods: A total of 350 food handlers' participant's stool samples who were not suspected to be infected with hookworms were studied. Conventional methods were applied to make an early diagnosis. Stool samples were collected from public health laboratories (the public health lab in the Medical Commission) of Khartoum State; Omdurman locality, Khartoum North locality and Khartoum locality between October 2016 and April 2017. Specific identification was made by PCR on specimens identified as positive by Baermann's technique, which were then sequenced and genotyped.

Results: The prevalence of hookworms in the stool samples of food-handlers was 1.43%. One larval specimen recovered by Baermann's technique was confirmed to be *Necator americanus* by PCR. PCR also confirmed that *Necator americanus* was the common species isolated from four further specimens. The results of DNA sequencing for *Necator americanus* were deposited in NCBI GenBank under the following accession numbers: sample 91, [MH035824](#); sample 92, [MH035825](#); sample 294, [MH035826](#); and sample 319 [MH035827](#).

Open Peer Review

Approval Status

	1	2
version 1 24 May 2018	 view	 view

1. **Luis Fernando Viana Furtado** , Federal University of Minas Gerais, Belo Horizonte, Brazil

Vivian Jordania da Silva, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

2. **Nicole Gottdenker**, University of Georgia, Athens, USA

Any reports and responses or comments on the article can be found at the end of the article.

Conclusion: PCR was found to be effective for confirmation of the diagnosis of hookworm infection and can aid the clinician in initiating prompt and appropriate antiparasite therapy.

Keywords

Hookworm, Genotyping, Molecular sequencing, *Necator americanus*, Sudan

This article is included in the **Pathogens** gateway.

Corresponding author: Tarig A. Gamar (tariquamer35@yahoo.com)

Author roles: **Gamar TA:** Data Curation, Investigation, Methodology, Resources, Visualization, Writing – Original Draft Preparation; **Musa HH:** Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Altayb HN:** Software, Writing – Original Draft Preparation, Writing – Review & Editing; **Mohamed MH:** Data Curation, Formal Analysis, Software; **Abakar AD:** Supervision, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2018 Gamar TA *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

How to cite this article: Gamar TA, Musa HH, Altayb HN *et al.* **Molecular characterization of hookworm spp. isolated from food handlers, Khartoum, Sudan: A cross-sectional study [version 1; peer review: 2 approved with reservations]** F1000Research 2018, 7:662 <https://doi.org/10.12688/f1000research.14683.1>

First published: 24 May 2018, 7:662 <https://doi.org/10.12688/f1000research.14683.1>

Introduction

Ancylostomatidae and strongyl nematodes were known to pose a burden among a variety of mammalian hosts, including humans¹. *Ancylostoma duodenale* and *Necator americanus* are the species recorded as being most responsible for human infection. They are found in mostly in warm and tropical areas², and infect around 1.3 billion people worldwide³. *A. duodenale* are located in Central, Eastern and Northern Africa, India, Australia and Europe⁴. Further, *N. americanus* is present in Sub-Saharan Africa, Eastern Asia and Southeast Asia⁴. A zoonotic species in Asia (*A. ceylanicum*), also causes human infection; however, this is of limited significance and its exact geological appropriation has not been depicted. The most widespread of all hookworms are *A. caninum*, a parasite that infects dogs, and has lately been affirmed to survive in the human gut (but without developing sexually)⁵. Laboratory diagnosis of hookworm infection, routinely based on the presence of eggs in faeces by direct microscopy and/or concentration techniques⁶. Sometimes after gathering, through faeces culture tests (after 24 hours), hookworm ova may have hatched and rhabditiform larvae might be visible; due to morphological similarity between the two, these larvae must be differentiated from *Strongyloides* larvae. The severity of disease can depend on the number of ova that are counted in faeces. Furthermore, in some cases, adult hookworms may be found⁶. Use of molecular techniques (such as PCR) for parasitic detection and identification is more accurate and effective than conventional methods, and requires DNA of the hookworms for detection. For example, identifying both types of internal transcript spacer (ITS1 and ITS2, individually) of ribosomal DNA (rDNA) are proven genetic markers of parasitic nematodes, including *A. duodenale* and *N. americanus*.

The aim of this study was to estimate the prevalence and molecular characterization of hookworms isolated from the stool of food handlers attending Public Health Laboratories in Khartoum state, Sudan, for annual check-ups.

Methods

Samples

A total of 350 stool samples were collected. A previously described formula was used to determine sample size⁷.

Food handlers working in food facilities in Khartoum State, annually medically checked in public health laboratories and willing to participate were included in this study irrespective of their age, gender and nationality. The public health laboratories were located in Khartoum State, Omdurman locality, Khartoum North locality and Khartoum locality, with sample collection conducted between October 2016 and April 2017. The age of participant ranged from 16 to 68 years, with an average age of 32 years; 46% of the participants were less than 29 years old compared with 54% of being 29 or above. The majority of participants were males (83.19%), with 16.81% being female. Distribution of samples according to the residence data showed that 101 participants were from Khartoum north (28.9%), 160 participants were from Omdurman (45.7%) and 89 participants were from Khartoum (25.4%).

In the first stage, the collected specimens were examined using a microscope (Olympus CX22, Japan), the formol-ether

concentration technique and Baermann's technique, as described previously⁸. Positive detected samples (those that included hookworm ova/larvae) were examined using PCR and DNA sequencing techniques for genotyping. Fresh stool specimens were collected from public health labs in Khartoum State.

Samples that were found to be positive for hookworms eggs by direct examination, or for larvae by Baermann's technique, were selected for PCR testing (five samples). The stool samples that were negative for parasites by direct smear or the formol-ether concentration technique on three consecutive stool samples were used as negative controls. For molecular examinations, all stool samples were preserved in 70% ethanol at -20°C. The third-stage larvae that were recovered by Baermann's technique (Filariform) were collected and preserved. The extracted DNA from filariform larvae were used as control DNA during the molecular assay using Biotechnology G-Spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Inc.), according to the manufacturer's protocol.

DNA extraction

DNA was extracted from stool as per manufacturer's instruction used iNtRON Biotechnology G-Spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Inc.) according to the manufacturer's instructions.

PCR amplification

One primer pair was used: RTHW1F (forward): 5'-GAT GAG CAT TGC WTG AAT GCC G-3') and RTHW1R (reverse): 5'-GCA AGT RCC GTT CGA CAA ACA G-3'⁹.

The partial ITS1, full-length 5.8S gene, and partial ITS2 ribosomal DNA regions were amplified from larvae and ova using PCR. Amplicon sizes were approximately 485 bp (if it typical to *N. americanus*) or 380 bp (if it typical to *Ancylostoma* spp.). The procedure for single-round PCR amplification was performed according to Maxime PCR premix kit (iNtRON Biotechnology, Inc.) REF technique. Briefly 5 µl of DNA extract was added to PCR premix (Maxime PCR premix kit i-Taq), containing i-Tag™ DNA polymerase, dNTP mixture and reaction buffer. Next, 2 µl primer (forward and reverse) was added alongside 13 µl of nuclease-free water. The reaction mixture was initially denatured at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 secs, annealing at 65°C for 30 secs and extension at 72°C for 30 sec. This was followed by a final extension step for 10 min at 72°C in a thermal cycler (SensoQuest GmbH, Germany).

Approximately 5µl of PCR product was electrophoresed on a 1.5% agarose gel (containing 1.5 µg/100 ml ethidium bromide) in Tris-borate-EDTA buffer, along with the tracking dye bromophenol blue, initially at 120 V and 35 A for 60 min. Thereafter, bands were visualized under UV light and an amplicon of 485 bp was considered positive for hookworm DNA.

DNA sequencing

DNA sequencing was carried out to confirm identification of the pathogen. Owing to the limited amount of DNA generated from one sample, only four samples of PCR products (485 bp) were sequenced using Sanger sequencing (Macrogen, Inc.,

Korea). The DNA fragment was 485 bp (if from *N. americanus*) or 380 bp (if it typical of *Ancylostoma* spp) from an internal sequence of the amplicon of single-round PCR were obtained using the specific primers. Two sequence fragments were generated for five samples, which were edited manually to correct possible base errors using **BIOEDIT** 7.09. They were then subsequently joined to reconstruct a fragment of 485 bp or 380 bp spanning genes for hookworm spp.

Bioinformatics analysis

DNA sequences were compared with the NCBI database to check DNA sequencing quality and specificity using the nucleotide BLAST server. Sequences were submitted in sequence alignment form to **Clustal W** (online tool) for multiple sequence alignment.

Sequences similarity and alignment

Firstly, before uploading the sequences to NCBI, we proof-read the nucleotide chromatogram using **Finch TV** software version 1.4.0 to ensure that all ambiguous sites were correctly called and to determine the overall quality. Next, nucleotides sequences were searched for sequence similarity using nucleotide BLAST¹⁰ Highly similar sequences were retrieved from NCBI and subjected to multiple sequence alignment using **BIOEDIT** software¹¹.

Ethical considerations

Samples were collected from participants after provision of informed consent. Ethical approval was obtained from the National Committee for Research, Ministry of Health, Khartoum State.

Results

In this study, the stool samples of 350 participants were investigated for hookworms; five samples were found to be

positive (1.43%) using the formol-ether concentration technique. One sample was found to be positive using Baermann's technique, which was used as the positive control. Five samples were found to be positive by PCR, as shown in **Figure 1**.

Four hookworm samples (91, 92, 294 and 319) were sequenced by Macrogen, Inc., Korea and the sequences uploaded to **NCBI Genbank** (accession numbers: **MH035824** (sample 91), **MH035825** (sample 92), **MH035826** (sample 294) and **MH035827** (sample 319). Using nucleotide BLAST, the sequence of samples 91, 92, 294 and 319 were queried for alignment. Samples 91 and 92 showed 100% similarity with *N. americanus* isolated genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA (**Figure 2**). Sample 294 showed 98% similarity to *N. americanus* isolated genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA (**Figure 3**). Sample 319 showed 97% similarity with *N. americanus* isolated genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA, (**Figure 4**).

Dataset 1. Complete positive/negative results for each technique used to identify parasites in every stool sample

<http://dx.doi.org/10.5256/f1000research.14683.d204176>¹²

Discussion

The present study indicates that the prevalence of hookworms in food-handlers who attended for annual check-ups in Khartoum State, Sudan was 1.43%. To the best of our knowledge, this is the first study to identify hookworm infection in Sudan using molecular techniques; it can therefore serve as a baseline for studies of hookworms in Sudan. Molecular techniques are more advantageous for hookworm identification as they are rapid and more sensitive. DNA was extracted from larva and ova using the iNtRON Biotechnology G-Spin™ Total DNA

Figure 1. 2% Agarose gel electrophoresis of 18S rRNA PCR products of Hookworm specimens. Lane M, 100 bp marker; lane 1, positive control 485 bp; lanes 2–5, positive samples; lane 6, negative control; lane 7, negative sample.

Figure 2. Query of sample 91 hookworm DNA using nucleotide BLAST. Red lines indicate high identity; sample 91 showed 100% identity to *Necator americanus* genes for 18S rRNA, ITS1, 5.8S rRNA and ITS2.

Figure 3. Query of sample 294 hookworm DNA using nucleotide BLAST. Red lines indicate high identity. Sample 294 showed 98% identity to *Necator americanus* genes for 18S rRNA, ITS1, 5.8S rRNA and ITS2.

Figure 4. Query of sample 319 hookworm DNA using nucleotide BLAST. Red lines indicate high identity. Sample 319 showed 97% identity to *Necator americanus* genes for 18S rRNA, ITS1, 5.8S rRNA and ITS2.

Extraction Kit. Partial ITS1, full-length 5.8S and partial ITS2 rDNA regions were amplified and then sequenced. Molecular analysis was used to confirm human infections with one species of human hookworms, namely, *N. americanus*. The nucleotide sequences were analyzed by nucleotide BLAST searching and DNA was aligned using Clustal W¹³. Amplicon sizes were approximately 485 bp (typical of *N. americanus*). Sequences showed extremely high similarities (97–100%) with hookworm sequences in the GenBank database. The 4 samples studied were *N. americanus*. They were similar to *N. americanus* LC036565.1 (Japan), KM891738.1 (China) and LC036563.1 (Southern Vietnam). All sequences obtained in this study were deposited in the GenBank database. The results also indicate that PCR may be considered as a sensitive method for confirming a diagnosis of hookworm infection, and can aid the clinician in initiating prompt and appropriate antiparasitic therapy.

The differences in hookworm species populations causing human disease in different areas may possibly be related to parasite manner, ethnicity, atmosphere, temperature, and ecological factors^{2,14}. The finding of *N. americanus* in Sudan goes well with other finding reported from Peninsular Malaysia¹⁵ and Thailand⁹, where *N. americanus* was more common than *A. ceylanicum*. Their findings also supported this study that *A. duodenale* infection was not found.

Conclusion

PCR is a valuable tool for the laboratory diagnosis of parasites. It was found to be effective, sensitive and confirmatory for the diagnosis of hookworm infection and can aid the clinician in initiating prompt and appropriate antiparasite therapy. We

confirmed that the major hookworm species infecting humans in Sudan is *N. americanus*.

Data availability

Dataset 1. Complete positive/negative results for each technique used to identify parasites in every stool sample. DOI: [10.5256/f1000research.14683.d204176](https://doi.org/10.5256/f1000research.14683.d204176)¹².

Sequence of sample 91, Accession number MH035824: <http://identifiers.org/ncbigi/GI:1356678983>.

Sequence of sample 92, Accession number MH035825: <http://identifiers.org/ncbigi/GI:1356678984>.

Sequence of sample 294, Accession number MH035826: <http://identifiers.org/ncbigi/GI:1356678985>.

Sequence of sample 294, Accession number MH035827: <http://identifiers.org/ncbigi/GI:1356678986>.

Competing interests

No competing interests were disclosed.

Grant information

The authors declared that no grants were involved in supporting this work.

Acknowledgements

The authors extend their heartfelt thanks to the participants and staff for their support.

References

- Anderson RC: **Nematode Parasites of Vertebrates: Their Development and Transmission.** (Cabi). 2000.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Beaver PC, Jung RC, Cupp EW, *et al.*: **Clinical Parasitology.** (Lea & Febiger). 1984.
[Reference Source](#)
- Crompton DW: **How much human helminthiasis is there in the world?** *J Parasitol.* 1999; **85**(3): 397–403.
[PubMed Abstract](#) | [Publisher Full Text](#)
- de Silva NR, Brooker S, Hotez PJ, *et al.*: **Soil-transmitted helminth infections: updating the global picture.** *Trends Parasitol.* 2003; **19**(12): 547–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Prociw P, Croese J: **Human enteric infection with *Ancylostoma caninum*: hookworms reappraised in the light of a “new” zoonosis.** *Acta Trop.* 1996; **62**(1): 23–44.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jayaram Paniker CK: **Textbook of Medical Parasitology.** (Jaypee Brothers Medical Publishers (P) Ltd). 2007.
[Publisher Full Text](#)
- Sathian B, Sreedharan J, Baboo SN, *et al.*: **Relevance of Sample Size Determination in Medical Research.** *Nepal J Epidemiol.* 2010; **1**(1): 4–10.
[Publisher Full Text](#)
- Garcia LS, Bruckner DA: **Diagnostic Medical Parasitology.** (American Society for Microbiology (ASM)). 1997.
[Reference Source](#)
- Phosuk I, Intapan PM, Thanchomnang T, *et al.*: **Molecular detection of *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, and *Necator americanus* in humans in northeastern and southern Thailand.** *Korean J Parasitol.* 2013; **51**(6): 747–49.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Altschul SF, Madden TL, Schäffer AA, *et al.*: **Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.** *Nucleic Acids Res.* 1997; **25**(17): 3389–402.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hall TA: **Bioedit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT.** In *Nucleic acids symposium series.* ([London]: Information Retrieval Ltd., c1979-c2000) 1999; 95–98.
[Reference Source](#)
- Gamar TA, Musa HH, Altayb HN, *et al.*: **Dataset 1 in: Molecular characterization of hookworm spp. isolated from food handlers, Khartoum, Sudan: A cross-sectional study.** *F1000Research.* 2018.
[Data Source](#)
- Thompson JD, Higgins DG, Gibson TJ: **CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice.** *Nucleic Acids Res.* 1994; **22**(22): 4673–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hoagland KE, Schad GA: ***Necator americanus* and *Ancylostoma duodenale*: Life History Parameters and Epidemiological Implications of Two Sympatric Hookworms of Humans.** *Exp Parasitol.* 1978; **44**(1): 36–49.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ngui R, Ching LS, Kai TT, *et al.*: **Molecular identification of human hookworm infections in economically disadvantaged communities in Peninsular Malaysia.** *Am J Trop Med Hyg.* 2012; **86**(5): 837–42.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 15 October 2018

<https://doi.org/10.5256/f1000research.15980.r36850>

© 2018 Gottdenker N. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Nicole Gottdenker

Department of Veterinary Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

This is a short data report on the molecular identification of *Necator americanus* in food handlers in Sudan which also serves as a 'proof of concept' for the molecular identification of hookworm species in feces. Although the report is not 'novel', it is new information for this region, and important information for public health officials and diagnostic laboratory scientists overall, and the data may be useful for those who study enteric parasites in humans in other regions.

I recommend someone that is an expert in the molecular diagnostics of hookworm infections to carefully evaluate the methods- to the best of my knowledge the methods are sound.

Major comments:

Please include values for the proportion infected and the 95% confidence intervals for all proportions infected/detected reported in your data. I think that the discussion section could be expanded a bit more to include other studies of molecular diagnostics of hookworm and compare the prevalence and detection rates of those studies. Since in Dataset 1 you have complete positive/negative results of each technique, you may also be able to do statistical tests of agreement.

In your conclusion, you state that 'We confirmed that the major hookworm species infecting humans in Sudan is *N. americanus*.' I think that a sample size of 350 stool samples is insufficient to make that conclusion. You can say that in your sample population, you confirmed that the major hookworm species infecting humans in Sudan is *N. Americanus*.

Minor comments:

First sentence Introduction- change 'were' to 'are'

Change 'age of participant' to 'age of participants'

Fig 1- is the low molecular weight band a primer dimer?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Disease ecology, wildlife pathology, theoretical disease ecology, vector borne and zoonotic diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 03 October 2018

<https://doi.org/10.5256/f1000research.15980.r38900>

© 2018 Furtado L et al. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Luis Fernando Viana Furtado

Molecular Parasitology Laboratory, Parasitology Department, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

Vivian Jordania da Silva

Molecular Parasitology Laboratory, Parasitology Department, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

The authors propose the analysis of the presence of hookworms in 350 samples of food handlers from Sudan. From the total of positive samples, the authors did a PCR with primers specific for hookworms, confirming the species found by sequencing. Although the work has relevance, some points can be improved.

Abstract

It is not necessary to mention the collection period of the samples in the abstract.

In the results of the abstract, I suggest that the authors mention the absolute number of infected persons after the percentage: "The prevalence of hookworms in the stool samples of food-handlers was 1.43% (5/350)."

It is not necessary to specify the accession numbers of the GenBank sequences in the abstract.

At the conclusion of the abstract, the authors do not say anything about the percentage of parasitized individuals. What is the impact of this prevalence on food handlers? What are the risks to consumers?

Introduction

The citation of reference 3 is outdated. The most current prevalence of hookworm infection can be seen in Pullan RL, Smith JL, Jasrasaria R, Brooker SJ 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors* 7:37.

Methodology

It is not clear to me whether all 350 samples were analyzed by formaldehyde-ether concentration technique and Baermann's technique. If all samples were analyzed by these two methods, this should also be clear in the abstract.

The concentrations of the PCR components and DNA must be provided. The authors only provide the volumes used, but not the concentration. For reproduction of the technique, other researchers need the same conditions.

I did not understand why the authors mentioned that the "amplicon of 485 bp was considered positive for hookworm DNA". What if the amplicon had 380 bp (corresponding to the species *Ancylostoma* spp.)?

Discussion

The authors should be more cautious about discussing molecular results. Although it is true that PCR is a more sensitive technique than conventional coproparasitological methods, the results of the work do not allow to conclude this. The authors do not screen all samples comparing the different methods. In addition, the authors could discuss the possibility of cross-reaction. How specific are these primers so that they can amplify only the genetic material of hookworms and not that of other nematodes? How feasible is the use of PCR for diagnosis in Sudan? Although PCR is a more sensitive technique, the ideal for the reality of many localities is still the combination of different coproparasitological methods, which, although requiring expertise, are inexpensive techniques.

Some molecular techniques such as RFLP-PCR and qPCR have already been standardized for the determination of hookworm species. The authors should discuss something about these other techniques.

Although the prevalence of hookworm infection has been low, the authors discuss nothing about the impact of parasitosis on food handlers. There are many papers that discuss the risks of this

infection. Some speculation can be made.

Conclusion

To conclude that *Necator americanus* is the most prevalent hookworm in Sudan based only on these results is very strong. The analysis of only 350 samples, with the molecular confirmation of only four of these samples does not allow to conclude something so strong.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research