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Molecular detection of intestinal helminths and protozoa among young children in Dosso Region, Niger

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RESEARCH NOTE

REVISED **Molecular detection of intestinal helminths and protozoa among young children in Dosso Region, Niger**
[version 2; peer review: 2 approved]

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Abstract

Eukaryotic parasites are significant contributors to childhood illness in Niger. While helminthiasis have received national attention through mass deworming efforts, the epidemiology of intestinal protozoa in Niger remains underexamined. This study employed real-time PCR diagnostics to describe the prevalence of two schistosomes, four soil-transmitted helminths, and one protozoan parasite in Boboye Department, Dosso Region. Prevalence was assessed using bulk stool specimens collected from a population-based sample of 86 children residing in 9 communities. Anthropometric measurements were used to calculate child growth z-scores and stool consistency was graded. Helminths were absent from the study population, with the exception of a single *Schistosoma haematobium* infection (1/86; 1.2%). *Giardia duodenalis* was the only protozoa present, detected in 65% (56/86) of children. Prevalence of *G. duodenalis* peaked in 2-year-olds with 88% (15/17) positivity. The population was generally undernourished, though growth indices did not differ significantly between children with and without *G. duodenalis* infection.

Keywords

Schistosoma, soil-transmitted helminths, protozoa, molecular diagnostics

Open Peer Review**Reviewer Status**

	Invited Reviewers	
	1	2
version 2		
(revision)		
20 Aug 2020	report	report
	↑	↑
version 1		
23 Apr 2020	report	report

- Olusola Ojurongbe** , Ladoke Akintola University of Technology, Osogbo, Nigeria
- Oumar Gaye**, University Cheikh Anta Diop de Dakar, Dakar, Senegal
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Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: **Aiemojoy K:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Software, Supervision, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Arzika AM:** Methodology, Project Administration, Supervision; **Cook C:** Data Curation, Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Lebas E:** Data Curation, Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Pilotte N:** Investigation, Writing – Review & Editing; **Grant JR:** Investigation, Writing – Review & Editing; **Williams SA:** Conceptualization, Investigation, Supervision, Writing – Review & Editing; **Lietman TM:** Funding Acquisition, Supervision, Writing – Review & Editing; **Keenan JD:** Funding Acquisition, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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REVISED Amendments from Version 1

In the second version we added additional references, reported the median Ct values and discussed possible explanations and implications for a high burden of *G. duodenalis* infection.

Any further responses from the reviewers can be found at the end of the article

Introduction

Eukaryotic parasites are significant contributors to childhood illness in Niger. In 2012, the World Health Organization estimated that 10–49% of Nigeriens lived with intestinal or urogenital schistosomiasis, while more than two-thirds of children required preventative chemotherapy for soil-transmitted helminths (STH)¹. In 2015, over 75% of pre-school aged children across Niger were targeted for preventative anthelmintic treatment². While mass deworming programs have drawn attention to the public health significance of helminthiasis, the epidemiology of intestinal protozoa in Niger is not well described³. However, limited data suggest that protozoa may cause an appreciable fraction of clinically evaluated enteric infections^{4,5}.

Real-time polymerase chain reaction (qPCR) assays targeting high-copy-number genetic elements have been validated

for many globally burdensome parasites and have been shown to provide greater sensitivity and specificity than traditional copromicroscopy^{6–8}. This study makes use of these significant strides in diagnostic accuracy to evaluate the age-dependent prevalence of seven eukaryotic pathogens in nine rural communities of the Niger River Valley.

Methods

Study background

We conducted a cross-sectional study to evaluate the prevalence of helminthiasis, schistosomiasis and intestinal protozoa infection in Boboye Department, Dosso Region, Niger. The study was nested within the *Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance* (MORDOR) trial, a cluster-randomized trial investigating the effects of community mass administration of azithromycin on child health and mortality (Clinicaltrials.gov ID [NCT02048007](https://clinicaltrials.gov/ct2/show/study/NCT02048007)). Details of the MORDOR study design are available elsewhere⁹.

This nested sub-study occurred in 9 of the 30 MORDOR study communities (see [Figure 1](#)) in Niger during a regularly scheduled study visit between May and June of 2017. These 9 communities were included because at the time this nested sub-study commenced there were only 9 study communities remaining for the annual site visit. All children residing in the study communities who were sampled for participation

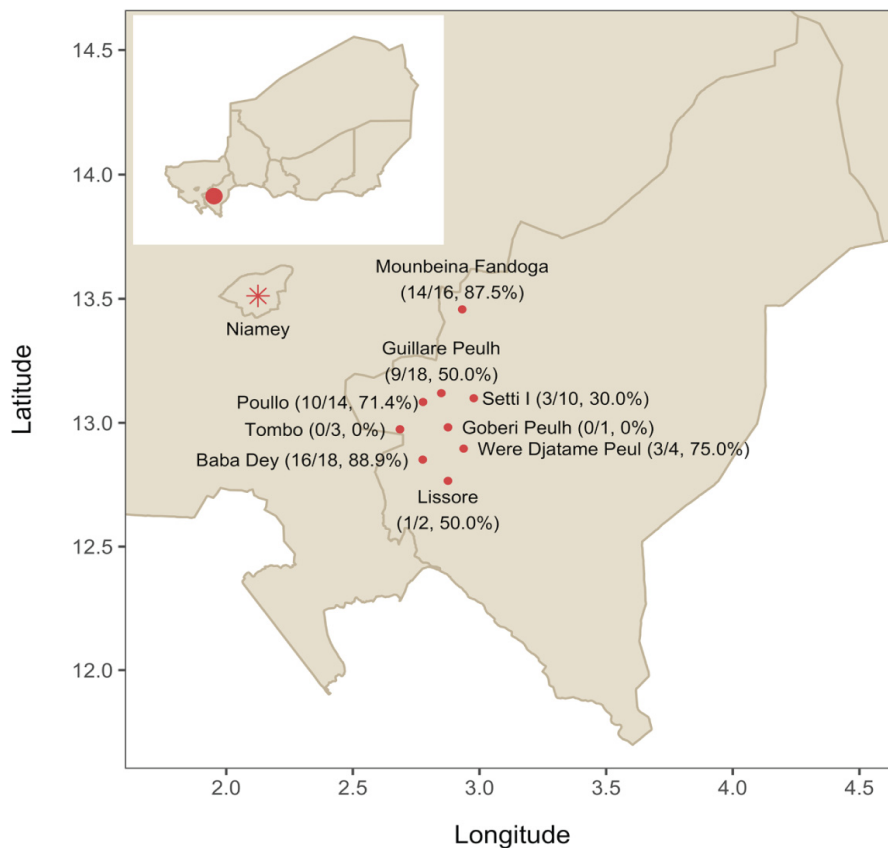


Figure 1. *Giardia duodenalis* prevalence by study site in Dosso Region, Niger. Study communities are indicated by red dots on the map. The prevalence is listed as the number of samples that tested positive for *Giardia duodenalis* over the total number of samples tested followed by the percentage.

in the parent trial were eligible to participate. In the 9 communities, 447 children age 0–4 years were eligible to participate and 354 participated in the MORDOR study visit and 86 were able to provide a same-day bulk stool sample.

Sample collection and analysis

On the day of the study visit, participating children gathered in a central location in the community. Trained field examiners performed three height and weight measurements per child in accordance with standard World Health Organization (WHO) protocols¹⁰. Stool was collected at the time of the study visit, by instructing caregivers to have their children defecate in a potty chair lined with a plastic bag. After defecation, the caregiver returned the stool sample to the field collection team. The field examiner then collected a 0.5-mL specimen and placed it in an empty sterile 2-mL tube. No media was added to the samples. The stool samples were immediately placed on ice and transported to a -20°C freezer by the end of the day.

Isolation of total DNA from bulk stool and rectal swabs followed a procedure optimized for the qPCR detection of intestinal helminths¹¹. Multi-parallel qPCR⁷ targeted the following species: the STH *Ancylostoma duodenale*⁶, *Ascaris lumbricoides*¹², *Necator americanus*⁶, and *Trichuris trichiura*⁶; the trematode flukes *Schistosoma haematobium*¹³ and *Schistosoma mansoni*¹³; and the protozoan parasite *Giardia duodenalis*¹⁴. All qPCR assays targeted highly repetitive non-coding elements with the exception of the *G. duodenalis* assay, which targets the small subunit ribosomal RNA gene. Procedures for sample collection, DNA isolation, qPCR, and quality control followed previously described protocols and standards¹¹.

The median height and weight measurements were used to calculate height-for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) *z*-scores according to WHO child growth standards¹⁵. We used generalized estimating equations

to evaluate differences in anthropometry *z*-scores according to *G. duodenalis* positivity adjusting for age and accounting for clustering by community. All analyses were run in R v3.5.3.

Ethical approval

Ethical committees from the Niger Ministry of Health and the University of California (San Francisco, CA, USA) granted approval for this study. Verbal informed consent was obtained in French from all caregivers. Verbal consent was obtained rather than written consent because of mixed literacy levels in the study population.

Results

Stool samples were collected from 86 children residing in 9 communities. The median age was 2 years old (IQR 1-4). Overall, 59.3% (51/86) of participants were female. The mean child growth *z*-scores were less than zero for all indicators (HAZ = -1.53 [SD 1.39], WAZ = -1.55 [SD 1.22], WHZ = -0.97 [SD 1.1]).

G. duodenalis infection was detected in 65% (56/86) children living in 7 of the 9 surveyed communities. The 2 communities for which no infections were observed, Goberi Peulh and Tombo, only contained 1 and 3 total participants, respectively. For the 7 communities in which *G. duodenalis* was detected, prevalence ranged from 30% (3/10) to 89% (16/18) (Figure 1). The prevalence of *G. duodenalis* increased with age, with 88.2% (15/17) of two-year-old children testing positive. The median Cq value for *G. duodenalis* positive stool was 29 (IQR 25.2, 31.7). Only 1 helminth infection was detected (*S. haematobium*) (Table 1).

Children who tested positive for *G. duodenalis* had a -0.18 (95% CI: -0.77–0.40) lower height-for-age *Z*-score, a 0.22 (95% CI: -0.3–0.75) higher weight-for-age *z*-score and a 0.52 (-0.07–1.1) higher weight-for-height *Z*-score. However, none of

Table 1. Prevalence of intestinal parasites among young children in Boboye Department, Dosso Region, Niger.

Variable	Age of child					
	< 1 year	1 year	2 years	3 years	4 years	Total
N children	11	20	17	13	25	86
Helminths						
<i>Ancylostoma duodenale</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ascaris lumbricoides</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Necator americanus</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Schistosoma haematobium</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	1 (1.2%)
<i>Schistosoma mansoni</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Trichuris trichiura</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Protozoa						
<i>Giardia duodenalis</i>	6 (54.5%)	9 (50.0%)	15 (88.2%)	10 (76.9%)	16 (76.0%)	56 (65.1 %)

these differences were statistically significant at the 0.05 level. Individual-level de-identified anthropometric, demographic and infection data are available (see *Underlying data*)¹⁶.

Discussion

We found that young children residing in the MORDOR study area in rural Niger had a high prevalence of *G. duodenalis* and a low prevalence of helminthic infections as measured by PCR in stool specimens. The population was generally undernourished, with all three anthropometric indices below average. Lower HAZ scores were observed among children positive for *G. duodenalis*, similar to prior observations in rural Amhara Region, Ethiopia¹⁷. However, there was no significant difference in growth indices between children with and without *G. duodenalis* infection, though this could be related to sample size.

The administrative Region of Dosso is situated in the Niger River Valley, a more densely populated region with higher rates of infectious disease than the majority of the nation¹⁸. In 2016, children 4 years of age and younger represented 81.1% of diarrhea cases, 43.7% of dysentery cases, and 37.7% of intestinal parasitisms recorded in Dosso Region, yet the etiological agents of these complaints remain unreported⁴. In other rural regions of sub-Saharan Africa the prevalence of giardia in young children has been reported to be as high as 56% when using a molecular assay on stool³. In a 2016 cross-sectional sero-survey in the Amhara region of Ethiopia over 80% of two year old children were seropositive for *G. intestinalis*¹⁹. Risk factors for giardia infection include close contact with animals and animal feces and manure, residing in a rural area, drinking contaminated water and eating raw fruit³. Given the high prevalence of *G. duodenalis* observed in the present study – albeit within a limited sample –the contributions of *G. duodenalis* infection to public health in the Niger River Valley warrant further investigation.

The low prevalence of STH and *S. mansoni* and *S. haematobium* in this cohort may relate to the success of national mass drug administration programs. In 2015, over 800,000 citizens of Dosso Region received praziquantel through mass drug

administration, including over 200,000 living in Boboye Department; over half a million received albendazole, with over 100,000 residing in Boboye Department⁴. However, without data on baseline prevalence and community-level anthelmintic distribution in this population, conclusions cannot be drawn. Another possible explanation for the low prevalence of *S. haematobium* may be that we tested stool rather than urine, the standard specimen type for this species. Though *S. haematobium* predominantly evacuates via urine, this species was detected in the feces of a single child. Ectopic elimination in the brain and intestine has been observed in *S. haematobium*, sometimes attributed to high parasite loads²⁰. Whether a greater number of children would have tested positive for *S. haematobium* by urine analysis cannot be known but could be an area of further research.

These findings indicate that *G. duodenalis* infections may be a significant contributor to child morbidity in the Dosso Region of Niger.

Data availability

Underlying data

Open Science Framework: Molecular detection of intestinal helminths and protozoa among young children in Dosso Region, Niger. <https://doi.org/10.17605/OSF.IO/FMTYH>¹⁶.

This project contains the following underlying data:

NigerParasitePCR.csv. (Demographic, anthropometric variables and infection status for each participant.)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

Acknowledgements

We would like to thank the children and guardians who participated in this study for their time and invaluable contributions. We would also like to acknowledge Jacqueline R. M. A. Maasch (University of Pennsylvania) for contributing the figures in this paper and for collaborating on data analysis.

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Open Peer Review

Current Peer Review Status:  

Version 2

Reviewer Report 02 September 2020

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Oumar Gaye

University Cheikh Anta Diop de Dakar, Dakar, Senegal

We have evaluated the revision version of the article submitted and can confirm that the corrections are appropriate.

Competing Interests: No competing interests were disclosed.

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.

Reviewer Report 21 August 2020

<https://doi.org/10.21956/gatesopenres.14376.r29414>

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Olusola Ojurongbe 

Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology, Osogbo, Nigeria

No further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Parasitology and genetics.

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.

Version 1

Reviewer Report 29 July 2020

<https://doi.org/10.21956/gatesopenres.14306.r29090>

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Oumar Gaye

University Cheikh Anta Diop de Dakar, Dakar, Senegal

Doudou Sow

University Gaston Berger of Saint-Louis, Saint-Louis, Senegal

Regarding the question 2 on methodology and results interpretation, authors should take into account comments below:

Methods section

1. How did authors select the 9 study communities among the 30 MORDOR communities? To be clarified.
2. Author must explain how did they choose the 86 children enrolled in this study among the 354 MORDOR participants, or it was an arbitrary choice? To be clarified.
3. Why did authors targeted *S. haematobium* in stool samples? *S. haematobium* is responsible of urogenital schistosomiasis and can be found in some cases in stool samples. Did they have previous data showing the importance of this parasite in stool samples?
4. In the reference 14, the authors mention positive controls but it is not clear what the positive controls were.
5. It is not considered appropriate to consider Ct values above 38 as negative, high Ct values can be an indicator of an (upcoming) problem of PCR contamination or non-specificity of the PCR.

Results section

1. Authors did not show any results on the intensity of infection. Authors should precise the median and/or the range of ct results to give an estimate of the intensity of infection.
2. Did authors compare real time PCR to microscopy results? If yes, results should be shown to support future epidemiological survey based on microscopy as PCR is expensive particularly in large surveys.

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

Yes

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

Partly

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

Yes

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

Yes

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

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: parasitology, molecular biology, epidemiology

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.

Reviewer Report 24 July 2020

<https://doi.org/10.21956/gatesopenres.14306.r29071>

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Olusola Ojurongbe 

Department of Medical Microbiology and Parasitology, Ladoké Akintola University of Technology, Osogbo, Nigeria

This manuscript reported a study carried out in Dosso Region, Niger on Molecular detection of intestinal helminths and protozoa among young children.

The authors reported high prevalence of *Giardia duodenalis* and generally undernourished population. The authors concluded that *G. duodenalis* infections may be a significant contributor to child morbidity in the Dosso Region of Niger. However, extremely low sample size of this manuscript makes it difficult to accept this conclusion. These, as well as other major concerns, must be addressed before this manuscript can be considered acceptable for indexing.

The presentation of the DNA extraction methodology is not clear. The author stated that “the Isolation of total DNA from bulk stool and rectal swabs followed a procedure optimized for the qPCR detection of intestinal helminths”. This procedure was not referenced and there was no mentioning of kit used. There are many procedure optimized for DNA extraction in stool. The author should properly explain what was done

The author also should briefly explain what he meant by “multi-parallel” in the result section.

The authors stated that “Goberi Peulh and Tombo, only contained 1 and 3 total participants” and no infection was observed in theses 2 communities. I will suggest that the names of these communities should not be listed as being free from parasitic infection as the sample size of 1 and 3 is too little to be considered.

“The prevalence of *G. duodenalis* increased with age, with 88.2% (15/17) testing positive” the author should correct this statement by including age “2 years old” in the sentence.

I will suggest that the author should not start discussion with “in conclusion”.

The author reported high prevalence of *G. duodenalis* in rural Niger. While the author attributed low STH and *S. mansoni* to the success of national mass drug administration programs, nothing was discussed as to why *G. duodenalis* infection was high. This should be discussed.

If according to the author there is a mass administration of PZQ that lead to the absence of STH and *S. mansoni*, it is not clear why the author is assuming that there will be a higher prevalence of SH in the same community if urine are to be tested. Both SM and SH are susceptible to PZQ. So the explanation of high load or ectopic egg elimination may not be tenable.

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

Yes

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

Partly

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

No

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

Yes

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

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Parasitology and genetics.

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.
