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Diagnosis of *Strongyloides stercoralis*: Detection of parasite-derived DNA in urine

Nilanjan Lodh[a,1], Reynaldo Caro[b,1], Shterna Sofer[c,1], Alan Scott[c], Alejandro Krolewiecki[b], Clive Shiff[c,*]

[a] *Marquette University, College of Health Sciences, Department of Clinical Laboratory Science, 561, North 15th Street, Milwaukee, WI 53233, USA*
[b] *Instituto de Investigaciones en Enfermedades Tropicales. Universidad Nacional de Salta, sede regional Oran, Alvarado 751, Orán (4530), Salta, Argentina*
[c] *Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, 615, N. Wolfe Street, Baltimore, MD 21205, USA*

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ABSTRACT

Detecting infections of *Strongyloides stercoralis* is arduous and has low sensitivity. Clinically this is a major problem because chronic infections may disseminate in the host and lead to a life threatening condition. Epidemiologically, *S. stercoralis* is often missed in surveys as it is difficult to identify by standard stool examination procedures. We present, for the first time, evidence that the infection can be detected in filtered urine samples collected and processed in the field and subsequently assayed for the presence of parasite DNA. Urine specimens (~40 mL) were collected from 125 test and control individuals living in rural and peri-urban regions of Northern Argentina. From the same individuals, fresh stool specimens were processed using three different copropological methods. Urine specimens were filtered in the field through a 12.5 cm Whatman No. 3 filter. The filters were dried and packed individually in sealable plastic bags with desiccant and shipped to a laboratory where DNA was recovered from the filter and PCR-amplified with primers specific to a dispersed repetitive sequence. Prevalence of *S. stercoralis* infection by stool culture and direct examination was 35/125 (28%), In contrast, PCR-based detection of parasite-specific trans-renal DNA in urine indicated that 56/125 (44.8%) carried the parasite. Of the patients that tested positive for urine-based parasite DNA, approximately half also tested positive in their stool specimens. There were 6.4% of cases where parasite larvae were seen in the stool but no DNA was amplified from the urine. As proof of principle, DNA amplification from urine residue reveals significantly more cases of *S. stercoralis* infection than the current standard stool examination techniques. Additional work is required to establish the relative utility, sensitivity and specificity of urine-based analysis compared to parasitological and nucleic acid detection from stool for clinical and epidemiological detection for *S. stercoralis* infection.

1. Introduction

Among the most neglected of the neglected tropical diseases is the infection caused by nematodes in the genus *Strongyloides*. Of the two species of *Strongyloides* that infect humans, *S. stercoralis*, which is mainly found in tropical and subtropical regions, is the most prevalent (Schad, 1989). Human infections with *S. fuelleborni* and *S. fuelleborni kellyi* are restricted to Africa and New Guinea, respectively (Pampiglione and Ricciardi, 1972; Ashford et al., 1992). The complicated life cycle of *Strongyloides* contributes to its persistence as a pathogen. Infection is initiated when the free-living infective third larval stage (L3) penetrate the skin. The larvae migrate, presumably via the blood and lungs, to the oropharyngeal area where they are swallowed and the parasite develops into reproductively active adult females in the small intestine where they release eggs. Within the intestinal tract, the first-stage larvae (L1) emerge from the eggs. The L1 s have two main fates. They can be released into the environment and undergo L2-L4 development into rhabditiform male and female worms and establish a single sexually reproducing, free-living cycle that generates large numbers of long-lived L3 parasites capable of spreading the infection. Alternatively, the L1 s can develop directly into an infective L3 within the intestine and initiate an autoinfection that, if uncontrolled as observed in immunocompromised individuals, can result in fulminant expansion of the parasite, multi-organ involvement and a fatal outcome. It is esti-

* Corresponding author.
E-mail address: cshiff1@jhu.edu (C. Shiff).
[1] These authors contributed equally to the work.

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Table 1
Demographics of population that provided specimens.

Age	Female	Male	Total
3–5	14	10	24
6–15	46	29	75
16–30	8	5	13
≥31	6	5	11
Total	74	49	123[a]

[a] 2 specimens were not included as age and sex was unrecorded, however 125 samples were used in the study which did not require this information.

mated that between 100 and 200 million individuals are infected with *Strongyloides* worldwide, however this is likely to be a serious underestimate because these infections are difficult to detect (Schar et al., 2013). The Kato-Katz and McMaster's procedures, the WHO recommended diagnostic techniques most commonly used to detect worm infections, focus on the identification of eggs in processed stool and hence miss larvae of *Strongyloides* spp. The low number of eggs or larvae released by females on a sporadic and unpredictable schedule compromises the sensitivity of these traditional diagnostic methods. Detection of specific cell-free DNA in urine appears to improve sensitivity over stool for some neglected tropical diseases (Ibironke et al., 2012; Lodh et al., 2014) as will be demonstrated here with *Strongyloides*.

Efforts to address this insensitivity gap have concentrated on serology to detect anti-*Strongyloides* antibodies and detection of parasite DNA in stool specimens (Pilotte et al., 2016). While antibody-based detection systems employing species-specific recombinant antigens can be a sensitive measure of exposure, the decline of antibody response over some months limits the utility of this approach for post-treatment assessment of incidence and prevalence. While DNA-based detection methods in stool specimens have been shown to be adequately sensitive (Basuni et al., 2011), the operational assumption that parasites are present in each stool sample may not be valid. An alternative approach that has shown validity for other pathogens is the detection of cell-free DNA in the urine (Ibironke et al., 2012). The utility of detecting parasite-specific trans renal DNA has been demonstrated for schistosomes (Ibironke et al., 2012) and *Plasmodium* (Mharakurwa et al., 2006).

The objective of this work is to demonstrate the feasibility of using a urine-based diagnostic test for *S. stercoralis*. To this end, a short repeat fragment of the *S. stercoralis* genome was tested for its practicality and diagnostic suitability among endemic communities in Northern Argentina.

2. Materials and methods

2.1. Study population

The study population was selected among rural and urban communities from Salta province, Argentina. Two rural villages, Solazuty and Rio Blanquito, (Fig. 1) are agricultural communities with open or limited sanitary facilities and a lack of potable water in the houses. The peri-urban community surrounds the city of San Ramon de la Nueva (population of ~74,000 (Fig. 1)) where the population has access to potable water from taps but in the periphery latrines are open and often there is alfresco defaecation. Participating individuals were among those attending at the outpatient laboratory of the Instituto de Investigaciones en Enfermedades Tropicales at the Universidad Nacional de Salta as well as individuals under coverage by the primary public health care system in communities participating in a programme for the control of soil transmitted helminths (STH) infections. Demographic data of the participants in this study are presented in Table 1.

Fig. 1. Map of Northern Argentina, indicating locations of the three communities that participated in the study. These are represented by stars. (Image Google Earth).

2.2. Ethical consent

Approval for this work was obtained from Commité de Ética, Colegio Médico de Salta, Salta, Argentina, and Johns Hopkins University (IRB number 6199).

2.3. Sample collection

Samples consisting of a single stool and a single urine sample were collected from members of the rural and periurban communities. Fresh stool specimens were taken to the University of Salta laboratory, and processed using sedimentation-concentration, Harada Mori and Baermann techniques for hookworm and *Strongyloides* identification as outlined previously (Garcia, 2001). Approximately 40 mL of urine was filtered through a 12.5 cm Whatman No. 3 filter disc. Filtration was done within 24 h after passing the urine specimen. The filter disc was dried under a fly proof cover and stored in a sealed plastic bag with desiccant at 4 °C and subsequently sent to Johns Hopkins University in Baltimore for processing and analysis for parasite DNA.

2.4. DNA detection

Filter papers were processed and analysed in a blinded fashion. Fifteen 1.0 mm discs were punched from the central portion of the filter paper and were transferred to a 1.5 mL Eppendorf tube and 800 μL of water was added to each tube. After incubation at 95 °C for 10 min the samples were subject to gentle agitation overnight at room temperature. Following the overnight stand, tubes were centrifuged at 4000 rpm to pack the paper and the supernatant was removed and processed from for DNA extraction using the