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Performance characteristics of diagnostic assays for schistosomiasis in Ontario, Canada

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Abstract

Introduction: Due to lower intensity of infection and greater intervals from last exposure, parasitologic detection methods for schistosomiasis are poorly sensitive in non-endemic areas, challenging accurate diagnosis.

Methods: We evaluated parasitologic *versus* indirect detection methods for schistosomiasis. We included specimens submitted for *Schistosoma* serology, and stool for ova and parasite microscopy. Three real-time PCR assays targeting *Schistosoma mansoni* and *S. haematobium* were performed. Primary outcome measures were sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), where both microscopy and serology were the composite reference standard against serum PCR.

Results: Of 8168 serum specimens submitted for *Schistosoma* serology, 638 (7.8%) were reactive and 6705 (82.1%) were non-reactive. Of 156,771 stool specimens submitted for ova and parasite testing, 46 (0.03%) were positive for eggs of *S. mansoni*. Four (0.5%) urine specimens were positive for eggs of *S. haematobium*. Combined serum PCRs targeting *S. mansoni* had a sensitivity and specificity of 27.8% (95% CI = 18.3–39.1%) and 100% (95% CI = 83.9–100%), respectively, with PPV of 100% (95% CI = 100%) and NPV of 26.9% (95% CI = 24.3–29.7%). The one serum sample positive for *S. haematobium* was also detectable by our *S. haematobium* PCR. No cross-reactivity was observed for all three PCR assays.

Conclusions: Although serology is highly sensitive, parasitologic tests signify active infection, but are limited by low population-level sensitivity, particularly in non-endemic settings. Although serum PCR offered no performance advantage over stool microscopy, its role in diagnostic parasitology should be pursued due to its high-throughput and operator-independent nature.

Keywords: diagnosis, PCR, schistosomiasis

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Introduction

Schistosomiasis, resulting from infection via freshwater exposure to the trematode parasite *Schistosoma*, is a neglected tropical disease of global clinical importance.[1–4] Severe morbidity and mortality in endemic areas arise due to chronic repeated exposure over years, but can be prevented with several transmission control measures and with early screening and treatment.[5–9] The principal species affecting humans are *Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, *S. haematobium*, and *S. intercalatum*, which lead to intestinal or hepatic schistosomiasis, causing portal hypertension and hepatic failure, and urogenital disease, causing a range of complications, including infertility and bladder carcinoma, respectively.[10,11] Chronic sequelae of schistosomiasis are due to the ability of *Schistosoma* eggs to

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remain within the host, where they incite tissue inflammation with resulting fibrosis or metaplasia.[2] Hence, the importance of transmission control measures including early and repeated treatment in areas of ongoing *Schistosoma* transmission.

In areas without transmission or with waning levels of transmission, presumptive and intermittent treatment without supportive diagnostics may be replaced with a test and treat strategy. Testing for schistosomiasis can be carried out for symptomatic patients, or for screening purposes, where an asymptomatic patient is tested based on their risk of exposure.[6] Given the increasing prevalence of migration to North America and Europe from areas of schistosomiasis risk,[5] it is essential to determine the diagnostic value of available tests to detect those with either symptomatic (fever, rash, chills, abdominal pain, and eosinophilia) or asymptomatic but active tissue disease.[12] The test of choice, particularly for asymptomatic screening, should be highly sensitive and thereby able to accurately exclude schistosomiasis.[6] Serology, or anti-*Schistosoma* antibody testing, is widely used for diagnosis and due to its high sensitivity, is especially useful for patients with low levels of parasite burden or even remote exposure to *Schistosoma*.[6,13,14] However, limitations of most serological testing include the inability to differentiate among the *Schistosoma* species and to distinguish between current and past infection, given that many individuals will remain serologically positive, even after treatment.[6] Direct parasitological testing, such as stool and urine microscopic examination for eggs, can detect active disease but are insensitive owing to the low number of eggs produced per worm couple per day, operator dependence, and reliance on advance technical expertise, which challenge technologists in non-endemic laboratories.[15] Novel molecular diagnostics, including polymerase chain reaction (PCR) are widely supplanting microscopy- and culture-based diagnostics in many areas of microbiology including parasitology.[16] By their very nature, molecular diagnostics are high-throughput, operator independent, and less labor intensive than microscopy for the detection of stool parasites.

As it is essential to understand the performance characteristics and limitations of our diagnostic tools to best serve our increasingly diverse patient population, we evaluated the performance of two *S. mansoni* and one *S. haematobium* real-time serum PCR assays compared with stool or urine microscopy and serology for the diagnosis of schistosomiasis at our reference laboratory.

Materials and methods

Study design and specimens

We performed a retrospective laboratory validation and included all specimens submitted to our reference laboratory for schistosomiasis serology, and stool specimens for ova and parasite (O&P) microscopic examination, between April 1, 2014 and December 31, 2017. Due to sample availability, a subset of 100 serum samples by convenience sampling, which were submitted for *Schistosoma* antibody detection with at least one corresponding stool sample submitted within 1 year of serum submission was evaluated by real-time PCR targeting *S. mansoni* and *S. haematobium*. Samples with indeterminate levels of antibody were excluded from the analysis of performance characteristics. Stool samples positive for *S. mansoni*, and urine samples positive for *S. haematobium* were also included in our validation.

Clinical microscopy and serology

Stool specimens submitted for O&P examination in sodium acetate acetic acid formalin (SAF) preservative were tested by formalin–ethyl acetate concentrate, iron–hematoxylin smear, and auramine–rhodamine fluorescence microscopy as per standard operating procedure. Stool samples that were positive for *Schistosoma* species were reported out as presence of eggs under standard reporting guidelines.[17] Urine specimens were centrifuged at 1500× g for 3 min, the supernatant were discarded and the sediment in the tube was used to prepare wet mounts for microscopic examination for *Schistosoma* eggs.

Schistosoma serology was performed with 5 μL of frozen serum diluted 1:40 using the commercial and Health Canada-approved SCIMEDX *Schistosoma* spp. Serology immunoglobulin G (IgG) kit (Microwell ELISA Kit Cat# SCHISTO-96, Dover, NJ) according to the manufacturer's protocol. This assay is unable to discriminate among the species of *Schistosoma*, and as per the manufacturer's validation, performance characteristics in the setting of *S. japonicum*

infection are unknown.[18] Absorbance reading (450 nm and reference filter at 620–650 nm) with optical density (OD) value of <0.2 was considered non-reactive; 0.2–0.4 was considered indeterminate; and >0.4 was considered reactive.

DNA extraction

DNA was extracted from 200 µL of serum or urine using Qiagen DNA Mini Kit (Germantown, MD) blood and body fluids protocol, with slight modification of incubation at 56°C for 1 hour and eluted with 60 µL Buffer AE. Qiagen Fast DNA Stool Mini Kit was used for DNA extraction from stool samples: 250 mg unpreserved frozen stool was mixed with 200 µL Buffer ASL and 1 mL InhibitEx before initiating five cycles of freeze-thawing with LN2 and 95°C heat block. Protocol for pathogen detection was subsequently followed and DNA was eluted with 60 µL Buffer AE. DNA was stored at −20°C prior to use.

Real-time PCR

Two *S. mansoni* and one *S. haematobium* real-time quantitative PCR (qPCR) assays were performed on all samples. The two assays targeting *S. mansoni* 121-bp tandem repeat sequence SM 1–7 (GenBank Accession# M61098) were conducted as follows: PCR 1 with 800 nM each of SRA1/SRS2 primers, and 300 nM of FAM-BHQ-labeled SRP probe;[13] and PCR 2 with 1000 nM each of F2/R2 primers, and 250 nM of FAM-BHQ-labeled PO2 probe,[16] with 12.5 µL Universal Taqman Master Mix (Thermofisher Scientific, Waltham, MA) and 5 µL DNA in a final volume of 25 µL. Similarly, *S. haematobium* PCR that targets the repetitive *DraI* sequence (GenBank Accession# DQ157698.1) was performed with 500 nM each of SH-FW and SH-RV primers and 250 nM of HEX-BHQ-labeled Sh-Probe.[19] Human beta-2-microglobulin PCR was performed as an extraction control as previously described.[20] All assays were run on Applied Biosystems® 7500HT Fast Real-Time PCR System with one cycle of 50°C for 2 min and 95°C for 10 min followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. All qPCR amplification curves were analyzed using a manual cycle threshold (Ct) of 0.2 and an automatic baseline. A result was called positive if the Ct value was <40 in the presence of a logarithmic amplification curve.

Cross-reactivity and limit of detection analysis

For the cross-reactivity analysis of serum *Schistosoma* PCR, a genomic DNA panel of other blood-borne parasitic pathogens, including *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *Babesia* spp. obtained from our malaria biobank housed at our reference laboratory, as well as commercial human DNA (Thermofisher Scientific, Waltham, MA) were included.

For the cross-reactivity analysis of *Schistosoma* stool PCR, true microscopy negatives and a panel other enteric parasites, including *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Clonorchis sinensis*, *Dicrocoelium dendriticum*, *Diphyllobothrium latum*, *Enterobius vermicularis*, *Strongyloides stercoralis*, *Taenia spp.*, *Blastocystis hominis*, *Cryptosporidium spp.*, *Cyclospora cayetanensis*, *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia lamblia* were obtained from our biobank of positive stool specimens.

Limit of detection (LOD) analysis for *S. mansoni* was performed using DNA extracted from an unpreserved frozen stool specimen containing *S. mansoni* and plotting Ct values by eggs count per gram weight. An unpreserved stool specimen was thawed and weighed, a small aliquot of stool was taken and the original tube was weighed again, with the difference being the weight of the stool that would be used to count the number of eggs to provide an egg count by weight. Equal volume of SAF was mixed with the stools and was fixed for 30 min. Slides were made with the whole mixture and *S. mansoni* eggs were counted. The total number of eggs counted was divided by the weight to determine eggs per gram stool. With the same stool sample, a defined weight of stool (42 mg) was taken for DNA extraction and eluted with 60 µL Buffer AE to calculate DNA volume (µL) by gram of stool. Combining the eggs count per gram stool and DNA volume per gram stool, eggs per DNA volume (µL) can be determined. Similarly, we used a defined volume (40 µL) of a frozen urine sample containing eggs of *S. haematobium* for microscopy to count the total number of eggs and determine eggs by urine volume (µL). 20 µL of urine were taken for DNA extraction and eluted with 60 µL of Buffer AE. Combining the eggs count per urine volume and DNA per urine volume, eggs per DNA volume (µL) can be determined.

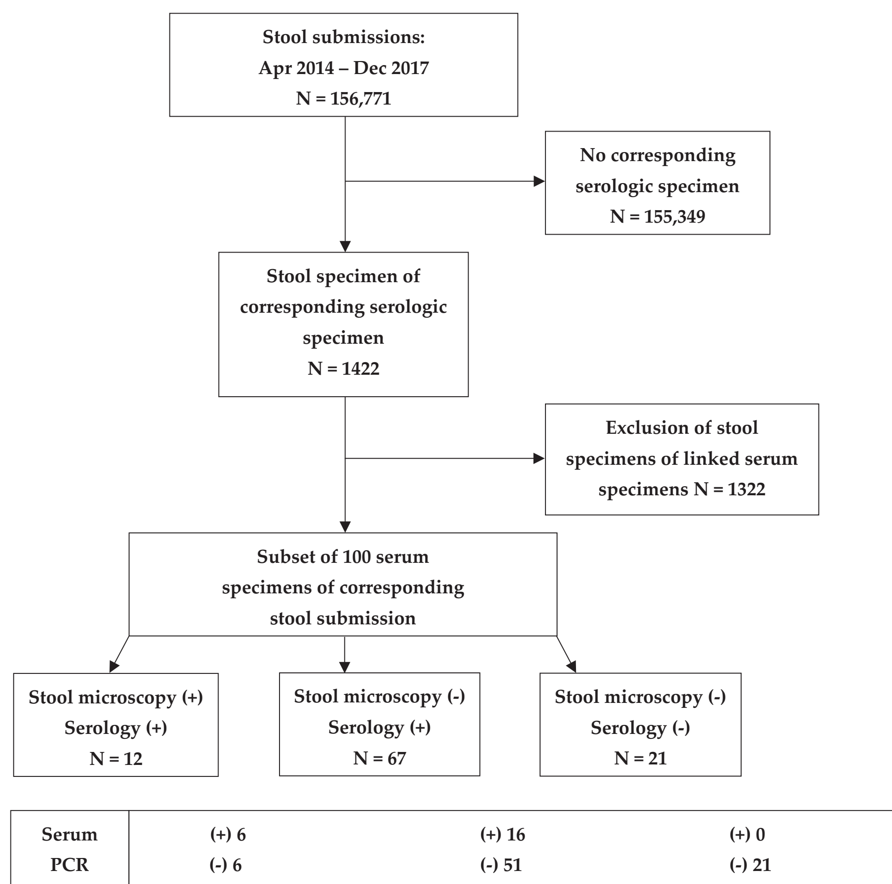

Figure 1. Flow diagram of specimens included in this study.

DNA containing *S. mansoni* (stool) and *S. haematobium* (urine) were serially titrated 1 in 10 and PCR assays run in triplicates. Mean Ct values were plotted against log number of eggs per gram stool or eggs per 100 µL urine to generate an equation to calculate LOD at Ct of 40.

Statistical analysis

Primary outcome measures were sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of serum PCR and were calculated using MedCalc Statistical Software version 18.5 (MedCalc Software Ltd, Ostend, Belgium; http://www.medcalc.org; 2018). Serum PCR was compared with a composite reference standard of serology and stool or urine microscopy. A secondary analysis of stool and urine PCR performance was also conducted, and again, outcome measures were sensitivity, specificity, PPV, and NPV compared with the composite of serology and stool or urine microscopy. Continuous variables were analyzed by *t*-test at power of 80% and alpha = 0.05 using GraphPad Prism version 5.04 (GraphPad Software, La Jolla, CA).

Results

Clinical diagnostic testing

Over the enrolment period, there were 156,771 stool specimens submitted to our reference laboratory for O&P microscopy testing, of which 46 specimens (0.03%) from 29 patients were positive for *S. mansoni* (Figures 1 and 2(a)).

In addition, there were 773 urine specimens submitted for microscopy, of which four specimens (0.5%) from three patients contained eggs of *S. haematobium*. For *Schistosoma* serology, there were 8168 specimens submitted for of which 638 (7.8%) were considered reactive, 825 (10.1%) were indeterminate, and 6705 (82.1%) were non-reactive. Mean OD of the 638 serum specimens reactive by *Schistosoma* serology was 1.20 ± 0.77 (*SD*)

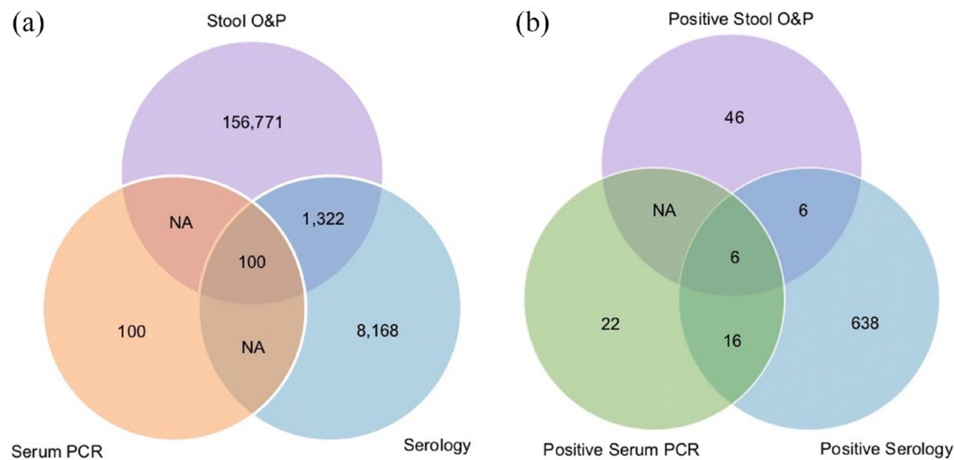

Figure 2. (a) Venn diagram demonstrating the relationship between available specimens for our diagnostic evaluation of schistosomiasis. (b) Venn diagram demonstrating the relationship between positive specimens in our diagnostic evaluation of schistosomiasis.

Table 1. Performance characteristics of *Schistosoma mansoni* qPCR assays compared with serology and microscopy composite reference.

Assay	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
S. mansoni qPCR 1 (Wichmann *et al.*[13])	27.8% (18.3–39.1%), 22/79	100% (83.9–100%), 21/21	100% (100%), 22/22	26.9% (24.3–29.7%), 21/78
S. mansoni qPCR 2 (Espirito-Santo *et al.*[16])	13.9% (7.1–23.6%), 11/79	100% (83.9–100%), 21/21	100% (100%), 11/11	23.6% (22–25.2%), 21/89
Combined qPCR	27.8% (18.3–39.1%), 22/79	100% (83.9–100%), 21/21	100% (100%), 22/22	26.9% (24.3–29.7%), 21/78

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; qPCR, quantitative PCR.

[median = 0.94, range = 0.41–4.08; interquartile range (IQR) = 0.58–1.61]. Of the total serologic specimens submitted, 1422 (17.4%) had a co-submission of at least one stool specimen for O&P microscopy within 1 year of serum submission (Figures 1 and 2(a)). In 13 patients with *S. mansoni*, both detected by stool O&P and reactive serology, mean OD was 2.04 ± 0.74 (*SD*) (median = 2.12, range = 0.92–3.23; IQR = 1.67–2.74). In one patient with *S. haematobium* detected by urine O&P and reactive serology, OD was 0.60. The remaining three samples only had urine O&P performed.

Serum PCR

The subset of 100 serum samples with corresponding stool submission for serum PCR validation comprised: Specimens reactive by both *Schistosoma* serology and positive by stool microscopy (*n* = 12); specimens reactive by serology but negative by stool microscopy (*n* = 67); and specimens negative by both serology and stool microscopy (*n* = 21) (Figure 1). Sensitivity of serum PCR 1 (Wichmann *et al.*[13] assay) was 27.8%, PCR 2 (Espirito-Santo *et al.*[16] assay) was 13.9% as compared with the composite reference of serology and stool microscopy. The combined qPCR assays for *S. mansoni* had a sensitivity and specificity of 27.8% (95% CI = 18.3–39.1%) and 100% (95% CI = 83.9–100%), respectively, with a PPV of 100% (95% CI = 100%) and an NPV of 26.9% (95% CI = 24.3–29.7%) (Table 1). All samples detected by Wichmann assay (22) were also detected by Espirito-Santo assay, but Espirito-Santo assay missed half (11) samples that were detected by Wichmann assay.

Of the 12 positive specimens detected by both serology and by stool O&P, only 50% ($n=6$) were positive by qPCR (Figure 1). Of the specimens reactive to serology but with negative stool O&P, 23.9% (16/67) were positive by qPCR. Finally, in the group of 21 serum specimens that were non-reactive serologically and had negative stool O&P, none ($n=0$) were qPCR positive. Therefore, serum PCR was more sensitive when the corresponding stool was also positive (50%, 6/12) compared with serological positives (27.8%, 22/79).

When using serology as the reference standard, combined PCRs had a higher sensitivity than stool microscopy with 27.8% compared with 15%. Real-time PCR targeting *S. haematobium* was negative in all serum samples tested ($n=100$), except for the one serum antibody positive specimen with corresponding *S. haematobium* positive urine O&P. There was no cross-reactivity of both *S. mansoni* serum PCR assays to *S. haematobium* and no cross-reactivity of *S. haematobium* PCR to *S. mansoni*. No cross-reactivity was observed between the three *Schistosoma* PCRs and biobanked specimens positive for *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, *Babesia* spp., or human DNA.

Stool PCR

Only three stool specimens positive for *S. mansoni* were available for stool PCR. All three were positive by both *S. mansoni* qPCR (100% sensitivity, 95% CI=29.2–100%) and negative by *S. haematobium* qPCR assay. There was no cross-reactivity of either *S. mansoni* stool PCR assay to common parasites found in stools: *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Clonorchis sinensis*, *Dicrocoelium dendriticum*, *Diphyllobothrium latum*, *Enterobius vermicularis*, *Strongyloides stercoralis*, *Taenia spp.*, *Blastocystis hominis*, *Cryptosporidium spp.*, *Cyclospora cayetanensis*, *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia lamblia*, or human DNA.

Limit of detection

Using a 10-fold serial dilution and extrapolating DNA volume used in the PCR to eggs per gram stool or eggs per 100 µL urine, LOD at Ct of 40 was 1.9×10^{-5} eggs per gram stool for Wichmann *S. masoni* assay and 2.2×10^{-5} eggs per gram stool for Espirito-Santo *S. mansoni* assay. Cnops *S. haematobium* PCR assay had an LOD of 1.2×10^{-5} eggs per 100 µL urine.

Serologic OD analysis

Stool egg positivity was correlated with higher OD values on *Schistosoma* serology (in those with reactive serology), with a mean of 1.80 ± 0.21 (*SE*) (range=0.55–2.9) compared with 1.26 ± 0.10 (*SE*) (range=0.41–3.78) with negative stool O&P ($p=0.0371$).

Similarly, serum *Schistosoma* PCR positivity was correlated with higher serologic OD values (in those with reactive serology), with a mean of 1.72 ± 0.14 (*SE*) (range=0.74–2.9) compared with 1.20 ± 0.11 (*SE*) (range=0.41–3.78) in PCR negatives ($p=0.0122$) (Figure 3).

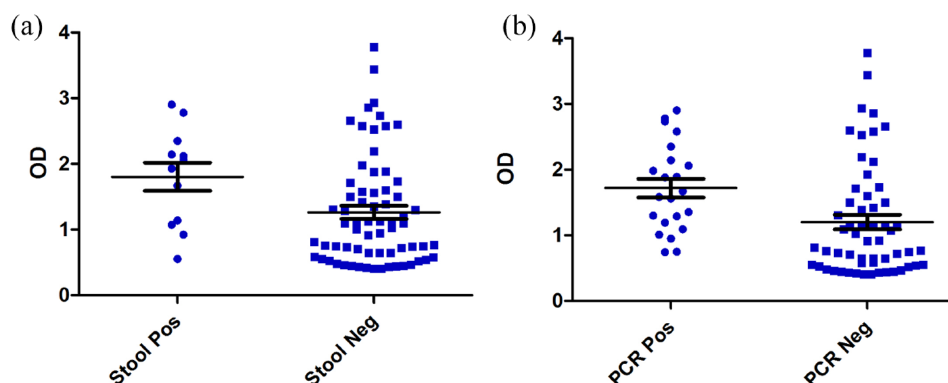

Figure 3. (a) Pan-*Schistosoma* reactive serologic OD values compared with stool microscopy and combined *S. mansoni* qPCR. (b) Mean OD value is indicated with error bars representing standard error.

Discussion

Schistosomiasis is a neglected tropical disease of great global significance[1] and with our increasingly diverse patient population, it is important to understand the performance characteristics and limitations of our diagnostic tools. In this study, we have evaluated the performance of two *S. mansoni* and one *S. haematobium* targeted PCR assays that can be utilized in the diagnosis and speciation of schistosomiasis. We have also considered a possible role for serological OD values in providing information about the burden of active schistosomiasis infection.

Serology remains the most sensitive diagnostic test for schistosomiasis

Indirect immunological assays that detect circulating anti-*Schistosoma* antibodies are widely employed in the laboratory diagnosis of schistosomiasis and are highly sensitive for detection of disease.[5] We demonstrated that the sensitivity of serum PCR was low compared with serology as well as to the composite reference of serology and stool microscopy. Serology therefore remains the most appropriate diagnostic assay and is especially important in patients who present asymptomatically or with few clinical symptoms. However, limitations of serological testing include the inability to identify *Schistosoma* species and to differentiate between current or past infection, thereby making it an imperfect test to check for disease resolution.[6,13] Furthermore, serological positivity can take up to 12 weeks, and therefore, serological diagnosis may not always be reliable in acute disease.[21]

Interestingly, we observed that higher serological OD values in *Schistosoma* antibody positive serum samples may have a correlation with microscopic stool and PCR positivity. Other studies have shown a possible role for antibody titer and OD values in the evaluation of schistosomiasis in human and animal studies.[22,23] One study showed that OD values for *S. japonicum* varied among different age groups, pointing toward a possible difference in exposure characteristics.[19] It has been previously demonstrated that certain variables, such as age and gender, are independently associated with *Schistosoma* infection,[24] and the study of OD values may potentially contribute to more detailed risk stratification of *Schistosoma* acquisition. The utilization of OD values may be an area of future investigation, especially as serological assays are near-universally used for the diagnosis of schistosomiasis.

Stool microscopy has low sensitivity to identify the burden of active schistosomiasis

In this study, stool microscopy had a very low sensitivity compared with serology. Other studies have also demonstrated low sensitivity and an inability to detect infection when there is a low egg burden.[7,25] Even in patients with a relatively high egg burden, there may be variability in the number of eggs that are shed in any given random sample of stool, therefore making it unreliable even in cases with high disease activity.[10] In addition, stool microscopy is operator dependent and laborious, involving multiple smears and staining protocols.[20,26] Such diagnostics are challenging to resource in both endemic and non-endemic countries alike.

PCR assays play a role in the speciation of schistosomiasis

In our study, *S. mansoni* and *S. haematobium* serum PCRs were highly specific and allowed identification of schistosomiasis to the species level. However, sensitivity was low compared with serology. Serum PCR by the Wichmann assay demonstrated higher sensitivity than serum PCR by the Espirito assay when compared with the composite reference of serology and stool microscopy. Although, both assays' target is the same toward the tandem repeat sequence SM 1–7, the primer and probe sequences are different and nucleotide content can have an effect on the PCR efficiency. With the higher sensitivity of the Wichmann PCR assay, and because all samples positive by the Espirito assay were also detected by the Wichmann assay, Wichmann PCR is sufficient to use as a standalone *S. mansoni* assay. *S. mansoni* stool PCR had high sensitivity and specificity compared with stool microscopy, but the number of stool samples available for our study was limited. Despite the low sensitivity of serum PCR, PCR methods have been utilized for diagnosis and can specifically differentiate between *Schistosoma* species which would be essential for risk stratification and appropriate management of schistosomiasis.[6] Addition of a bead beating step during DNA extraction could also increase DNA yield and PCR sensitivity. Although the drug of choice, praziquantel, targets all human species effectively, species identification is important for appropriate clinical risk stratification for chronic

sequelae, such as periportal fibrosis and squamous cell carcinoma of the bladder.[27]

Limitations

First, the limitations of this study include lack of full clinical linkage of specimens to medical records, preventing us from knowing the treatment status of patients whose specimens were included. It is possible that the long interval of inclusion around a serum specimen in our study may have led to inclusion of post-treatment stool specimens, which would potentially underestimate the performance of both serum and stool PCR. In addition, not all serum samples were available for PCR, and thus, our selection of samples could induce a selection bias and underestimate or overestimate the sensitivity. Second, by necessity, we included stool specimens submitted for general O&P examination rather than solely for the purpose of detecting *Schistosoma* as we have no *Schistosoma*-specific stool diagnostic test available. Moreover, neither circulating cathodic antigen (CCA) nor circulating anodic antigen (CAA) assays are licensed or commercially available in Canada, nor are these diagnostic tools used in accredited Canadian hospital or reference laboratories. As such, these assays were not included in this validation study. Given that Canada is non-endemic for human schistosomiasis, our number of positive specimens by microscopy is low compared with those positive serologically, raising the probability of our serologic assay detecting remote or past infection. In addition, many serum specimens submitted could reflect asymptomatic screening of migrants who would not have had a corresponding stool specimen submitted, which may have introduced selection bias with regards to stool microscopy. Third, without full clinical, travel and migration details, and treatment information, we were unable to estimate the true accuracy of serology for active schistosomiasis. Finally, the LOD values for our qPCR assays were relatively low compared with those documented in other studies.[17,20] It is possible that specimen integrity negatively influenced our results as the microscopy counts for LOD were conducted using frozen banked samples. In future studies, this can be corrected by obtaining a fresh prospective specimens for LOD analysis.

In our study, we demonstrated high specificity and PPV of serum PCR, thus highlighting a role for PCR in the speciation of schistosomiasis, once presence of disease is confirmed. Given the limitations of serological tests for schistosomiasis, there is a potential role for PCR, serologic OD values and other molecular techniques for the diagnosis and management of schistosomiasis. Nevertheless, serology remains the most sensitive diagnostic test for schistosomiasis especially for patients who present asymptomatically or with few clinical symptoms.

Conclusion

We reiterate that serology remains the most sensitive diagnostic test for schistosomiasis in our population but does not differentiate between current or past disease, nor does it provide causative *Schistosoma* species information. In our study, serum PCR detected more serologically positive samples when compared with stool microscopy, however, offered no overall performance advantage. There remains a large gap in the diagnostic tools used to detect active schistosomiasis. To improve clinical management, as well as to uphold anti-parasitic stewardship, it is important to continue to investigate available and newly arising techniques for the diagnosis of schistosomiasis. Despite the low sensitivity of serum PCR, its role in diagnostic parasitology should be pursued due to its high-throughput and operator-independent nature.

Declarations

Ethics approval and consent to participate

The study was a standard internal laboratory validation of a new assay using surplus discard clinical specimens. Per the Code of Federal Regulations, Title 45, Part 46, the use of deidentified diagnostic specimens for verification and laboratory validation purposes is not considered human subject research and is therefore exempt from human subject considerations.

Consent for publication

Not applicable.

Author contributions

Rachel Lau: Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing – original draft; Writing – review & editing.

Leila Makhani: Formal analysis; Investigation; Validation; Visualization; Writing – original draft; Writing – review & editing.

Osaru Omoruna: Data curation; Formal analysis; Investigation; Validation; Writing – review & editing.

Celine Lecce: Data curation; Formal analysis; Validation; Writing – review & editing.

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Karamjit Cheema: Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing – review & editing.

Andrea K. Boggild: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision, Writing – original draft, Writing – review & editing

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Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

All data are included in this manuscript.

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