Comparison of Loop-Mediated Isothermal Amplification and Real-Time PCR Assays for Detection of *Strongyloides* Larvae in Different Specimen Matrices

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ABSTRACT *Strongyloides stercoralis* can cause disease that ranges from asymptomatic chronic infection to fatal hyperinfection. Diagnosis from stool can be challenging because the most sensitive conventional tests require live larvae to be effective and there can be low larval output in chronic infection. Nucleic acid amplification tests (NAAT) have been developed to complement existing diagnostic methods. We compared a recently developed loop-mediated isothermal amplification (LAMP) assay with a real-time PCR that has previously been validated with larval microscopy. The limits of detection—quantified using serial dilutions of DNA extracts from single *Strongyloides ratti* third-stage (L3) larvae spiked into approximately 250 μ l of 5 different *S. stercoralis*-negative stool specimens—were 10^{-3} (1/5 replicates) and 10^{-2} (1/5 replicates) dilutions for PCR and LAMP, respectively. PCR was positive for 4/5 replicates at 10^{-2} . LAMP was compared to PCR using extracts from 396 stool specimens collected in Bangladesh and Australia, of which 53 were positive and 343 were negative by PCR. The positive percentage agreement of LAMP was 77.3% (95% score confidence interval [CI], 64.5 to 86.6). The negative percentage agreement was 100% (95% CI, 98.9 to 100). In a preliminary investigation, PCR and LAMP assays were positive using DNA extracted from serum (PCR, 3/16 extracts; LAMP, 2/16 extracts) and bronchoalveolar lavage fluid (PCR and LAMP, 2/2 extracts), demonstrating proof of concept. Compared to PCR, the lower number of positive results using the LAMP assay may have been due to reaction inhibitors and DNA degradation, and strategies to improve the LAMP assay are discussed.

KEYWORDS PCR, *Strongyloides*, bronchoalveolar lavage, diagnosis, loop-mediated isothermal amplification, nucleic acid amplification tests, polymerase chain reaction, serum, stool, strongyloidiasis

Strongyloides stercoralis is a microscopic nematode that can cause a fatal hyperinfection where large numbers of larvae spread throughout the body (1). It is endemic in the tropics and subtropics, and has recently been estimated to infect at least 370 million people worldwide (2). Acquisition typically occurs where there is contact with fecally contaminated soil, allowing infective-stage larvae to penetrate the skin, but it may also occur in health institutions (1). Chronic autoinfection may last for many

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decades, and thus disease can persist in areas where sanitation has since improved or in people who have migrated from areas where *Strongyloides* is endemic to areas where it is nonendemic (1, 3).

The gold standard for *Strongyloides* diagnosis is the morphological identification of adults or larvae in clinical specimens (4). Diagnosis is often based on the detection of larvae in stool specimens or serological tests (4). *Strongyloides* may also be identified in histopathological specimens (4). Hyperinfection is usually triggered by immunosuppression, which can reduce the sensitivity of serological tests, but in these cases large numbers of larvae will be detected in stool, respiratory secretions, and body fluids (1, 4, 5). At the other end of the disease spectrum, serology may be positive in the context of negative stool tests, as the diagnosis of asymptomatic chronic infection can be complicated by low larval output (6).

The most sensitive conventional techniques for the detection of *Strongyloides* larvae in stool are dependent on live larvae and include agar plate culture, Harada culture, and the Baermann technique (4). These methods can be compromised in routine laboratory practice, due to preservatives or refrigeration causing larval death (7). While nucleic acid amplification tests (NAAT) do not require live larvae, their effectiveness may be reduced due to low numbers of larvae, reaction inhibition, and nucleases (8, 9). Real-time PCR based on the primers developed by Verweij et al. is the most characterized method, and clinical validation studies have demonstrated sensitivities from 33% to 93% and specificities of 85% to 99%, depending on the comparator methods (10–15). A recent meta-analysis of clinical validation studies calculated a PCR sensitivity of 71.8% compared to morphological identification, while a study comparing PCR to a comprehensive range of stool culture and concentration methods determined a PCR sensitivity of >90% (16, 17). Loop-mediated isothermal amplification (LAMP) is another NAAT that has been adapted for *Strongyloides* diagnosis (18, 19). The LAMP method is rapid and can be performed with simple, cost-effective equipment (20, 21). However, the evaluations of *Strongyloides* LAMP assays have primarily been laboratory validations (18, 19).

To further evaluate the performance of the LAMP assay, we compared it to a real-time PCR assay (using primers developed by Verweij et al.) using spiked *Strongyloides ratti* larvae to determine the limit of detection, and DNA extracted from clinical stool specimens from Dhaka in Bangladesh, regional northern Queensland, and metropolitan Sydney, Australia (10, 12, 22). In cases of hyperinfection patients may be seronegative, have a paralytic ileus, and be unable to produce a fecal sample, so we also performed a preliminary investigation into the use of serum and bronchoalveolar lavage specimens for *Strongyloides* NAAT (1).

MATERIALS AND METHODS

Samples and DNA extraction methods. DNA was extracted from the stool samples using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA). DNA was eluted from the spin column using the supplied C6 (Tris) buffer or Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]; Sigma-Aldrich, St. Louis, MO). Tris-EDTA was recommended in the manufacturer's instructions for cases where DNA degradation is a concern. Previous use of the PowerSoil DNA isolation kit with the *Strongyloides* PCR assay was associated with a higher limit of detection (12).

Laboratory cultured *S. ratti* third-stage (L3) larvae were isolated from rat feces using the Baermann technique (23). A single larva in water was aspirated into a 10- μ l pipette and then spiked into approximately 250- μ l aliquots of human stool from 5 separate people, previously confirmed to be negative for *Strongyloides* by agar plate culture, PCR, and LAMP (18). DNA was eluted using Tris-EDTA buffer. *Strongyloides ratti* larvae were used instead of *S. stercoralis*, as *S. ratti* larvae were available in culture, are nonpathogenic to humans, and contain the conserved target sequences of the PCR and LAMP assays.

DNA extracts from 156 stool specimens collected in a survey of *S. stercoralis* infection in Dhaka and DNA extracts from 219 clinical stool specimens from regional northern Queensland were obtained from -20°C storage (12, 22). The stool specimens from Dhaka were collected as part of a research project and transported frozen on dry ice (without preservative) to Sydney, where the DNA was extracted (12). The clinical stool specimens from Queensland were transported to a diagnostic laboratory in Townsville, Queensland, some over long distances with the possibility of fluctuations in temperature (22). Specimens were stored at 4°C for 1 to 7 days prior to DNA extraction. DNA was extracted in Townsville and transported to Sydney with freeze-thawing and periods at room temperature. Both sets of specimens were eluted using the C6 (Tris) buffer. DNA was also extracted from clinical stool specimens from metropolitan Sydney, which were stored unpreserved at -20°C . Six were positive on single agar plate

culture for *S. stercoralis* and 15 were negative. The six agar-positive stool specimens were from 5 male patients between the ages of 51 and 76 years old who had either resided in or travelled to areas where *Strongyloides* is endemic. Stool specimens were collected from one patient before and after ivermectin therapy. The details of any immunosuppressive therapy were not available. DNA was eluted in Tris-EDTA buffer.

Previously *Strongyloides* antibody-positive serum specimens from a survey of *Strongyloides* infection in Dhaka, Bangladesh, were taken from -20°C storage (24). They had been stored for >3 years. Seven specimens were from participants who were stool culture-positive for *S. stercoralis* and 7 were from stool culture-negative participants. Serum was also collected from 2 patients from Sydney, Australia, with *S. stercoralis* hyperinfection, which was confirmed on the basis of larvae identified on bronchoalveolar lavage fluid by direct microscopy. One patient was a 27-year-old man with Crohn's disease and HIV, and the other was a 60-year-old man with ulcerative colitis and pemphigus vulgaris (25). Both were treated with azathioprine and corticosteroids and spent time in areas where *S. stercoralis* is endemic. The serum and bronchoalveolar lavage fluid from the patient with Crohn's disease were stored at -20°C for approximately 3 years prior to DNA extraction, and the serum and bronchoalveolar lavage fluid from the patient with ulcerative colitis were stored at -20°C for approximately 1 month prior to extraction. The NucliSENS easyMag system (bioMérieux, Marcy-l'Etoile, France) was used to extract DNA from 250 μl of serum, according to the manufacturer's instructions. DNA was extracted from 250 μl of the bronchoalveolar lavage fluid containing *S. stercoralis* using the High Pure DNA template preparation kit (Roche, Basel, Switzerland) according to the manufacturer's instructions.

Approval for the use of clinical specimens from Bangladesh was obtained from the National Research Ethics Committee, Bangladesh Medical Research Council (BMRC/NREC/2007-2010/1256) (12). Approval for the use of stool specimens from northern Queensland, Australia, was obtained from the Townsville Hospital and Health Service Human Research Ethics Committee (HREC/14/QTHS/4) (22). Approval for the use of clinical specimens from metropolitan Sydney, Australia, was obtained from Human Research Ethics Committee, Sydney West Area Health Service [HREC2014/9/6.3 (4907) QA].

Real-time PCR assay method. The real-time PCR method used the primers and probe designed by Verweij et al., which target the small subunit rRNA gene, primers Stro18S-1530F (GAATCCAAGTAAAC GTAAGTCATTAGC) and Stro18S-1630R (TGCCTCTGGATATTGCTCAGTTC) and probe Stro18S-1586T (carboxyfluorescein [FAM]-ACACACCGGCGCTGCTGC-black hole quencher 1 [BHQ1]) (10, 12). The primers and probe were manufactured by Sigma-Aldrich and purified by high-performance liquid chromatography (HPLC). The PCR mixture contained 25 μl of HotStarTaq master mix (catalogue number 203446; Qiagen, Hilden, Germany). The final concentrations of other components of the reaction mixture were 300 nM forward and reverse primers, 200 nM probe, 5 mM MgCl_2 , and 0.1 mg/ml of bovine serum albumin (BSA) (Promega, Alexandria, Australia). Molecular biology purity H_2O and 10 μl of DNA extract (or H_2O for nontemplate controls) made up a reaction volume of 50 μl .

The reaction mixture was amplified using a Rotor-Gene 6000 instrument (Corbett Research, Sydney, Australia) with the following protocol: 15 min at 95°C , then 40 cycles of 15 s at 95°C for denaturation, 30 s at 62°C for annealing, and 30 s at 72°C for extension. This was followed by 5 min at 72°C as a final extension and then cooling to room temperature. All PCR products in the study were sequenced using the primers, and, while they did not cover the full target, they matched *Strongyloides* spp. identified by NCBI *blastn* analysis.

LAMP assay method. The LAMP assay used primers which targeted the 28S ribosomal subunit gene: forward outer primer (F3), GTGTAGGCTGGCGTAGT; backward outer primer (B3), TTCAATTTAGCTTAG GACC; forward inner composite primer (FIP; F1c-F2), GCTACTATCACCAAGATCTGCAC-GCATTGAAGGTTA TAAGCGTAAG; backward inner composite primer (BIP; B1c-B2) ACACAAGTGAGAATCTTGTTGGAC-CTAAC TCACAGTCAAATGATGT; and loop backward primer (LB), CGAAGTGAAAAGGGTTTCACG (18). The primers were manufactured by Sigma-Aldrich and purified by HPLC.

The amounts of primers in the reaction mixture were as follows: FIP and BIP, 40 pmol; F3 and B3, 20 pmol; and LB, 20 pmol. The final concentration of other components of the reaction mixture were as follows: ThermoPol buffer [$1\times$ dilution consisting of 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 0.1% Triton X-100, 2 mM Mg_2SO_4 , New England Biolabs, Ipswich, MA], 0.8 M betaine, deoxynucleoside triphosphates (dNTPs) 1.4 mM each, 6 mM Mg_2SO_4 , BSA 0.1% (wt/vol), polyvinylpyrrolidone (PVP) 1% (wt/vol), and 15 μM Syto 82 fluorescent dye (Life Technologies, Carlsbad, CA). Initially the mixture was made up to a volume of 19 μl with molecular biology purity H_2O , and 5 μl of DNA extract was added, except in the case of nontemplate controls, where an additional 5 μl of H_2O was added.

The reaction mixture was then heated to 95°C and allowed to cool to room temperature. Eight units (1 μL) of *Bst* (large fragment) DNA polymerase (New England Biolabs) were added to each tube, making the total volume of the reaction mixture 25 μl . Tubes were then pulse centrifuged and heated to 60°C for a period of 60 min in a Rotor Gene 6000 instrument (Corbett Research), with an increase in fluorescence, indicative of amplification, detected through the yellow channel (excitation, 530 nm; detection, 555 nm). The mixture was then heated to 95°C for 3 min to inactivate the DNA polymerase. Fluorescence was visible with the naked eye under normal white lighting conditions (18). Reaction products were also visualized using agarose gel electrophoresis and had the characteristic "ladder pattern" (18).

Comparison of PCR and LAMP assays. The DNA extracts from single *S. ratti* larva were serially diluted in TE buffer, with 5 replicates made. These were tested with LAMP and PCR to determine the respective limits of detection and to indicate the highest cycle threshold (C_T) or longest time to amplification for a known positive specimen.

TABLE 1 PCR and LAMP assay limits of detection based on DNA extracted from single *Strongyloides ratti* L3 larvae spiked into 5 separate stool specimens

Assay result	Serial dilution of DNA extracts				
	Neat	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
PCR (no. positive/no. tested)	5/5	5/5	4/5	1/5	0/5
C_T^a					
Median	28.39	31.84	35.67	35.83	
Range	3.46 (26.38–29.84)	3.13 (29.84–32.97)	2.45 (34.71–37.16)		
LAMP (no. positive/no. tested)	5/5	5/5	1/5	0/5	0/5
Reaction time (min)					
Median	29	35	44		
Range	19 (27–46)	22 (32–54)			

^a C_T , cycle threshold.

The stool DNA extracts from Dhaka, Sydney, and Queensland were tested by trained operators who were blind to sample identity. As the comparison was between NAAT, percentage agreements rather than sensitivity and specificity were calculated, in accordance with CLSI and FDA recommendations (26, 27). Score confidence intervals (CI) were calculated using Wilson's method (27). Comparison was also made between the DNA extracts from serum and bronchoalveolar lavage fluid.

When each PCR and LAMP assay was performed, negative (H₂O nontemplate) controls were used, and in the case of clinical specimens, stool spiked with *S. ratti* were used as positive controls. Results for positive and negative controls were as expected for all reactions. The duration of time between testing particular DNA extracts with the LAMP and PCR assays was less than 2 weeks.

RESULTS

Limits of detection based on *Strongyloides ratti* larvae spiked into stool. The maximum limits of detection, median PCR C_T values and ranges, and median LAMP reaction times and ranges are listed in Table 1. The maximum limit of detection for PCR was 10 times greater (10⁻³) than that of the LAMP assay (10⁻²). PCR was positive for 4 of 5 replicates at 10⁻².

Clinical stool specimens from Dhaka (Bangladesh), Sydney (Australia), and northern Queensland (Australia). The results of the comparison between the LAMP and PCR assays using DNA extracted from 396 stool specimens collected in Dhaka, Sydney, and regional northern Queensland are summarized in Tables 2 and 3. The total positive percentage agreement was 77.3% (95% CI, 64.5 to 86.6) and the negative percentage agreement was 100% (95% CI, 98.9 to 100). The median PCR C_T values and ranges for the DNA extracts that were also positive using the LAMP assay were lower than the median C_T values and ranges for the total number of PCR-positive extracts.

For the stool specimens collected in Dhaka, the positive percentage agreement was 91.6% (95% CI, 64.6 to 98.5) and the negative percentage agreement was 100% (95% CI, 97.4 to 100). For the stool specimens from Sydney, the positive percentage agreement was 83.3% (95% CI, 43.7 to 97.0) and the negative percentage agreement was 100% (95% CI, 79.6 to 100). For the Queensland stool specimens, the positive percentage agreement was 71.4% (95% CI, 55.0 to 83.7) and the negative percentage agreement was 100% (95% CI, 97.8 to 100).

TABLE 2 Comparison of PCR and LAMP assays using DNA extracts from stool collected in Dhaka (Bangladesh), Sydney (Australia), and northern Queensland (Australia)

LAMP result	PCR result ^a						Total
	Positive			Negative			
	Dha	Syd	Qld	Dha	Syd	Qld	
Positive ^b	11	5	25	0	0	0	41
Negative ^c	1	1	10	144	15	184	355
Total	53			343			396

^aDha, Dhaka, Bangladesh; Syd, Sydney, Australia; Qld, northern Queensland, Australia.^bTotal positive percentage agreement, 41/53 = 77.3% (95% score confidence interval [CI] [27], 64.5 to 86.6).^cTotal negative percentage agreement, 343/343 = 100% (95% CI, 98.9 to 100).

TABLE 3 Median values and ranges for PCR cycle thresholds and LAMP reaction times, using DNA extracts from stool collected in Dhaka (Bangladesh), Sydney (Australia), and northern Queensland (Australia)^a

Assay result	Total	Dha	Syd	Qld
PCR C_T^b (total)				
Median	32.31	30.29	24.81	34.11
Range	21.91 (16.34–38.25)	15.23 (23.02–38.25)	10.41 (20.74–31.16)	20.79 (16.34–37.13)
PCR C_T (LAMP+)				
Median	31.24	29.89	23.15	33.09
Range	20.78 (16.34–37.12)	10.18 (23.03–33.20)	6.12 (20.74–26.86)	20.78 (16.34–37.12)
LAMP reaction time (min)				
Median	31	32	29	29
Range	41 (16–57)	14 (28–42)	16 (28–44)	41 (16–57)

^aPCR cycle thresholds reported for total positive extracts and LAMP-positive (LAMP+) extracts. Dha, Dhaka, Bangladesh; Syd, Sydney, Australia; Qld, northern Queensland (Australia).

^b C_T , cycle threshold.

Detection in serum and bronchoalveolar lavage specimens. The results of the PCR and LAMP assays on DNA extracted from serum are summarized in Table 4. DNA extracts from the bronchoalveolar lavage fluid were positive by both the PCR and LAMP assays.

DISCUSSION

The accurate diagnosis and treatment of strongyloidiasis is important for epidemiological reasons, the management of symptoms, and the prevention of complications, including low birth weight in infants and malnutrition (1, 28–30). The screening of patients with risk factors prior to immunosuppression is necessary to prevent hyperinfection (1, 31). Studies that measured the clinical sensitivity of the PCR assay designed by Verweij et al. compared to morphological identification have provided a benchmark for comparison with the LAMP assay (10–15, 17). The PCR and LAMP assays both targeted components of the ribosomal cistron, which is a single transcription unit (32).

In this study, the limit of detection using serial dilutions of extracts from spiked single *S. ratti* larva was 10^{-2} for the LAMP assay and 10^{-3} for the PCR assay. Almost all of the positive stored stool extracts, excluding 1 sample each for the LAMP and PCR assays, fell within the range of C_T values or LAMP reaction times that were recorded for the stool specimens spiked with *S. ratti* larvae. The range of reaction times or C_T values for known positive results is necessary for determining result cutoffs, as extended reactions have been associated with false-positive results in PCR and LAMP assays (33–35).

The greater number of positive serially diluted DNA extracts was also associated with a greater number of PCR-positive DNA extracts from Dhaka, Sydney, and northern Queensland, and lower median C_T values and ranges for the LAMP-positive extracts. These results indicated that the LAMP assay was less effective, but also raised the possibility that there may have been nonspecific amplification with the PCR primers to account for the difference. However, the PCR and LAMP primers have both been tested

TABLE 4 *Strongyloides* PCR and LAMP assay results using DNA extracted from serum

Serum	Positive microscopy		Negative microscopy stool ^b (n = 7)
	BAL fluid ^c (n = 2)	Stool (n = 7)	
PCR-positive C_T^d	2 (31.44 and 31.83)	1 ^a (34.53)	0
LAMP positive reaction time (min)	1 (33)	1 ^a (38)	0

^aPositive serum was from the same study participant, who was stool positive by LAMP and PCR (C_T , 25.63).

^bStool microscopy negative study participants were *Strongyloides* antibody positive, indicating current or past infection.

^cBAL, bronchoalveolar lavage.

^d C_T , cycle threshold.

for analytical specificity using DNA extracts from a wide range of organisms found in stool, and there was 100% negative agreement between the PCR and LAMP assay in this study (10, 12, 18). While the amplified PCR sequences did not cover the full target, they matched *Strongyloides* spp. The PCR primers have previously demonstrated a clinical specificity of >85% (10–15). In these studies, if the comparator methods relied on the presence of live larvae, nonviable larvae may lead to a “true positive” PCR result being labeled “false positive” (10).

In a previous study, the *Strongyloides* LAMP assay was able to detect <10 copies of DNA when a plasmid containing the target sequence was serially diluted in Tris-EDTA (18). This indicated that the reaction had a high analytical sensitivity in controlled circumstances. When diagnostic specimens are used, the decreased limit of detection and lower number of positive specimens may be related to reaction inhibition or increased degradation of the longer LAMP target (184 bp), compared to the PCR target (101 bp) (8, 9, 36, 37). The difference between the positive percentage agreement in stool DNA extracts from Queensland (71.4%) compared to those from Dhaka (91.6%) and Sydney (83.3%) may have been related to the transportation and storage of the stool specimens and the elution of DNA in Tris buffer, rather than in Tris-EDTA, leaving the LAMP target more susceptible to nucleases (38, 39). Other components of stool, tannic and humic acids bound to the DNA template, had a greater inhibitory effect on longer target sequences when investigated using PCR (9). Accordingly, geographical variation in diet and stool composition may also have accounted for some of the difference in percentage agreement between the stool extracts (8, 40). Due to low volumes of the stored DNA extracts, it was not possible to determine if extract dilution would reduce the effect of inhibitors (8, 18). Repeated freeze-thawing and bead beating can fragment DNA, but these processes have less impact on sequences as short as the targets of both assays (36, 37). While the PowerSoil DNA isolation kit extraction method utilizes bead beating, it was an effective method for *Strongyloides* DNA extraction in a previous comparison, leading to high analytical sensitivity (12). If the amount of target DNA is close to the assay limit of detection, the effects of sampling error, inhibitors, and DNA degradation may lead to a negative result (8). The efficiency of a *Salmonella enterica* serovar Typhi LAMP assay was reportedly unaffected by the addition of 1 μ l of decanted feces into a reaction volume of 25 μ l; however, a relatively large amount of target DNA (1,000 copies) was used in the study (41).

DNA degradation would be mitigated by DNA extraction as close to the time of specimen collection as possible and by DNA elution in Tris-EDTA. Where there will be a delay in DNA extraction, the use of ethanol or transport buffers to stabilize DNA may minimize degradation (17, 42–44). A shorter duration of bead beating may also reduce DNA fragmentation. The limit of detection of the LAMP assay using *S. ratti* larvae spiked into stool also reflects the capacity of DNA extraction methods to remove reaction inhibitors (12). If an effective extraction method could be incorporated into the *Strongyloides* assay, like those in the LAMP assays for *Mycobacterium tuberculosis* and influenza virus, this would simplify the assay and facilitate its use in resource-limited settings (20). The primers that were used in the *Strongyloides* LAMP assay were designed with a comparatively short target sequence (with 5 primers), and the options for primer sets were limited by the AT-rich genome (18, 45). The primers were found to be specific (18) compared to trial primer sets that also amplified *Candida parapsilosis* DNA despite specificity *in silico* (unpublished data).

The process of preheating the reaction mixture and extract to 95°C prior to the addition of DNA polymerase increases the sensitivity of the LAMP assay (18, 46). A homologue of *Bst* (large fragment) DNA polymerase, *Bst* 3.0 (catalogue number M374S; New England Biolabs) has been reported to have good performance in whole blood despite polymerase inhibitors (47). While this is yet to be compared with standard *Bst* (large fragment) using stool extracts, there may be benefit to its use. Lyophilization may not necessarily increase assay sensitivity, but it can contribute to the stability of reagents and facilitate diagnostics in settings where resources are limited (48, 49). Syto 82 intercalating dye is an effective means of detecting amplified DNA without the need

to open the reaction tube, minimizing the risk of contamination (18, 50, 51). Based on previous data, a lower concentration of Syto 82 ($<15 \mu\text{M}$) could be used to visually detect target amplification while increasing reaction efficiency (18). Amplicon detection methods, such as hybridization probes, may improve assay specificity with high analytical sensitivity (52, 53). Multiplexed LAMP assays for stool pathogens could include primers that target internal control DNA (8, 52, 53). These could be used to identify reaction inhibition and improve quality assurance.

Strongyloides DNA has previously been detected in urine, and we detected DNA in the sera of 2 patients with hyperinfection and another person with a comparatively low C_T (25.63) on stool PCR (19, 54). These preliminary results indicate that the diagnosis of strongyloidiasis on the basis of serum NAAT would be relatively insensitive but may be useful if stool is not available due to paralytic ileus. The detection of DNA in bronchoalveolar lavage fluid was expected, considering the visible larvae on microscopy, and further investigation will be required to determine the sensitivity of NAAT in respiratory secretions.

Operators were blind to sample identity, except when testing the bronchoalveolar and serum specimens from the 2 patients known to have *Strongyloides* hyperinfection. While the study used stored material with selective sampling and was not designed to calculate clinical sensitivity and specificity, it identified the need for further optimization of the *Strongyloides* LAMP assay prior to a field trial (26, 27).

The potential for low numbers of larvae in stool requires that NAAT for *Strongyloides* are highly sensitive. While not as effective as PCR in this study, the ease of use of LAMP assays is suited to the diagnosis of strongyloidiasis, and further research and development would be valuable. The NAAT detection of *Strongyloides* in non-stool specimens may be clinically useful, and additional study is warranted.

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M.R.W. designed, planned and performed experimental work, and wrote and revised the manuscript. R.K. and V.A. performed experimental work and edited the manuscript. G.J.R., Y.S., M.W., and R.S.B. provided samples for experimental work and edited the manuscript. G.L.G. and R.L. supervised experimental work, secured funding, and edited the manuscript.

R.S.B. coauthored the manuscript in his personal capacity and in his capacity as an adjunct academic at Central Queensland University.

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We have no conflicts of interest to declare.

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