Recombinase Polymerase Amplification Compared to Real-Time Polymerase Chain Reaction Test for the Detection of *Fasciola hepatica* in Human Stool

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Abstract. *Fasciola hepatica* is the most widely distributed trematode infection in the world. Control efforts may be hindered by the lack of diagnostic capacity especially in remote endemic areas. Polymerase chain reaction (PCR)-based methods offer high sensitivity and specificity but require expensive technology. However, the recombinase polymerase amplification (RPA) is an efficient isothermal method that eliminates the need for a thermal cycler and has a high deployment potential to resource-limited settings. We report on the characterization of RPA and PCR tests to detect *Fasciola* infection in clinical stool samples with low egg burdens. The sensitivity of the RPA and PCR were 87% and 66%, respectively. Both tests were 100% specific showing no cross-reactivity with trematode, cestode, or nematode parasites. In addition, RPA and PCR were able to detect 47% and 26% of infections not detected by microscopy, respectively. The RPA adapted to a lateral flow platform was more sensitive than gel-based detection of the reaction products. In conclusion, the *Fasciola* RPA is a highly sensitive and specific test to diagnose chronic infection using stool samples. The *Fasciola* RPA lateral flow has the potential for deployment to endemic areas after further characterization.

INTRODUCTION

Fasciola hepatica is the most widely distributed trematode with millions of people infected worldwide.¹ The highlands of Bolivia and Peru are hyperendemic areas with a prevalence of up to 70% determined by stool microscopy. Furthermore, the areas that are endemic and hyperendemic for fascioliasis are expanding as better data are emerging from diverse countries. In northwest Ethiopia where only sporadic fascioliasis had previously been described, 3.3% of children were found infected in 2013.² In some provinces of Vietnam, a prevalence between 2% and 11% have been reported.³ Similarly, new endemic areas have been described in Iran and Kyrgyzstan.^{4,5} Nonetheless, accurate data on the distribution and burden of fascioliasis are still limited. Difficulties in assessing the epidemiology of *Fasciola* stem from the focal distribution of the infection even in endemic areas and the poor performance of currently available diagnostic methods.⁶

Fascioliasis surveillance by stool microscopy is highly dependent on personnel training and motivation. In addition, microscopy suffers from poor sensitivity.⁶ Although egg concentration techniques may achieve higher sensitivity, they still require testing of multiple specimens and most are not standardized to provide quantitative results.^{7–9} The Kato-Katz test is a quantitative technique recommended for diagnosis, but it is insensitive in low-burden infections.¹⁰ Therefore, as endemic areas of *Fasciola* expand, improved diagnostic capacity is needed. Coproantigen detection in the stool by enzyme-linked immunosorbent assay (ELISA) is highly sensitive and specific.¹¹ A version using the MM3 monoclonal antibody has been successfully used to test human fecal samples, but no commercial version is available and the potential for point-of-care use has not been tested.¹² The ideal diagnostic method should require minimal personnel

training, be deployable to resource-limited settings, provide semiquantitative or quantitative results, and be highly sensitive to detect low-burden infections.

Isothermal DNA amplification methods have been touted as alternative diagnostic techniques that might be more widely applicable than polymerase chain reaction (PCR)-based tests that use expensive thermal cycling equipment and require extensive personnel training. In particular, recombinase polymerase amplification (RPA) uses a highly efficient displacement polymerase that amplifies detectable products at a constant temperature of 37°C in as little as 20 minutes.¹³ Several tests using RPA have demonstrated high sensitivity and specificity for detecting small amounts of viral, bacterial, and parasite DNA.^{14–16} The objective of this study was to perform the characterization of an RPA and real-time PCR tests to detect *Fasciola* DNA in stools and compare their sensitivity and specificity using microscopy-tested human samples as the comparator. This study provides the first proof of concept for the future adaptation of the *Fasciola* RPA to semiquantitative point-of-care diagnosis.

METHODS

Ethics statement. Samples were collected as part of an ongoing epidemiologic study of fascioliasis in the Peruvian highlands. In this study, three stool samples per subject were tested by microscopy including the Kato-Katz and Lumbrellas rapid sedimentation tests for the presence of helminth eggs and larvae.^{17,18} Participants in the epidemiologic study were also tested for *F. hepatica* serum antibodies using FAS2 ELISA (Bionoma, Lima, Peru). The study used de-identified stool samples of those consenting to the use of their discarded specimens for future studies. Samples were banked at the Universidad Peruana Cayetano Heredia and University of Texas Medical Branch Collaborative Research Center in Cusco, Peru. The study protocol was reviewed by the Universidad Peruana Cayetano Heredia and University of Texas Medical Branch Institutional Review Boards.

Specimen procurement. Adult parasites and eggs of *F. hepatica* were obtained from naturally infected cattle livers

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and gallbladders that had been condemned at a local slaughterhouse in Cusco. *Echinococcus granulosus*, *Diphyllobothrium* spp., *Strongyloides stercoralis*, *Taenia solium*, and *Schistosoma mansoni* specimens were obtained from discarded clinical or research laboratory materials. De-identified human stool samples with and without *F. hepatica* eggs were obtained from research specimens banked at -70°C without preservation solutions.

Sample processing and DNA extraction. Extraction of DNA from adult parasites was performed using commercially available QiaAmp DNA Stool Mini Kit (Qiagen, Valencia, CA). Freshly collected parasite specimens were washed repeatedly with phosphate-buffered saline, cut into approximately 200 mg aliquots, and resuspended in the manufacturer's provided lysing buffer. The resuspended specimens were exposed to three cycles of freezing and heating (-70°C for 5 minutes and 90°C for 5 minutes) and then subjected to DNA extraction following the manufacturer's instructions.

Stool aliquots of 200 mg were used for all experiments and DNA extraction procedures. All stool specimens were placed in screw cap tubes and suspended in 1.2 mL lysis buffer supplied with the DNA Extraction Kit (Qiagen), exposed to five cycles of freezing and heating, incubated at 95°C for 3 hours, and subjected to DNA extraction using QiaAmp DNA Stool Mini Kit according to manufacturer's instructions. DNA concentration and quality was assessed by spectrophotometry using NanoDrop 2000 (Thermo Scientific, Wilmington, DE).

RPA test for *F. hepatica*. Five sets of primers were designed using the Primer-BLAST software (National Center for Biotechnology Information) targeting the highly repeated *Fasciola ITS1* region of the *rRNA* gene (GenBank AJ243016). Primers were tested with DNA extracted from adult *Fasciola* parasites using commercially available Twist Amp Basic Kits (TwistDx, Cambridge, United Kingdom). The RPA test for *F. hepatica* (FAS-RPA) reaction composition and conditions were optimized to use the primer pair with the shortest and more sensitive reaction (Table 1). The final reaction composition was set in a 50 μL volume using the supplied dry reagents, 29.5 μL of rehydration buffer, 2.4 μL of 10 μM forward and reverse primers, 3.5 μL of 280 mM magnesium acetate, and 12.2 μL of extracted DNA solution at concentrations between 10 and 75 ng/ μL . The reaction was incubated at 38°C in a heat block for 30 minutes. Adult *F. hepatica* DNA was used as positive control, human DNA was used as negative control, and DNase-free water was used as the non-template control in all the reactions. The reaction products were visualized in 1.5% agarose gel electrophoresis stained with ethidium bromide. Samples were considered positive if a product of ~300 bp was detected, the positive control showed the same band size, and the negative and non-template controls showed no bands.

TABLE 1

Primer and probe sequences for the FAS-RPA and FAS-PCR tests

	Primer/Probe	Sequence
FAS-RPA	Forward	3'-atcattacctgaaaatctactctcaca-5'
	Reverse	3'-agtgtaaatgtagtaataaagcagttc-5'
	Probe	FAM-cgaacactgatggggtgcctacctgtatga [THF]actccgatggatgcttgctg-C3
FAS-PCR	Forward	3'-tgatactccgatggtatgcttg-5'
	Reverse	3'-acgtatggtcaagaccaggtt-5'

FAS-PCR = real-time polymerase chain reaction for *Fasciola hepatica*; FAS-RPA = recombinase polymerase amplification test for *F. hepatica*.

The RPA reaction was tested for its analytic limit of detection (LOD) with 5-fold dilutions of purified adult *Fasciola* DNA. The LOD was set at the lowest DNA concentration detected by duplicate reactions. The analytical specificity of the FAS-RPA was evaluated with DNA purified from stool specimens containing *Ascaris lumbricoides*, hookworm, *Trichuris trichiura*, *Hymenolepis nana*, and *Giardia intestinalis* and DNA extracted from *S. mansoni*, *S. stercoralis*, *Diphyllobothrium* spp., *Echinococcus granulosus*, and *T. solium* parasite specimens.

Real-time PCR for *F. hepatica*. Five PCR primer sets were design targeting the *Fasciola ITS1* region of the *rRNA* gene (GenBank AJ243016) using the Primer-BLAST software. The PCR primer sets were used to standardize a real-time PCR test to detect *F. hepatica* DNA in stools. The standardization process for the real-time PCR for *F. hepatica* (FAS-PCR) followed the same steps and used the same specimens described for the FAS-RPA. Briefly, the primer sets were tested with DNA extracted from adult *Fasciola* parasites; those with the maximum sensitivity and specificity were selected for further testing (Table 1). The final conditions were set in a 20 μL volume containing 10 μL Sso Advanced Universal SYBR Green Supermix (BioRad, Hercules, CA), 0.5 μL of a 10- μM solution of each forward and reverse primer, 4 μL DNase-free water, and 4 μL of purified DNA at concentrations between 10 and 75 ng/ μL . The reactions were run in duplicate on a CFX96 Touch Real-Time PCR System (BioRad), with the following cycling conditions: 50°C for 3 minutes, 98°C for 5 minutes, (98°C for 15 seconds, 64°C for 30 seconds, 72°C for 30 seconds) for 40 cycles followed by melting point analysis. Adult *F. hepatica* DNA was used as positive control, human DNA was used as negative control, and DNase-free water was used as the non-template control in all the reactions. The FAS-PCR was considered positive if the melting point of the product was the same as the melting point of the positive control DNA sample ($\pm 0.5^{\circ}\text{C}$) in both replicates before the 40th cycle and the negative and non-template controls were negative. The LOD was evaluated with several fold dilutions of purified *Fasciola* DNA. The real-time PCR specificity was tested with DNA extracted from helminth specimens and stool containing other parasites described in RPA test for *F. hepatica* section.

Gold standard and testing of de-identified human stool samples. A total of 102 human stool samples were selected from banked specimens. A positive specimen was defined as a stool sample with *Fasciola* eggs detected by microscopy. A negative specimen was define as a stool sample with no *Fasciola* eggs detectable by Lumbreras rapid sedimentation and Kato-Katz tests, no *Fasciola* eggs in the other two specimens provided by the same subject, and a negative Fas2 ELISA for *Fasciola* antibodies. False-negative specimen was defined as a stool sample without *Fasciola* eggs by Lumbreras rapid sedimentation test and Kato-Katz test, but obtained from a subject with at least one other stool sample positive for *Fasciola* by microscopy. The sensitivity and specificity of FAS-RPA and FAS-PCR were calculated using the composite gold standard (Lumbreras rapid sedimentation/Kato-Katz test/Fas2 ELISA as defined above). The sensitivity of the methods was compared using the McNemar's χ^2 test. The correlation between number of eggs per gram of stool in the Kato-Katz test and FAS-PCR Ct values was also evaluated. Results were considered statistically significant if $P < 0.05$.

TABLE 2
Sensitivity and specificity of FAS-RPA and FAS-PCR compared with microscopy

	Microscopy positive (N)	Microscopy negative (N)	Sensitivity	McNemar's χ^2	Specificity
FAS-RPA positive	36	0	87.8%	$P = 0.04$	100%
FAS-RPA negative	5	42			
FAS-PCR positive	27	0	65.9%		100%
FAS-PCR negative	14	42			

FAS-PCR = real-time polymerase chain reaction for *Fasciola hepatica*; FAS-RPA = recombinase polymerase amplification test for *F. hepatica*.

FAS-RPA detection by lateral flow. As a proof of concept, FAS-RPA was adapted to lateral flow detection of the reaction products. The LOD of the lateral flow FAS-RPA was determined by testing several dilutions of adult *Fasciola* DNA. Then we evaluated the performance of the lateral flow assay using 21 positive and 21 negative clinical samples to *F. hepatica* tested previously by FAS-RPA, FAS-PCR, and microscopy. The commercially available Twist Amp RPA Kit (TwistDx) was used to prepare a mix containing the supplied dry reagents, 29.5 μ L of rehydration buffer, 2.1 μ L FAS-RPA forward primer (5 μ M), 2.1 μ L FAS-RPA biotinylated reverse primer (5 μ M), 0.6 μ L of the Twist Amp FAM-labeled probe (2.5 μ M) (Table 1), 10 μ L sample DNA template (20–100 ng/ μ L), 2.7 μ L DNase-free water, and 3 μ L of magnesium acetate. The reaction mix was incubated at 38°C for 30 minutes. Positive RPA reactions generated DNA products labeled with FAM and biotin that were detected using commercially available lateral flow strips (Ustar Biotechnologies, Hangzhou, China). The strips contain an anti-FAM gold-labeled antibody that binds to the products as they flow to the test band that immobilizes them with an anti-biotin-specific antibody generating a positive band. The control band traps the unbound gold particles giving a positive control band at the end of the strip. After incubating the reaction mixture, a dilution of 1/25 was made using 4 μ L of the RPA products with 96 μ L of lateral flow assay buffer provided by the manufacturer in a 1.5-mL tube. Then, the lateral flow strip was immersed in the tube and the solution was allowed to flow through the strip for 10 minutes, after which the test was interpreted. The test was positive if a red band was apparent in the inferior part of the test strip and the negative control specimen failed to show a band.

RESULTS

FAS-RPA characterization. The FAS-RPA test showed a high sensitivity, detecting *Fasciola* DNA at concentrations as low as 1.6 pg/ μ L (Figure 1A). FAS-RPA did not produce any amplification with DNA of *S. mansoni*, *T. solium*, *A. lumbricoides*, hookworm, *T. trichiura*, *H. nana*, and *S. stercoralis* specimens (Figure 1B). Similarly, there was no amplification when *E. granulosus*, *Diphylobothrium* spp., and *Giardia* spp. specimens were tested (data not shown).

FAS-PCR characterization. The FAS-PCR test also showed a high sensitivity, detecting *Fasciola* DNA at a concentration of 1.6 pg/ μ L (Figure 2A). FAS-PCR did not produce amplification curves when tested with specimens of *S. mansoni*, *T. solium*, *A. lumbricoides*, hookworm, *T. trichiura*, *H. nana*, and *S. stercoralis* (Figure 2B). Similarly, there was no amplification when *E. granulosus*, *Diphylobothrium* spp., and *Giardia* spp. positive specimens were tested (data not shown).

Evaluation of clinical specimens. A total of 102 de-identified human samples were evaluated by FAS-RPA and FAS-PCR. Forty one samples were *Fasciola* positive with a low burden of infection (geometric mean of 18 eggs/g of stool, arithmetic mean 66.3 eggs/g of stool [± 45.0]). Forty two were *Fasciola* negative with or without other helminths, and 19 were false-negative specimens. The sensitivity of FAS-RPA was 87.8% and the sensitivity of FAS-PCR was 65.9% (Table 2). The McNemar's χ^2 test showed that the difference between the sensitivity of both tests was statistically significant ($P = 0.04$). No false-positive results were detected by any of the tests giving a specificity of 100%. When testing false-negative samples, FAS-RPA was able to detect *Fasciola* DNA in nine of the 19 samples (47.3%), while FAS-PCR was able to detect DNA in five samples (26.3%) (Table 3). PCR Ct values did not significantly correlate with egg counts by the Kato-Katz method (Spearman's rho = -0.21 , $P = 0.24$).

FAS-RPA lateral flow. The lateral flow detection of the RPA products showed sensitive and specific results. The LOD of the lateral flow assay was 1 pg/ μ L (Figure 3A). Of the 21 *Fasciola* positive samples tested by FAS-RPA lateral flow, 20 (95.2%) were detected by lateral flow and one failed to show a band. Similarly, of the 21 *Fasciola*

FIGURE 1. Recombinase polymerase amplification test for *Fasciola hepatica* (FAS-RPA) characterization. Agarose gel electrophoresis of FAS-RPA reactions. (A) FAS-RPA limit of detection showing ~300-bp products using *F. hepatica* DNA at concentration between 10 ng/ μ L and 1.6 pg/ μ L. Additional unspecific bands are observed at the 600-bp level. (B) Evaluation of FAS-RPA specificity showing amplification of *F. hepatica* DNA but not DNA extracted from trematode, cestode, and nematode parasites. Fh = *F. hepatica*; Scm = *Schistosoma mansoni*; Ts = *Taenia solium*; Al = *Ascaris lumbricoides*; Hkw = hookworm; Tt = *Trichuris trichiura*; Hn = *Hymenolepis nana*; Ss = *Strongyloides stercoralis*; Hs = *Homo sapiens*; NTC = no-template control; 100 bp = DNA ladder.

FIGURE 2. Real-time polymerase chain reaction for *Fasciola hepatica* (FAS-PCR) characterization: amplification and melt curves. (A) FAS-PCR reactions using *F. hepatica* DNA at concentrations between 10 ng/ μ L and 1.6 pg/ μ L. (B) Evaluation of FAS-PCR specificity showing amplification of *F. hepatica* DNA, but no amplification with trematode, cestode, and nematode parasites' DNA (no amplification curves appear in the figure). *Fh* = *F. hepatica*; *Scm* = *Schistosoma mansoni*; *Ts* = *Taenia solium*; *Al* = *Ascaris lumbricoides*; *Hwk* = hookworm; *Tt* = *Trichuris trichiura*; *Hn* = *Hymenolepis nana*; *Ss* = *Strongyloides stercoralis*; *Hs* = *Homo sapiens*; NTC = no-template control.

negative samples tested, 19 (90.4%) were negative by lateral flow and two were positive (Figure 3B).

DISCUSSION

The diagnosis of *Fasciola* infection relies on morphologic examination and quantitation of eggs by stool microscopy. The low sensitivity of microscopy usually requires testing multiple specimens and may not detect low-burden infections.^{7–10} Most of the quantitative microscopy tests are not standardized to use preserved stool, making testing of samples from remote areas problematic.¹⁹ Our results on the characterization of FAS-RPA and FAS-PCR tests showed that both tests are sensitive and specific to detect low concentrations of *Fasciola* DNA in the stool. The sensitivity of FAS-RPA test outperformed FAS-PCR, but both were more sensitive than microscopy for false-negative samples. The differences between the sensitivity of FAS-RPA and FAS-PCR might be related to the higher susceptibility of PCR to inhibitors that may be present in complex samples like stool. The lateral flow detection of FAS-RPA products increased the sensitivity of the test maybe by capturing the double-labeled

products and concentrating them in the test line of the lateral flow strip allowing an easier visualization of the results.

Highly sensitive PCR tests has been reported for the identification of *Fasciola* infection in intermediate hosts.^{20,21} Recently, Martinez-Valladares and Rojo-Vasquez reported on a conventional PCR test and a LAMP test that showed a LOD of 10 pg/ μ L of *Fasciola* DNA and detected 100% of the infections in sheep with a mean 84.4 ($\pm$ 54) eggs/g of stool.²² Few studies have validated PCR tests to diagnose *Fasciola* using a large number of human clinical stool samples. Le and others²³ reported on a duplex PCR test that detected 100% of *Fasciola gigantica* infections in Vietnam. However, only 13 human stool samples were tested, and data on stool microscopy methods and egg burden were not reported.²³ Although the LOD of FAS-PCR was much lower than in other studies, the sensitivity of FAS-PCR was 66% using stool samples with low burdens of eggs (geometric mean 18 eggs/g of stool). In addition, FAS-PCR was able to detect more infections in microscopy false-negative samples. This sensitivity seemed low compared with the results for conventional PCR reported by other authors. It is possible that our real-time PCR was susceptible to inhibitors in human stool or that DNA extraction was incomplete despite our efforts to lyse the hard *Fasciola* eggs. Another possibility is that other studies did not use the same stringent criteria for sample classification ascertainment, which may have led to missing very low burdens of infection and misclassification of samples. Still, FAS-PCR is likely to be more sensitive than commonly used microscopy tests like Kato-Katz. The reported sensitivity of one Kato-Katz test to detect *Fasciola* eggs is approximately 65% in moderate- and high-burden areas. However, the Kato-Katz test sensitivity is decreased in light infections as was the case of the

TABLE 3

Sensitivity and specificity of the FAS-RPA and FAS-PCR tests using microscopy-negative samples of subjects with fascioliasis

	Subjects with <i>Fasciola</i> (N)	Sensitivity	McNemar's χ^2
FAS-RPA positive	9	47.3%	$P = 0.12$
FAS-RPA negative	10		
FAS-PCR positive	5	26.3%	
FAS-PCR negative	14		

FAS-PCR = real-time polymerase chain reaction for *Fasciola hepatica*; FAS-RPA = recombinase polymerase amplification test for *F. hepatica*.

FIGURE 3. Recombinase polymerase amplification test for *Fasciola hepatica* (FAS-RPA) lateral flow. (A) Limit of detection for FAS-RPA products detected by lateral flow using serial 10-fold dilutions of *F. hepatica* DNA. The lowest DNA concentration detected was 1 pg/ μ L. (B) FAS-RPA products detected by lateral flow using clinical samples with *F. hepatica* eggs. (C) FAS-RPA products detected by lateral flow using clinical samples without *F. hepatica* eggs. NTC = no-template control; PC = positive control.

samples tested in our study.²⁴ We had assumed that FAS-PCR Ct values could be used to estimate the parasite burden in stool samples. However, the Ct values did not correlate with egg counts estimated by Kato-Katz test. It is possible that the distribution of eggs was not uniform in the stools, and aliquots taken from different areas of the specimen could have different egg burdens. Alternatively, incomplete lysis of *Fasciola* eggs during DNA extraction could explain this finding. Further optimization of FAS-PCR to improve sensitivity and quantitative results is needed.

The preliminary results of the FAS-RPA showed a high analytical and clinical sensitivity. The sensitivity in positive stool specimens was higher than that reported for most microscopy tests in single specimens and significantly higher than that of FAS-PCR. Also, the FAS-RPA detected *Fasciola* eggs in half of the microscopy false-negative stool specimens. Importantly, the increased sensitivity of stool testing by FAS-RPA may help to detect *Fasciola* eggs very early or very late in chronic infection when egg production is low. At the individual level, this may lead to an earlier diagnosis, and at the population level to increase accuracy of prevalence and control programs' impact estimates. Notably, the FAS-RPA showed 100% specificity and did not produce false-positive results when tested with relevant related parasites like *S. mansoni* and helminth parasites prevalent in the area.

The lateral flow detection of FAS-RPA was not only feasible, but also decreased the LOD of the test from 1.6 to 1 pg/ μ L. When evaluating the clinical samples, the lateral flow format allowed the detection of two additional cases of *Fasciola* infection. Thus, FAS-RPA lateral flow may be a

step closer to have a highly sensitive and specific deployable test to diagnose fascioliasis in endemic areas.

The coproantigen ELISA tests is highly sensitive for *Fasciola* diagnosis, and commercial assays are already in use for veterinary purposes. The MM3 coproantigen ELISA uses a monoclonal antibody to detect *Fasciola* excretory/secretory antigens in the stool.^{11,12,25} This test has been evaluated for field diagnosis in humans showing a sensitivity of 94% and specificity between 93% and 98% depending on the parasite burden.¹² This test is able to detect *Fasciola* antigens in stool early in the infection and several days before eggs are detected by microscopy.²⁵ Robles-Perez and others²⁶ compared the coproantigen ELISA test with conventional PCR to detect *Fasciola* infection in sheep. PCR was able to detect *Fasciola* infection on week 2 after experimental infection, and both tests showed higher sensitivity than microscopy.²⁶ However, the sensitivity of conventional PCR outperformed the coproantigen ELISA.²⁶ These tests have not been compared for the diagnosis of human fascioliasis, but given the performance reported by other authors it is likely that PCR-based methods will be at least as sensitive as antigen-based tests.^{22,23,26} Importantly, the lateral flow FAS-RPA test showed significantly more sensitivity than FAS-PCR and probably has more potential for point-of-care use than currently used coproantigen ELISA platforms.

The capability to deliver quantitative results is a desirable characteristic that limits the applicability of the FAS-RPA lateral flow in its current format. Several quantitative RPA tests have been reported recently using real-time devices and solid-phase reactions for result detection.^{27,28} Future experiments will focus on adapting FAS-RPA to a low-cost, device-based, semiquantitative reading of results.

In conclusion, FAS-PCR and FAS-RPA (lateral flow) are highly sensitive and specific tests to detect *Fasciola* DNA in stools. These tests will add to the tools available to study the epidemiology of this infection. Further optimization of FAS-RPA will potentially generate a point-of-care test easy to deploy to resource-poor settings. This work will need to be complemented with field-applicable stool DNA extraction methods.

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