Supplementary data.

Figure S1

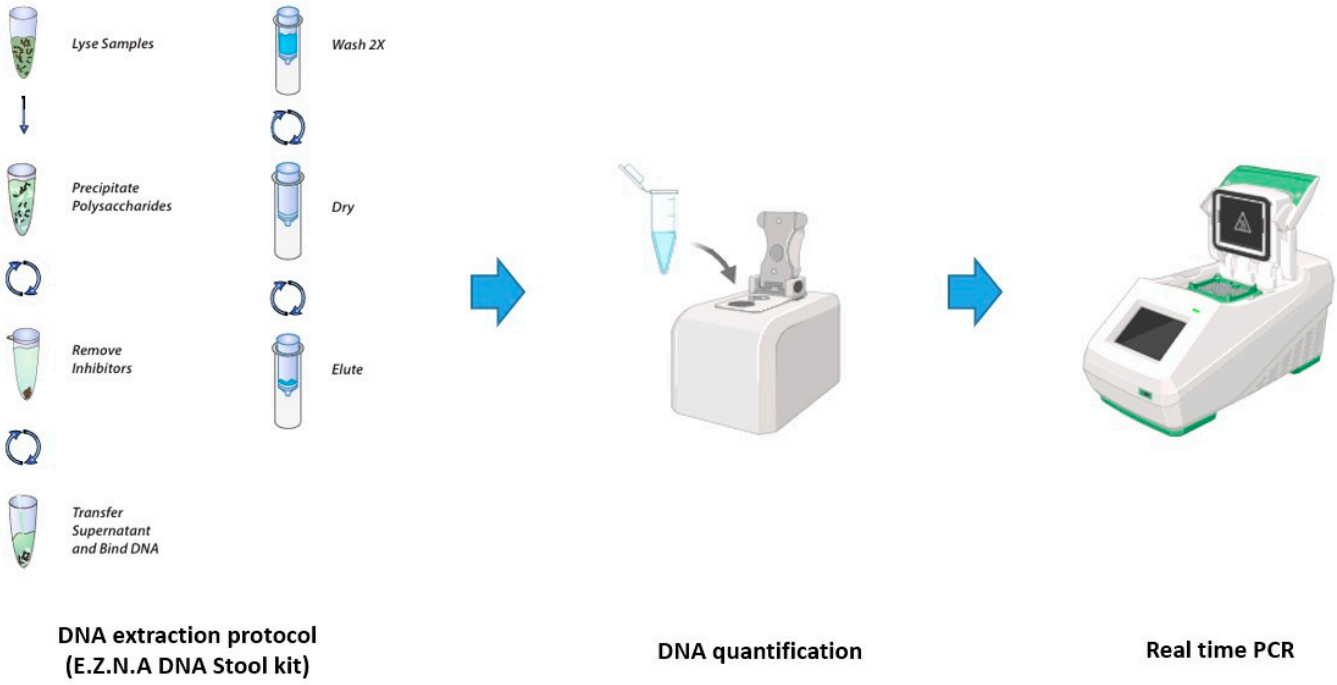


Figure S1. Workflow for the Molecular Detection of *F. hepatica* in Clinical Samples. Schematic representation of the laboratory pipeline, including the DNA extraction protocol, followed by spectrophotometric DNA quantification, and qPCR amplification targeting the ITS1 gene of *F. hepatica*.

Table S1.

Limit of detection of spiked eggs in feces	DNA concentration (ng/uL)	Average DNA concentration and standard deviation	Ct1	Ct2	Ct3	Average Ct and standard deviation per sample	Average Ct and standard deviation per egg count
5 eggs/g of feces	213.1	184.9 ± 25.1	30.55	31.45	32.77	31.59 ± 1.12	34.66 ± 2.14
5 eggs/g of feces	187.6	—	34.7	35.08	38.67	36.15 ± 2.19	—
5 eggs/g of feces	176.8	—	33.74	36.1	NA	34.92 ± 1.67	—
5 eggs/g of feces	199.9	—	34.96	35.17	36.16	35.43 ± 0.64	—
5 eggs/g of feces	147.1	—	36.09	35.09	NA	35.59 ± 0.71	—
10 eggs/g of feces	217.6	207.82 ± 10.56	28.08	28.1	28.15	28.11 ± 0.04	27.88 ± 0.68
10 eggs/g of feces	205.9	—	27.44	27.45	27.72	27.53 ± 0.15	—
10 eggs/g of feces	195.1	—	26.87	27.22	27.11	27.06 ± 0.17	—
10 eggs/g of feces	193.3	—	27.53	27.75	27.93	27.74 ± 0.20	—
10 eggs/g of feces	212.2	—	28.72	28.91	29.29	28.97 ± 0.29	—
15 eggs/g of feces	372.1	345.68 ± 55.33	29.21	29.38	30.04	29.54 ± 0.44	30.89 ± 0.97
15 eggs/g of feces	379.7	—	30.61	30.96	31.01	30.86 ± 0.22	—
15 eggs/g of feces	288.1	—	30.47	31.07	31.29	30.94 ± 0.42	—
15 eggs/g of feces	284.8	—	30.18	31.05	31.13	30.79 ± 0.53	—
15 eggs/g of feces	403.7	—	32.38	32.38	32.41	32.39 ± 0.02	—

Table S1. Evaluation of the limit of detection (LOD) using fecal samples spiked with varying egg concentrations (5, 10, and 15 eggs/g of feces). Bars represent the average values, error bars indicate the standard deviation (SD), and individual data points (N=5 biological replicates per concentration level) are overlaid as dots. Ct1, Ct2, and Ct3 represent technical replicates for each sample. Note: Concentrations lower than 5 eggs/g of feces were not detected after 40 PCR cycles.

Figure S2.

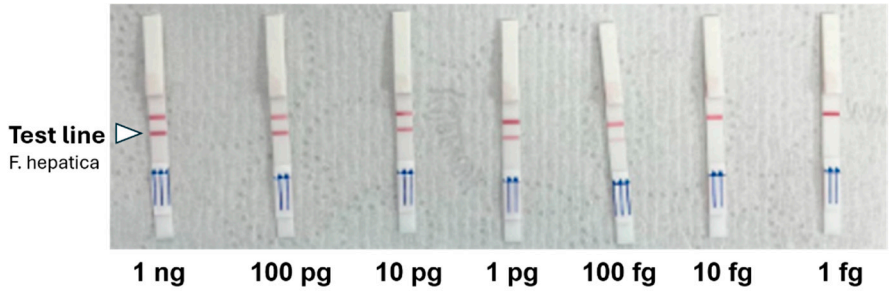


Figure S2. Lateral flow assay (LFA) sensitivity using serial dilutions of *Fasciola hepatica* DNA. The image displays seven lateral flow strips corresponding to template DNA concentrations ranging from 1 ng to 1 fg (left to right). Arrows indicate the test line specific to *F. hepatica*. Results were captured immediately following the 2-minute reaction development time.

Figure S3.

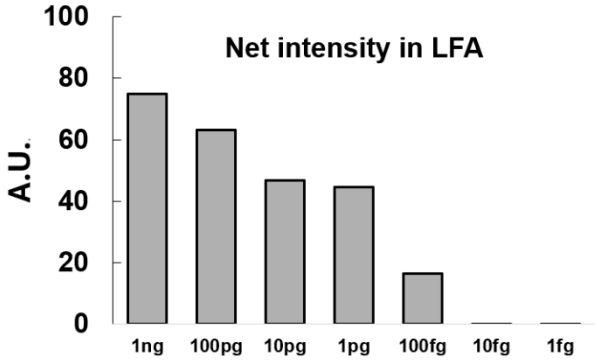


Figure S3. Quantitative net band intensity analysis of the lateral flow strips. The graph shows the net signal intensity (arbitrary units, A.U.) of the test lines measured using ImageJ software across serial Fasciola hepatica DNA dilutions ranging from 1 ng to 1 fg. Reactions were developed for 2 minutes prior to image capture and subsequent densitometric analysis. A positivity threshold of 17 A.U. (background-subtracted net intensity) was established from this titration with signals below 17 A.U. classified as negative and used to define both the limit of detection and positivity in clinical samples.

Table S2.

Detection Limit	qPCR CTs Replicates			Visual Result / Amplification
1 ng	17.88	17.84	17.63	Yes
100 pg	21.25	21.28	21.46	Yes
10 pg	24.82	24.86	24.69	Yes
1 pg	28.18	28.35	28.36	Yes
100 fg	31.12	31.46	32.35	Yes
10 fg	33.28	33.84	34.62	No
1 fg	NA	NA	41.21	No
0.1 fg	NA	NA	NA	No

Table S2. Determination of the analytical limit of detection (LOD) using serial dilutions of template DNA. The table presents the cycle threshold (Ct) values across technical replicates (columns 2–4) and the corresponding visual/amplification outcomes (Yes/No) for DNA concentrations ranging from 1 ng to 0.1 fg. The reaction was allowed to fully develop for 2 minutes before visual interpretation and immediate image capture for analysis.

Huayllapata

Method	Frequency	95% CI
Microscopy	16/129	12.40% (7.26% - 19.36%)
qPCR	21/129	16.3% (10.9% - 23.6%)
LAF	24/129	18.6% (12.8% - 26.2%)

Table S3. Detection frequency, percentage, and 95% confidence intervals (CI) for Microscopy, qPCR, and LAF methods in Huayllapata (N = 129). Data represents the number of positive detections out of the total samples tested per method.

Churo

Method	Frequency	95% CI
Microscopy	7/58	12.07% (4.99% - 23.30%)
qPCR	10/58	17.2% (9.6% - 28.9%)
LAF	7/58	12.1% (6.0% - 22.9%)

Table S4. Detection frequency, percentage, and 95% confidence intervals (CI) for Microscopy, qPCR, and LAF methods in Churo (N = 58). Data represents the number of positive detections out of the total samples tested per method.

Sayllapata

Method	Frequency	95% CI
Microscopy	7/53	13.21% (5.48% - 25.34%)
qPCR	8/53	15.1% (7.9% - 27.1%)
LAF	15/53	28.3% (18.0% - 41.3%)

Table S5. Detection frequency, percentage, and 95% confidence intervals (CI) for Microscopy, qPCR, and LAF methods in Sayllapata (N = 53). Data represents the number of positive detections out of the total samples tested per method.

Accha

Method	Frequency	95% CI
Microscopy	8/59	13.56% (6.04% - 24.98%)
qPCR	10/59	16.9% (9.5% - 28.5%)
LAF	21/59	35.6% (24.6% - 48.3%)

Table S6. Detection frequency, percentage, and 95% confidence intervals (CI) for Microscopy, qPCR, and LAF methods in Accha (N = 59). Data represents the number of positive detections out of the total samples tested per method.

Quisicancha

Method	Frequency	95% CI
Microscopy	4/38	10.53% (2.94% - 24.80%)
qPCR	10/38	35.7% (20.7% - 54.2%)
LAF	6/38	21.4% (10.2% - 39.5%)

Table S7. Detection frequency, percentage, and 95% confidence intervals (CI) for Microscopy, qPCR, and LAF methods in Quisicancha (N = 38). Data represents the number of positive detections out of the total samples tested by method.

Piscohuata

Method	Frequency	95% CI
Microscopy	4/28	14.29% (4.03% - 32.67%)
qPCR	8/28	28.6% (15.3% - 47.1%)
LAF	11/28	39.3% (23.6% - 57.6%)

Table S8. Detection frequency, percentage, and 95% confidence intervals (CI) for Microscopy, qPCR, and LAF methods in Piscohuata (N = 28). Data represents the number of positive detections out of the total samples tested by method.

Table S9

Profile	Microscopy (A)	qPCR (B)	PCR-LFA (C)	(*N*)	Frequency	IC95 (%)
All Positive	+	+	+	32	8.8%	6.28 - 12.11
B/C Only	-	+	+	21	5.8%	3.79 - 8.63
A/B Only	+	+	-	3	0.8%	0.28 - 2.39
A/C Only	+	-	+	2	0.5%	0.15 - 1.98
A Only	+	-	-	9	2.5%	1.30 - 4.62
B Only	-	+	-	11	3.0%	1.69 - 5.32
C Only	-	-	+	29	7.9%	5.59 - 11.18
All Negative	-	-	-	258	70.7%	65.82 - 75.12
Total				365	100.0%	

Table S9. Distribution and Concordance of *F. hepatica* Detection Profiles among 365 Samples Using Microscopy, qPCR, and miiniPCR-LFA. The table compares the diagnostic performance of three methods for the detection of *Fasciola hepatica*: Microscopy (A), quantitative Polymerase Chain Reaction (qPCR) (B), and Polymerase Chain Reaction-Lateral Flow Assay (PCR-LFA) (C). Positivity (+) and negativity (−) for each method are displayed across eight distinct "Profiles" (combinations of test results), along with the number of samples (*N*), percentage frequency (%), and 95% Confidence Interval (95% CI).

Figure S4.

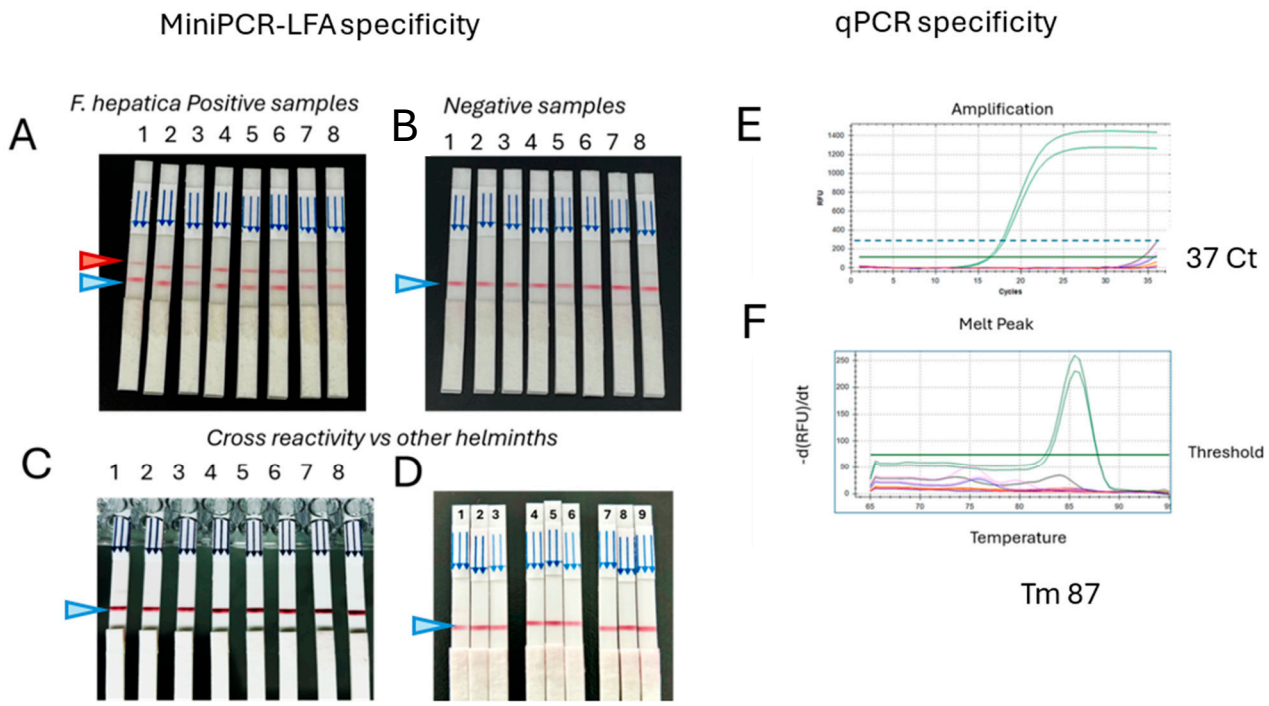


Figure S4. (A) Repeatability assay using positive *Fasciola hepatica* samples (1-8, representing replicate tests), showing consistent positive band formation. **(B)** Negative control samples (1-8), displaying only control lines without false-positive amplification. All positive test bands (indicated by the red arrowhead) exceeded the colorimetric densitometry threshold of 17 A.U. (equivalent to ~100 fg of target DNA) established in Figure S3, while control lines of the LFA assay are indicated by the blue arrowhead.**(C)** Cross-reactivity evaluation against other helminth species (1: *Trichuris*, 2: *Strongyloides*, 3: *Diphyllobothrium*, 4: *Echinococcus*, 5: *Uncinaria*, 6: *Taenia*, 7: H_2O blank, 8: *Ascaris*), demonstrating no cross-reactivity with non-target parasites.**(D)** Additional specificity testing performed in triplicate (1-3 non-template blanks, 4-6: *Calicophoron*, 7-9: *Ascaris*) noting that faint bands observed in lanes 1 and 8 did not pass the densitometry threshold shown in Figure S3. (**E**) qPCR amplification curves showing positive *F. hepatica* samples (green lines) crossing the fluorescence threshold, with non-target negative samples remaining below the 37 °C threshold line. **(F)** Melting curve analysis of the samples evaluated in panel E, where only the positive *F. hepatica* amplicon exhibits a specific melt peak Tm=87 °C, while all negative control samples fail to pass the fluorescence threshold.

Table S10.

Parameter	Posterior Median	Posterior Mean	95% CrI	R^	ESS
True Prevalence	14.6%	14.7%	11.2%–18.7%	1.0000	7,695
Coprological Microscopy Sensitivity	58.6%	58.5%	44.8%–71.8%	1.0000	9,237
Coprological Microscopy Specificity	97.5%	97.4%	95.6%–98.9%	1.0002	12,024
Stool qPCR Sensitivity	93.1%	92.2%	80.1%–99.3%	1.0000	7,981
Stool qPCR Specificity	97.6%	97.6%	95.4%–99.5%	1.0005	6,451
miniPCR-LFA Sensitivity	89.2%	88.4%	75.6%–97.2%	1.0002	6,543
miniPCR-LFA Specificity	92.8%	92.8%	89.8%–95.4%	1.0003	9,759

Table S10. Posterior medians, means, 95% credible intervals (95% CrI), Gelman–Rubin convergence statistics (R^), and Effective Sample Sizes (ESS) were estimated across 4 independent MCMC chains (20,000 iterations per chain, 5,000 warm-up draws) under a conditional-independence three-test BLCA model (N = 365) using Beta (6, 94) for prevalence, Beta (99, 1) for specificity of all three assays (Coprological Microscopy, Stool qPCR, and miniPCR-LFA), and uninformative Beta (1, 1) priors for sensitivity.

Parameter	Primary: Prev Beta(6,94) + Sp Beta(99,1)	Sensitivity 1: Prev Beta(6,94) + Sp Beta(95,1)	Sensitivity 2: Prev Beta(10,90) + Sp Beta(99,1)	Sensitivity 3: Prev Beta(20,80) + Sp Beta(99,1)
True Prevalence	14.6% (11.2%–18.7%)	14.6% (11.2%–18.7%)	15.8% (12.2%–20.3%)	19.2% (14.9%–24.8%)
Coprological Microscopy Sensitivity	58.6% (44.9%–71.7%)	58.7% (44.8%–71.8%)	57.6% (43.9%–71.1%)	54.2% (40.0%–68.5%)
Coprological Microscopy Specificity	97.5% (95.6%–98.8%)	97.5% (95.5%–98.8%)	97.5% (95.6%–98.9%)	97.7% (95.8%–99.1%)
Stool qPCR Sensitivity	93.2% (79.7%–99.4%)	93.2% (80.2%–99.4%)	92.3% (78.1%–99.2%)	88.3% (68.8%–98.3%)
Stool qPCR Specificity	97.6% (95.4%–99.5%)	97.6% (95.3%–99.4%)	97.8% (95.5%–99.7%)	98.4% (96.0%–99.9%)
miniPCR-LFA Sensitivity	89.2% (76.1%–97.2%)	89.3% (76.3%–97.5%)	88.1% (75.0%–96.9%)	84.9% (71.6%–95.4%)
miniPCR-LFA Specificity	92.8% (89.8%–95.4%)	92.7% (89.7%–95.4%)	93.0% (90.0%–95.7%)	93.6% (90.4%–97.2%)
Max $\hat{R}$ (across all parameters)	1.0002	1.0003	1.0005	1.0018
Min Effective Sample Size (ESS)	6,650	7,053	5,656	3,242

Table S11. Posterior medians and 95% credible intervals (95% CrI) from conditional-independence three-test Bayesian Latent Class Analysis (BLCA) across N=365 participants. Uninformative Beta (1,1) priors were placed on test sensitivities across all models, while specificity priors (Sp) and prevalence priors (Prev) were varied as indicated. Diagnostics are reported across 4 Markov chains (20,000 iterations and 5,000 warm-up draws per chain; 60,000 total post-warmup draws).

BLCA Convergence Diagnostics and Prior Sensitivity Analysis. Markov chain Monte Carlo (MCMC) convergence for all three-test conditional-independence BLCA models (N=365) was evaluated across 4

independent chains (20,000 iterations per chain, 5,000 warm-up draws, yielding 60,000 post-warmup draws per model) using the Gelman–Rubin convergence statistic ($\hat{R}$) and Effective Sample Size (ESS). Across all four prior specifications, chains mixed well and converged cleanly, with maximum $\hat{R} \leq 1.0018$ and minimum ESS≥3,242 across all prevalence, sensitivity, and specificity parameters.

In prior sensitivity analyses with uninformative Beta (1,1) sensitivity priors, changing the specificity prior across all three assays from Beta (99,1) to Beta (95,1) under a Beta (6,94) prevalence prior resulted in virtually identical posterior medians and 95% credible intervals (within ≤0.1 percentage points for all parameters). Varying the prevalence prior from Beta (6,94) (~6%) to Beta (10,90) (~10%) and Beta (20,80) (~20%) while retaining Beta (99,1) specificity priors yielded posterior prevalence medians of 15.8% (95% CrI: 12.2%–20.3%) and 19.2% (95% CrI: 14.9%–24.8%), respectively, with modest, expected shifts in estimated sensitivities (e.g., miniPCR-LFA sensitivity: 89.2% under Prev Beta (6,94) vs. 88.1% under Prev Beta (10,90) and 84.9% under Prev Beta (20,80)) and consistent specificities across assays.

Table S12.

Parameter	Posterior Mean	Approximate 95% CrI
True Prevalence (π)	21.5%	17.2% – 26.3%
Sensitivity - Microscopy (A)	51.3%	39.8% – 62.8%
Sensitivity - qPCR (B)	76.9%	66.5% – 86.0%
Sensitivity - miniPCR-LFA (C)	85.9%	76.8% – 93.2%
Specificity - Microscopy (A)	97.8%	95.9% – 99.1%
Specificity - qPCR (B)	98.3%	96.6% – 99.4%
Specificity - miniPCR-LFA (C)	96.2%	93.8% – 98.1%
Covariance - covBC D=1	+0.028	-0.015 – +0.075
Covariance - covBC D=0	+0.002	-0.002 – +0.008

Table S12. Approximate posterior estimates from the three-test Bayesian latent class analysis (BLCA) incorporating pairwise conditional covariance between qPCR (B) and miniPCR-LFA (C) across N=365 stool samples. (following Dendukuri et al. (2009) [25]), with conditional independence assumed for coprological microscopy (A). Models were specified using an informative prevalence prior (Beta (6,94)), informative specificity priors across all three assays (SpA, SpB, SpC Beta (99,1)), and uninformative sensitivity priors (SeA, SeB, SeC Beta (1,1)). covBC D=1 and covBC D=0 denote conditional covariance between qPCR and PCR-LFA among latently infected (D=1) and uninfected (D=0) subjects, respectively.