ARTICLE

Opportunistic fecal sampling and PCR testing for canine schistosomiasis japonica surveillance in endemic settings

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ABSTRACT

Among the challenges in animal surveillance for *Schistosoma japonicum* are the difficulty of collecting samples from free-roaming dogs and the low sensitivity of traditional diagnostics. This study evaluated opportunistic environmental fecal sampling combined with PCR as a surveillance tool for canine schistosomiasis japonica in selected endemic barangays of the northern Philippines. Dog stool samples (n = 72) were opportunistically collected in Gonzaga, Cagayan (6:00–8:00 AM) and analyzed using microscopy and PCR. All samples were examined for ova using the formalin–ether concentration technique (FECT). In parallel, DNA was extracted and screened using an internal control PCR; eligible extracts were then tested using an endpoint PCR assay targeting the *S. japonicum* SjR2 retrotransposon. While FECT detected no *S. japonicum* ova, PCR detected parasite DNA in 19.12% (13/68) of eligible samples. These findings provide proof of concept for non-invasive molecular surveillance using environmental dog fecal samples and suggest that this approach may help identify areas with canine shedding in endemic settings alongside existing One Health surveillance and control efforts.

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INTRODUCTION

Schistosomiasis japonica, a major neglected tropical disease (NTD), persists as a public health challenge, particularly because of the zoonotic nature of its causative agent (**Colley et al. 2024**). In the Philippines, *Schistosoma japonicum* is endemic in at least 28 provinces and infects a wide range of mammalian reservoirs that perpetuate its life cycle (**Yao et al. 2025**). Domestic dogs are recognized as highly significant reservoirs because of their close interaction with human communities and evidence of substantial parasite gene flow between hosts (**Carabin et al. 2015**; **Rudge et al. 2008**). Despite national control efforts, interrupting transmission remains difficult because of inadequacies in animal surveillance (**Tabilin et al. 2025**). In particular, reliance on traditional diagnostic techniques such as Kato–Katz often yields low sensitivity for detecting light-intensity infections in animal hosts (**Angeles et al. 2025**; **Cai et al. 2019**). Consequently, these methods significantly underestimate true prevalence, obscuring

KEYWORDS

Schistosoma japonicum, non-invasive sampling, fecal DNA, canine schistosomiasis, molecular diagnosis, neglected tropical disease

risk from animal populations and hindering effective public health planning (Lin et al. 2008; Tabilin et al. 2025). Effective control therefore requires a One Health approach that integrates human, animal, and environmental health surveillance (Díaz et al. 2023).

To overcome this diagnostic gap, more sensitive and field-appropriate tools are urgently needed. Molecular diagnostics such as polymerase chain reaction (PCR) offer a powerful alternative that can detect minute quantities of parasite DNA in fecal samples, thereby identifying infections that microscopy consistently misses (**Cai et al. 2019**; **Fung et al. 2012**; **Wu et al. 2010**). When paired with opportunistic, non-invasive sampling of feces from the environment, PCR becomes a practical and scalable approach for surveillance of free-roaming animal populations without direct animal handling.

This study evaluated non-invasive fecal sampling combined with endpoint PCR as a surveillance tool for *S. japonicum* in canine populations in endemic settings. The study was conducted in Gonzaga, Cagayan, a recently recognized endemic focus for schistosomiasis in the Philippines (**Leonardo et al. 2015**). By directly comparing the diagnostic yield of microscopy and PCR, this study provides proof of concept for adopting enhanced surveillance methods to better inform and strengthen schistosomiasis control programs.

MATERIALS AND METHODS

Sample collection and processing

Opportunistic sampling of fresh canine stools was conducted over three consecutive mornings (6:00–8:00 AM) in Gonzaga, Cagayan. Samples were collected from four schistosomiasis-endemic barangays—Tapel (n = 11), Magrafil (n = 20), Sta. Maria (n = 17), and Sta. Cruz (n = 16)—and one non-endemic barangay, SMART (n = 8), located in the town center. Licensed veterinarians and veterinary technicians identified freshly deposited dog feces (i.e., moist, warm, with an intact mucus film) and distinguished them from older feces, ensuring that only specimens defecated that morning were selected. Each specimen was placed in a pre-labeled, clean resealable plastic bag. On the day of collection, specimens were divided into two 1 g aliquots for microscopic examination and molecular diagnostics. The aliquot for microscopy was processed the same day and fixed in a Mini Parasep® SF (Apacor Ltd., United Kingdom) stool concentrator tube containing 3.3 mL of 10% buffered formalin with Triton X-100. The second aliquot was placed in sterile 5.0 mL tubes, packaged according to standard triple-packaging procedures (**World Health Organization 2020**), transported on ice to the laboratory, and stored at −80°C for at least one week before downstream processing. For processing, samples were thawed at 4°C and homogenized with 1 mL of sterile 1× PBS, after which a 300 µL suspension was used for DNA extraction.

Copro-parasitological microscopic analyses

The formalin-preserved aliquots were processed the formalin-ether concentration technique (FECT) using Mini Parasep® SF (Apacor Ltd., United Kingdom), following the manufacturer's protocol. The final sediment from each sample was examined by light microscopy to screen for schistosome ova using established morphological criteria. Any gastrointestinal parasites detected during examination were documented as ancillary findings but were not the primary focus of this study. To ensure accuracy and minimize bias, two independent examiners assessed each slide using the systematic examination of all fields (**World Health Organization 2019**).

Genomic DNA extraction and quality control

Total genomic DNA was extracted from a 200 µL aliquot of the fecal–PBS suspension using the DNeasy PowerSoil Pro Kit (QIAGEN, Germany), which is optimized for high-yield DNA purification from inhibitor-rich fecal matrices. The manufacturer's protocol was followed, with a minor modification: bead-beating was performed using a BeadBug 6 Six-Position Homogenizer (Benchmark Scientific, USA) at 3000 rpm for two 30-second intervals to ensure efficient disruption of parasite ova. DNA was eluted in 100 µL of AE buffer, and concentration and purity were quantified by spectrophotometry (A260/A280 ratio).

To verify DNA integrity and confirm that samples were of canine origin, an internal amplification control (IAC) PCR assay was used to amplify a conserved 102-bp region of the canine mitochondrial NADH dehydrogenase subunit 5 (ND5) gene. Amplification used the primer pair Dog_MT_F (5′-GGCATGCCTTTCCTTACAGGATTC-3′) and Dog_MT_R (5′-GGGATGTGGCAACGAGTGTAATTATG-3′) (**Caldwell and Levine 2009**).

Each 25 µL reaction contained 5.0 µL of 5× Green GoTaq® Flexi Buffer (Promega), 2.0 µL of 25 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs, 1.0 µL each of 10 µM primers, 0.125 µL of GoTaq® DNA Polymerase (5 U/µL), and 5.0 µL of DNA template. Thermal cycling conditions were as follows: 95°C for 3 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds; and a final extension at 72°C for 5 minutes. Amplicons were visualized on a 1.5% agarose gel in 0.5× TBE buffer stained with GelRed (Biotium, USA) and run at 100 V for 20 minutes. Only extracts with successful ND5 amplification were considered eligible for downstream *S. japonicum* SjR2 PCR testing; IAC-negative extracts were excluded. No fixed minimum concentration or purity cutoff was enforced.

Molecular diagnosis of *S. japonicum* elements

Detection of *S. japonicum* genetic material was performed using a diagnostic endpoint PCR assay designed to amplify a 200-bp fragment of the *S. japonicum*–associated non–long terminal repeat (non-LTR) retrotransposon SjR2-like sequence. Primers were used as previously described (**Liu et al. 2023**): k141_29_F (5′-GGTCCGCGAGACGAAAGTCACCAATGGTTA-3′) and k141_29_R (5′-TGCAGAAAATTAGGGAGCGAAGATATGATTTGA-3′).

Each 12.5 µL reaction contained 6.25 µL of 2× GoTaq® Hot Start Green Master Mix (Promega), 3.05 µL of PCR-grade nuclease-free water, 0.5 µL each of 10 µM k141_29 forward and reverse primers, 0.2 µL of 1% bovine serum albumin (BSA), and 2.0 µL of DNA template (2–110 ng/µL). Thermal cycling consisted of 94°C for 5 minutes; 40 cycles of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 40 seconds; and a final extension at 72°C for 10 minutes. Amplicons were visualized under the same electrophoresis conditions as for the IAC assay.

Amplicon verification and statistical analyses

After endpoint PCR, nine SjR2-positive amplicons (selected based on the strongest visible 200-bp bands) were pooled into three sequencing reactions (three 10 µL amplicons per pool), with each pool comprising samples from the same barangay and processed in a single PCR run. Pooled products were submitted to the University of the Philippines System Philippine Genome Center DNA Sequencing Core Facility for purification and bidirectional Sanger sequencing. Chromatograms were analyzed in Geneious Prime® (Biomatters Ltd., New Zealand) for quality trimming (Phred score > 20) and assembly of bidirectional reads into consensus sequences. Sequence identity was validated using nucleotide BLAST against the GenBank non-redundant (nr/nt) database. Positivity was calculated as the number of PCR-positive samples divided by the number of samples that passed the IAC assay.

RESULTS

Comparative diagnostic yields

The two assays produced divergent results. Among the 72 canine fecal samples examined using FECT, none were positive for *S. japonicum* ova. However, FECT detected other parasites (**Table 1**), with hookworm ova as the most prevalent finding (27.78%). After quality control, 68 of 71 extracted DNA samples passed the IAC assay, and endpoint PCR detected *S. japonicum* DNA in 19.12% (13/68) of eligible samples.

Barangay-level distribution and molecular confirmation

Positivity was highest in Magrafil (11/20; 55.00%), followed by Sta. Maria (2/17; 11.76%). No positive samples were detected in the remaining barangays (Table 1). No positive samples were detected in the other three barangays, as detailed in **Table 1**. To confirm the identity of the amplified DNA, nine of the PCR-positive amplicons were selected for sequencing based on the strongest visible bands and pooled into three sequencing reactions (three amplicons per pool). BLASTn analysis of the pooled consensus sequences against the NCBI nucleotide database returned *S. japonicum* clone S11A non-LTR retrotransposon SjR2-like (AF412215) as the top hit for all three pools, with very high pairwise identities of >99.5% for all three pooled samples. E-values were extremely low ($\leq 4.19 \times 10^{-93}$), providing robust molecular confirmation that the endpoint PCR amplicons correspond to the target *S. japonicum* SjR2 sequence.

Table 1: Barangay-level distribution of PCR results for *S. japonicum* in canine fecal samples, Gonzaga, Cagayan (n=72)

Barangay	samples, n	Microscopy Testing, n [%]*			PCR Testing, n [%]	
		S. japonicum	Hookworms	Other Parasites**	IAC-positive	*Sj* PCR-positive
Tapel	11	0	4 [36.36]	0	9 [81.82]	0
Magrafil	20	0	8 [40.00]	2 [10.00]	20 [100.00]	11 [55.00]
Sta. Maria	17	0	2 [11.76]	3 [17.65]	17 [100.00]	2 [11.76]
Sta. Cruz	16	0	5 [31.25]	0	14 [87.50]	0
SMART	8	0	1 [12.50]	0	8 [100.00]	0
OVERALL	72	0	20 [27.78]	5 [6.94]	68 [94.44]	13 [19.12]

**Counted positive if either examiner detected it.*
***Toxocara canis, Trichuris vulpis, Eimeria sp.*

DISCUSSION

This study demonstrated the utility of combining opportunistic environmental fecal sampling with PCR testing as an effective surveillance tool for canine schistosomiasis in an endemic setting. The approach proved highly effective, identifying *S. japonicum* DNA in canine stool samples collected non-invasively from the environment. This stands in contrast to conventional microscopy (through FECT) where no samples were positive for *S. japonicum* ova. Representative PCR-positive amplicons (n=9; pooled into three sequencing reactions) were confirmed by DNA sequencing, supporting the specificity of the endpoint PCR assay.

The use of PCR testing for improved surveillance

The observed difference between diagnostic outcomes (0% positivity by microscopy versus 19.12% by PCR among eligible extracts) is consistent with evidence that copro-parasitological techniques can be insufficient for the effective surveillance of low intensity infections in animal reservoir hosts. Negative microscopy results could be conceivably attributed to several biological and sampling factors: schistosome egg output in dogs is highly variable and can be intermittent, meaning a single fecal sample may not contain eggs even in an infected animal **(Lin et al. 2008; Yu et al. 2007)**. Schistosomiasis transmission also typically follows an "over-dispersed" pattern, where many hosts harbor light infections with low or even undetectable egg shedding, while a small subset contributes disproportionately to environmental contamination **(Churcher et al. 2005; Lu et al. 2010; Poulin 2021)**. Consequently, stronger degrees of overdispersion have often been associated with more resilient parasite-host systems alongside greater difficulty in eradicating infection **(Churcher et al. 2005)**. By detecting minute quantities of parasite DNA from the high-copy SjR2 retrotransposon, the PCR tool circumvents intermittent egg shedding, offering a more sensitive presence-detection approach than microscopy alone.

At the same time, it is important to recognize that fecal heterogeneity and subsampling can still influence molecular detection. In this study, stool specimens were partitioned into aliquots and downstream DNA extraction was performed from a processed subsample, which introduces the possibility that eggs/DNA may be unevenly distributed across aliquots. In the context of intermittent shedding, this may contribute to false negatives even when PCR is used. Thorough homogenization helps mitigate this risk, but replicate extractions/PCR replicates and/or increased input mass where feasible may further improve detection in low-intensity infections.

Implications for a One Health approach to schistosomiasis control

Dogs in schistosomiasis-endemic foci are recognized reservoirs that can maintain zoonotic transmission **(Yao et al. 2025; Carabin et al. 2015)**. Empirical studies in Samar showed dog infection levels correlate with human incidence, and modeling identified dogs as significant contributors to human schistosomiasis risk **(Carabin et al. 2015)**. Likewise, population genetics indicate *S. japonicum* freely moves between dogs and humans **(Rudge et al. 2008)**. Monitoring canine populations for infection, therefore, serves as a vital surveillance tool. Specifically, employing a non-invasive fecal sampling strategy paired with molecular diagnostics makes this approach fundamentally more practical and scalable than invasive methods, as it bypasses the logistical and ethical challenges of capturing and handling free-roaming animals.

The utility and complexity of this approach are demonstrated in Gonzaga, Cagayan. Previous environmental surveys in

schistosomiasis-endemic barangays (Tapel, Magrafil, Sta. Maria, Cabiraoan, and Sta. Cruz) have documented numerous snail habitats, with at least two sites in Magrafil and Sta. Maria yielding *Oncomelania hupensis quadrasi* infected with *S. japonicum* cercariae **(Leonardo et al. 2015; Manalo et al. 2023)**. However, the detection of *S. japonicum* DNA exclusively in Magrafil and Sta. Maria may be attributed to several factors: (1) the patchy distribution and low abundance of infected snails across surveyed sites **(Manalo et al. 2023)**; (2) variations in dog movement, behavior, and exposure to contaminated water sources; and (3) the possibility that fecal PCR may still under-detect low-intensity infections depending on sample matrix effects and infection intensity **(He et al. 2018; Wang et al. 2011)**. Furthermore, the timing of sample collection relative to transmission seasonality or recent exposure could similarly influence detection. For instance, sampling during a low-transmission season might result in lower observed prevalence, as infections could be older and shed less parasite DNA, whereas sampling after a peak transmission period (often associated with the rainy season and increased water contact) could yield comparatively higher detection rates **(Manalo et al. 2023)**. Thus, while environmental conditions conducive to transmission exist in several barangays, active transmission may currently be sustained or detectable only in Magrafil and Sta. Maria.

In Gonzaga, these foci are characterized by specific environmental and social factors. Soil and water analyses indicate that areas of perpetual wetness (e.g., irrigated rice paddies, marshes, slow streams) with silty-loam soil replete with organic matter and stable water retention favor snail persistence **(Manalo et al. 2023)**. Poor sanitation similarly contributes to persistence as schistosomiasis distribution is tightly linked to lack of hygiene, infrastructure, and poverty **(Yao et al. 2025)**. This is exemplified by cercarial seeding into snail habitats owing to low latrine coverage and open defecation (human or canine) **(Manalo et al. 2023**; **Tabilin et al. 2025)**. Moreover, Gonzaga's endemic barangayas were only recently recognized as endemic foci (first reported in 2002, declared endemic in 2004), suggesting that snail-human-animal/snail-animal-human transmission is a recent development tied to land use and migration. For example, laborers moving from endemic provinces to Gonzaga could have introduced the parasite, which then found suitable snail habitats and reservoir hosts that include dogs **(Belizario et al. 2005; Leonardo et al. 2015)**. This is especially concerning given that dogs constitute the overwhelming majority of companion animals in Philippine households and are often allowed to roam freely in rural communities **(Chaudhari et al. 2022; Rudge et al. 2008)**. Pinpointing these environmental and zoonotic niches is critical for transforming general surveys into more actionable tools for targeted public health resource deployment.

Therefore, schistosomiasis control must follow a One Health framework since it is insufficient to treat only humans. China's long-term integrated program offers a model: over ~70 years of combined human mass drug administration, snail habitat management, and livestock treatment reduced human cases by >99% **(Wang et al. 2021)**. The Philippine control program has historically focused on human MDA, while recent reviews highlight that this human-centric strategy underestimates ongoing risk given low coverage with less than a third of people treated annually and cure rates only ~50% **(Olveda et al. 2016)**. Consequently, untreated infected animals continue to contaminate waterbodies. The Philippines' current National Schistosomiasis Control and Elimination Program (SCEP) has begun to address this gap by training veterinarians and establishing animal diagnostic labs in endemic provinces; as of 2018, six endemic provinces had set up animal schistosomiasis laboratories for testing livestock **(Philippine Department of Health 2020)**. However, gaps remain, as even in China where goats and dogs are often not targeted, consistently challenging elimination **(Wang et al. 2021)**. A global review emphasizes that "snail control, treatment of livestock, WASH (Water, Sanitation, and Hygiene), and health education" are all important components of integrated 'One Health' interventions **(Walker and Webster 2023)**. In Gonzaga and similar settings, this means coordinating veterinary and public health actions such as regular deworming of dogs (along with other companion and farm animals), environmental snail control, sanitation improvements, and community education about reducing water contact. Improving latrine coverage and hygiene can directly cut exposure risk, while engaging local vets in routine animal screening ensures that zoonotic sources are addressed.

Diagnostic complementarity and methodological considerations

Beyond schistosomiasis, FECT identified other gastrointestinal parasites (**Table 1**), underscoring a complementary advantage of routine microscopy: it can screen broadly for multiple parasites in a single examination. While targeted PCR provides improved sensitivity for low-intensity *S. japonicum* infections, integrating copro-parasitology can yield additional actionable information for One Health programming (e.g., canine deworming programs addressing multiple helminths and protozoa), especially in resource-limited endemic settings.

All three pooled consensus sequences showed near-perfect identity to known *S. japonicum* SjR2-like sequences, supporting assay specificity within local contexts. This aligns with genetic studies in the Philippines showing high parasite diversity but no deep geographic clustering, suggesting that human and dog-derived parasites are genetically indistinguishable **(Moendeg et al. 2017; Rudge et al. 2008)**. This genetic homogeneity implies that a diagnostic target like SjR2 is a reliable marker for surveillance across different regions.

A potential limitation of the non-invasive sampling strategy is the possibility that positive PCR signals could originate from environmental DNA contamination rather than true host infection. However, several factors suggest that this is unlikely. First, free nucleic acids degrade rapidly in soil and water because of UV exposure and microbial activity, making persistence of high-quality environmental DNA improbable **(Sirois and Buckley 2019; Zulkefli *et al*. 2019)**. Second, restricting collection to freshly voided feces minimized ground contact time, reducing opportunities for contamination. It is therefore more plausible that detected *S. japonicum* DNA originated from a small number of eggs within the fecal matrix that fell below the detection threshold of conventional microscopy.

Another limitation of opportunistic sampling is that individual dogs were not identified; therefore, multiple samples could have originated from the same animal. Because defecation was not directly observed, repeat sampling of the same individual cannot be ruled out. Each specimen was treated as a unique defecation event, which is an inherent limitation of environmental, non-invasive collection. Future surveillance could reduce this uncertainty by incorporating direct observation where feasible, increasing spacing between collection points, and/or sampling across additional days.

Based on these findings, canine fecal PCR could be used as a sentinel surveillance tool in endemic barangays. Given positivity of ~19% overall (and >50% in some barangays), we propose semiannual sampling of at least 30–50 dogs per survey area as a statistically robust target. This sample size provides high confidence (95.8–99.5%) of detecting at least one positive case if true prevalence rate exceeds 10%. Surveillance should be complemented by geo-referenced human serologic surveys and malacological or eDNA monitoring of snail habitats, particularly during the rainy season when transmission peaks. These activities should be paired with integrated interventions, including biannual mass dog deworming with praziquantel and community education on the role of dogs in transmission. Future studies should validate

this stool PCR assay across a gradient of endemic settings and report predictive performance (e.g., PPV/NPV) under differing infection and tranmission intensities. Finally, adopting quantitative real-time PCR (qPCR) may further improve sensitivity and provide quantitative data on infection burden.

CONCLUSION

This study demonstrates that non-invasive fecal sampling combined with PCR is a sensitive and practical tool for canine schistosomiasis surveillance. PCR revealed a 19.12% positivity proportion that microscopy missed entirely, highlighting its ability to better capture infection burden in reservoir hosts. Adopting this enhanced surveillance approach is a critical step toward implementing a One Health framework by providing the animal reservoir data needed to interrupt disease transmission and advance schistosomiasis control and elimination efforts in the Philippines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Hans Webster P. Labordo: Writing – Original Draft, Writing – Review & Editing, Visualization, Validation, Methodology, Investigation, Formal Analysis, Data Curation. **Amina C. Kunting:** Writing – Original Draft, Writing – Review & Editing, Visualization, Validation, Project Administration, Methodology, Investigation, Formal Analysis. **Daria L. Manalo:** Writing – Review & Editing, Supervision, Resources, Methodology, Investigation. **Raffy Jay C. Fornillos:** Conceptualization, Funding Acquisition, Formal Analysis, Writing – Review & Editing, Supervision, Resources, Methodology, Investigation. **Juvren Romvic A. Batalon:** Writing – Review & Editing, Supervision, Resources, Methodology, Investigation. **Efraim P. Panganiban:** Writing – Review & Editing, Supervision, Resources, Methodology, Investigation. **Mark Joseph M. Espino:** Writing – Review & Editing, Supervision, Resources, Methodology, Investigation. **Ian Kendrich C. Fontanilla:** Conceptualization, Funding Acquisition, Formal Analysis, Writing – Review & Editing, Supervision, Resources. **Lydia R. Leonardo:** Conceptualization, Funding Acquisition, Formal Analysis, Writing – Review & Editing, Supervision, Resources. **Ian Kim B. Tabios:** Conceptualization, Funding Acquisition, Formal Analysis, Writing – Review & Editing, Supervision, Resources, Project Administration, Investigation.

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