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Molecular identification of zoonotic hookworms in environmentally collected canine fecal samples in Northern Philippines

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Short Report

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Abstract

Background

Hookworm infections remain an important public health and veterinary concern in tropical and subtropical regions, particularly in communities with close human-animal interactions and limited sanitation. Dogs are recognized as reservoirs of zoonotic hookworms, yet species-level infection data in endemic areas remain lacking because the widely utilized microscopic examination of stool samples cannot differentiate morphologically indistinguishable hookworm eggs.

Methods

Environmentally deposited canine fecal samples (n = 72) were collected from five barangays in Gonzaga, Cagayan, Northern Philippines. Samples were examined using formalin-ether concentration technique (FECT) and endpoint PCR targeting the ITS region, followed by Sanger sequencing for species identification.

Results

Hookworm ova were detected by coproscopy in 20 samples (27.8%), while the PCR assay yielded 24 positive samples (33.3%). Three hookworm species were identified using nBLAST alignment, with *Ancylostoma ceylanicum* as the predominant species, alongside single detections of *A.* caninum and *N. americanus*, the latter likely reflecting environmental contamination or coprophagic passage rather than true canine infection. There were no mixed-species infections identified.

Conclusion

The predominance of the zoonotic hookworm *A. ceylanicum* in environmentally collected canine feces highlights the role of free-roaming dogs in zoonotic hookworm transmission in shared environments. Molecular species identification provided greater resolution than microscopy alone, underscoring the value of integrating PCR-based approaches with conventional coproscopy to strengthen zoonotic hookworm surveillance and better inform One Health interventions.

Introduction

Hookworm infection is an important public health and veterinary problem in tropical and subtropical regions where environmental conditions favor the development, growth, and survival of the infective larvae in soil (George et al. 2016). In the Philippines, soil-transmitted helminthiasis (STH) remains a neglected tropical disease (Mationg et al. 2021) prevalent in rural and peri-urban settings with limited sanitation infrastructure and frequent human-animal interactions. Hookworm infections have been

traditionally attributed to soil contamination from human feces (George et al. 2016); however, infections in dogs caused by *Ancylostoma caninum*, *A. ceylanicum*, and *Uncinaria stenocephala* are also widespread. Of particular concern is *A. ceylanicum*, a zoonotic species capable of completing its life cycle in the human small intestine and causing patent infections that clinically resemble those caused by human hookworms *A. duodenale* and *Necator americanus*.

N. americanus was classified historically as an obligate human parasite, but the recent study of Boyko et al. (2020) has cultured larvae from viable human hookworm eggs isolated from canine and pig fecal samples. Similarly, *A. duodenale*, the highly pathogenic anthroponotic hookworm, has been detected in domestic dogs in Latin America, which indicates a potential role for canine transport hosts in high-prevalence areas (Mawrie et al. 2026). Meanwhile, *A. ceylanicum* is a well-established zoonotic threat in the Asia-Pacific region, where dogs act as primary reservoirs, with prevalence rates ranging from 24% to 92% across various countries in Southeast Asia, such as Thailand, Laos, and Malaysia (Inpankaew et al. 2014).

The widely available and most commonly utilized microscopy-based approaches are insufficient for reliable species-level identification, as hookworm eggs of different species are morphologically indistinguishable. This results in underestimation of zoonotic hookworm diversity and incorrect attribution of environmental contamination sources (Dongmo et al. 2024). This study used a three-stage endpoint PCR followed by Sanger sequencing on environmentally collected canine fecal samples from Gonzaga, Cagayan, Northern Philippines, to complement conventional microscopic examination and enable species identification of zoonotic hookworms.

Materials and Methods

We examined a total of 72 environmentally deposited canine fecal samples collected by a previous study on schistosomiasis from five barangays within the municipality of Gonzaga, Cagayan, Northern Philippines (Supplemental Method). These samples were originally collected for the detection of canine schistosomiasis japonica. A veterinarian assisted in distinguishing fresh canine feces during field collection based on collection timing and gross physical characteristics (i.e., moist, warm, with intact mucus film). Stool samples were divided into two aliquots; the first aliquot was processed for coproscopy using the Mini Parasep® SF stool concentrator kit (Apacor Ltd., United Kingdom) following the manufacturer's protocol, while the second aliquot was suspended in 95% ethanol and stored at -20 ºC prior to DNA extraction and downstream PCR. DNA was extracted using DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Formalin-ether concentration technique (FECT) showed the presence of hookworm ova in 20 samples (27.8%). Three PCR assays were conducted sequentially: first, canine ND5 PCR to verify the host origin and DNA amplifiability using the Dog MT_F/R primer pair; second, RTHW1 F/R primer pair PCR for screening of hookworm DNA (*Anyclostoma* spp.: 380 bp; *Necator americanus*: 485 bp); and third, RTGHF1 and RTABCR1 primer pair PCR (*A. caninum*: 545 bp; *A. ceylanicum*: 673 bp) for species identification (see

Supplementary Method). Of the 72 samples screened, 24 (33.3%) yielded amplicons of the expected size. Subsequent Sanger sequencing (Macrogen, Inc., Seoul, Republic of Korea) was successful for 18 of these positive samples (75.0%). The other six amplicons could not be sequenced despite multiple attempts. Sequencing failure in several amplicons was likely due to low DNA yield and low amounts of purified PCR product serving as template DNA (< 10 ng). Three hookworm species were identified using nBLAST alignment with reference sequences: sixteen sequences as the zoonotic intestinal hookworm *A. ceylanicum*, one sequence as the zoonotic canine hookworm *A. caninum*, and one sequence as the anthroponotic hookworm *N. americanus* (Table 1). There were no mixed-species infections recorded in the samples.

Results and Discussion

Table 1
Species identification based on nBLAST results of the ITS region sequences.

Sample Code	Identification based on BLAST match			GenBank Accession Number
	Closest match	% Identity	Species	
MGF-13	OQ772250.1	99.8%	*Necator americanus*	SRR37325607
SRT-04	PX590271.1	98.7%	*Ancylostoma caninum*	SRR37325606
SCZ-11	OR342818.1	100.0%	*Ancylostoma ceylanicum*	SRR37325597
SCZ-15	OR826944.1	100.0%	*A. ceylanicum*	SRR37325596
TPL-05	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325595
TPL-08	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325594
TPL-09	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325593
STM-03a	PQ326416.1	100.0%	*A. ceylanicum*	SRR37325592
SRT-05	DQ381541.2	99.0%	*A. ceylanicum*	SRR37325591
MGF-01	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325590
MGF-02	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325605
STM-03b	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325604
STM-07	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325603
STM-08	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325602
STM-10	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325601
STM-11	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325600
STM-13	DQ381541.2	100.0%	*A. ceylanicum*	SRR37325599
STM-14	DQ381541.2	99.0%	*A. ceylanicum*	SRR37325598

A. ceylanicum was the most commonly identified hookworm species in the collected dog stool samples from the environment, contributing additional evidence to its importance as a locally relevant zoonotic species (Inpankaew et al. 2014). In Southeast Asia, *A. ceylanicum* has been increasingly documented in both dogs and humans, particularly in rural communities where shared environments are common (Inpankaew et al. 2014). Studies from neighboring ASEAN countries have similarly demonstrated that *A. ceylanicum*, rather than *A. caninum*, predominates in free-roaming dog populations (Kladkempetch et al. 2020; Tahar et al. 2025), with parallel detection in human infections, reinforcing its role as a reservoir-transmitted hookworm. These findings align with recent reports that recognize it as an endemic burden (Inpankaew et al. 2014; Tenorio 2023) and suggest that the species is widespread but frequently

overlooked due to gaps in molecular surveillance. Although this study did not directly assess soil, canine feces are major contributors to environmental hookworm larvae and to potential human exposure (Mationg et al. 2021; Nath et al. 2023).

The identification of *N. americanus* in a single environmental canine fecal sample is more plausibly attributable to environmental contamination of human origin, where free-roaming dogs have access to human fecal material and can act as mechanical vectors or engage in coprophagy (Boyko et al. 2020; Bourgoin et al. 2022; Mawrie et al. 2026). Such infections are not considered part of the parasite's normal transmission cycle, and there is little evidence that dogs function as sustained reservoir hosts in most settings. Instead, canine detection is more consistent with reverse zoonotic exposure, including coprophagic behavior or mechanical passage of eggs following ingestion of contaminated material (Boyko et al. 2020; Mawrie et al. 2026). Its presence may therefore serve as an indicator of shared environmental exposure and limited sanitation infrastructure in the study area (Calvopina et al. 2024).

A. caninum was also identified in only one sample. Unlike *A. ceylanicum*, which readily infects both humans and animals, *A. caninum* is primarily maintained within canine populations and is rarely associated with human disease (Nath et al. 2023). The long-standing assumption that *A. caninum* is the principal canine hookworm in the Philippines is increasingly challenged by emerging molecular surveillance. Our findings, consistent with Aula et al. (2022), demonstrate a shift toward *A. ceylanicum* as the predominant species. This transition is of significant public health concern, as *A. ceylanicum* is the only common canine hookworm molecularly verified to establish patent infections in humans within high-interaction communities (Tenorio 2023). This observed prevalence mirrors the wider Southeast Asian context, where *A. ceylanicum* has surpassed *A. caninum* as the most frequently detected hookworm in domestic dog populations (Tahar et al. 2025). The comparatively low frequency of *A. caninum* relative to *A. ceylanicum* may reflect methodological factors influencing detectability and identification in fecal-based surveillance, including differences in parasite burden, egg shedding, and mixed-species infections (Geurden et al. 2008; Levecke et al. 2009).

Methodological considerations, such as primer-template compatibility and DNA quality, may have influenced the observed species distribution. The primers targeting the ITS region used here were previously validated for *A. ceylanicum* and *A. caninum* (Inpankaew et al. 2014). However, in Sanger sequencing, mixed-template samples can preferentially amplify DNA from the more abundant species, resulting in PCR amplification bias. Variations in parasite burden, DNA preservation, and environmental sample quality are recognized challenges in molecular analyses of field-collected feces and may contribute to underrepresentation of less abundant species (Geurden et al. 2008; Levecke et al. 2009; Sumboh et al. 2025).

Furthermore, Sanger sequencing generates a single consensus sequence and may mask low-abundance species in mixed infections (Geurden et al. 2008; Levecke et al. 2009). Future research employing next-generation sequencing (NGS) would allow for deep amplicon sequencing (Satam et al. 2023) and

improved identification of minority variants, providing a more refined understanding of hookworm diversity and transmission dynamics in the region.

While the current study utilized ITS-based markers for definitive species identification, future research should incorporate mitochondrial protein-coding genes (e.g., Cox1, Cyt b, or NAD5) to facilitate high-resolution phylogenetic analysis. Their relatively low intraspecific polymorphism limits their utility in resolving fine-scale evolutionary relationships or geographical lineages. Transitioning to mitochondrial loci (Hu et al. 2002) would enable the construction of robust cladograms, allowing for a clearer understanding of where Philippine canine hookworm isolates stand phylogenetically in relation to global strains.

Although conventional coproscopy remains essential for routine screening and community-level surveillance, its inability to distinguish hookworm species limits assessment of zoonotic risk and transmission patterns. The three-stage PCR approach used in this study was not intended to replace microscopy but to complement it by enabling species-level identification. The identification of zoonotic species such as *A. ceylanicum* in environmentally deposited canine feces demonstrates the added value of PCR-based methods in revealing infections that cannot be resolved by morphology alone. This additional resolution is particularly important in shared human-animal environments, where accurate identification of contamination sources is needed to guide targeted control measures.

Differences between microscopy and PCR results, including samples that were coproscopy-positive but PCR-negative, likely reflect variation in DNA quality, environmental degradation, and the presence of non-viable eggs. These findings indicate that microscopy and molecular assays measure different aspects of infection and therefore provide complementary information. When used together, coproscopy enables rapid population-level screening, while PCR and sequencing allow confirmatory species identification for epidemiological surveillance. Together, these findings highlight the value of integrating species identification with conventional coproscopy to strengthen zoonotic hookworm surveillance and support evidence-based One Health interventions in endemic communities.

The detection of zoonotic hookworms in canine feces collected from communal environments underscores dogs' role as reservoirs of environmental contamination and potential human exposure in settings with close human-animal contact. Integrating molecular species identification with routine coproscopic surveillance can therefore enhance risk assessment, clarify transmission pathways, and support targeted One Health control strategies.

Conclusion

This study provides molecular evidence of zoonotic hookworm species circulating in environmentally deposited canine feces in Northern Philippines, with *Ancylostoma ceylanicum* identified as the predominant species. By enabling species-level identification, endpoint PCR followed by Sanger sequencing addressed a key limitation of conventional coproscopy: its inability to differentiate morphologically similar hookworm eggs. Importantly, the PCR approach used was not intended to

replace microscopy but to complement it by adding epidemiologically relevant information on zoonotic species composition.

Abbreviations

ASEAN
Association of Southeast Asian Nations
BLAST
Basic Local Alignment Search Tool
FECT
Formalin-ether concentration technique
ITS
Internal transcribed spacer
LGU
Local Government Unit
nBLAST
Nucleotide Basic Local Alignment Search Tool
ND5
NADH dehydrogenase subunit 5
NGS
Next-generation sequencing
PCR
Polymerase chain reaction
STH
Soil-transmitted helminthiasis

Declarations

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AUTHOR CONTRIBUTIONS

I.K.B.T., O.A.G.T., L.R.L., and **J.C.B.T.** conceptualized the study, designed the experimental framework, and provided overall project supervision. **A.C.K., M.J.M.E., E.P.P.** and **D.L.M.** were responsible for field investigation, including the collection, transport, and parasitological examination of stool samples. **A.J.A.D.M., A.C.K.,** and **G.I.C.** performed the methodology, including DNA extraction and PCR analysis, and managed data curation. Sequence analysis and interpretation were conducted by **A.J.A.D.M., A.C.K., I.K.B.T.** and **J.C.B.T.**. The original draft was prepared by **A.J.A.D.M.** and **G.I.C.**, and all authors contributed to the review, editing, and final approval of the manuscript.

ETHICAL DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY

All DNA sequence data used in this study are available in GenBank under the accession numbers listed in Table 1.

References

1. Aula OP, McManus DP, Weerakoon KG, Olveda R, Ross AG, Rogers MJ, Gordon CA (2020) Molecular identification of *Ancylostoma ceylanicum* in the Philippines. Parasitology 147:1718–1722. https://doi.org/10.1017/S0031182020001547
2. Bourgoin G, Callait-Cardinal M-P, Bouhsira E, Polack B, Bourdeau P, Ariza CR, Carassou L, Lienard E, Drake J (2022) Prevalence of major digestive and respiratory helminths in dogs and cats in France: results of a multicenter study. Parasites Vectors 15:314. https://doi.org/10.1186/s13071-022-05368-7
3. Boyko RH, Harrison LM, Humphries D, Galvani AP, Townsend JP, Otchere J, Wilson MD, Cappello M (2020) Dogs and pigs are transport hosts of *Necator americanus*: molecular evidence for a zoonotic mechanism of human hookworm transmission in Ghana. Zoonoses Public Health 67:474–483. https://doi.org/10.1111/zph.12708

4. Caldwell JM, Levine JF (2009) Domestic wastewater influent profiling using mitochondrial real-time PCR for source tracking animal contamination. J Microbiol Methods 77:17–22. https://doi.org/10.1016/j.mimet.2008.11.007
5. Calvopina M, Aguilar-Rodríguez D, DeGroot A, Cevallos W, Lee GO, Lopez A, Nutman TB, Levy K, Eisenberg J, Sears WJ, Cooper PJ (2024) Anthroponotic and zoonotic hookworm DNA in an indigenous community in coastal Ecuador: potential cross-transmission between dogs and humans. Pathogens 13:609. https://doi.org/10.3390/pathogens13080609
6. Dongmo CN, Jatsa HB, Tchuente L-AT (2024) Morphological identification of hookworm species in five regions of Cameroon. Helminthologia 61:224–231. https://doi.org/10.2478/helm-2024-0025
7. George S, Levecke B, Kattula D, Velusamy V, Roy S, Geldhof P, Sarkar R, Kang G (2016) Molecular identification of hookworm isolates in humans, dogs and soil in a tribal area in Tamil Nadu, India. PLoS Negl Trop Dis 10:e0004891. https://doi.org/10.1371/journal.pntd.0004891
8. Geurden T, Geldhof P, Levecke B, Martens C, Berkvens D, Casaert S, Vercruysse J, Claerebout E (2008) Mixed *Giardia duodenalis* assemblage A and E infections in calves. Int J Parasitol 38:259–264. https://doi.org/10.1016/j.ijpara.2007.07.016
9. Hu M, Chilton NB, Gasser RB (2002) The mitochondrial genomes of the human hookworms, *Ancylostoma duodenale* and *Necator americanus* (Nematoda: Secernentea). Int J Parasitol 32:145–158. https://doi.org/10.1016/S0020-7519(01)00316-2
10. Inpankaew T, Schär F, Dalsgaard A, Khieu V, Chimnoi W, Chhoun C, Sok D, Marti H, Muth S, Odermatt P, Traub RJ (2014) High Prevalence of *Ancylostoma ceylanicum* Hookworm Infections in Humans, Cambodia, 2012. Emerg Infect Dis 20:976–982. https://doi.org/10.3201/eid2006.131770
11. Kladkempetch D, Tangtrongsup S, Tiwananthagorn S (2020) *Ancylostoma ceylanicum*: the neglected zoonotic parasite of community dogs in Thailand and its genetic diversity among Asian countries. Anim (Basel) 10:2154. https://doi.org/10.3390/ani10112154
12. Levecke B, Geldhof P, Claerebout E, Dorny P, Vercammen F, Cacciò SM, Vercruysse J, Geurden T (2009) Molecular characterisation of *Giardia duodenalis* in captive non-human primates reveals mixed assemblage A and B infections and novel polymorphisms. Int J Parasitol 39:1595–1601. https://doi.org/10.1016/j.ijpara.2009.05.013
13. Mationg MLS, Williams GM, Tallo VL, Olveda RM, Aung E, Alday P, Reñosa MD, Daga CM, Landicho J, Demonteverde MP, Santos ED, Bravo TA, Bieri FA, Li Y, Clements ACA, Steinmann P, Halton K, Stewart DE, McManus DP, Gray DJ (2021) Soil-transmitted helminth infections and nutritional indices among Filipino schoolchildren. PLoS Negl Trop Dis 15:e0010008. https://doi.org/10.1371/journal.pntd.0010008
14. Mawrie UG, Manuel M, Marbaniang PJ, Sangriang IL, Laxmanan S, Ajjampur SSR, Sarkar R (2026) Zoonotic transmission of soil-transmitted helminths in a setting with close human-animal interaction: a cross-sectional pilot study from Meghalaya, India. Am J Trop Med Hyg 114:138–141. https://doi.org/10.4269/ajtmh.25-0401

15. Nath TC, Eom KS, Choe S, Park H, Lee D (2023) Molecular evidence of hookworms in public environment of Bangladesh. Sci Rep 13:133. https://doi.org/10.1038/s41598-022-26813-8
16. Pappalardo P, Hemmi JM, Machida RJ, Leray M, Collins AG, Osborn KJ (2025) Taxon-specific BLAST percent identity thresholds for identification of unknown sequences using metabarcoding. Methods Ecol Evol 16:2380–2394. https://doi.org/10.1111/2041-210X.70147
17. Satam H, Joshi K, Mangrolia U, Waghoo S, Zaidi G, Rawool S, Thakare RP, Banday S, Mishra AK, Das G, Malonia SK (2023) Next-generation sequencing technology: current trends and advancements. Biology (Basel) 12:997. https://doi.org/10.3390/biology12070997
18. Sumboh JG, Agyenkwa-Mawuli K, Schwinger E, Donkor IO, Akorli J, Dwomoh D, Ashong Y, Osabutey D, Ababio FO, Nusbaum O, Humphries D, Cappello M, Koram KA, Kwofie SK, Wilson MD (2025) Investigating environmental determinants of hookworm transmission using GPS tracking and metagenomics technologies. Am J Trop Med Hyg 112:561–570. https://doi.org/10.4269/ajtmh.24-0384
19. Tahar AS, Habeebur-Rahman SP, Yaman K, Ngui R, Lim LLA, Edwin J, Saboden NS, Manggi T, Tan CS (2025) Genetic diversity of *Ancylostoma ceylanicum* and first molecular detection of *Ancylostoma braziliense* in stray dogs from Sarawak, Malaysia. Sci Rep 15:14586. https://doi.org/10.1038/s41598-025-99092-8
20. Tenorio JCB (2023) Canine hookworms in the Philippines-very common but very much neglected in veterinary research. Front Vet Sci 10:1297962. https://doi.org/10.3389/fvets.2023.1297962

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