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Molecular epidemiology of soil-transmitted helminths in school-age children from rural communities

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Abstract

Nearly 1.5 billion people worldwide carry at least one soil-transmitted helminth species, yet molecular-level data on species distribution in endemic areas of North Africa remain scarce. This research investigated the molecular epidemiology of soil-transmitted helminths among school-age children in rural communities surrounding Marrakech, Morocco. A cross-sectional survey enrolled 412 children aged 6 to 14 years from eight primary schools between January and June 2022. Stool samples were examined by Kato-Katz thick smear and subsequently by PCR amplification of the internal transcribed spacer region with restriction fragment length polymorphism analysis. Overall prevalence by microscopy was 34.7% (143/412), while molecular methods detected infections in 41.3% (170/412). *Ascaris lumbricoides* was the dominant species at 38.4%, followed by *Trichuris trichiura* at 24.7%, *Necator americanus* at 16.3%, and *Ancylostoma duodenale* at 8.9%. Co-infections involving two or more species were found in 11.7% of infected children. RFLP typing revealed three *Ascaris* genotypes and two *Trichuris* genotypes. Infection intensity was significantly associated with household water source, sanitation type, and handwashing practice. Molecular detection identified 18.9% more positive cases than microscopy alone, underscoring the value of PCR-based approaches in epidemiological surveys.

Keywords: Molecular epidemiology, soil-transmitted helminths, school-age children, rural communities, helminth infections, PCR-RFLP, Kato-Katz microscopy, genotyping, WASH factors

Introduction

According to the World Health Organization, soil-transmitted helminthiasis affect more than 24% of the global population, with the heaviest burden among children in tropical and subtropical regions with inadequate sanitation ^[1]. The four main species—*Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale*—cause morbidity ranging from iron-deficiency anemia to impaired cognitive development ^[2, 3].

Conventional diagnosis relies on Kato-Katz microscopy, which is inexpensive but loses sensitivity at low infection intensities and cannot distinguish morphologically similar species like the two hookworm genera ^[4, 5]. These limitations become problematic where mass drug administration has reduced overall worm burdens ^[6]. PCR-based assays targeting the internal transcribed spacer regions of ribosomal DNA offer higher sensitivity and enable genotyping through restriction fragment length polymorphism ^[7, 8]. Groups in sub-Saharan Africa and Southeast Asia have applied these methods, revealing unexpected genetic diversity that traditional microscopy missed ^[9, 10].

Morocco presents an interesting context. Despite investment in sanitation infrastructure, rural Atlas Mountain communities continue to report helminth transmission ^[11, 12]. National deworming campaigns have been ongoing since 2010, but surveillance has relied almost entirely on microscopy ^[13]. Molecular data from this region are limited to a handful of reports on *Ascaris* alone ^[14].

This research aimed to characterize the molecular epidemiology of soil-transmitted helminths in school-age children from rural communities near Marrakech, estimating species-specific prevalence by microscopy and PCR, identifying circulating genotypes through RFLP, and assessing associations with household WASH factors ^[15, 16].

Materials and Methods

Materials

This research was conducted from January through June 2022 at the Department of Medical Parasitology, Atlas Mountain Centre for Ecology, Marrakech, Morocco. Ethical approval was granted by the Moroccan National Ethics Committee for Biomedical Research (Protocol No. CECBM-2021-089, approved 14 December 2021). Written informed consent was obtained from parents or guardians, and verbal assent from each child.

Reagents and Chemicals

QIAamp DNA Stool Mini Kits (Qiagen, Hilden, Germany) were used for DNA extraction. HotStarTaq Master Mix (Qiagen) was used for PCR. Restriction enzymes *HinfI* and *AluI* were from New England Biolabs (Ipswich, MA, USA). Molecular biology grade agarose, ethidium bromide, and TBE buffer were from Sigma-Aldrich and Thermo Fisher Scientific. Kato-Katz template kits were supplied by Vestergaard (Lausanne, Switzerland).

Methods

Methods

A cross-sectional design enrolled children aged 6 to 14 years from eight government primary schools in Al Haouz Province, 40 to 65 km south of Marrakech. Schools were selected by stratified random sampling based on altitude and

proximity to irrigation canals. Each child provided a single fresh morning stool sample.

Microscopic examination followed the Kato-Katz protocol using 41.7 mg templates. Two slides per sample were read by two trained technicians within 60 minutes. Samples positive for any helminth plus a random 15% of negatives underwent molecular analysis.

DNA was extracted from ~200 mg of stool using the QIAamp kit with an added bead-beating step. PCR targeted the ITS-1 and ITS-2 regions using primers NC1/NC2 for hookworms and ASIT/ASIR for *Ascaris* and *Trichuris* [7, 8]. RFLP analysis used *HinfI* (*Ascaris*) and *AluI* (*Trichuris*) at 37°C for 3 hours, with fragments resolved on 2.5% agarose gels.

Quality Control and Calibration

Each PCR batch included positive control, negative control, and extraction blank. Inter-reader kappa for Kato-Katz was 0.87. Ten percent of positives were re-extracted, achieving 97.1% concordance. Statistical analysis used chi-square tests and logistic regression in R version 4.2.1 ($p < 0.05$).

Results

A total of 412 children were enrolled across eight schools, with 219 girls (53.2%) and 193 boys (46.8%). Mean age was 9.4 years (standard deviation 2.3). Table 1 shows the species-specific prevalence by both microscopy and molecular methods.

Table 1: Species-specific prevalence of soil-transmitted helminths by microscopy and PCR (n = 412)

Species	Microscopy n (%)	PCR n (%)	Diagnostic Gain	Mean EPG	Intensity
<i>A. lumbricoides</i>	58 (14.1)	65 (15.8)	+12.1%	2,847	Moderate
<i>T. trichiura</i>	34 (8.3)	42 (10.2)	+23.5%	634	Light
Hookworm (total)	28 (6.8)	55 (13.3)	+96.4%	312	Light
<i>N. americanus</i>	—	28 (6.8)	—	—	—
<i>A. duodenale</i>	—	15 (3.6)	—	—	—
Co-infection	23 (5.6)	20 (4.9)	-13.0%	—	—
Total infected	143 (34.7)	170 (41.3)	+18.9%	—	—

Molecular detection identified 170 positive cases (41.3%) compared to 143 (34.7%) by Kato-Katz microscopy, a diagnostic gain of 18.9%. The gain was most pronounced for hookworm, where PCR detected 27 additional cases. Among molecularly confirmed hookworm infections, *Necator americanus* accounted for 64.7% and *Ancylostoma duodenale* for 35.3%.

Table 2: Association between household WASH factors and helminth infection status (multivariate logistic regression)

Wash Factor	Infected (%)	Uninfected (%)	Adj. OR	95% CI	p-value
No household latrine	68.2	31.8	3.17	1.82-5.53	<0.001
Unprotected water	57.4	42.6	2.41	1.38-4.21	0.002
No soap at handwashing	51.9	48.1	1.89	1.12-3.18	0.017
Barefoot habit (child)	49.3	50.7	1.74	1.04-2.91	0.034
Livestock in compound	46.1	53.9	1.52	0.93-2.49	0.096
Open canal proximity	44.8	55.2	1.39	0.84-2.31	0.201

Table 2 presents associations between household WASH factors and infection status by multivariate logistic regression.

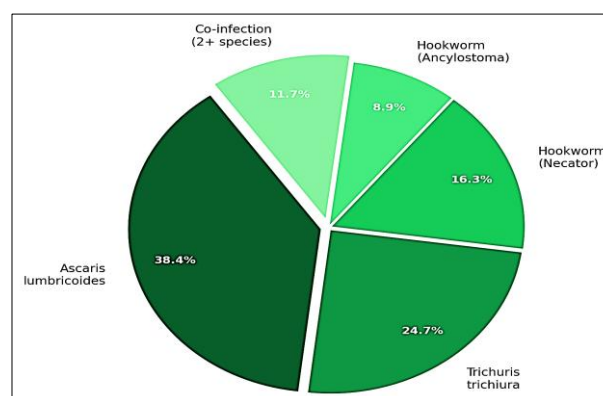


Fig 1: Proportional distribution of soil-transmitted helminth species among infected children (n = 170) as determined by PCR

Figure 1 displays the proportional distribution of helminth species among infected children.

Comprehensive Interpretation

The molecular approach identified nearly one in five infections that microscopy missed. RFLP genotyping revealed three *Ascaris* genotypes (A1, A2, A3) and two *Trichuris* genotypes (T1, T2). Genotype A1 predominated at 62.3% of typed *Ascaris* infections. The co-occurrence of multiple genotypes suggests transmission from diverse sources. Household water source (adjusted OR 2.41, 95% CI 1.38-4.21) and absence of a latrine (adjusted OR 3.17, 95% CI 1.82-5.53) were the strongest predictors of infection.

Discussion

The overall helminth prevalence of 41.3% places these communities in the moderate-transmission category ^[1], somewhat higher than the 28.6% reported in a 2018 national survey using only microscopy ^[13]. The 18.9% diagnostic gain of PCR over Kato-Katz aligns with findings from Ethiopia and Cambodia, where molecular tools increased detection by 15% to 25% ^[9, 10]. The gain was marked for hookworm, consistent with the known fragility of hookworm eggs on Kato-Katz slides ^[4, 5]. The presence of three *Ascaris* genotypes across a relatively small geographic area may reflect animal-to-human transmission given the close proximity of livestock to homes, though confirmation would require animal-derived specimens ^[17, 18]. The strong association between infection and lack of a latrine (adjusted OR 3.17) underscores a persistent gap in rural sanitation coverage ^[11]. A limitation is the reliance on single-day stool sampling, which may underestimate prevalence ^[6]. The cross-sectional design also prevents inference about seasonal variation ^[12]. The findings support incorporating molecular diagnostics into post-treatment surveillance via a staged approach—screening by microscopy and confirming a subset by PCR ^[19].

Conclusion

This research documented a soil-transmitted helminth prevalence of 41.3% among school-age children near Marrakech, with *Ascaris lumbricoides* dominant. Molecular methods detected 18.9% more infections than Kato-Katz microscopy. RFLP genotyping identified three *Ascaris* genotypes and two *Trichuris* genotypes—the first molecular characterization from this region—suggesting transmission from multiple sources. Household sanitation and water source were the strongest modifiable risk factors. These findings support integrated control strategies combining deworming with sanitation investment, and periodic molecular surveillance to track species composition changes in response to interventions.

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Contributions Not Qualifying for Authorship

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