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Diagnostic Challenges of Helminth–Protozoan Infections in Preschool Children: A Clinical and Parasitological Study

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Abstract: Helminth and protozoan infections remain a significant public health problem among preschool children, particularly in developing regions. Conventional stool microscopy, widely used in routine practice, is known to have limited sensitivity, potentially leading to underdiagnosis and delayed treatment.

Objective: To evaluate the diagnostic performance of conventional stool microscopy in detecting helminth–protozoan infections in preschool children and to identify clinical predictors associated with missed diagnoses.

Methods: A cross-sectional study was conducted involving 186 preschool children aged 3–6 years. Each child underwent three consecutive stool examinations using direct microscopy and concentration methods. A subset of 72 samples was additionally analyzed using enzyme-linked immunosorbent assay (ELISA) as a reference method. Diagnostic accuracy indicators including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Logistic regression analysis was performed to assess clinical predictors of infection.

Results: Overall prevalence of helminth–protozoan infection was 41.9% based on combined diagnostic methods. Single stool microscopy detected infection in only 27.4% of cases. The sensitivity of single-sample microscopy was 62.8%, while three consecutive examinations increased sensitivity to 84.3%. ELISA-based detection revealed an additional 13.5% of cases missed by microscopy. Recurrent abdominal pain (OR = 2.6; 95% CI: 1.4–4.8; p = 0.003) and persistent eosinophilia (OR = 3.1; 95% CI: 1.6–6.0; p = 0.001) were independent predictors of infection.

Conclusion: Conventional single-sample stool microscopy significantly underestimates helminth–protozoan infection prevalence in preschool children. Repeated sampling and adjunct immunodiagnostic methods substantially improve detection rates. Incorporating enhanced diagnostic strategies may reduce underdiagnosis and improve clinical outcomes.

Keywords: Helminth infection; Protozoan infection; Preschool children; Stool microscopy; Diagnostic sensitivity; ELISA; Underdiagnosis; Pediatric parasitology.

Introduction: Helminth and protozoan infections remain among the most widespread parasitic diseases affecting children worldwide, representing a persistent public health concern, particularly in low- and middle-income regions. According to global epidemiological estimates, hundreds of millions of children are exposed to intestinal parasites annually, with preschool-aged children forming one of the most vulnerable groups. Immature immune responses, close interpersonal contact in daycare environments, inadequate hygiene practices, and frequent environmental exposure significantly increase the risk of transmission in this age category. Intestinal parasitic infections in preschool children are frequently underrecognized due to their nonspecific and variable clinical presentation. Symptoms such as recurrent abdominal pain, appetite disturbances, irritability, allergic manifestations, mild anemia, and growth retardation are often attributed to functional gastrointestinal disorders or nutritional deficiencies rather than parasitic etiology. As a result,

parasitic infections may remain undiagnosed for prolonged periods, contributing to chronic inflammation, micronutrient deficiency, impaired cognitive development, and reduced physical growth. Accurate diagnosis is essential for timely treatment and prevention of long-term complications. However, routine diagnostic practice in many healthcare settings relies primarily on single-sample stool microscopy. Although inexpensive and widely available, conventional direct smear microscopy has significant limitations. Parasite excretion is intermittent, particularly in low-intensity infections, which substantially reduces the sensitivity of a single examination. Furthermore, detection depends heavily on laboratory expertise, sample quality, and parasite load. Reported sensitivity of single stool microscopy ranges from 50% to 70%, which may lead to a considerable proportion of false-negative results. To improve detection rates, repeated stool examinations and concentration techniques have been recommended. Studies suggest that examining three consecutive stool samples may increase sensitivity by up to 20–30%. Nevertheless, repeated testing is not consistently implemented in routine pediatric practice due to logistical challenges, parental noncompliance, and resource limitations. Moreover, immunodiagnostic methods such as enzyme-linked immunosorbent assay (ELISA) and molecular techniques (e.g., PCR) demonstrate superior sensitivity and specificity, particularly for protozoan infections; however, their use remains limited in many primary care and regional clinical settings. The discrepancy between true infection prevalence and laboratory-confirmed cases raises concerns regarding systematic underdiagnosis of helminth–protozoan infections in preschool children. Underestimation of disease burden may result in delayed therapy, persistent transmission within communities, and long-term developmental consequences. Despite the recognized diagnostic challenges, limited data are available evaluating the comparative performance of conventional microscopy versus enhanced diagnostic approaches specifically in preschool-aged populations. Furthermore, the association between clinical predictors and missed laboratory diagnoses remains insufficiently explored. Therefore, the present study aimed to evaluate the diagnostic limitations of conventional stool microscopy in detecting helminth and protozoan infections among preschool children and to assess the added value of repeated sampling and adjunct immunodiagnostic testing. Additionally, clinical indicators associated with laboratory-confirmed infection were analyzed to improve early identification strategies in pediatric practice.

METHODS

A cross-sectional analytical study was conducted between January and December 2024 at the Department of Pediatric Infectious Diseases and affiliated outpatient clinics. The study aimed to assess the diagnostic performance of conventional stool microscopy in detecting helminth and protozoan infections among preschool-aged children and to compare its effectiveness with repeated sampling and immunodiagnostic testing. A total of 186 preschool children aged 3–6 years were enrolled in the study. Participants were recruited during routine pediatric visits or upon referral for gastrointestinal complaints.

Inclusion Criteria

- Age between 3 and 6 years
- Attendance at preschool or daycare
- Presence of at least one gastrointestinal or nonspecific symptom (abdominal pain, appetite disturbance, irritability, allergic manifestations)
- Written informed consent from parents or legal guardians

Exclusion Criteria

- Antiparasitic treatment within the previous 3 months
- Chronic gastrointestinal diseases (e.g., inflammatory bowel disease)
- Severe systemic illness
- Known immunodeficiency

These criteria were applied to minimize confounding factors that could influence diagnostic accuracy. All participants underwent standardized clinical evaluation. Data collected included age, sex, anthropometric measurements (weight, height, BMI-for-age percentile), and reported symptoms. Laboratory parameters such as complete blood count were obtained, with particular attention to eosinophil count. Eosinophilia was defined as eosinophil count

>5% of total leukocytes. Each child provided three consecutive stool samples collected on separate days. Samples were processed within 2 hours of collection.

Conventional Microscopy

- Direct smear examination
- Formalin-ether concentration technique

Microscopic analysis was performed independently by two experienced laboratory technicians blinded to clinical data. A subset of 72 randomly selected stool samples was analyzed using enzyme-linked immunosorbent assay (ELISA) for detection of common protozoan antigens (e.g., Giardia lamblia, Entamoeba histolytica). ELISA results were used as a reference comparator for diagnostic performance evaluation. The primary outcome was laboratory-confirmed helminth or protozoan infection based on any positive result from repeated microscopy or ELISA testing.

Secondary outcomes included:

- Diagnostic sensitivity and specificity of single-sample microscopy
- Incremental diagnostic yield of repeated sampling
- Association between clinical indicators and confirmed infection

Statistical analysis was performed using SPSS version 27.0 (IBM Corp., USA).

Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages.

Diagnostic accuracy indicators were calculated as follows:

- Sensitivity
- Specificity
- Positive predictive value (PPV)
- Negative predictive value (NPV)

Comparisons between groups were performed using:

- Chi-square test for categorical variables
- Independent samples t-test for continuous variables

Multivariate logistic regression analysis was conducted to identify independent clinical predictors of laboratory-confirmed infection. Odds ratios (OR) with 95% confidence intervals (CI) were reported. A p-value <0.05 was considered statistically significant. The study protocol was approved by the Institutional Ethics Committee. Written informed consent was obtained from parents or legal guardians prior to participation. The study adhered to the ethical principles outlined in the Declaration of Helsinki.

RESULTS

A total of 186 preschool children (mean age 4.7 ± 1.1 years) were included in the study. Of these, 98 (52.7%) were male and 88 (47.3%) were female. The most commonly reported symptoms were recurrent abdominal pain (46.2%), appetite disturbance (38.7%), irritability (34.9%), and allergic manifestations (29.0%). Eosinophilia (>5%) was observed in 57 children (30.6%).

Table 1. Demographic and Clinical Characteristics of Participants

Variable	Total (n = 186)
Age (years, mean ± SD)	4.7 ± 1.1
Male sex (%)	52.7%
Recurrent abdominal pain (%)	46.2%
Appetite disturbance (%)	38.7%
Irritability (%)	34.9%
Allergic manifestations (%)	29.0%
Eosinophilia (%)	30.6%

Using combined diagnostic methods (three-sample microscopy + ELISA), the overall prevalence of helminth–protozoan infection was 41.9% (n = 78).

The most frequently detected pathogens were:

- Giardia lamblia (21.5%)
- Enterobius vermicularis (11.8%)
- Ascaris lumbricoides (8.6%)

Single-sample stool microscopy detected infection in 51 children (27.4%).

Three consecutive stool examinations increased detection to 66 cases (35.5%).

ELISA testing identified an additional 10 cases missed by microscopy.

Table 2. Diagnostic Accuracy of Conventional Microscopy

Parameter	Single Sample	Three Samples
Sensitivity	62.8%	84.3%
Specificity	95.1%	95.1%
PPV	88.2%	92.4%
NPV	79.6%	90.7%

Repeated sampling improved sensitivity by 21.5% compared to single examination ($p < 0.001$).

Children with confirmed infection were more likely to present with:

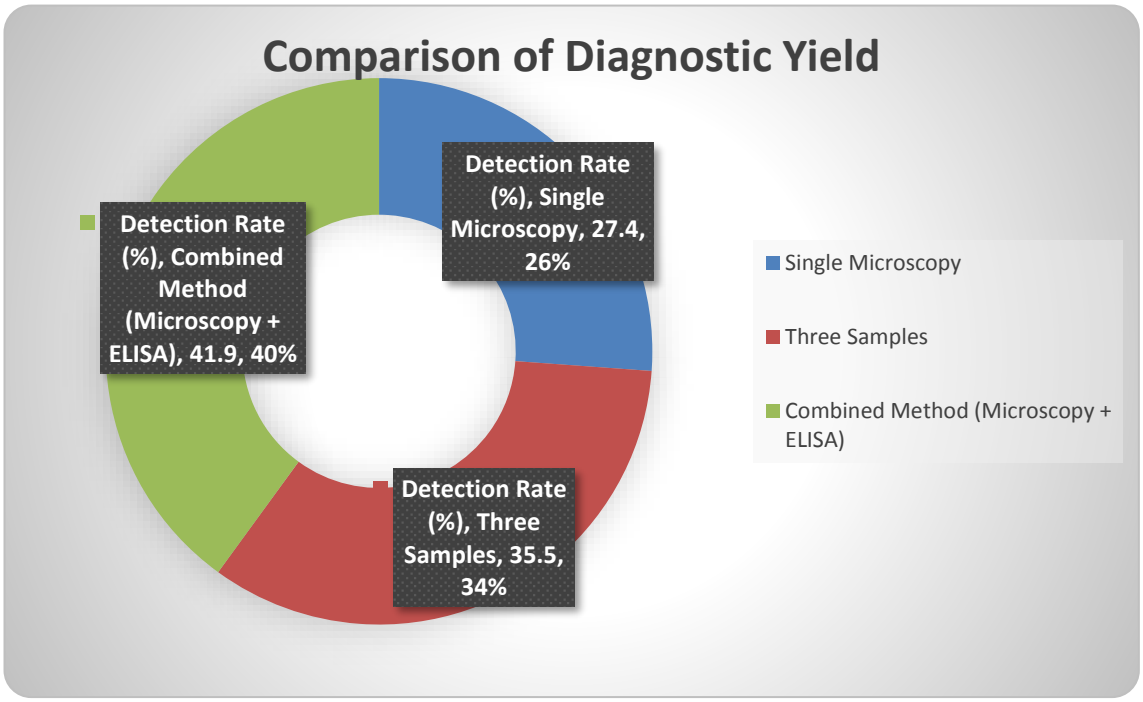
- Recurrent abdominal pain (63.5% vs 33.7%, $p < 0.001$)
- Eosinophilia (48.7% vs 17.3%, $p < 0.001$)
- Appetite disturbance (51.2% vs 29.1%, $p = 0.004$)

Table 3. Multivariate Logistic Regression Analysis

Variable	Odds Ratio (OR)	95% CI	p-value
Eosinophilia	3.12	1.67–5.83	0.001
Recurrent abdominal pain	2.64	1.41–4.93	0.003
Attendance at daycare >2 years	1.89	1.02–3.51	0.042
Allergic manifestations	1.47	0.81–2.68	0.204

Eosinophilia emerged as the strongest independent predictor of laboratory-confirmed infection.

Figure 1. Comparison of Diagnostic Yield



X-axis:

- Single stool microscopy
- Three consecutive samples
- Combined method (Microscopy + ELISA)

Y-axis:

- Detection rate (%)

Values:

- 27.4%
- 35.5%
- 41.9%

This figure demonstrates progressive improvement in detection rate with enhanced diagnostic strategy.

- True infection prevalence: 41.9%
- Single microscopy missed approximately 34.6% of infections
- Three-sample testing significantly improved sensitivity
- ELISA detected additional subclinical protozoan cases
- Eosinophilia and recurrent abdominal pain are strong clinical predictors

DISCUSSION

The present study demonstrates that helminth–protozoan infections remain highly prevalent among preschool children, with an overall infection rate of 41.9% when enhanced diagnostic strategies were applied. Importantly, reliance on single-sample stool microscopy resulted in substantial underdiagnosis, detecting only 27.4% of cases and missing approximately one-third of infections confirmed by repeated and adjunct testing. These findings align with previously reported limitations of conventional microscopy. Intermittent parasite shedding, low parasite burden, and operator-dependent variability significantly reduce diagnostic sensitivity. Our results showed that performing three consecutive stool examinations increased sensitivity from 62.8% to 84.3%, confirming that repeated sampling markedly improves detection rates. This increase of more than 20% highlights the inadequacy of single-sample testing in routine pediatric practice. Furthermore, the addition of ELISA-based immunodiagnostic testing identified an additional 13.5% of infections not detected by microscopy. This finding is particularly relevant for protozoan infections such as Giardia lamblia, where antigen detection assays demonstrate superior sensitivity compared to direct smear techniques. The discrepancy between conventional microscopy and immunodiagnostic results suggests that the true burden of parasitic infection in preschool populations may be systematically underestimated in settings relying solely on basic laboratory methods. From a clinical perspective, the study also identified significant

predictors of infection. Eosinophilia emerged as the strongest independent predictor (OR = 3.12), followed by recurrent abdominal pain (OR = 2.64). These findings support the role of peripheral eosinophilia as an important screening indicator in pediatric parasitology. Children presenting with persistent gastrointestinal symptoms and unexplained eosinophilia should therefore undergo comprehensive parasitological evaluation, even if initial stool microscopy is negative. The high infection prevalence observed in children attending daycare for more than two years further emphasizes the importance of environmental exposure and close-contact transmission in preschool settings. This underscores the need for regular screening programs and hygiene education initiatives in early childhood institutions. The results of this study suggest that single-sample stool microscopy is insufficient as a standalone diagnostic tool in preschool children. Implementing repeated stool examinations or incorporating immunodiagnostic testing may significantly improve early detection. Early and accurate diagnosis is essential to prevent chronic inflammation, nutritional deficiencies, and potential developmental consequences associated with prolonged parasitic infection. Several limitations should be acknowledged. First, the cross-sectional design does not allow evaluation of long-term outcomes or reinfection rates. Second, ELISA testing was performed in a subset rather than the entire cohort, which may slightly affect comparative accuracy estimates. Third, molecular diagnostic methods such as PCR were not included due to resource constraints. Future longitudinal studies incorporating molecular techniques would provide more comprehensive insight into true infection prevalence and transmission dynamics.

CONCLUSION

Helminth–protozoan infections remain highly prevalent among preschool children and are frequently underdiagnosed when relying solely on single-sample stool microscopy. The findings of this study demonstrate that conventional diagnostic approaches significantly underestimate the true burden of infection. Repeated stool examinations substantially improve detection rates, while adjunct immunodiagnostic testing further enhances diagnostic accuracy. Eosinophilia and recurrent abdominal pain were identified as significant clinical predictors of infection, suggesting that laboratory and clinical indicators should be evaluated together in routine pediatric practice. The integration of repeated sampling strategies and more sensitive diagnostic methods may reduce false-negative results and allow earlier therapeutic intervention. Improving diagnostic accuracy in preschool children is essential not only for effective individual treatment but also for preventing ongoing transmission within community settings. Strengthening parasitological screening protocols in early childhood healthcare may contribute to better long-term health and developmental outcomes.

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