

Original Article

# Frequency of Giardiasis in Pediatric Patients Presenting with Iron-Deficiency Anemia

Hafsa Faiz<sup>1</sup>, Zille Huma<sup>1</sup>, Muhammad Ahsan Fareed<sup>2</sup>, Abid Hussain<sup>2</sup>, Shanza Tariq<sup>3</sup>, Saad Elahi<sup>4</sup><sup>1</sup> Department of Pediatrics, Allied Hospital-I, Faisalabad, Pakistan<sup>2</sup> Faisalabad Institute of Cardiology, Faisalabad, Pakistan<sup>3</sup> Rehman Maternity Home & General Hospital, Faisalabad, Pakistan<sup>4</sup> Department of Neurology, Allied Hospital-I, Faisalabad, Pakistan\*Corresponding author: Hafsa Faiz, [hfaiz155399@gmail.com](mailto:hfaiz155399@gmail.com)**"Cite this Article"** Received: 20 October 2025; Accepted: 16 December 2025; Published: 31 December 2025**Author Contributions:** Concept: HF; Design: HF and ZH; Data Collection: HF, ZH, AH, ST, and SE; Analysis: MAF; Drafting and Critical Revision: HF, ZH, MAF, AH, ST, and SE. **Ethical Approval:** The study was approved by the Institutional Ethical Review Committee of Allied Hospital Faisalabad via approval number No48.ERC/FMU/2021-22/220. **Informed Consent:** Written informed consent was obtained from parents or legal guardians. Standard-of-care management was not withheld; participants testing positive for Giardia were managed per hospital treatment protocols; **Conflict of Interest:** The authors declare no conflict of interest. **Funding:** No external funding; **Data Availability:** Available from the corresponding author on reasonable request; **Acknowledgments:** N/A.

## ABSTRACT

**Background:** Giardia duodenalis is a common enteric protozoan in children and may coexist with nutritional anemia through diarrhea, intestinal dysfunction, and impaired nutrient absorption. **Objective:** To determine the frequency of Giardia duodenalis infection among pediatric patients with laboratory-confirmed iron-deficiency anemia and identify associated clinical correlates. **Methods:** This single-center cross-sectional study enrolled 385 children aged 6 months to 12 years with confirmed iron-deficiency anemia at the Department of Pediatric Medicine, Allied Hospital-I, Faisalabad. Stool specimens were examined using microscopy and antigen testing, with polymerase chain reaction performed in a subset where available. Giardia positivity was defined as a positive result by any performed diagnostic modality. Demographic, clinical, hematologic, and biochemical variables were analyzed using SPSS version 26. Associations were assessed using chi-square testing and multivariable logistic regression. **Results:** Giardia duodenalis was detected in 171 children, giving a composite prevalence of 44.4% (95% CI 39.5-49.4%). Diarrhea was more frequent in Giardia-positive children than Giardia-negative children (42.7% vs 19.6%; unadjusted OR 3.05, 95% CI 1.94-4.80). In adjusted analysis, diarrhea (aOR 3.02, 95% CI 1.91-4.79;  $p < 0.001$ ) and male sex (aOR 1.64, 95% CI 1.07-2.51;  $p = 0.022$ ) were independently associated with Giardia positivity. Hemoglobin and ferritin were not independently associated with infection status. **Conclusion:** Giardia infection was common among children with iron-deficiency anemia, particularly in those with diarrhea, but was not independently associated with anemia severity or ferritin level. Targeted stool testing should be considered in symptomatic pediatric IDA patients in endemic settings. **Keywords:** Giardia; Iron-Deficiency Anemia; Pediatrics; Diarrhea; Malabsorption; Stool Testing; Pakistan.

## INTRODUCTION

Iron-deficiency anemia remains one of the most frequent micronutrient disorders in childhood and is particularly burdensome in low- and middle-income countries, where nutritional insufficiency, recurrent infections, socioeconomic deprivation, and limited access to preventive health services commonly coexist. In children, iron deficiency is clinically important because it may impair physical growth, neurocognitive development, immune competence, school readiness, and overall functional health, especially when anemia occurs during early developmental periods (1-3). Although inadequate dietary iron intake and increased physiological requirements are central contributors, intestinal infections may further aggravate iron deficiency by disturbing nutrient absorption, increasing gastrointestinal losses, and sustaining inflammatory or malabsorptive states. Among these infections, Giardia duodenalis is clinically relevant because it commonly affects children, is transmitted through contaminated water and

poor sanitation, and may remain asymptomatic or present with diarrhea, abdominal pain, anorexia, vomiting, failure to thrive, or recurrent gastrointestinal disturbance (4-7).

The biological plausibility linking *Giardia* infection with iron deficiency is supported by its effects on the small intestinal mucosa. *Giardia* may impair brush-border function, alter epithelial integrity, reduce absorptive surface efficiency, and interfere with micronutrient uptake, particularly in children with symptomatic or persistent infection. Clinical and experimental literature has suggested that giardiasis may contribute to malabsorption and poor nutritional recovery in selected patients, and some studies have reported improvement in hematologic or iron-related indices after antiparasitic treatment (6-10). However, the relationship between *Giardia* carriage and iron-deficiency anemia is not uniform across populations. Community-based studies have reported variable, null, or even inverse associations after adjustment for confounding factors, suggesting that the observed relationship may depend on infection intensity, diagnostic method, host nutritional status, inflammation, dietary intake, sanitation exposure, and whether infection is symptomatic or asymptomatic (11-13). Therefore, *Giardia* should be considered a potential coexisting and clinically relevant condition in children with iron-deficiency anemia rather than an established causal explanation in every case.

In Pakistan and similar endemic settings, both pediatric iron-deficiency anemia and enteric parasitic infections are common, yet hospital-based data describing the frequency of *Giardia* infection among children presenting with laboratory-confirmed iron-deficiency anemia remain limited. This gap is important because children attending tertiary pediatric services may represent a clinically enriched population with more severe anemia, recurrent symptoms, or unresolved nutritional problems. At the same time, facility-based prevalence estimates require cautious interpretation because they may not reflect community prevalence and may be influenced by referral patterns, symptom severity, and diagnostic practices. Clarifying the burden of *Giardia* infection in this clinical group may help clinicians decide when stool testing should be incorporated into the evaluation of iron-deficiency anemia, particularly in children with diarrheal symptoms or poor response to iron therapy.

The present study was therefore conducted to determine the frequency of *Giardia duodenalis* infection among pediatric patients with laboratory-confirmed iron-deficiency anemia presenting to a tertiary care hospital in Faisalabad, Pakistan. The secondary objective was to identify demographic, clinical, and laboratory correlates of *Giardia* positivity, including age, sex, residence, diarrhea, hemoglobin, ferritin, and inflammatory status. The study addressed the research question: among children aged 6 months to 12 years with confirmed iron-deficiency anemia, what proportion have *Giardia duodenalis* detected on stool testing, and which clinical factors are independently associated with *Giardia* positivity?

## MATERIALS AND METHODS

This single-center cross-sectional observational study was conducted in the Department of Pediatric Medicine, Allied Hospital-I, Faisalabad, Pakistan, over a 12-month recruitment period defined in the approved study protocol. The cross-sectional design was selected to estimate the frequency of *Giardia duodenalis* infection among children with laboratory-confirmed iron-deficiency anemia and to examine associated clinical correlates at the time of presentation. Because exposure and outcome were assessed simultaneously, the study was designed to evaluate co-occurrence and associations rather than causal relationships between giardiasis and iron-deficiency anemia.

Children aged 6 months to 12 years presenting to the pediatric department with clinical features prompting anemia evaluation, including pallor, poor feeding, lethargy, reduced activity, or related symptoms, were screened for eligibility. Participants were included if they met laboratory criteria for iron-deficiency anemia, defined as hemoglobin below World Health Organization age-specific thresholds with ferritin consistent with depleted iron stores. Ferritin was interpreted using a threshold of <12 ng/mL in the absence of biochemical inflammation and <30 ng/mL when inflammation was present, with C-reactive protein measured to support interpretation of iron status (14,15). Children were excluded if they

had received antiparasitic therapy or iron therapy within the preceding 4 weeks, had a known hemoglobinopathy, chronic inflammatory disease, chronic renal disease, acute blood loss, or if parental or guardian consent was not provided. Consecutive eligible patients were enrolled to reduce selection by investigator preference; however, because the sample was drawn from a tertiary-care hospital, the study population was considered clinically enriched and not intended to represent community prevalence.

After written informed consent was obtained from parents or legal guardians, demographic and clinical information was collected using a standardized data collection form. Recorded variables included age, sex, place of residence, socioeconomic indicators where available, presenting gastrointestinal symptoms, symptom duration, medication history, and relevant comorbid conditions. Diarrhea was operationally recorded as caregiver-reported increased stool frequency or loose/watery stools at or near presentation. Other gastrointestinal variables included abdominal pain, anorexia, vomiting, and anal pruritus. Laboratory variables included hemoglobin concentration, ferritin level, C-reactive protein, and stool testing results for *Giardia duodenalis*. Residence was categorized as urban or rural and was included as a potential contextual variable because sanitation, water exposure, and healthcare access may differ by locality.

Caregivers were provided standardized instructions for early-morning stool collection. Each stool specimen was collected in a sterile, pre-labeled container, avoiding urine or water contamination, and transported to the hospital laboratory according to institutional procedures. Stool testing included direct microscopy for *Giardia* cysts or trophozoites and stool antigen testing for *Giardia duodenalis*. Polymerase chain reaction testing was performed where available as an additional confirmatory molecular method in a subset of specimens according to laboratory resource availability and clinical-laboratory workflow. To ensure reproducibility of case classification, participants were classified as *Giardia*-positive if any performed diagnostic modality—microscopy, antigen testing, or polymerase chain reaction—was positive. Participants were classified as *Giardia*-negative when all performed tests were negative. Because polymerase chain reaction was not performed for all participants, its result was not treated as a universal reference standard; the study therefore used a pragmatic composite diagnostic definition reflecting real-world hospital testing. Test-specific positivity was reported separately to allow interpretation of diagnostic yield by modality.

The primary outcome was the frequency of *Giardia duodenalis* infection among children with laboratory-confirmed iron-deficiency anemia, expressed as the proportion of participants meeting the composite *Giardia*-positive definition. Secondary outcomes included associations between *Giardia* positivity and demographic, clinical, and laboratory variables, particularly diarrhea, sex, age, residence, hemoglobin, ferritin, and inflammatory status. Potential confounding was addressed analytically by including clinically relevant variables in multivariable regression rather than relying only on unadjusted comparisons. Age, sex, residence, diarrhea, hemoglobin, and ferritin were selected a priori because of their biological or clinical relevance to infection risk, anemia severity, and healthcare presentation. C-reactive protein was used to support interpretation of ferritin and to conduct a sensitivity analysis restricted to participants with CRP  $\leq 5$  mg/L, thereby reducing the risk that inflammation-related ferritin elevation would obscure depleted iron stores.

The sample size was calculated using a single-population proportion formula with a 95% confidence level, anticipated *Giardia* prevalence of 51% based on previously reported data among iron-deficient children, and a 5% margin of error, yielding a required sample of 385 participants (11). Data were entered into a secure database and checked for completeness, internal consistency, and range errors before analysis. Analyses were performed using SPSS version 26. Continuous variables were summarized as mean  $\pm$  standard deviation when approximately normally distributed and as median with interquartile range when skewed. Categorical variables were summarized as frequencies and percentages. The overall *Giardia* prevalence was reported with a 95% confidence interval using binomial methods. Between-group comparisons according to *Giardia* status were performed using Student's t-test or the Mann-Whitney U

test for continuous variables and the chi-square test or Fisher's exact test for categorical variables, as appropriate. Multivariable logistic regression was used to estimate adjusted odds ratios with 95% confidence intervals for independent correlates of Giardia positivity. Missing values, if encountered, were assessed by variable and handled using complete-case analysis for the relevant comparison or model; denominators were reported where they differed from the total sample. A two-sided p-value <0.05 was considered statistically significant.

Bias and data-quality safeguards included consecutive recruitment of eligible patients, use of predefined eligibility criteria, standardized caregiver instructions for stool collection, laboratory processing according to institutional operating procedures, duplicate checks for a subset of laboratory records, and use of a prespecified composite diagnostic definition for Giardia classification. Diagnostic misclassification was minimized by reporting test-specific positivity separately and by interpreting microscopy, antigen testing, and polymerase chain reaction within their practical clinical context. The study was approved by the Institutional Ethical Review Committee of Allied Hospital Faisalabad via approval number No48.ERC/FMU/2021-22/220. Written informed consent was obtained from parents or legal guardians before enrollment. Standard clinical care was not withheld, and children with positive Giardia results were managed according to hospital treatment protocols. De-identified participant-level data and analysis code were retained securely and made available from the corresponding author on reasonable request, subject to institutional data-sharing policies.

## RESULTS

The analytic sample included 385 children with laboratory-confirmed iron-deficiency anemia. The mean age was  $5.03 \pm 3.14$  years, and 202 participants were male, representing 52.5% of the cohort. Mean hemoglobin concentration was  $8.87 \pm 1.33$  g/dL, while median ferritin was 8.2 ng/mL with an interquartile range of 6.2–10.1 ng/mL. The median C-reactive protein level was 3.0 mg/L, indicating that most participants did not have marked biochemical inflammation at presentation.

*Table 1. Baseline Characteristics of Children With Iron-Deficiency Anemia*

Characteristic	Overall Sample (n = 385)
Age, years, mean $\pm$ SD	5.03 $\pm$ 3.14
Male sex, n (%)	202 (52.5%)
Hemoglobin, g/dL, mean $\pm$ SD	8.87 $\pm$ 1.33
Ferritin, ng/mL, median (IQR)	8.2 (6.2–10.1)
C-reactive protein, mg/L, median (IQR)	3.0 (1.2–5.0)

Using the composite diagnostic definition of positivity by any performed Giardia test, Giardia duodenalis was detected in 171 of 385 children, giving an overall prevalence of 44.4% with a 95% confidence interval of 39.5% to 49.4%. Test-specific positivity varied by diagnostic modality. Microscopy detected Giardia in 98 children, corresponding to 25.5% of the full sample, while stool antigen testing detected Giardia in 150 children, corresponding to 39.0%. Polymerase chain reaction testing was performed in a subset of 88 participants and was positive in 37, giving a subset positivity rate of 42.0%. Because PCR was not performed universally, test-specific results are reported separately and were not treated as a universal reference standard.

*Table 2. Diagnostic Yield of Giardia Testing*

Diagnostic Test	Denominator	Positive, n	Positivity Rate	Interpretation
Direct microscopy	385	98	25.5%	Lowest observed yield; may miss low-burden or intermittent shedding
Stool antigen test	385	150	39.0%	Higher diagnostic yield than microscopy
PCR	88	37	42.0%	Performed in subset only; not generalizable to full cohort
Composite Giardia positivity	385	171	44.4%	Positive by any performed diagnostic modality

Clinical symptom patterns differed substantially by Giardia status. Diarrhea was reported in 73 of 171 Giardia-positive children compared with 42 of 214 Giardia-negative children, corresponding to 42.7%

versus 19.6%. This association was statistically significant, with an unadjusted odds ratio of 3.05 and 95% confidence interval of 1.94–4.80. Abdominal pain was also more frequent among Giardia-positive children, occurring in 35.1% compared with 21.0% of Giardia-negative children, with an unadjusted odds ratio of 2.03 and 95% confidence interval of 1.29–3.20. Anorexia was reported in 17.5% of Giardia-positive children and 8.4% of Giardia-negative children, showing a significant unadjusted association. Vomiting and anal pruritus were numerically more frequent in Giardia-positive children, but their confidence intervals crossed unity, indicating statistical uncertainty.

**Table 3. Gastrointestinal Symptoms by Giardia Status**

Symptom	Giardia-Positive (n = 171), n (%)	Giardia-Negative (n = 214), n (%)	Unadjusted OR (95% CI)	p-value
Diarrhea	73 (42.7%)	42 (19.6%)	3.05 (1.94–4.80)	<0.001
Abdominal pain	60 (35.1%)	45 (21.0%)	2.03 (1.29–3.20)	0.003
Anorexia	30 (17.5%)	18 (8.4%)	2.32 (1.24–4.32)	0.044
Vomiting	15 (8.8%)	10 (4.7%)	1.96 (0.86–4.48)	0.087
Anal pruritus	8 (4.7%)	3 (1.4%)	3.45 (0.90–13.22)	0.058

Laboratory comparisons showed no statistically significant difference in anemia severity or iron-store markers between Giardia-positive and Giardia-negative children. Mean hemoglobin was  $8.83 \pm 1.34$  g/dL in Giardia-positive participants and  $8.90 \pm 1.32$  g/dL in Giardia-negative participants, with a p-value of 0.315. Median ferritin was slightly lower in Giardia-positive children, at 8.0 ng/mL compared with 8.4 ng/mL in Giardia-negative children, but this difference was not statistically significant. C-reactive protein values were also similar between groups, suggesting that systemic inflammatory status did not materially differ by Giardia classification in this cohort.

**Table 4. Laboratory Comparisons by Giardia Status**

Variable	Giardia-Positive (n = 171)	Giardia-Negative (n = 214)	Mean/Median Difference	p-value
Age, years, mean $\pm$ SD	5.10 $\pm$ 3.10	5.00 $\pm$ 3.17	+0.10 years	0.720
Hemoglobin, g/dL, mean $\pm$ SD	8.83 $\pm$ 1.34	8.90 $\pm$ 1.32	-0.07 g/dL	0.315
Ferritin, ng/mL, median (IQR)	8.0 (6.0–9.4)	8.4 (6.4–10.1)	-0.4 ng/mL	0.204
C-reactive protein, mg/L, median (IQR)	3.1 (1.5–5.2)	3.0 (1.0–4.9)	+0.1 mg/L	0.820

In multivariable logistic regression adjusting for age, sex, residence, diarrhea, hemoglobin, and ferritin, diarrhea remained the strongest independent correlate of Giardia positivity. Children with diarrhea had approximately three-fold higher adjusted odds of Giardia detection compared with children without diarrhea, with an adjusted odds ratio of 3.02 and 95% confidence interval of 1.91–4.79. Male sex was also independently associated with Giardia positivity, with an adjusted odds ratio of 1.64 and 95% confidence interval of 1.07–2.51. Age, urban residence, hemoglobin, and ferritin were not independently associated with Giardia positivity after adjustment.

**Table 5. Multivariable Logistic Regression Predicting Giardia Positivity**

Predictor	Adjusted OR	95% CI	p-value
Age, per 1-year increase	0.99	0.90–1.09	0.850
Male sex vs female	1.64	1.07–2.51	0.022
Urban residence vs rural residence	1.10	0.70–1.73	0.680
Diarrhea, yes vs no	3.02	1.91–4.79	<0.001
Hemoglobin, per 1 g/dL increase	1.10	0.93–1.29	0.255
Ferritin, per 1 ng/mL increase	0.94	0.86–1.03	0.200

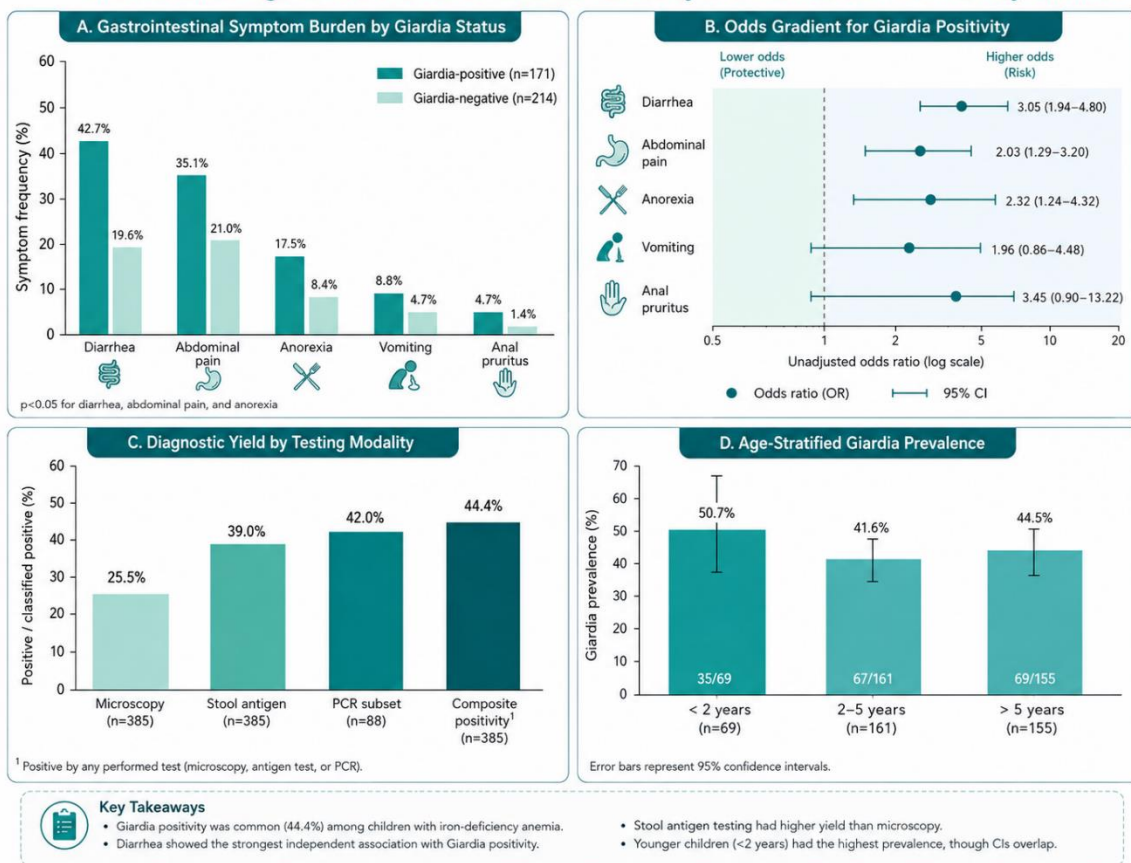
Age-stratified analysis showed that Giardia prevalence was numerically highest among children younger than 2 years, with 35 of 69 children testing positive, corresponding to 50.7%. Prevalence was 41.6% among children aged 2–5 years and 44.5% among children older than 5 years. Although younger children had the highest point estimate, confidence intervals overlapped across age strata, indicating no clear age-gradient in Giardia prevalence. In the sensitivity analysis restricted to participants with CRP  $\leq 5$  mg/L, Giardia was detected in 149 of 350 children, corresponding to a prevalence of 42.6% with a 95% confidence interval of 37.5%–47.8%. Within this low-inflammatory subgroup, ferritin did not differ significantly by Giardia status, supporting the primary finding that Giardia positivity was not independently associated with measured iron-store severity in this cross-sectional dataset.

**Table 6. Age-Stratified and CRP-Restricted Sensitivity Analyses**

Analysis Group	Giardia Positive / Total	Prevalence	95% CI	Interpretation
Age <2 years	35/69	50.7%	39.2%–62.2%	Highest point estimate
Age 2–5 years	67/161	41.6%	34.3%–49.3%	Lower point estimate
Age >5 years	69/155	44.5%	36.9%–52.4%	Intermediate point estimate
CRP ≤5 mg/L subgroup	149/350	42.6%	37.5%–47.8%	Similar to overall prevalence
Ferritin comparison in CRP ≤5 mg/L subgroup	—	—	—	No significant difference by Giardia status; p = 0.463

Overall, the results demonstrate that *Giardia duodenalis* was common among children presenting with iron-deficiency anemia, affecting nearly 4 in every 10 to 5 in every 10 children depending on diagnostic framing. The most clinically consistent association was with diarrhea, which remained independently associated with *Giardia* positivity after adjustment. Male sex also showed an independent association. In contrast, hemoglobin and ferritin did not differ significantly by *Giardia* status in either unadjusted or adjusted analyses, indicating that while *Giardia* infection was frequent in this IDA cohort, the available cross-sectional data do not establish a measurable independent association with anemia severity or iron-store depletion.

**Clinical and Diagnostic Patterns of Giardia Positivity in Pediatric Iron-Deficiency Anemia**



**Figure 5. Clinical and Diagnostic Patterns of Giardia Positivity in Pediatric Iron-Deficiency Anemia.**

Description: The figure demonstrates that *Giardia duodenalis* was detected in 44.4% of children with iron-deficiency anemia and was most strongly associated with diarrheal symptoms, which occurred in 42.7% of *Giardia*-positive children compared with 19.6% of *Giardia*-negative children. Abdominal pain and anorexia were also more frequent among infected participants, while vomiting and anal pruritus showed weaker associations. Diagnostic yield varied by testing modality, with stool antigen testing (39.0%) and PCR in the tested subset (42.0%) identifying substantially more infections than microscopy

alone (25.5%). Age-stratified analysis showed the highest prevalence among children younger than 2 years (50.7%), although confidence intervals overlapped across age groups, indicating no definitive age-dependent difference in infection frequency.

## DISCUSSION

This cross-sectional study found that *Giardia duodenalis* was detected in 171 of 385 children with laboratory-confirmed iron-deficiency anemia, corresponding to a composite prevalence of 44.4%. The finding indicates that *Giardia* infection was common in this tertiary-care pediatric IDA cohort, although the facility-based design means that this estimate should be interpreted as a clinical-service prevalence rather than a community prevalence. The most consistent clinical correlate was diarrhea, which remained independently associated with *Giardia* positivity after adjustment for age, sex, residence, hemoglobin, and ferritin. Male sex was also independently associated with higher odds of *Giardia* detection. In contrast, hemoglobin and ferritin were not significantly associated with *Giardia* positivity in unadjusted comparisons or in the adjusted model, and the CRP-restricted sensitivity analysis produced concordant findings. These results support the clinical relevance of considering *Giardia* in symptomatic children with IDA, while also showing that *Giardia* positivity in this dataset did not independently explain the severity of anemia or iron-store depletion.

The high frequency of *Giardia* detection is biologically plausible in a pediatric population from an endemic, resource-limited setting where exposure to contaminated water, sanitation limitations, and recurrent enteric infections may contribute to intestinal parasite transmission. *Giardia* is a recognized cause of diarrhea and malabsorptive gastrointestinal illness in children, and symptomatic infection may interfere with nutrient intake and absorption through epithelial disruption, brush-border dysfunction, and altered intestinal metabolism (4-7). However, the present findings should be interpreted carefully because the study was not designed to establish causality between *Giardia* and IDA. The absence of a statistically significant association with hemoglobin or ferritin suggests that *Giardia* infection may coexist with IDA in many children without necessarily being the dominant measurable determinant of anemia severity at presentation. This interpretation is consistent with prior literature showing mixed associations between giardiasis and iron deficiency, with some clinical reports suggesting hematologic improvement after treatment and other community-based studies showing null or inconsistent relationships after accounting for confounding factors (8-13).

Diarrhea showed the strongest and most precise association with *Giardia* positivity. Children with diarrhea had approximately three-fold higher adjusted odds of *Giardia* detection, indicating that diarrheal symptoms may be a useful clinical marker for targeted stool evaluation in children with IDA. Abdominal pain and anorexia were also more frequent in *Giardia*-positive children in unadjusted analyses, while vomiting and anal pruritus showed higher point estimates but weaker statistical certainty. These findings align with the recognized clinical spectrum of giardiasis, which commonly includes diarrhea, abdominal discomfort, anorexia, and variable gastrointestinal disturbance (16-18). The independent association with male sex may reflect sex-related differences in exposure patterns, healthcare-seeking behavior, outdoor activity, hygiene practices, or unmeasured household and environmental risks; however, this study did not collect sufficient behavioral or environmental data to determine the mechanism behind this association. Future studies should include water source, sanitation, household crowding, daycare or school exposure, dietary intake, and helminth co-infection to better explain sex-related and environmental risk patterns.

Diagnostic modality influenced the measured yield of *Giardia* detection. Direct microscopy identified fewer positive cases than stool antigen testing, while PCR showed a high positivity rate in the subset tested. This pattern is expected because microscopy is operator-dependent and may miss intermittent shedding or low parasite burden, whereas antigen-based and molecular assays generally improve detection in clinical settings (19-21). The use of a pragmatic composite diagnostic definition

strengthened clinical case detection but also requires transparent interpretation because PCR was not performed universally and diagnostic overlap between microscopy, antigen testing, and PCR was not available. Therefore, the reported 44.4% prevalence should be understood as composite positivity by any performed test rather than positivity by a single reference standard. Future studies should apply the same diagnostic protocol to all participants and report test overlap, agreement statistics, and diagnostic accuracy measures where feasible.

The study has several strengths. It included a relatively large pediatric IDA cohort, used predefined laboratory criteria for iron-deficiency anemia, measured CRP to support ferritin interpretation, and incorporated multivariable regression with clinically relevant covariates. The analysis also included sensitivity testing among participants with CRP  $\leq 5$  mg/L, which reduced the likelihood that inflammation-related ferritin changes distorted interpretation of iron stores. Nevertheless, several limitations must be acknowledged. The cross-sectional design prevents temporal or causal inference, so the study cannot determine whether Giardia preceded, contributed to, or merely coexisted with IDA. Consecutive sampling from a tertiary-care hospital may have enriched the sample for symptomatic or more clinically complex children and may limit generalizability to the wider pediatric community. PCR testing was performed only in a subset, creating potential verification bias. The study did not fully measure dietary iron intake, socioeconomic deprivation, sanitation exposure, water source, helminth co-infection, prior nutritional supplementation beyond the exclusion window, or household-level infection risk, all of which may confound the relationship between giardiasis and iron status. Finally, although ferritin was interpreted with CRP support, other inflammatory markers and more detailed iron indices were not included.

Clinically, these findings support targeted consideration of Giardia testing in children with IDA, especially when diarrhea or other gastrointestinal symptoms are present. The data do not justify a strong causal claim that Giardia is responsible for IDA severity, nor do they prove that routine screening of all children with IDA would improve hematologic outcomes. A more appropriate implication is that stool testing may be useful in symptomatic IDA patients in endemic settings, particularly when anemia is recurrent, refractory, or accompanied by gastrointestinal symptoms. Prospective studies are needed to determine whether detection and treatment of Giardia improves iron absorption, ferritin recovery, hemoglobin response, growth outcomes, and recurrence of anemia. Randomized or well-controlled interventional designs comparing iron therapy alone with iron therapy plus antiparasitic treatment in confirmed Giardia-positive IDA cases would provide stronger evidence for treatment policy and screening recommendations.

## CONCLUSION

Giardia duodenalis was common among children presenting with laboratory-confirmed iron-deficiency anemia in this tertiary-care pediatric cohort, with composite positivity observed in 44.4% of participants. Diarrhea and male sex were independently associated with Giardia positivity, whereas hemoglobin and ferritin were not significantly associated with infection status after adjustment. These findings suggest that Giardia should be considered in the diagnostic assessment of children with IDA who present with diarrheal or gastrointestinal symptoms in endemic settings. However, because the study was cross-sectional and facility-based, the results demonstrate clinical co-occurrence and associated factors rather than causality. Prospective diagnostic and interventional studies are required to determine whether systematic Giardia detection and treatment can improve iron repletion, hematologic recovery, and child health outcomes.

## REFERENCES

1. World Health Organization. Anaemia. WHO fact sheet. 2024. Available from: <https://www.who.int/news-room/fact-sheets/detail/anaemia>. Accessed 10 Oct 2025.

2. DeLoughery TG. Iron deficiency anemia. *Med Clin North Am.* 2017;101(2):319-32. doi:10.1016/j.mcna.2016.09.004.
3. Leung AKC, Lam JM, Wong AHC, Hon KL, Li X. Iron deficiency anemia: an updated review. *Curr Pediatr Rev.* 2024;20(3):339-56. doi:10.2174/1573396320666230727102042.
4. Centers for Disease Control and Prevention. About Giardia infection. 2024. Available from: <https://www.cdc.gov/giardia/about/index.html>. Accessed 10 Oct 2025.
5. Iolascon A, Andolfo I, Russo R, Sanchez M, Busti F, Swinkels DW, et al. Recommendations for diagnosis, treatment, and prevention of iron deficiency and iron deficiency anemia. *Hemasphere.* 2024;8(7):e108. doi:10.1002/hem3.108.
6. De Vizia B, Poggi V, Conenna R, Fiorillo A, Scippa L. Iron malabsorption in giardiasis. *Gut.* 1985;26(9):945-8.
7. Giallourou N, Arnold J, Stensvold CR, Elshaghabee FME, Nielsen HV, Nielsen DS, et al. Giardia hinders growth by disrupting nutrient metabolism independent of inflammatory enteropathy. *Nat Commun.* 2023;14(1):2840. doi:10.1038/s41467-023-38363-2.
8. Monajemzadeh M, Monajemzadeh S, Zarkesh MR, Mirsattari D. Comparison of iron and hematological indices in Giardia lamblia infection before and after treatment. *Med Sci Monit.* 2008;14(1):CR19-23.
9. Sousa D, Sousa B, Tavares M, Pereira F, Oliveira A. Malabsorption due to chronic giardiasis as a presenting symptom of common variable immunodeficiency. *Cureus.* 2020;12(12):e12201. doi:10.7759/cureus.12201.
10. Khalil Q, Imran M, Nasir A, Ahmad N. Giardiasis: an unusual cause of iron deficiency anemia. *J Ark Med Soc.* 2012;108(13):294-6.
11. Danquah I, Gahutu JB, Zeile I, Musemakweri A, Mockenhaupt FP. Reduced prevalence of Giardia duodenalis in iron-deficient Rwandan children. *Trop Med Int Health.* 2014;19(5):563-7. doi:10.1111/tmi.12284.
12. Morais MB, Suzuki HU, Corral JN, Machado NL, Neto UF. Asymptomatic giardiasis does not affect iron absorption in children with iron deficiency anemia. *J Am Coll Nutr.* 1996;15(5):434-8. doi:10.1080/07315724.1996.10718621.
13. Gutiérrez L, Bartelt LA. Current understanding of Giardia lamblia and pathogenesis of stunting and cognitive deficits in children. *Curr Trop Med Rep.* 2024;11(1):28-39. doi:10.1007/s40475-024-00314-2.
14. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva: World Health Organization; 2011. Available from: <https://www.who.int/publications/i/item/WHO-NMH-NHD-MNM-11.1>. Accessed 10 Oct 2025.
15. World Health Organization. WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva: World Health Organization; 2020.
16. Dunn N, Juergens AL. Giardiasis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK513239/>. Accessed 10 Oct 2025.
17. Khattak I, Khan W, Ahmad S, Khan MA, Ullah F, Ullah I, et al. Individual and community-level risk factors for giardiasis in children under five years of age in Pakistan: a prospective multi-regional study. *Children (Basel).* 2023;10(6):1087. doi:10.3390/children10061087.

18. Rahmat ZS, Mahmood S, Rasheed H, Asif M, Zahid A. The rise of diarrheal illnesses in the children of Pakistan and key determinants. *Health Sci Rep.* 2023;6:e1043. doi:10.1002/hsr2.1043.
19. Emisiko J, Wamachi A, Onyango R, Mwinzi P. Comparison of microscopy and PCR for detection of *Giardia lamblia* and *Entamoeba histolytica* in human stool specimens in a resource-limited setting in Western Kenya. *Ethiop J Health Sci.* 2020;30(6):891-8. doi:10.4314/ejhs.v30i6.6.
20. Vicente B, Simao M, Pereira A, Santos H, Costa M. Systematic review of diagnostic approaches for human *Giardia duodenalis* detection. *Diagnostics.* 2024;14(4):364. doi:10.3390/diagnostics14040364.
21. Weinreich F, Hahn A, Frickmann H, Dekker D, Poppert S, Köller T. Comparative evaluation of real-time screening PCR assays for *Giardia duodenalis* and of assays discriminating assemblages A and B. *Microorganisms.* 2022;10(7):1310. doi:10.3390/microorganisms10071310.