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Beyond Parasite Detection: Association between Intestinal Parasitic Infections, Hematological Profiles, and Gastrointestinal Symptoms among Children: A Cross-Sectional Study

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ABSTRACT:

Background: Intestinal parasites are frequently detected in children living in endemic settings, but pathogen detection does not necessarily correspond to gastrointestinal illness or measurable systemic disturbance. This study examined whether parasite-centered infection phenotypes were associated with gastrointestinal symptoms and routine hematological profiles among children in rural Madagascar.

Methods: A secondary cross-sectional analysis included 242 children younger than 15 years from three rural communities. Infection status was classified as pathogen-negative, parasite-only, bacterial-only, or mixed/complex. Firth bias-reduced logistic regression estimated adjusted associations with gastrointestinal symptoms, and multivariable linear

models with HC3 robust standard errors evaluated six hematological outcomes. Age group, sex, and study site were included as covariates, and false-discovery-rate correction was applied to hematological tests.

Results: Eighty-six children (35.5%) were pathogen-negative, 68 (28.1%) had parasite-only infection, 42 (17.4%) had bacterial-only infection, and 46 (19.0%) had mixed/complex infection. Gastrointestinal symptoms were recorded in 68 children (28.1%). Parasite-only infection was not associated with symptom status after adjustment (adjusted OR 1.01, 95% CI 0.42–2.44). Infection phenotype was not associated with white blood cell count, lymphocytes, granulocytes, hemoglobin, hematocrit, or platelets after multiplicity correction (all $q \geq 0.552$). None of the six hematological markers independently predicted gastrointestinal symptoms (all $q \geq 0.460$).

Conclusion: In this endemic pediatric population, parasite detection alone did not identify children with gastrointestinal symptoms or a distinct routine hematological profile. Gastrointestinal symptoms and standard blood-count indices therefore appear to be poor proxies for intestinal parasitic carriage, supporting laboratory-based diagnosis and more species- and intensity-specific assessment of parasite-related morbidity.

INTRODUCTION

Intestinal parasitic infections are still frequently observed in children in many low and middle-income countries where there is frequent environmental exposure due to poor sanitation, unsafe water and fecal contamination (Hajissa et al., 2022; Sartorius et al., 2021; Strunz et al., 2014). The burden is not limited to clinical manifestation of the disease as children may be infected with protozoa and helminths yet show no clinical signs at the time of testing (Kpene et al., 2020; Wilczyńska et al., 2026). This distinction is clinically important because the presence of parasites can reflect active infection, latent infection or repeated exposure and these different states may manifest with a different pattern of symptoms (Bartelt & Platts-Mills, 2016; Muhsen & Levine, 2012; Wilczyńska et al., 2026).

The gastrointestinal response to an infection thus offers one dimension of the host response to infection (Muhsen & Levine, 2012). Hematological indices may provide complementary data as some parasites can also have an impact on the level of hemoglobin (due to chronic blood losses and/or due to nutritional effects) and inflammatory or immune responses may also affect leukocytes-related parameters (Byrne et al., 2021; Loukas et al., 2016; Yanola et al., 2018). The degree of changes, on the other hand, depends on the type of parasites, intensity of the infections, nutritional status and the simultaneous presence of other infections (Byrne et al. 2021; Fauziah et al. 2022; Jourdan et al. 2018). Studies based solely on hemoglobin may thus fail to detect small trends within each of the white-cell fractions, the hematocrit or platelets (Richert et al., 2024; Yanola et al., 2018).

Madagascar is a convenient country to study these associations because the rates of intestinal parasitism, mixed enteric infections and asymptomatic carriage are known to occur in rural communities (Richert et al., 2024; Wilczyńska et al., 2026). Wilczyńska et al. (2026) reported that 242 children from three rural communities were tested for stool microscopy and molecular methods in the 2025 community survey in addition to the regular testing of hematology. The source study found co-infection and environmental exposure to be important factors and had a high burden of enteric pathogens, with the main limitation of its hematological comparisons being that they were mainly unadjusted across the infection categories (Wilczyńska et al., 2026).

A more focused analysis to see whether there is any association between parasite defined infection phenotypes and gastrointestinal symptoms or routine blood indices independent of demographic and geographic differences is therefore required. The main aim of the current study was to estimate the relationship of two intestinal infection phenotypes (parasite-only, mixed/complex) with the gastrointestinal symptom status and six hematological parameters (WBC, lymphocytes, granulocytes, Hb, HCT, platelets) with pathogen-negative children as the main reference population. An additional aim was to identify if the hematological measures on their own were related to gastrointestinal symptom status, independent of infection phenotype. It was hypothesized that those infection phenotypes with parasites would be correlated with an increased risk for

gastrointestinal symptoms and actual changes in hematological parameters and selected hematological parameters would be independently associated with the appearance of gastrointestinal symptoms.

Literature Review

Epidemiology of intestinal parasitism among children is highly variable in endemic areas due to differences in the level of exposure, sanitation, age distribution, diagnostic tools and the ecology of the parasites among children (Hajissa et al., 2022; Sartorius et al., 2021; Strunz et al., 2014). The infection caused by soil transmitted helminthes is still strongly associated with environmental contamination and repeated exposure, and the infection caused by protozoal organisms (e.g. giardiasis) may be present without causing disease in high exposure environments (Bartelt & Platts-Mills, 2016; Jourdan et al., 2018). This is of importance when clinical symptoms are used as the indicator of infection as a positive stool test may either indicate clinically important infection or subclinical infection or simply carriage in a child with other causes of clinical symptoms (Wilczyńska et al., 2026; Muhsen & Levine, 2012).

The clinical presentation is also variable. Although acute and chronic diarrhea, gastrointestinal discomfort and longer term consequences are all possible outcomes of infection with *Giardia*, it is also common for children to remain subclinical, particularly in high prevalence settings (Bartelt & Platts-Mills, 2016; Halliez & Buret, 2013; Muhsen & Levine, 2012). This makes it difficult to diagnose the organism in endemic communities since its presence does not imply that it is the cause of a child's symptoms (Bartelt & Platts-Mills, 2016). However, this can get complicated if multiple enteric organisms are identified at the same time, as there may be co-infection, which can make it hard to detect the clinical effects of each organism individually, and simple infected/uninfected comparisons are not as straightforward to interpret (Wilczyńska et al., 2026).

Hematological effects of intestinal parasites also vary dependent upon the organism. Hookworm causes chronic blood loss from the intestine which can lead to a decrease in haemoglobin concentrations; this is dependent on the worm load, the iron status, age and background nutrition (Byrne et al., 2021; Loukas et al., 2016). Other intestinal parasites can cause growth defects, nutrient uptake and/or inflammatory reactions, but do not lead to a consistent anemia phenotype (Fauziah et al., 2022; Halliez & Buret, 2013; Jourdan et al., 2018). In Madagascar, Richert et al. (2024) identified high levels of parasitic infections among children, but did not see strong associations between the presence of parasites and abnormalities in routine blood parameters, suggesting that the presence of a parasite in the blood does not always result in changes in blood parameters in community-level samples.

Overall, the literature indicates that symptoms and routine (hematological) markers are inadequate and may be complementary indicators of parasite associated morbidity (Byrne et al., 2021; Richert et al., 2024; Yanola et al., 2018). Much of the available evidence is based on unadjusted comparisons, single haematological outcomes or binary definitions of infection, which do not differentiate between a parasite-only infection and bacterial or mixed enteric infection (Richert et al., 2024; Wilczyńska et al., 2026). An adjusted phenotype-based analysis can then help determine if the signal from parasitic infection is independent of geographic or demographic variation and multiplicity control can help mitigate the risk of over-interpreting the finding of a significant signal from any one blood index.

METHODOLOGY

Study design and data source

This study was a secondary cross-sectional analysis of de-identified individual-level data collected by Wilczyńska et al. (2026) in a community-based survey. The source survey took place between August 2025 in Manerinerina, Bekoratsaka and Antanadava in the Northwest of Madagascar. The present analysis was designed to ask a more limited question than the source report: are there any intestinal infection phenotypes that are associated with gastrointestinal symptoms and common hematological parameters? Reporting was done according to the STROBE recommendations (von Elm et al., 2007) for reporting of observational studies.

Participants and clinical data

In the source survey, children under the age of 15 years were considered eligible when the epidemiological questionnaire was filled out, and a stool sample was provided, with the parent or legal guardian giving written consent (Wilczyńska et al., 2026). From 246 children registered in the study, four children were excluded due to lack of complete or matched study records, leaving 242 children. The variables available for the participants were age group, sex, study site, infection classification, status

of gastrointestinal symptom, selected environmental variables and 6 hematological indices. Status of gastrointestinal symptoms was measured as a binary variable. One participant had non-numeric values in all of the hematological fields, and was excluded from analyses that included blood measurements; no substitutions were made.

Infection phenotype and outcomes

Pathogen-negative, parasite-only (including single-parasite and multiple-parasite), bacterial-only (including single-bacterial and multiple-bacterial), and the mixed/complex infection (including the parasite–bacteria co-infection and more than two pathogens detected) were a priori aggregated from the original infection classification to reduce the sparse categories, while maintaining clinically meaningful distinctions. Children who were not exposed to pathogens were used for comparison. Main clinical endpoint was the occurrence of gastrointestinal symptoms. Hematological parameters evaluated were white blood cell count (WBC), lymphocyte count, granulocyte count, hemoglobin (HGB), hematocrit (HCT) and platelet count (PLT). Age group, sex and study site were prespecified adjustment variables since each might affect the pattern of infection, reporting of symptoms or hematological distributions.

Laboratory procedures

The laboratory procedures were carried out based on the source procedure (Wilczyńska et al., 2026). The venous blood was analyzed in turn by Mindray BC-3000 Plus automated hematology analyzer on site. One faecal sample was taken from each child. Parasitological examination was performed by direct Lugol-stained smear, Fülleborn flotation and sedimentation; and molecular assays for selected intestinal protozoa and bacterial enteropathogens. Microscopy was complemented with real-time PCR which extended the range of detection for those infections where microscopy alone was available, especially for protozoal infections (Oliva et al., 2025; Wilczyńska et al., 2026).

Statistical analysis

Categorical variables were presented in a tabular format with counts, and percentages. Several variables of blood count were right skewed and so were summarized using medians and interquartile ranges in the descriptive analyses. Wilson 95% confidence intervals (CI) were used to report the prevalence of gastrointestinal (GI) symptoms. Pearson's chi-square was used for the unadjusted analysis of the association between infection phenotype and gastrointestinal symptoms.

For the primary symptom model, adjusted odds ratios (aORs) and 95% confidence intervals were computed using Firth bias-reduced logistic regression with the infection phenotype as the main predictor, and age group, sex, and study site as covariates. In the conventional maximum-likelihood logistic regression (Heinze & Schemper, 2002), gastrointestinal symptoms were quite strongly clustered by study site, and Firth's correction was used. The contribution of infection phenotype was tested overall by a joint Wald test. For sensitivity analysis, symptom model was re-run on children from Manerinerina (where almost all symptom observations were made) adjusting for age group and sex.

Multivariable linear regression models were fit for each hematological outcome using infection phenotype, age group, sex and study site as predictors. WBC, lymphocytes and granulocytes were log transformed due to strong right skew, and reported as adjusted percentage differences from pathogen negative children, based on log transformed coefficients. HGB, HCT and PLT were modeled as they are on their original scales. For all linear models, Heteroscedasticity consistent HC3 standard errors were used. The two-by-two joint Wald test was used to test the overall effect of infection-phenotype for each blood marker; and the Benjamini–Hochberg procedure was used to control the false discovery rate for the six primary hematological tests (Benjamini & Hochberg, 1995).

Each of the six Firth logistic models included one standardized hematological marker in addition to infection phenotype, age group, sex and study site, and were examined to investigate whether any of the hematological markers were independently associated with the symptomatic presentation. Results were presented as aORs for every one SD increase in the marker and corrected for the six marker tests using the Benjamini–Hochberg method. All tests were two tailed. Effect estimates and 95% confidence intervals were highlighted in addition to p values and statistical significance was considered as $p < 0.05$ and $q < 0.05$ (false-discovery-rate) for families of hematological tests.

Ethical considerations

The source study received approval from the Service de Santé Publique du District d'Ambatoboeny, Ministry of Public Health, Madagascar (Approval No. 24/04 AB 2024), and written informed consent was obtained from a parent or legal

guardian for each participating child (Wilczyńska et al., 2026). The present study used de-identified data made publicly available with the original publication and involved no new participant contact or specimen collection.

RESULTS

Study population and infection phenotypes

The analysis included 242 children: 86 (35.5%) were pathogen-negative, 68 (28.1%) had parasite-only infection, 42 (17.4%) had bacterial-only infection, and 46 (19.0%) had mixed/complex infection. The sample contained equal numbers of girls and boys (121 each). Children aged 5–10 years formed the largest age group (110/242, 45.5%), followed by those younger than 5 years (93/242, 38.4%) and those older than 10 years (39/242, 16.1%). Gastrointestinal symptoms were recorded in 68 children (28.1%). Symptom reporting was strongly geographically clustered: 67 of 68 symptomatic children were from Manerinerina, one was from Antanadava, and none was from Bekoratsaka. Participant characteristics by infection phenotype are shown in Table 1.

Table 1. Participant characteristics according to infection phenotype.

Characteristic	Overall	Pathogen-negative	Parasite-only	Bacterial-only	Mixed/complex
Participants, n	242	86	68	42	46
Age group					
<5 years	93 (38.4%)	34 (39.5%)	22 (32.4%)	16 (38.1%)	21 (45.7%)
5–10 years	110 (45.5%)	33 (38.4%)	41 (60.3%)	17 (40.5%)	19 (41.3%)
>10 years	39 (16.1%)	19 (22.1%)	5 (7.4%)	9 (21.4%)	6 (13.0%)
Sex					
Female	121 (50.0%)	47 (54.7%)	31 (45.6%)	19 (45.2%)	24 (52.2%)
Male	121 (50.0%)	39 (45.3%)	37 (54.4%)	23 (54.8%)	22 (47.8%)
Study site					
Manerinerina	130 (53.7%)	51 (59.3%)	41 (60.3%)	19 (45.2%)	19 (41.3%)
Bekoratsaka	86 (35.5%)	27 (31.4%)	22 (32.4%)	13 (31.0%)	24 (52.2%)
Antanadava	26 (10.7%)	8 (9.3%)	5 (7.4%)	10 (23.8%)	3 (6.5%)
GI symptoms present	68 (28.1%)	25 (29.1%)	20 (29.4%)	14 (33.3%)	9 (19.6%)

Note. Percentages are column percentages. Mixed/complex includes parasite–bacteria co-infection and the source category with >2 detected pathogens.

Gastrointestinal symptoms and infection phenotype

Unadjusted gastrointestinal symptom prevalence was 29.1% (25/86) in pathogen-negative children, 29.4% (20/68) in the parasite-only group, 33.3% (14/42) in the bacterial-only group, and 19.6% (9/46) in the mixed/complex group (Figure 1). The overall unadjusted association was not statistically significant ($\chi^2=2.33$, $df=3$, $p=0.508$). Model-standardized symptom probabilities were 27.2% for pathogen-negative, 27.2% for parasite-only, 38.3% for bacterial-only, and 24.6% for mixed/complex infection, after adjustment for age group, sex, and study site.

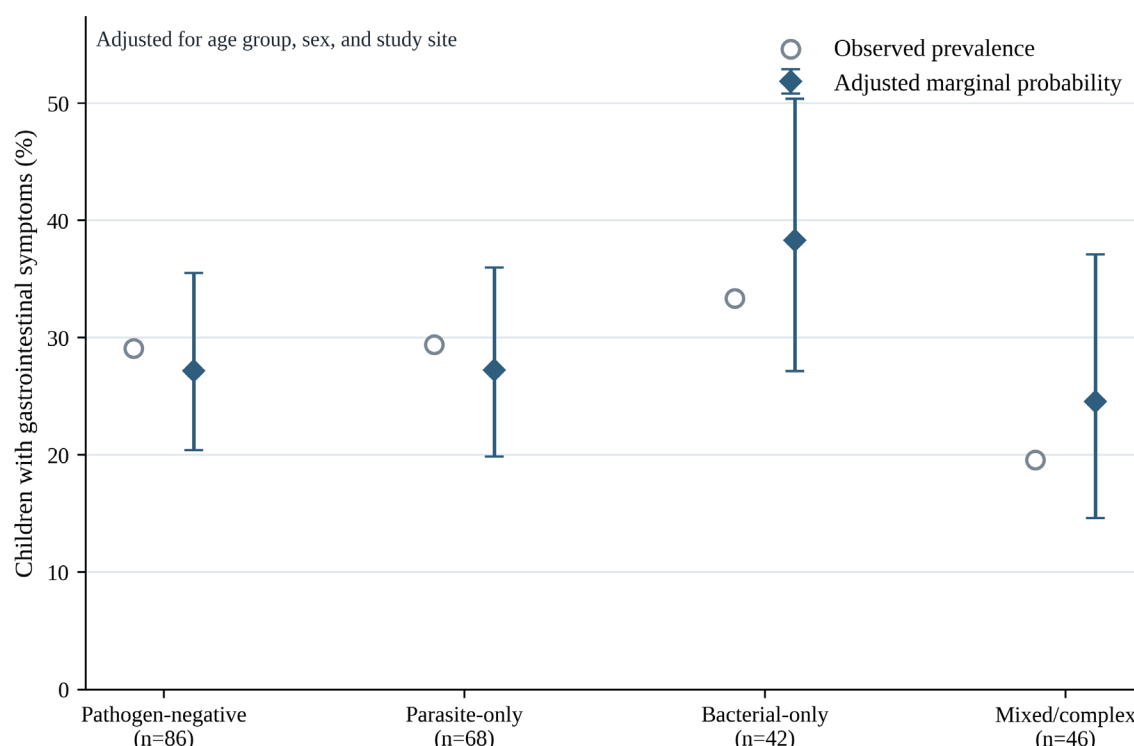


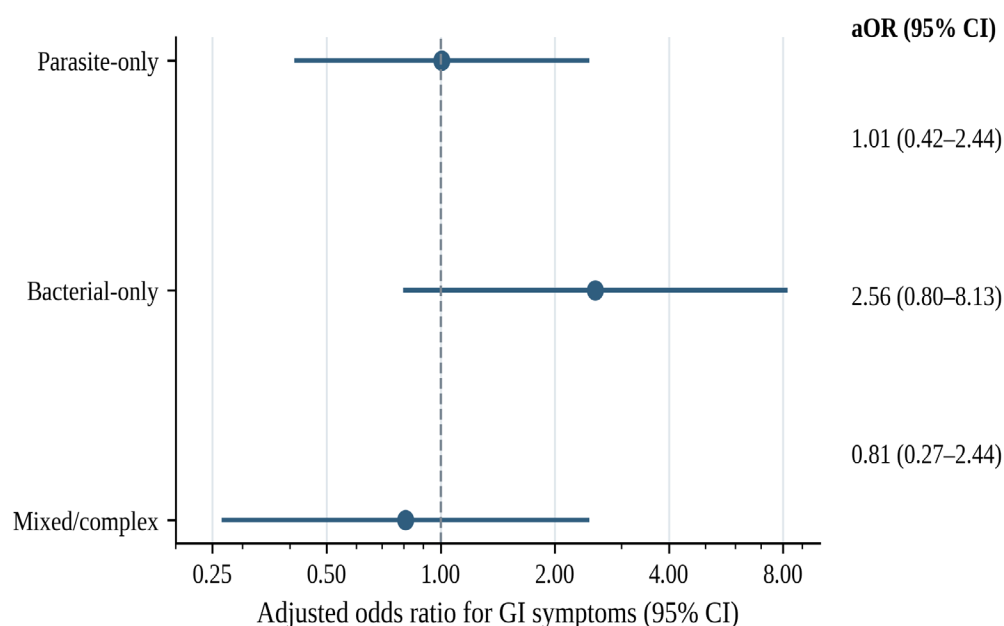
Figure 1. Observed and adjusted probability of gastrointestinal symptoms by infection phenotype. Open circles show observed prevalence; diamonds show standardized marginal probabilities from the Firth logistic model adjusted for age group, sex, and study site, with model-based 95% confidence intervals.

Table 2. Adjusted association between infection phenotype and gastrointestinal symptoms.

Infection phenotype	Adjusted OR	95% CI	p value
Parasite-only	1.01	0.42–2.44	0.988
Bacterial-only	2.56	0.80–8.13	0.111
Mixed/complex	0.81	0.27–2.44	0.704

Note. Reference group: pathogen-negative. Firth logistic regression adjusted for age group, sex, and study site. Joint Wald p value for infection phenotype = 0.339.

After adjustment for age, sex, and study site, infection phenotype was not associated with gastrointestinal symptom status as a group (joint Wald $p=0.339$). Parasite-only infection showed essentially the same odds of symptoms as the pathogen-negative reference (aOR 1.01, 95% CI 0.42–2.44, $p=0.988$). The bacterial-only estimate was higher but imprecise and did not reach statistical significance (aOR 2.56, 95% CI 0.80–8.13), while the mixed/complex estimate was below 1 with a wide confidence interval (aOR 0.81, 95% CI 0.27–2.44).



Overall phenotype test: $p=0.339$

Figure 2. Adjusted association between infection phenotype and gastrointestinal symptoms. Forest plot shows Firth logistic regression odds ratios and 95% confidence intervals adjusted for age group, sex, and study site; pathogen-negative children are the reference group.

The sensitivity analysis restricted to Manerinerina gave a similar pattern: parasite-only aOR 1.04 (95% CI 0.42–2.58), bacterial-only aOR 2.30 (95% CI 0.69–7.69), and mixed/complex aOR 0.81 (95% CI 0.26–2.51). This site-restricted analysis did not reveal a parasite-specific association that was masked by the geographic clustering of symptoms.

Hematological profiles across infection phenotypes

Hematological analyses included 241 children because one bacterial-only record lacked interpretable values for all six blood indices. In adjusted models, infection phenotype was not associated with WBC, lymphocyte count, granulocyte count, hemoglobin, hematocrit, or platelet count after false-discovery-rate correction (all $q \geq 0.552$). The parasite-only group showed a 5.1% lower adjusted WBC count and a 6.7% lower granulocyte count than pathogen-negative children, but both confidence intervals included the null. Hemoglobin and hematocrit differences were small across all infection phenotypes, and no platelet contrast was statistically significant (Table 3; Figure 3).

Table 3. Adjusted differences in hematological parameters by infection phenotype.

Marker	Contrast vs pathogen-negative	Adjusted effect	95% CI	p value	Global q
WBC	Parasite-only	-5.1%	-14.0% to 4.7%	0.298	0.552
WBC	Bacterial-only	4.1%	-9.6% to 19.9%	0.576	0.552
WBC	Mixed/complex	10.1%	-3.1% to 25.0%	0.140	0.552
Lymphocytes	Parasite-only	-2.6%	-12.3% to 8.3%	0.627	0.861
Lymphocytes	Bacterial-only	3.3%	-10.9% to 19.8%	0.663	0.861
Lymphocytes	Mixed/complex	8.0%	-7.2% to 25.7%	0.317	0.861
Granulocytes	Parasite-only	-6.7%	-19.0% to 7.4%	0.332	0.552

Marker	Contrast vs pathogen-negative	Adjusted effect	95% CI	p value	Global q
Granulocytes	Bacterial-only	6.3%	-12.2% to 28.7%	0.530	0.552
Granulocytes	Mixed/complex	12.2%	-4.6% to 32.1%	0.165	0.552
Hemoglobin	Parasite-only	0.10 g/dL	-0.34 to 0.53	0.662	0.905
Hemoglobin	Bacterial-only	0.14 g/dL	-0.29 to 0.57	0.525	0.905
Hemoglobin	Mixed/complex	0.13 g/dL	-0.31 to 0.58	0.550	0.905
Hematocrit	Parasite-only	0.27 pp	-0.89 to 1.43	0.653	0.905
Hematocrit	Bacterial-only	0.39 pp	-0.75 to 1.54	0.503	0.905
Hematocrit	Mixed/complex	0.41 pp	-0.75 to 1.58	0.487	0.905
Platelets	Parasite-only	$14.4 \times 10^9/L$	-18.9 to 47.7	0.397	0.861
Platelets	Bacterial-only	$-9.1 \times 10^9/L$	-45.7 to 27.6	0.628	0.861
Platelets	Mixed/complex	$20.8 \times 10^9/L$	-15.4 to 57.0	0.260	0.861

Note. Models adjusted for age group, sex, and study site with HC3 robust standard errors. WBC, lymphocyte, and granulocyte counts were log-transformed and are reported as percentage differences. HCT effects are percentage-point differences. Global q values apply to the joint three-degree-of-freedom infection-phenotype test for each marker after Benjamini–Hochberg correction.

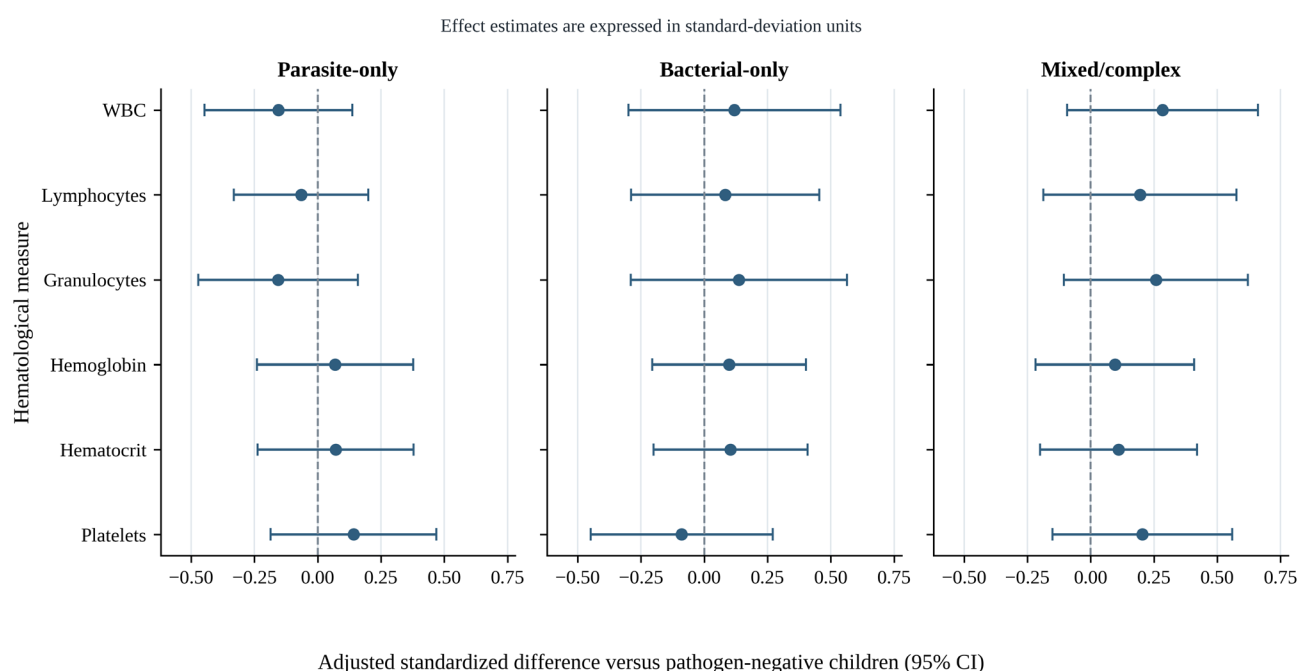


Figure 3. Adjusted standardized hematological differences across infection phenotypes. Each panel shows regression coefficients and 95% confidence intervals in standard-deviation units relative to pathogen-negative children, adjusted for age group, sex, and study site.

Hematological markers and gastrointestinal symptoms

Table 4. Adjusted association of hematological markers with gastrointestinal symptoms.

Marker	Adjusted OR per 1 SD	95% CI	p value	q value
WBC	1.25	0.82–1.90	0.307	0.460
Lymphocytes	1.08	0.71–1.65	0.711	0.827
Granulocytes	1.24	0.84–1.82	0.276	0.460
Hemoglobin	1.28	0.84–1.95	0.252	0.460
Hematocrit	1.37	0.88–2.12	0.165	0.460
Platelets	1.04	0.72–1.50	0.827	0.827

Note. Separate Firth logistic models were fitted for each standardized marker and adjusted for infection phenotype, age group, sex, and study site. *q* values were obtained with the Benjamini–Hochberg procedure across the six marker tests.

None of the six hematological markers was independently associated with gastrointestinal symptom status after adjustment for infection phenotype, age, sex, and study site (all $q \geq 0.460$). The largest point estimate was observed for hematocrit (aOR 1.37 per 1-SD increase, 95% CI 0.88–2.12), but the confidence interval was compatible with both lower and higher symptom odds. WBC, granulocytes, hemoglobin, lymphocytes, and platelet count likewise showed no statistically supported association with symptomatic presentation (Table 4; Figure 4).

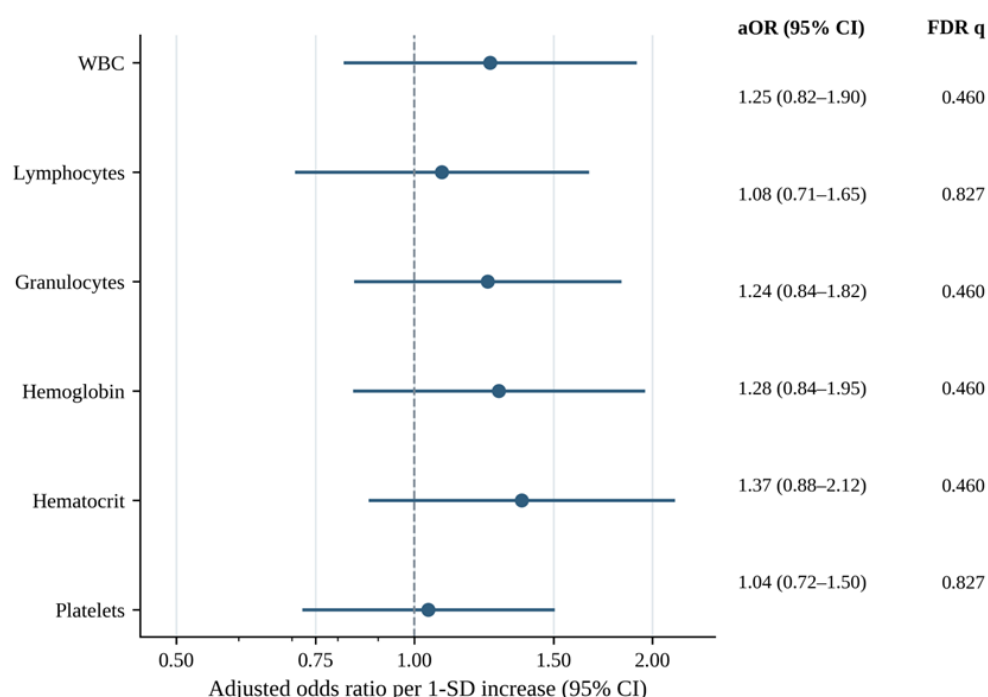


Figure 4. Adjusted associations between hematological markers and gastrointestinal symptoms. Forest plot shows odds ratios per one-standard-deviation increase in each marker with 95% confidence intervals; models adjusted for infection phenotype, age group, sex, and study site. False-discovery-rate-adjusted *q* values are shown alongside estimates.

Summary of primary findings

Across the prespecified analyses, parasite-only infection was not associated with increased gastrointestinal symptom odds, and no reproducible parasite-related shift was identified across the six routine hematological measures. Routine blood indices also

did not distinguish symptomatic from asymptomatic children after infection phenotype and core demographic factors were considered. Taken together, the prespecified models did not identify a statistically supported association linking parasite-only infection, gastrointestinal symptom status, and routine hematological variation at this cross-sectional time point.

DISCUSSION

The current study assessed if there was an independent association between parasite-specific intestinal infection phenotypes with gastrointestinal symptoms and routine hematological parameters in children from the rural villages of the northwestern Madagascar. There are three findings that are highlighted. First, there was no increased risk of gastrointestinal symptoms in the parasite only infected group when adjusted for age, sex and study site. Second, no hematological parameter measured routinely on the six parameters analyzed had a reproducible difference after Fdr correction. Third, after taking into account the infection phenotype and demographic factors the blood indices in themselves failed to discriminate symptomatic children from asymptomatic children post infection. These findings do not imply that intestinal parasites are clinically insignificant, but do show that in a very exposed paediatric population, discovering a parasite and its presence does not equal to the presence of a significant disease and that it is not synonymous with a readily measurable abnormality in normal blood counts.

The symptom results are generally similar to those obtained by Wilczyńska et al. (2026) who found a high prevalence of intestinal pathogens and asymptomatic carriers in the same rural population. In the aforementioned study, the prevalence of at least one intestinal pathogen was 64.9% of the children, with the presence of parasites in 46.7% and *Giardia intestinalis* being the most prevalent pathogen, found in 43.4% of the children (Wilczyńska et al., 2026). Significantly, most of the infections detected - including mixed infections - did not have noticeable signs of illness. The same association was found in the previous report when detailed categories of infection and symptom were cross-tabulated, unadjusted for age. The current analysis focuses on a different question: can broader clinically interpretable infection phenotypes be predictive of binary gastrointestinal-symptom outcome provided in the publicly available participant-level data, while controlling for age, sex and geographic site? As part of that, the association was not supported. The gap is not, then, merely a contradiction of the original report, but a change of analysis or adjustment.

This is important since its occurrence was highly clustered by study site. 68 children were classified as having gastrointestinal symptoms of which 67 were from Manerinerina. This type of clustering may result in unreliable conventional logistic estimates and can lead to spurious conclusions when comparing crude estimates. To minimize small-sample and separation bias, Firth regression was therefore used and study site was added to the regressed model. The sensitivity analysis performed on Manerinerina yielded no increase in the odds of any symptom with any of the parasites. This consistency indicates that the apparent null association with the parasite-only group was not entirely due to between-site imbalance; however, high geographic clustering of symptoms is still a major concern and may be due to various factors related to the local epidemic, reporting of symptoms or data collection methods that are difficult to distinguish in a cross-sectional secondary analysis.

This high prevalence of *Giardia* also offers a potential explanation for the lack of association between presence of *Giardia* and current symptoms. In 2012, Muhsen and Levine performed a systematic review and meta-analysis and found evidence for the association between *Giardia* and paediatric diarrhoea to be variable between study designs and epidemiological settings. Recent reviews have highlighted the same clinical paradox, with *Giardia* being found to cause symptomatic or persistent disease in children; however, children in high prevalence areas are often found to carry *Giardia* without symptoms of acute diarrhea (Bartelt & Platts-Mills, 2016; Halliez & Buret, 2013). Thus, the current estimate of prevalence for parasite-only infection can be accommodated in a context where the stool result is not necessarily a good predictor of the cause of a child's signs and symptoms when only one cross-sectional examination is performed.

Additionally, there was no increase in symptoms in the mixed/complex infection group and the point estimate was less than 1. This is not to be read as proof of co-infection as being protective. The confidence intervals were large, the combinations were heterogeneous and comprised biologically very different combinations, and the study was not set up to estimate organism-specific interactions. Wilczyńska et al. (2026) stressed that is often the case in these communities that more than one parasite and bacteria are detected and that the co-infection is often not noticed. In endemic locations, clinical impact of co-detection is likely to be more dependent on pathogen type and quantity as well as immune response and nutritional status of the host and time of exposure, rather than quantity of organisms alone. However, rare categories had to be collapsed to achieve statistical stability of the adjusted models and this will decrease the power to detect specific combinations which may have importance in clinical practice.

The results of the haematological examination are in good agreement with earlier studies done in Madagascar. Richert et al. (2024) in an earlier study conducted on 208 children from Ambatoboeny District revealed the presence of a high prevalence of intestinal parasites, but did not observe any relationship between anemia and low hematological parameters among the children. Similarly, the source study for the current analysis also found no significant Kruskal–Wallis differences between groups of infections for mean values of WBC, lymphocytes, granulocytes, hemoglobin, hematocrit or platelets (Wilczyńska et al., 2026). The present models have been further modified to adjust for all three variables (age, sex and study site) simultaneously and also control for false discovery rate across the six hematological outcomes. Even then, the more focused approach, there was no statistically supported shift related to the parasites. These small reductions in the counts of WBC and granulocytes in the parasite-only group were imprecise and did not withstand the multiplicity correction, and hemoglobin and hematocrit estimates were very close to the pathogen-negative reference.

This finding is in contrast to the results of similar studies in populations with greater prevalence of helminth infections, or more clinically selected giardiasis. Hookworm has the potential to lead to chronic intestinal blood loss and iron-deficiency anemia especially if the infection intensity is high (Byrne et al., 2021; Loukas et al., 2016). In turn, Jourdan et al. 2018 highlighted the fact that morbidity caused by STH infections is highly dependent on species, intensity of infection, age of the host and nutritional status. Monajemzadeh and Monajemzadeh (2008) on the other hand, reported alterations in the iron and haematological indices of children with clinically evident *Giardia* infection. Such differences highlight the need for infection composition and clinical selection differences when comparing haematological results from different studies.

Other paediatric studies also indicate a differential effect of haematological effects between organisms and markers. However, intestinal parasitic infection was not found to be associated with anemia and iron deficiency in Karen children in Thailand (Yanola et al., 2018). Eosinophils were not available as a separate variable in the available data and granulocyte is too general a measure to be equivalent to eosinophilic responses due to helminths. On the other hand, Monajemzadeh and Monajemzadeh (2008) indicated that hemoglobin, hematocrit, ferritin and iron indices levels improved following treating the children infected with *Giardia*. That study compared a clinically defined infected group longitudinally, and contained measurements of iron status which are not included here. These differences highlight an important limitation: the lack of change in routine CBC parameters does not rule out nutritional and/or inflammatory effects of parasites that can be identified by more specific biomarkers, by follow-up analysis, or by species-specific analysis.

The other models which associated blood indices with gastrointestinal symptoms, gave a similar general result. No one of the six markers could predict symptom status after adjustment. The largest point estimate was for hematocrit, but that effect was still consistent with little effect and a moderate positive association, and was not significant after multiplicity correction. This clinically means that, when using these data, routine blood counts including the white blood cell count, the differential white blood cell components, hemoglobin, hematocrit and platelet count should not be used to identify which children are currently symptomatic after exposure to an intestinal pathogen. This corroborates the general finding of most enteric infections in endemic areas being subclinical and the variability of host response to the organism, intensity of infection, nutritional status, previous exposure to the organism and concurrent diseases (Fauziah et al., 2022; Kpene et al., 2020). Routine hematology may be helpful for the assessment of anemia or systemic illness in a single child, but is not recommended here as screening tests for the presence of intestinal parasites.

The study has a number of methodological strengths. The number of participants with parasite-only infection was compared to those with bacterial-only and mixed/complex infections; all positive stool samples were not treated as equivalent. Firth bias reduction has been applied to the symptom model to overcome the near-separation of the sites caused by site clustering, and the result of the principal symptom has been tested by the sensitivity analysis based on the sites. Hematological models employed relatively large standard errors and multiplicity was corrected by the Benjamini–Hochberg procedure instead of utilizing a string of uncorrected p values. Throughout, the emphasis was on effect estimates and confidence intervals which is important in a small sample where the lack of statistical significance does not imply the absence of biological effect.

There are also a number of limitations which limit interpretation. The cross-sectional design does not allow to determine whether the pathogens were detected before symptoms or hematological changes, and intermittent shedding of parasites might result in absence of pathogens in a single stool sample. Although the public participant-level data do not allow for separate analysis of symptom type (diarrhea, abdominal pain, vomiting and constipation), symptom duration or severity. The parasite load and intensity of infection were not available on a species-specific level, and could therefore not be included in the present phenotype models. The parasite-only phenotype also included mixed samples, which comprised of organisms

with different/pathogenic or unknown pathogenic potential, reducing species specific clinical effects (Wilczyńska et al., 2026). Also, nutritional status, iron indices, eosinophil counts, inflammatory biomarkers and other infections that could have an impact on the blood count were not available for the present analysis. One of the participants had uninterpretable haematological values. Furthermore, there were small sample sizes within some phenotype groups, resulting in large confidence intervals, and loss of power to identify small effects. The high proportion of symptom positive observations made in one study site suggests that geography and/or measurement may still be confounded, despite the adjustment. Lastly, the three rural communities might not necessarily be representative of children in other endemic country or in other parts of Madagascar.

These restrictions help to outline the next step in research. Future research should be associated with quantitative or semi-quantitative assessment of parasite burden, parasite species and should include longitudinal recording of gastrointestinal symptoms. Eosinophils, ferritin, transferrin saturation, C-reactive protein, nutritional assessment and evaluation for other significant causes of anemia would have been useful to accompany the hematological assessment. Such work would help identify if specific parasites or strong infections have a significant systemic impact even though there is no overall impact of the number of parasites carried. The findings of the present study should be a warning to use either present gastrointestinal symptoms or routine blood counts as a proxy of intestinal parasitic infection in children living under the sustained exposure in clinical and public health practices. Laboratory confirmation is important when carriage is of clinical or epidemiological significance; health significance of a positive diagnosis should be interpreted in the context of the severity of symptoms, nutritional status of the recipient and characteristics of the organism.

CONCLUSION

Among children from three rural communities in northwestern Madagascar, intestinal parasite detection was not independently associated with gastrointestinal symptoms or a distinct pattern across six routine hematological measures. Symptom odds were similar for parasite-only infection as pathogen-negative and routine blood indices did not differentiate between children who were symptomatic or not when adjusted for infection phenotype, age or sex and study site. These findings match those of high asymptomatic carriage found in the source-population, and earlier data from Madagascar that revealed weak relationship between the positivity of the parasite and the conventional hematological abnormalities. Results should not be interpreted to mean that intestinal parasites do not have health effects: species, intensity of infections, nutritional status and chronicity may influence the health effects and these are not necessarily measured by a binary exposure or the standard blood count. Thus, in endemic paediatric situations, clinical manifestations and routine laboratory haematology data are not enough to serve as indicators of parasite carriage and laboratory diagnosis is key in parasite detection and epidemiological surveillance.

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